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Molecular Genetic Analysis of Durable Adult Plant Leaf Rust Resistance of the Austrian Winter Wheat Cultivar “Capo”

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Kurzfassung

Titel: Molekulargenetische Analyse der dauerhaften Resistenz der österreichischen Winterweizensorte „Capo“ gegenüber Braunrost im Erwachsenenstadium

Braunrost ist eine durch *Puccinia triticina* (früher *Puccinia recondita* f. sp. *tritici*) hervorgerufene, weit verbreitete Pilzkrankheit von Weizen, die zu beträchtlichen Ertragsverlusten führt. Capo ist eine wichtige österreichische Winterweizensorte, die kaum anfällig für *P. recondita* ist, obwohl sie seit mehr als 20 Jahren großflächig angebaut und häufig in Zuchtprogrammen verwendet wird. Frühere Tests haben gezeigt, dass Capo das Resistenzgen *Lr13* (*Lr* für ’leaf rust’) enthält, das allein aber in großen Teilen Europas nicht mehr länger wirksam ist. Ziel der vorliegenden Untersuchung war es, genauere Kenntnisse über die Vererbung der quantitativen und dauerhaften Resistenz im Erwachsenenstadium von Capo zu erlangen.

Drei von Capo abgeleitete Populationen wurden in künstlich inokulierten Feldversuchen auf mehreren Standorten in Mittel- und Osteuropa in drei bis sechs Jahren auf Braunrostresistenz getestet. Darüber hinaus wurden von diesen drei Populationen Entwicklungs- und Wuchsmerkmale sowie weitere auftretende Krankheiten erhoben. Die Population Isengrain/Capo wurde mit fast 700 molekularen Markern genetisch charakterisiert und eine Kopplungskarte erstellt. In einem ersten Validierungsschritt wurden einige Marker auch für eine zweite Population eingesetzt. Zusätzlich wurden die in wiederholten Bonituren gesammelten Daten zum Braunrostbefall dazu verwendet, die Wiederholbarkeit von Bewertungen durch dieselbe bzw. durch verschiedene Personen zu beurteilen.

In der Population Isengrain/Capo wurden mehrere quantitativ wirkende Effekte (QTL für ’quantitative trait loci’) entdeckt. Der wirksamste von Capo vererbte QTL wurde am kurzen Arm von Chromosom 3B kartiert und erklärte bis zu 15 % der phänotypischen Varianz. Dieser QTL konnte auch in der zweiten Population gefunden werden und war eng mit dem Marker *Xbarc75* gekoppelt. Der wirksamste QTL stammte von der anfälligen Sorte Isengrain und erklärte bis zu 50 % der phänotypischen Varianz. Die wahrscheinlichste Position ist das Markerintervall *XS26M14_4–Xwmc557.1* am langen Arm von Chromosom 7B. In einer nachfolgenden Haplotypenanalyse konnte nicht eindeutig geklärt werden, ob der Effekt auf 7B dem Gen *Lr14a* entspricht. Außerdem wurden mehrere QTL für andere Merkmale gefunden. Der QTL auf Chromosom 3B war auch gegenüber Gelbrost (*Puccinia striiformis* f. sp. *tritici*) wirksam.

Die entdeckten genetischen Marker für diese neuen Resistenz-QTL gegen Braunrost sind die ersten, die für mitteleuropäisches Zuchtmaterial geeignet sind. Markergestützte Selektion beschleunigt die Resistenzzüchtung und ermöglicht somit die Entwicklung von Sorten mit kombinierter und dadurch dauerhafter Braunrostresistenz.

Abstract

Leaf rust caused by *Puccinia triticina* (formerly *Puccinia recondita* f. sp. *tritici*) is a commonly occurring fungal wheat disease and leads to significant yield loss. Capo is an important Austrian winter wheat cultivar hardly susceptible to *P. recondita* despite extensive cultivation and wide use in breeding programs for more than 20 years. Previous tests have shown that Capo carries *Lr13*, a leaf rust resistance (*Lr*) gene which on its own is no longer effective against leaf rust in large parts of Europe. The aim of the study at hand was to elucidate the genetics of Capo's quantitative and durable adult plant resistance by means of molecular mapping.

Three Capo derived populations were tested for leaf rust resistance in artificially inoculated field experiments at several Middle and Eastern European locations during three to six seasons. These three populations were also assessed for developmental and morphological traits and further occurring diseases. The Isengrain/Capo population was genotyped with almost 700 molecular markers and a linkage map was calculated. In a first validation step some of the markers were also applied to a second population. Furthermore leaf rust data collected from repeated assessments were used to evaluate the inter-rater and intra-rater reproducibility.

In the Isengrain/Capo population several quantitative trait loci (QTL) for leaf rust were identified. The most effective Capo derived QTL was located on the short arm of chromosome 3B and accounted for up to 15 % of the phenotypic variance. This QTL was also detected in the second population and tightly linked to marker *Xbarc75*. The most effective QTL originated from the susceptible parent Isengrain and contributed up to 50 % of the phenotypic variance. The most likely position is the marker interval *XS26M14_4–Xwmc557.1* on the long arm of chromosome 7B. A subsequent haplotype analysis did not definitely clarify whether the effect on 7B corresponds to the gene *Lr14a*. Furthermore QTL for several other traits were identified. Notably the QTL on chromosome 3B was also effective against yellow rust (*Puccinia striiformis* f. sp. *tritici*).

The identified genetic markers for tagging these new leaf rust resistance QTL are the first suitable for Central European breeding material. Marker-assisted selection accelerates resistance breeding and enables the development of lines with combined and thus durable leaf rust resistance.

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Abbreviations

α	type I error probability or risk of the first kind (rejecting a true null hypothesis)
AFLP	amplified fragment length polymorphism
AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety)
ANOVA	analysis of variance
APR	adult plant resistance
AUDPC	area under the disease progress curve
β	type II error probability or risk of the second kind (rejecting a true alternative hypothesis)
BAES	Bundesamt für Ernährungssicherheit (Federal Office for Food Safety)
BARC	Beltsville Agricultural Research Centre
BBCH	coding system for plant growth stages (Maier 2001)
cM	centiMorgan
CTAB	cetyltrimethylammoniumbromide
CV	coefficient of variation
DArT	diversity arrays technology
df	degrees of freedom
DNA	deoxyribonucleic acid
DON	deoxynivalenol
FAO	Food and Agriculture Organization of the United Nations
F_x	x. filial-generation
$F_{x:y}$	F_x derived F_y generation
GWM	Gatersleben-Wheat-Microsatellite
IFA-Tulln	Department for Agrobiotechnology in Tulln
kb	kilo-base pair (1 kb = 1,000 bp)
LOD	logarithm of odds
LR	leaf rust*Lr* leaf rust resistance gene
LSD	least significant difference
NIL	near isogenic line
PCR	polymerase chain reaction
Pm	powdery mildew resistance gene
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
R-gene	race-specific resistance gene
RIL	recombinant inbred line
SSR	simple sequence repeat (or microsatellite)
Sr	stem (black) rust resistance gene
Stb	*Septoria tritici* blotch resistance gene
Yr	yellow (stripe) rust resistance gene

1 Introduction and Problem Description

Leaf rust caused by the fungus *Puccinia triticina* (formerly *Puccinia recondita* f. sp. *tritici*) is a regularly occurring cereal disease throughout the world. It can not just infect hexaploid or bread wheat (*Triticum aestivum* ssp. *aestivum*), but also durum wheat (*T. turgidum* ssp. *durum*) and triticale (x *Triticosecale*) and the wheat's immediate ancestors (Roelfs et al. 1992). Yield losses of 50 % and higher are possible if infection occurs at an early developmental stage (Huerta-Espino et al. 2011). In Austria losses of up to 20% are possible, especially in the eastern region due to the dry Pannonian climate (Cate and Besenhofer 2009). Development and cultivation of less susceptible wheat varieties is an economically – and moreover environmentally – sound method to reduce yield losses.

More than 70 genes conferring resistance to leaf rust (*Lr*) have been identified (McIntosh et al. 2013), but just a few have shown to provide longer lasting protection despite intensive cultivation on large acreage and are still effective in (Central) Europe (Mesterházy et al. 2002, Vida et al. 2009). Ideally a cultivar's resistance should be durable. Durable or stable resistance keeps effective for a long period of time as no physiologic races of the pathogen have emerged that are able to overcome the resistance (Birch 2001b).

The Austrian winter wheat cultivar Capo was registered in 1989 and is still the most important Austrian quality winter wheat variety regarding the certified area for the seed production and the amount of produced seeds (BAES 2012b, 2013a). A reason is its unique combination of above average yield, good bread making quality and low to medium susceptibility to various diseases. It seems to possess durable resistance to leaf rust. Since its registration more than 20 year ago, the official rating by the Austrian Agency for Health and Food Safety (AGES) dropped just from 2 to 4 in 2012 on a 1 (= absent/ very low) to 9 (= very strong) scale (BAES 2013b). Anyhow, it has been difficult if not impossible to find this resistance again in the offspring. Furore, a near relative of Capo (see Table 3), was scored with 6 in 2010 (BAES 2011), only two registered relatives were rated better than Capo: Peppino (registered in 2008) was rated with 3, Philipp (2005) with 2 (BAES 2013b).

The genetic of Capo's resistance is yet not well understood. In seedling tests Capo was susceptible, indicating that it possesses adult plant resistance only (Winzeler et al. 2000). It was postulated that Capo has *Lr13* and some additional yet unknown *Lr* gene(s), as *Lr13* on its own is not effective in Europe (Mesterházy et al. 2002). Several combinations of *Lr13* with other leaf rust resistance genes have been reported to be more effective than the individual genes alone (Kolmer 1992a, 1996, Kolmer and Liu 2001, Park et al. 2002). The combination of *Lr13* with *Lr34* was reported to provide the most durable resistance throughout the world (Kolmer 1996). In a preceding diploma project it was not verified definitely, whether Capo carries *Lr34*, but it is clearly not the exclusive source of resistance (Matiasch 2005).

Markers linked to the traits of interest facilitate breeding. Morphological markers have only limited availability, whereas molecular markers (DNA markers) are numerous (Jones et al.

1997). Especially in the case of resistance breeding molecular markers are a very powerful tool. On the one hand it is often difficult to establish reliable inoculation and scoring methods and for some diseases natural infection does not occur regularly. On the other hand molecular markers allow the fast screening of large plant numbers at an early seedling stage (Young 1999). Whether a breeding line contains just one effective resistance allele or a combination of desired alleles which increases the durability of resistance can not be determined on the field, but with molecular markers. Thus, molecular markers enable the so-called “gene pyramiding”.

2 Specific Aims and Experimental Approaches

2.1 Specific Aims

The objectives of this study were:

- To detect the chromosomal regions of the Austrian winter wheat cultivar Capo that are responsible for its long lasting adult plant resistance against leaf rust by means of molecular mapping.
- To quantify the additive and non-additive effects of these regions involved in leaf rust resistance.
- To identify molecular markers tightly linked to these regions.
- To find possible relationships between leaf rust resistance and other traits such as day of heading and plant height.
- To analyze whether the loci responsible for leaf rust resistance are only effective in the detected crossing population or in other Capo offspring, too.

2.2 Experimental Approaches

To achieve these aims, 240 recombinant inbred lines (RILs) from a cross of Capo and the susceptible French winter wheat cultivar Isengrain were evaluated for leaf rust resistance in replicated field experiments with artificial inoculation at several locations in the years 2004 to 2009. In addition, 233 RILs from an Arina/Capo and 201 RILs from a Furore/Capo cross were tested in some of these years. Day of heading, plant height and – if occurring – other plant diseases (e.g. powdery mildew severity, *Septoria* leaf blotch severity) or environmental influences such as lodging severity or frost heaving severity were evaluated.

The population Isengrain/Capo was used for the identification of those chromosomal regions that contribute to leaf rust resistance or other evaluated plant characters (quantitative trait loci, QTL). Therefore the RILs were in parallel genotyped with molecular markers: A genetic map was constructed with microsatellite (simple sequence repeats, SSR) and amplified fragment length polymorphism markers (AFLP). The joint analysis of the phenotypic data of the field experiments and these marker data by simple interval mapping enabled the detection of QTL for several traits including leaf rust resistance in the population Isengrain/Capo.

Additionally the population Arina/Capo was characterized with some SSR, preferably in the region of a detected QTL for leaf rust severity inherited from Capo. As far as possible due to the very low number of markers, a linkage map was also constructed for this RIL population. The combined analysis of field and marker data was performed by single point analysis of variance and simple interval mapping.

The main research in the lab was carried out between December 2005 and November 2009.

3 State of the Art

3.1 Wheat

The term “wheat” comprises grain crops from the genera *Triticum* and *Aegilops*. Five biological species belong to the genus *Triticum*: *T. monococcum*, *T. urartu*, *T. turgidum*, *T. timopheevii*, *T. aestivum* (Zohary et al. 2012). *T. aestivum* and *T. turgidum* are most important in present-day agriculture, *T. monococcum* was historically a wheat species of great importance (Feuillet et al. 2008). Several different classifications of wheat exist. Tables of current and historical classifications of *Triticum* and *Aegilops* as well as comparisons between the most frequently used classifications are available at the Wheat Genetic and Genomic Resources Center of the Kansas State University (http://www.k-state.edu/wgrc/Taxonomy/taxintro.html). This monograph uses the names according to the classification by van Slageren (1994). Names of other genera of the Poaceae family are used according to the USDA classification (http://plants.usda.gov/classification.html). Table 2 provides a compilation of the *Triticum* and *Aegilops* species mentioned in the monograph. The common names are taken from the GRIN Taxonomy of Plants (http://www.ars-grin.gov/cgi-bin/npgs/html/queries.pl?language=en).

Wheat (*Triticum aestivum* ssp. *aestivum* and *T. turgidum* ssp. *durum*) is among the three most important cereals worldwide. In 2011 wheat was grown on more than 220 million hectares, accounting for almost 32 % of the world’s cereal production area, being more than the area for the cultivation of maize and rice (24 %). Due to the lower average yield, the produced quantity of more than 704 million tons lagged behind that of maize (883 m. t) and rice (723 m. t). In the European Union wheat was grown in 2011 on 26 million hectares corresponding to 46 % of the total area for cereal cultivation, giving 140 million tons of grain or 48 % of the gross cereal production. In Austria wheat covered 42 % of the cereal growing area, giving 31 % of the total cereal production, but according to yield lagged with 1.8 million tons behind maize with 2.5 million tons. The world’s main wheat producing countries according to the produced quantities are China, India, the Russian Federation, the United States of America and France; according to the area of cultivation India, the Russian Federation, China, the United States of America, Kazakhstan and Australia (FAOSTAT 2013).

The oldest archeological evidence comes from the Fertile Crescent and confirms the domestication of wheat for more than 10,000 years. The hexaploid bread wheat most likely originated from the south-western corner of the Caspian Belt about 8,000 to 7,000 years ago (Zohary et al. 2012). In Fig. 1 and Table 1 the origination of wheat is displayed. *Triticum urartu* with the genomic constitution A^uA^u and a yet unknown *Aegilops* species from the *Sitopsis* section closely related to *Ae. speltoides* with the genomic constitution SS hybridized and formed the first polyploid wheat *T. turgidum* (genomic constitution BBAA). Domestication and selection resulted in emmer and durum wheat. A further hybridization step between *T. turgidum* ssp. *dicoccum* and *Ae. tauschii* having the genomic constitution DD gave rise to the hexaploid *T. aestivum* bread wheat (Feuillet et al. 2008).

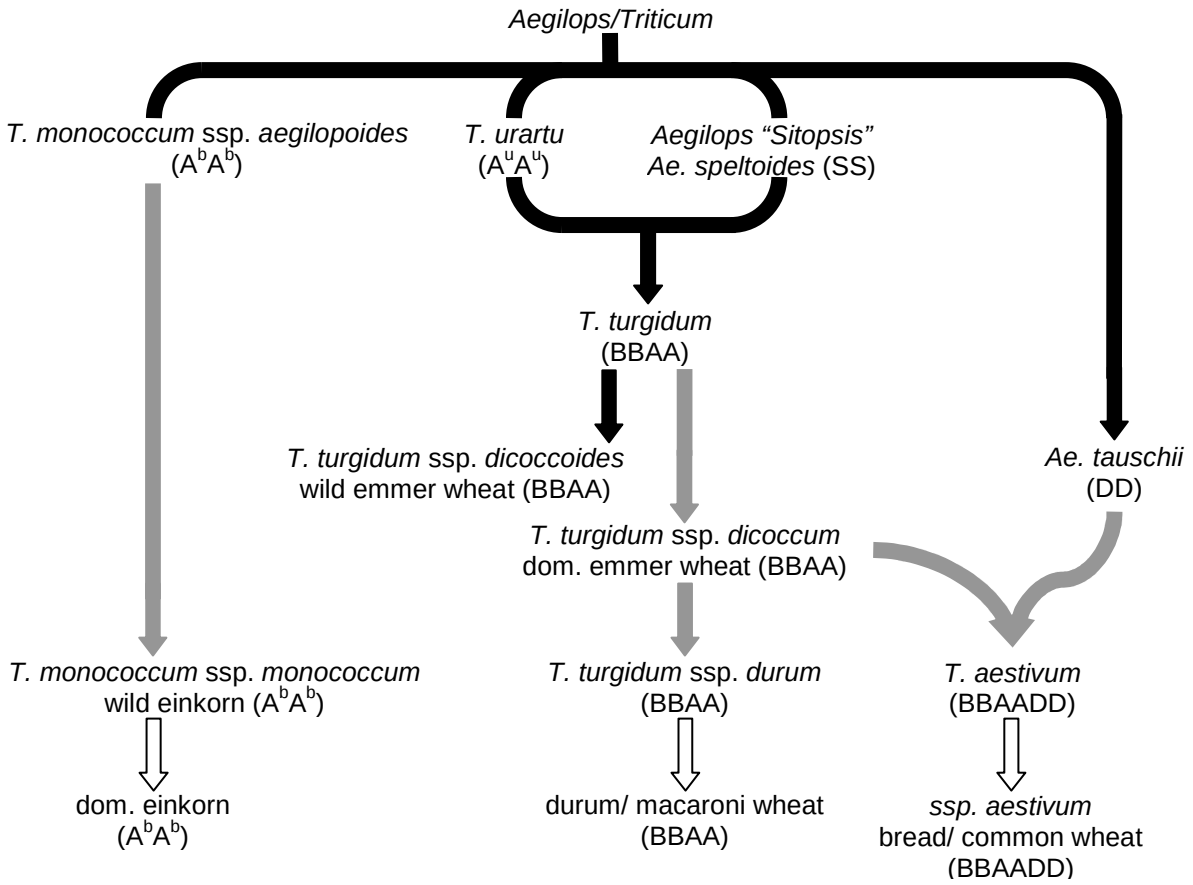

Fig. 1 **Origination of wheat: Evolutionary hybridization (black), domestication (grey) and selection steps (white arrows) (modified after Feuillet et al. 2008 and van Slageren 1994)**

Table 1 **Classification of cultivated wheats, closely related wild species (*Triticum* sp.) and the ancestors of hexaploid bread wheat (modified after van Slageren 1994 and Zohary et al. 2012). For the common names see Table 2.**

chromosome number	species	genomic constitution	wild brittle, hulled	domesticated non-brittle, hulled	domesticated free-threshing
diploid	*Ae. speltoides*	SS	all	-	-
(2*n* = 14)	*Ae. tauschii*	DD	all	-	-
	T. monococcum	A^bA^b	ssp. *aegilopoides*	ssp. *monococcum*	-
	T. urartu	A^uA^u	all	-	-
tetraploid	*T. turgidum*	BBAA	ssp. *dicoccoides*	ssp. *dicoccum*	ssp. *carthlicum*
(2*n* = 28)				ssp. *paleocolchicum*	ssp. *durum*
					ssp. *polonicum*
					ssp. *turanicum*
					ssp. *parvicoccum* [extinct]
					ssp. *turgidum*
hexaploid	*T. aestivum*	BBAADD	-	ssp. *spelta*	ssp. *aestivum*
(2n = 42)				ssp. *macha*	ssp. *compactum*
					ssp. *sphaerococcum*

Table 2 ***Triticum*** **and** ***Aegilops*** **species discussed in this monograph: classification by van Slageren (1994) vs. classification by Kimber and Sears (1987) and their common names**

van Slageren (1994)	Kimber and Sears (1987)	common name	genome
Ae. geniculata	*T. ovatum*	ovate goat grass	UUMM
Ae. kotschyi	*T. kotschyi*		UUSS
Ae. neglecta	*T. neglectum*	three-awn goat grass	UUMM
Ae. peregrina	*T. peregrinum*		SSUU
Ae. sharonensis	*T. sharonense*		$S^{sh}S^{sh}$
Ae. speltoides var. *ligustica*	*T. speltoides*	goat grass	DD
Ae. speltoides var. *speltoides*	*T. speltoides*	goat grass	DD
Ae. tauschii	*T. tauschii*	Tausch's goat grass	DD
Ae. triuncialis	*T. triunciale*	barbed/ jointed goat grass	UUCC
Ae. umbellulata	*T. umbellulatum*		UU
Ae. ventricosa	*T. ventricosum*	belly-shape hard grass	DDNN
T. aestivum ssp. *aestivum*	*T. aestivum*	bread/ common wheat	BBAADD
T. aestivum ssp. *compactum*	*T. aestivum*	club wheat	BBAADD
T. aestivum ssp. *macha*	*T. aestivum*	macha wheat	BBAADD
T. aestivum ssp. *spelta*	*T. aestivum*	spelt/ dinkel wheat	BBAADD
T. aestivum ssp. *sphaerococcum*	*T. aestivum*	Indian dwarf wheat	BBAADD
T. monococcum ssp. *aegilopoides*	*T. monococcum*	wild einkorn	A^bA^b
T. monococcum ssp. *monococcum*	*T. monococcum*	domesticated einkorn	A^bA^b
T. timopheevii ssp. *armeniacum*	*T. timopheevii*	Timopheev's wheat	GGAA
T. timopheevii ssp. *timopheevii*	*T. timopheevii*	Timopheev's wheat	GGAA
T. turgidum ssp. *carthlicum*	*T. turgidum*	Persian wheat	BBAA
T. turgidum ssp. *dicoccoides*	*T. turgidum*	wild emmer	BBAA
T. turgidum ssp. *dicoccum*	*T. turgidum*	domesticated emmer	BBAA
T. turgidum ssp. *durum*	*T. turgidum*	durum/ macaroni wheat	BBAA
T. turgidum ssp. *paleocolchicum*	*T. turgidum*	Georgian emmer	BBAA
T. turgidum ssp. *polonicum*	*T. turgidum*	Polish wheat	BBAA
T. turgidum ssp. *turanicum*	*T. turgidum*	Khorossan/ Oriental wheat	BBAA
T. turgidum ssp. *turgidum*	*T. turgidum*	rivet/ poulard wheat	BBAA
T. urartu	*T. monococcum*	red wild einkorn	A^uA^u

All of the about 500 species in 30 genera from the Triticeae tribe listed in the NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi) belong to the gene pool of wheat. Depending on the closeness of the genomic relationship, they are either part of the primary, secondary or tertiary gene pool of polyploid wheats. The primary gene pool is most easily to exploit as the species hybridize directly with cultivated wheat. It comprises hexaploid and tetraploid cultivars and landraces, early domesticated species and wild species with a polyploid BBAA genome as well as the diploid ancestors with the AA and the DD genome. Species sharing at least one homologous genome with cultivated wheat are classed among the secondary gene pool. If the gene of interest is located on one of the homologous genes, it can be introgressed into wheat by means of homologous recombination. Polyploid *Triticum* and *Aegilops* species such as *T. timopheevii* with the GGAA genome and diploid *Aegilops* species from the *Sitopsis* section with the SS genome are part of the secondary gene pool of wheat. For gene transfer from non-homologous chromosomes or species of the tertiary gene pool special methods such

as irradiation, callus culture mediated translocation or gametocidal chromosomes are necessary. Species of the tertiary gene pool – diploids as well as polyploids – share none of the genomes with wheat. In the case of wheat important species belong to the genera *Secale* (RR) and *Thinopyrum* (EE), also including important perennials (Mujeeb-Kazi and Rajaram 2002, Feuillet et al. 2008).

The gene centers of these species are the regions of choice when searching for interesting traits to be introgressed into wheat such as disease resistance. Dvorak et al. (2011) performed molecular studies of wild and domesticated emmer, hexaploid wheat and *Aegilops tauschii*. Their results indicate today's center of diversity of domesticated emmer in the Mediterranean and of wheat in Turkey, which do not coincide with Vavilov's centers of crop origin, due to gene flow from the ancestors subsequent to crop origin.

3.2 Leaf Rust of Wheat

The causal organism for leaf rust of wheat (*Triticum aestivum* ssp. *aestivum* and *T. turgidum* ssp. *durum*) is the fungus *Puccinia triticina*. Formerly it was named *Puccinia recondita* (Poelt 1985) with the forma specialis (f. sp.) *tritici* infecting wheat, durum, triticale (x *Triticosecale*) and the immediate ancestors of wheat (Roelfs et al. 1992). Further details of the taxonomic history are given in Bolton et al. (2008) and of the genus *Puccinia* in Poelt (1985) and Poelt and Zwetko (1997).

Taxonomy of *Puccinia triticina* (Bolton et al. 2008):

- kingdom: Fungi
- phylum: Basidiomycota
- class: Urediniomycetes
- order: Uredinales
- family: Pucciniaceae
- genus: *Puccinia*

Leaf rust occurs wherever wheat is grown. It is the most common and the most widely distributed rust disease of wheat, more frequent than stem rust (*P. graminis* f. sp. *tritici*) and yellow rust (*P. striiformis* f. sp. *tritici)* of wheat (Knott 1989 and Bolton et al. 2008). Its importance depends on the resistance of the predominant cultivars, the climate and the weather in the particular year (Knott 1989). Serious infection before tillering can result under extreme conditions in yield reductions of up to 90 %. Epidemics of the 20th century with yield reductions of up to 50 % have been reported from the United States of America, Canada, Mexico, Chile, South Africa and Egypt. In North-Western Europe the importance of leaf rust increased with the growing intensity (McIntosh et al. 1995, Hoffmann and Schmutterer 1999). In Austria leaf rust occurs especially in the eastern region with Pannonian climate. In years with warm weather losses of late varieties can reach 20 % (Cate and Besenhofer 2009).

3.2.1 Symptoms of Leaf Rust

Throughout the whole vegetation period, but intensified after stem elongation, symptoms of leaf rust infection develop (Cate and Besenhofer 2009). Preferably on the upper surface of the leaves circularly shaped small (about 1-2 mm in diameter) orange-brown to orange-red randomly scattered pustules, rarely arranged in rings, appear that can be wiped off (Fig. 7 on page 44). These are the uredia. Frequently these pustules are surrounded by a chlorotic halo (Knott 1989, Parry 1990, Hoffmann and Schmutterer 1999, Kolmer 2009). In the case of severe infection, pustules can cover almost the entire leaf surface (Knott 1989), and not only leaf blades and leaf sheaths (Hoffmann and Schmutterer 1999), indeed the cereal head can become infected (Murray et al. 1998). Later in the season black elongated telia appear on the lower leaf surface (Hoffmann and Schmutterer 1999).

Infection with leaf rust increases transpiration and reduces photosynthetic activity because of chlorosis, withering starting from the leaf tips, and premature defoliation, thus resulting in a decreased number of kernels per head as well as a lower thousand kernel and hectoliter weight due to shriveling of kernels. Furthermore protein content can be negatively affected (Knott 1989, Hoffmann and Schmutterer 1999, Bolton et al. 2008). If plants are heavily infested early in the season, the rooting system develops badly, growth can lag behind and tillering is reduced. Winter wheat infected in autumn can be more prone to frost heaving (Roelfs et al. 1992, Hoffmann and Schmutterer 1999). According to estimations from the United States regarding infections at the early dough stage, an increase in leaf rust severity by 1 % results in a yield loss growth of 0.42 % (Murray et al. 1998).

3.2.2 Epidemiology

Puccinia triticina is an obligate parasite that can grow on host plants only (Börner 2009). The mycelium grows in the intercellular space and haustoria take nutrients from the plant cells. Frequently the infected tissue remains green, whereas the surrounding tissue ages prematurely due to the loss of nutrients (Hoffmann and Schmutterer 1999). The fungus is macrocyclic and heteroecious (McIntosh et al. 1995). Macrocyclic fungi have five spore types: urediniospores, teliospores, basidiospores, pycniospores and aeciospores. Heteroecious means that there is an alternate host. In regions too cold for overwintering as mycelium or in the urediniospores stage, but sufficient for the teliospores, the alternate hosts are important (Knott 1989). The alternate hosts do not only provide local inoculum for infection of adjacent wheat crops, but by hosting the sexual stage of the life cycle they facilitate the development of new pathotypes. Amongst the alternate hosts of *Puccinia triticina* are species of *Thalictrum*, *Anchusa*, *Isopyrum* and *Clematis* (McIntosh et al. 1995). Fig. 2 gives the life cycle of leaf rust.

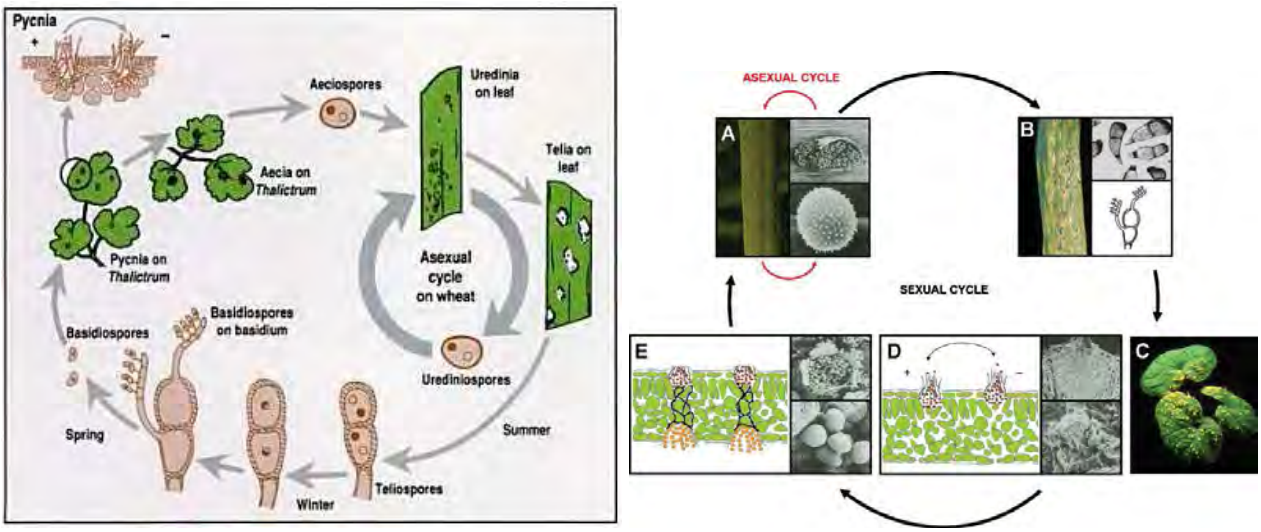

Fig. 2 Life cycle of leaf rust (left: Roelfs et al. 1992, right: Bolton et al. 2008)

Urediniospores can be carried by wind over long distances, up to several thousand kilometers. Depending on the temperature the dikaryotic urediniospores (two genetically different nuclei) can survive on stubble and dry plant parts between one to several weeks. In temperate to subtropical climate leaf rust can overwinter in the urediniospore stage. They are resistant to temperature and remain alive for months under snow. Urediniospores deposited by the wind from distant areas cause initial infections in the spring. At temperatures of 10-28°C and if free water, e.g. because of dew development, is available for about three hours, germination of the urediniospores is possible. The optimal temperature for the infection process is 16°C. In this case germination happens within one hour. Within three hours the germ tube grows until it reaches a stoma and an appressorium develops pushing an infection peg through the stoma. In the substomatal cavity vesicle develop within eight hours. From the vesicle infection hyphae grow producing haustoria mother cells. Then penetration pegs push into cells of the host and haustoria form within twelve hours. In the darkness infection develops faster. About one third of germinated urediniospores result in an infection. After about 5-8 days pustules – the uredia – become visible. In case of optimal conditions sporulation – the production of urediniospores – starts after 7-14 days. Increasing temperatures decrease latency time with an optimum at 25°C. Over a period of about three weeks one uredinium can produce about 3,000 spores per day. Thus, within two weeks heavy infestation can occur especially high in the crop canopy, spreading rapidly horizontally. Volunteer grain can be an infection reservoir and winter wheat can already be infected in the autumn. Frequently infections late in the autumn are not visible as no urediniospores are produced, but the fungus overwinters as mycelium being the reason for endemic occurrence. *Puccinia triticina* does not necessarily require an alternate host, but can survive and proliferate with the urediniospores producing asexual life cycle only, if the conditions allow survival of urediniospores.

Later in the season when leaf senescence starts or under unfavorable conditions brown to black two-celled dikaryotic teliospores appear. In cold climates this is the overwintering stage of the fungus and in the Mediterranean climate the stage surviving the hot and dry summers. Generally after a dormancy period of several weeks with alternate periods of freezing and thawing, or wetting and drying, the teliospores germinate forming a basidium at temperatures of 7-27°C with an optimum at 10-16°C. In the mature teliospores the two nuclei have undergone karyogamy and fused to a diploid nucleus. Now undergoing meiosis four haploid basidiospores develop on a sterigma. After mitosis each basidiospore contains two identical haploid nuclei.

The basidiospores are distributed by the air but just for short distances of a few meters. They can only infect very young plants or organs of the alternate host. The various species of possible alternate hosts differ in susceptibility. Germinating rapidly, the produced infection peg penetrates directly into epidermal cells. On the upper surface of the leaves bottle-shaped pycnia develop under the epidermis. Approximately 7-14 days after the infection, the pycnia open and honeydew leaks that contains the pycniospores. They are the male gametes of which two different mating types (+ and -) exist. Thus, this

fungus is heterothallic. Furthermore the pycnia contain flexuous hyphae that are the female gametes. For a successful fertilization the opposite mating types are required. The pycniospores can be transferred to other pycnia by rain splash, dew, direct contact (e.g. leaves rubbing together in the wind), or by insects attracted by the honeydew. The developing mycelium is dikaryotic and culminates as an aecium which becomes visible further 7-10 days later on the lower leaf surface directly below the pycnium. The dikaryotic aeciospores are produced in long chains in the aecial horns. One aecium can contain several aecial horns, the number depending on the number of fertilizations occurred in the pycnium. Thus, the aeciospores of a single aecium differ genetically between different aecial horns, but within one aecial horn they are genetically identical.
The aeciospores – when wetted and dried – are forcibly discharged and can be carried by the wind over relatively short distances and infect adjacent wheat fields. On infected wheat leaves urediniospores are produced, thus the lifecycle is completed (Knott 1989, Roelfs et al. 1992, Hoffmann and Schmutterer 1999).

3.2.3 Control of Leaf Rust

3.2.3.1 Agronomic Measures

Control of leaf rust can be achieved by cultural methods, but just to a lesser extent than the use of resistant varieties or chemicals (Knott 1989). One aspect is the appropriate use of fertilizers. Excessive application of nitrogen fertilizer and the use of plant growth regulators (CCC compounds) increase susceptibility to leaf rust (BFL 2000). Too dense wheat stands should be avoided (Hoffmann and Schmutterer 1999). Controlling the timing, frequency and amount of irrigation can help decrease leaf rust infections in some regions.
Furthermore, the green bridge ought to be removed to reduce the chance of epidemics due to endogenous inoculum (Roelfs et al. 1992). Disposal of crop debris (Murray et al. 1998), thorough stubble working and tillage to control volunteer plants – in some areas several times – minimizes leaf rust survival between the wheat-growing seasons (Roelfs et al. 1992, Cate and Besenhofer 2009). Delayed planting of winter wheat reduces inoculum transfer from nearby fields of spring or late winter wheat cultivars. On the other hand, in areas where wind transported inoculum arrives late in the season, early planting allows the plants to mature before leaf rust infections can become serious (Knott 1989). Spring and winter wheat should be spatially divided. Regarding the prevailing wind direction, fields of early maturing cultivars should be placed downwind of late varieties. The eradication of alternate hosts generally is economically not feasible. They rather give the possibility of sexual reproduction and thus occurrence of new pathotypes than being a reservoir for epidemics causing inoculum (Roelfs et al. 1992).

3.2.3.2 Chemical Control

In areas with intense production and high yields (70 dt/ha and above), the application of chemicals can be economically reasonable (Knott 1989, Hoffmann and Schmutterer 1999). In Austria the economic threshold for an application of rust fungicides in wheat is generally reached if the infected leaf area of the three uppermost leaves exceeds 2 % or more than 30 % of the plants show symptoms of leaf rust. The most susceptible developmental stage and thus the time of application starts with the formation of the second node (BBCH 32) and lasts until after the end of heading (BBCH 59) (BFL 2000). Registered fungicides include different agents from the substance groups triazole, strobilurin and pyrazole (BAES 2013c, PPDB 2013). One to several applications are necessary, depending on the weather, the length of the growing season and the fungicide (Knott 1989). Fungicides need to be sprayed immediately after the development of the first leaf rust symptoms to ensure success. Usually fungicides have a combined action against several leaf pathogens. Frequently the “secondary effect” of a fungicide application against powdery mildew, *Septoria* or head diseases is sufficiently controlling a development of leaf rust later in the season (Meinert and Mittnacht 1992, Hoffmann and Schmutterer 1999). Disadvantages of chemical rust control are not only the costs and possible environmental hazards, but also the potential development of fungicide resistant rusts as has occurred with other fungal pathogens. Besides, if environmental conditions are favorable for the development of leaf rust, fungicides may provide inadequate protection (Roelfs et al. 1992).

3.2.3.3 Biological Control

From different species (insects, bacteria, fungi and plants) suppressing effects on the development of *Puccinia triticina* has been reported, but there has not been any large-scale utilization yet.

In several studies *Mycodiplosis* larvae were observed feeding on spores of rust fungi, among other, of the Uredinales order (e.g. Powell 1971, Henk et al. 2011).

The saprophytic bacterium *Erwinia herbicola* provided nearly complete protection to *Puccinia triticina* due to antibiosis (Kempf and Wolf 1989). Strains of the bacteria *Pseudomonas* can suppress leaf rust of wheat by producing antibiotics, hydrogen cyanide and siderophores (Levy et al. 1989, Flaishman et al. 1996). An isolate of *Bacillus velezensis* showed a control of *Puccinia triticina* of more than 70 % compared to solely spraying the detergent (Roh et al. 2009).

Endophytic fungi (*Chaetomium* sp. A, *Chaetomium* sp. B and *Phoma* sp.) reduced the density of rust pustules. In this study it was impossible to fully elucidate the mechanisms (Dingle and McGee 2003). The authors suggested that most probably the endophytes induced defense mechanisms in the wheat plants. In another experiment Park et al. (2005) revealed that *Chaetomium globosum* produces antifungal substances. Spencer and Atkey (1981) found evidence for the parasitism of *Verticillium lecanii* on spores of *Puccinia triticina*.

Yoon et al. (2010) studied the one-day protective activities of *Prunella vulgaris* (common self heal) extracts against several fungal diseases. In the greenhouse the methanol extract had 83 % of the effect against *Puccinia triticina* compared to the fungicide flusilazole.
Some further predators preying on the urediniospores are listed by Fleming (1980). He tried to model the influence on rust development by an effective natural enemy complex and specifies the identified priorities for research.

3.2.3.4 Genetic Resistance

The most effective method of biological rust control is the cultivation of resistant varieties (Knott 1989). For breeding leaf rust resistant spring bread wheat varieties by CIMMYT (International Maize and Wheat Improvement Center) since 1973, Marasas et al. (2003) estimated that the benefit-cost ratio was 27:1. Genetic resistance is an ecologically and for the farmer furthermore economically sound strategy of rust control. In organic farming the use of resistant cultivars is – apart from agronomic measures and biological control – the only possibility for reducing leaf rust infection.

3.3 Breeding Wheat for Leaf Rust Resistance

Breeding wheat for resistance to leaf rust requires the identification of resistance sources, determining the components of resistance and their phenotypic and genetic characteristics such as the plant developmental stage at which they are effective and whether they are useful against a wide range of leaf rust isolates. In order to enhance the chance of durable resistance either the future development of the rust population needs to be predicted or several genetically diverse resistance sources need to be combined which can be facilitated by molecular mapping. Seeking for genetic diversity in resistance is not just important in breeding of a new cultivar, but perhaps even more important in selecting cultivars for cultivation in a certain region (McIntosh et al. 1995).

3.3.1 Sources of Resistance

Puccinia triticina can infect hexaploid wheat (*Triticum aestivum* ssp. *aestivum*), tetraploid durum wheat (*T. turgidum* ssp. *durum*), wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), domesticated emmer wheat (*T. turgidum* ssp. *dicoccum*), triticale (x *Triticosecale*), *Aegilops speltoides* and *Ae. cylindrica*. It seems that leaf rust infecting *T. aestivum*, *T. turgidum* and *Aegilops* are different *formae speciales* (Bolton et al. 2008). These plant species belong to the primary or secondary gene pool of wheat, but also resistances from the tertiary gene pool can be introgressed (Feuillet et al. 2008). All these species are possible sources of leaf rust resistance.

More than 70 genes for leaf rust resistance (*Lr*) have been identified. Most of them have been found in *T. aestivum* cultivars. Some have been obtained from other *Triticum* as well as from *Aegilops* (goat grass), *Secale* (rye), *Thinopyrum* and *Elymus* (wheat grass) species (Roelfs et al. 1992, McIntosh et al. 1995, Kolmer 2007, McIntosh et al. 2013):

- *Aegilops geniculata*
- *Ae. kotschyi*
- *Ae. neglecta*
- *Ae. peregrina*
- *Ae. sharonensis*
- *Ae. speltoides*
- *Ae. tauschii*
- *Ae. triuncialis*
- *Ae. umbellulata*
- *Ae. ventricosa*
- *Elymus trachycaulis*
- *Secale cereale*
- *Triticum aestivum* ssp*. spelta*
- *T. monococcum*
- *T. monococcum* ssp*. monococcum.*
- *T. timopheevii* ssp*. armeniacum*
- *T. timopheevii* ssp*. viticulosum*
- *T. turgidum* ssp*. dicoccoides*
- *T. turgidum* ssp*. dicoccum*
- *T. turgidum* ssp*. durum*
- *Thinopyrum intermedium*
- *Thinopyrum ponticum*

3.3.2 Genetics of Resistance

Leaf rust can infect wheat if it has the particular gene(s) for virulence corresponding to the *Lr* gene(s) of the cultivar. This gene-for-gene relationship has first been established for flax and flax rust (*Melampsora lini*) by Harold H. Flor, who conducted his studies already in

the 1940s. Several exceptions from the existence of two possible alleles in leaf rust (either for virulence or for avirulence) for each resistance gene in the host have been detected: For the *Lr2* locus three alleles exist (*Lr2a*, *Lr2b* and *Lr2c*), but it seems that virulence/ avirulence to them can not segregate independently. The *Lr3* locus has likewise three different possible alleles (*Lr3*, *Lr3bg* and *Lr3ka*), but leaf rust races can be virulent to one, two or three of them. Similar virulence/ avirulence to the two alleles of the *Lr14* locus (*Lr14a* and *Lr14b*) are inherited independently (Kolmer 1996). Whether *Lr14a* and *Lr14b* can be considered as true alleles, has not been definitely clarified as it was possible to combine both in one single line (Dyck and Samborski 1970). Furthermore, the expression of avirulence in leaf rust as well as the expression of resistance in wheat can range from completely dominant to recessive, depending on the genotype (homozygous or heterozygous) of the corresponding resistance and avirulence gene (Bolton et al. 2008). Whether an *Lr* gene is expressed dominantly or recessively can also depend on the genetic background. This has been observed e.g. for *Lr2b* and *Lr2c*. The dominance of *Lr23* was furthermore temperature dependent (Kolmer 1996).

Virulence genes in the rust population can disappear if *Lr* genes are not present in the wheat population, but occur in high frequencies if extensively used in agriculture. Frequently resistance from alien sources is present in just a small population. Thus, there is little contact with leaf rust and the selection pressure low, and initially these sources of resistance are effective against a wide range of rust races. But resistance in the wheat's wild relatives is genetically and physiologically similar to resistance of cultivated wheat and thus not necessarily more useful or durable (Knott 1989, Roelfs et al. 1992). Whether or not virulence genes disappear from the rust population as they become unnecessary when no longer present in the grown cultivars, depends on the penalty on fitness associated with the particular virulence (Wilde et al. 2002, Agrios 2005).

Most of the already identified *Lr* genes confer race-specific resistance (R-genes) (Bolton et al. 2008). Thus, by definition, these genes do not provide durable resistance as at least one physiological race of *P. triticina* has emerged that was able to infect wheat carrying these genes (Birch 2001b). Race-specific leaf rust resistance frequently results in a rapid cell death around the point of infection, known as hypersensitive response, causing characteristic infection types (Bolton et al. 2008). McIntosh et al. (2001) described the infection types of more than 40 *Lr* genes. Race-specific *Lr* genes are effective from the seedling to the adult plant stage, although the effectiveness can vary with the developmental stage: Two examples are *Lr12* and *Lr13*. Whereas *Lr13* is already effective at the stage of leaf development (about three leaf stage), *Lr12* is most effective at the end of stem elongation (flag leaf stage) (Knott 1989).

One of just a few race non-specific *Lr* genes is *Lr34*. Regardless of the tested leaf rust isolates the resistance was the same and did not involve hypersensitive reactions (Bolton et al. 2008). Resistance associated with *Lr34* is characterized by fewer numbers of uredinia also being smaller in size, and a longer latent period compared to susceptible cultivars, thus meeting the definition of slow rusting or partial resistance (Kolmer 1996). *Lr34* is an adult plant resistance gene, expressed during the grain-filling stage. It is most

effective in the flag leaf and stimulates senescence-like processes. Despite extensive cultivation of cultivars carrying *Lr34*, virulence toward *Lr34* has not increased and the resistance has remained durable for more than 50 years (Krattinger et al. 2009). Other slow rusting resistance genes are *Lr46* (Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011; Hiebert et al. 2010) and *LrP* (Singh RP et al. 2011).

The effectiveness of *Lr* genes can not just be influenced by the inoculum density and the plant developmental stage, but also by temperature. Whereas infection of plants carrying certain *Lr* genes increases with increasing temperatures (e.g. *Lr11*, *Lr18*), it decreases if plants have others (e.g. *Lr3*, *Lr16*, *Lr17*, *Lr23*), but temperature sensitivity is often dependent on leaf rust isolate and plant developmental stage. Additionally interactions between temperature and light influencing the expression of resistance have been observed. Modifiers, inhibitors (e.g. for *Lr23*) and suppressors are further possible influences on *Lr* genes. Sometimes it is rather the whole genetic background that affects resistance than just a single modifier. In the case of *Lr27* and *Lr31* both genes need to be present to condition resistance, being the only clear example of complementary genes. More frequently interactions between resistance genes are observed: The combination of several resistance alleles results in a higher level of resistance than is conditioned by the most effective individual gene. Examples are combinations of the adult plant resistance genes *Lr13* and *Lr34* either with one another or with other *Lr* genes such as *Lr33* (Roelfs 1988, Kolmer 1996). Nevertheless, even the resistance of some varieties carrying *Lr13* and a second R-gene broke down just a few years after their release in northwestern Mexico in the 1970s and 1980s as virulent races emerged. The still improper use of R-genes results in “boom-and-bust” cycles and the necessity of cultivar replacement frequently even shortly after release. To enhance resistance durability, at least two effective R-genes need to be combined (Singh 2012).

Two leaf rust resistance genes have proven to be durable till date: *Lr34* and *Lr46* (Priyamvada et al. 2011). Both are conferring slow rusting or partial resistance, expressed at the adult plant stage without hypersensitive reaction (Kolmer 1996, Singh et al. 1998, Martínez et al. 2001, Krattinger et al. 2009). This is mostly in congruence with McIntosh’s general concept of a durable (or race non-specific) source of rust resistance already stated in 1992: “that it may be controlled by more than a single gene; that it is more likely to operate at the adult-plant stage rather than at both the juvenile (seedling) and adult stages; and that it confers a non-hypersensitive response to infection” (McIntosh 1992, p. 523). Latest by the 1980s there was evidence that most likely gene combinations of several genes all having small, additive effects provide durable resistance, whereas single genes conferring durable resistance are rare (Roelfs 1988, Knott 1989). If 4-5 genes conferring slow rusting or partial resistance are combined, acceptable levels of long lasting resistance can be achieved, even if the single genes provide insufficient protection. Although breeding for partial resistance is – especially at the beginning – more laborious for various reasons, it should be preferred as this kind of resistance is considered to be less likely to be overcome by virulent races und thus durable and less prone to “boom-and-bust” cycles compared to an improper usage of R-genes. Pyramiding several partial

resistance genes (and possibly additional R-genes) is facilitated by the identification of tightly linked molecular markers and genomic selection (Singh 2012).
Components of quantitative resistance are the infection efficiency (the number of lesions per deposited spores), the latent period (frequently measured by the number of days from inoculation to sporulation), the lesion size and the spore production per lesion. Azzimonti et al. (2013) were the first to consider several components of quantitative resistance (infection efficiency, latent period, lesion size, sporulation rate per lesion, sporulation rate per unit of sporulating tissue) altogether. Combining factors for different components of quantitative resistance makes it more difficult for the pathogen to adapt.
The terms race non-specific, general, horizontal, partial, minor gene, quantitative, polygenic, adult-plant, field and durable resistance have often been used interchangeably. Likewise race-specific, differential, vertical, strong, major/ R gene, qualitative, and mono-/ oligogenic have synonymously been used for the second kind of true resistance, i.e. genetically controlled resistance of a host to a pathogen (Agrios 2005). Although this appears to be generally correct, exceptions have been detected: Partial (or slow rusting) resistance is not necessarily non-specific (Knott 1989). *Lr34* is a major gene, but conferring quantitative resistance (Kolmer 1996). The review about durable resistance in wheat by Priyamvada et al. (2011) also focuses on the various terms used to characterize resistance.
The opposite of true resistance is apparent resistance, plants appearing to be resistant due to disease escape or tolerance. Wheat can escape rust infection e.g. because of stomata opening that late in the day that germ tubes of rust spores have already desiccated. A possibility also used in wheat breeding is to select for earliness, helping to escape rust diseases (Agrios 2005).
Tolerant plants show no harmful physiologic effects despite infection. The pathogen can multiply, but neither disease symptoms nor yield or quality losses are observed, thus it can be regarded as a kind of disease resistance without pathogen resistance. Selecting for tolerance has to be regarded with caution as tolerant plants provide a reservoir of inoculum and thus increase disease pressure on other varieties (Birch 2001a+c). Different levels of tolerance to leaf rust have been observed e.g. by Andenow et al. (1997) in Ethiopian landraces and by Herrera-Foessel et al. (2006) in CIMMYT genotypes of *Triticum turgidum*.

3.3.3 Disease Assessment

Disease assessment or phytopathometry is the process of measuring disease intensity quantitatively. It is always aimed at having a quantitative measure that is related to yield losses. Disease assessment can be done on a single sample unit (a plant or part of a plant) or on a population of plants; at one point in time during plant development or at several time points. As disease is the result of an interaction between a host and a pathogen, there are two populations that can be assessed: In pathogen assessment numbers of pathogens (e.g. spores) per unit (area or volume) are measured. Disease

assessment in the narrower sense is the observation of visible symptoms caused by the pathogen. Results and the correlation to yield losses may be different. The term scoring is defined as the activity of assigning each item of the sample to the appropriate class (Zadoks and Schein 1979, Nutter and Schultz 1995, Nutter 2001).
Response is a qualitative measure for describing symptoms. A lesion can be classified as necrotic, chlorotic, poorly sporulating, or richly sporulating. Disease intensity is a quantitative measure for the amount of disease in a population. Three terms have to be distinguished: disease prevalence, incidence and severity (often used synonymously with intensity). Disease prevalence is the proportion of geographic sampling units (e.g. regions, fields) where a disease has been detected, divided by the total number of sampling units observed. Disease incidence is the number of plant units (e.g. whole plants, leaves) with disease symptoms, expressed as a proportion of the total number of sampling units assessed. Disease severity is the term for the quantification of the amount of disease symptoms on a single sampling unit, e.g. percentage of infected leaf area (Horsfall and Cowling 1978, Zadoks and Schein 1979, Nutter and Schultz 1995, Nutter 2001).

3.3.3.1 Keys for Disease Assessment

Various keys have been developed for scoring disease severity. They can be divided into two classes: Descriptive keys (in some cases with pictures) for different types of disease symptoms and levels, and standard area diagrams. Field or descriptive keys are verbal and numerical descriptions of disease severity classes. They aim at fast visual assessment of diseases on whole plants, plots, or fields (Zadoks and Schein 1979). Standard area diagrams or diagrammatic scales are pictorial representations consisting of a set of schemes that depict the true value of disease severity on individual sampling units, expressed as a percentage of the total surface area (Lindow 1983, Nutter and Esker 2001). They differ in the number of classes and whether these classes are equally distributed over the total range or unequally because of other assumptions. Either intermediate values are interpolated (Sherwood et al. 1983) or only values shown by the key are “allowed” ratings. The first scale to measure disease severity was developed by Cobb (1892 cited in Horsfall and Cowling 1978). His sketches showed five degrees of rust infection from 1 to 50 % of diseased leaf area. A still widely used key for assessment of leaf rust severity is the modified Cobb scale. The maximum of tissue area that can be covered by pustules is arbitrarily set to 37 % as only about one third of leaf area can be occupied by rust uredinia. Therefore there are two sets of numbers for disease severity: One for the actual percentage (up to 37 %) or true severity and a second for the relative severity as the percentage of maximum tissue covered by rust pustules (up to 100 %) (Peterson et al. 1948 cited in Zadoks and Schein 1979 and Roelfs et al. 1992). James (1971) published similar standard area diagrams in black and white for various diseases of cereal and forage crops including leaf rust of cereals together with detailed description of their usage.

Horsfall and Heuberger (1942 cited in Horsfall and Cowling 1978) were using the McKinney index for assessing disease severity caused by *Alternaria solani* on tomatoes (*Solanum lycopersicum*) for many years. The McKinney index is a quantitative, numerical rating: 0.00 = no disease, 0.75 = very slight, 1.00 = slight, 2.00 = moderate, 3.00 = abundant. They found out that their data assessed with the McKinney index fitted well to the counts of stem end rot and dead leaves, that loss was linear with disease grade and that the agreement between observers was good. When Horsfall and Barratt (1945 cited in Horsfall and Cowling 1978) wanted to improve the McKinney index, they came across the Weber-Fechner law. It states that an observed intensity is proportional to the logarithm of the intensity of the stimulus (true intensity) (Horsfall and Cowling 1978, Lindow 1983, Forbes and Jeger 1987).
Horsfall and Barratt were confident that this law can also be applied to visual assessment of disease severity (Nita et al. 2003). Thus, Horsfall and Barratt (1945 cited in Horsfall and Cowling 1978) developed a scale with disease grades placed logarithmically instead of arithmetically. In addition they realized that it is always the smaller amount that is observed: Below 50 % infection it is the proportion of diseased area, beyond 50 % the proportion of healthy tissue. Therefore grades downwards and onwards from 50 % were set in that way that they covered half the range of the previous class. On a double-logarithmic scale with 50 % as median, these twelve classes (0, 0-3, 3-6, 6-12, 12-25, 25-50, 50-75, 75-88, 88-94, 94-100, 100 %) are equally distributed (Lindow 1983). That means, whereas the classes in the mid range cover 25 % (50-25 and 50-75 %), classes at both ends of the scale cover just a few percent difference. Hau et al. (1989) pointed out that experienced assessors would use finer grades in direct assessments than the Horsfall-Barratt scale.
Other scales are used for assessing disease response/ reaction types (Zadoks and Schein 1979). A widely used scale was developed by McNeal et al. (1971 cited in Zadoks and Schein 1979 and Roelfs et al. 1992). Disease symptoms are described for 10 levels of infection types from immune to very susceptible and these classes are either coded with symbols or numbers.

3.3.3.2 Suitability of Assessment Keys

Large (1966 cited in Zadoks and Schein 1979, p. 253) stated that “No standard diagram can show all the differing distributions of lesions that can make up a percentage cover. What the observer really has to do is to visualize what area the lesions would cover if he could gather them all together, and then to estimate this area as a percentage of the total area of the leaf.”
This is the problem of all field keys and rating scales: How well can they mirror disease severities occurring in the experiments, are there sufficient intervals to represent all of the disease developmental stages? Furthermore are they easy and fast to use and applicable to a wide range of different conditions (Godoy et al. 1997)? Which one(s) is (are) the best with regard to repeatability of disease assessment, negligible differences in scoring

between raters, and foremost, results of which scoring aid approach are next to the true severity values and – in the end – are the best prediction of yield losses?

Baird and Noma (1978 cited in Hebert 1982 and Hau et al. 1989) proposed four different types of stimulus-response curves:

- linear function: Response (R) and stimulus (S) increase linearly. $R = a\,S$
- logarithmic function or Weber-Fechner law: Linear increase of response due to a logarithmic increase of the stimulus. $R = a \log S$
- exponential function: The response increases logarithmically as the stimulus increases linearly. $R = e^{aS}$
- power function or Stevens' law: The stimulus and response increase logarithmically. $R = S^a$, $\log R = a \log S$

Hebert (1982) urged to compare different scales for these assumptions in the same experiment to find out the best and to determine whether the use of a scale can improve the accuracy of estimates at all. In addition it must be tested, whether the scale can be used for assessing actual disease severity or rather for classifying into disease severity categories only.

Several studies have been conducted in order to find the most suitable scales or diagrammatic keys for the assessment of disease severity in various host-pathogen systems.

Hau et al. (1989) tested data from a previous experiment (Kranz 1970 cited in Hau et al. 1989) for the four possible stimulus-response curves. They proved a linear relationship only for untransformed data or for the logarithmic transformation of both stimulus and response, i.e. power function or Steven's law. The Weber-Fechner law was not valid for these data.

Redman and Brown (1964) tested raters' abilities to distinguish percentages in disease severity. Their experiments substantiated the Horsfall-Barratt rating system. Between 0-5 % and 95-100 % disease severity the amount of accurate estimates was largest, whereas between 40-60 % it was lowest. But they regarded percentages determined from the rating numbers as more meaningful and developed a series of tables for the easy transformation.

Others who tested the raters' abilities to distinguish differences in disease severity were Hau et al. (1989). Assessors had to estimate the severity level of two schematic leaves at one time. The left picture had a lower, higher or the same level as the right one. In the latter case, it was only rotated by 180°. The highest severity levels in the experiment were 30 %, differences between the leaves to be compared varied between 0 and 8 %. As they observed that the distribution pattern of points on the schematic leaves influenced discriminability, they summarized severity levels to pairs of three. Hau et al. (1989) showed that the ability to distinguish minor differences in disease severity decreased with increasing disease level. As the least necessary difference did not further increase with disease severity from 8 % infected leaf area onwards, they assumed a proportional relationship only below a constant threshold, thus rejecting the validity of the Weber law in the case of disease assessment at least when regarding the total range of infection levels.

Koch and Hau (1980) discovered in their direct assessment studies without the use of severity scales that certain values were preferred by raters. They are primarily dependent on the ability to differentiate levels of disease severity, but also based on the decimal system. These values, which they call “knots”, are obviously best suitable to be used in standard diagrams. Hau et al. (1989) proved these knots with data from a previous experiment by Kranz (1970 cited in Hau et al. 1989). As Koch and Hau (1980) detected furthermore that with growing disease severity differences between values used for estimates became larger, they tested whether the knots fit with the Weber-Fechner law. With logit (logistic) and logarithmic transformations they showed that their knots were placed well upon a straight line, thus corroborating the Weber-Fechner law. Koch and Hau (1980) assumed that minor deviations can be explained with the familiarity to the decimal system. This fact should obviously not be ignored when constructing a scoring scheme.

Having used the Horsfall-Barratt scale for 35 years, Hollis (1984, p. 145) declared that “the contention of Horsfall and Barratt that the eye sees in logarithms remains a valid perception of the Weber-Fechner law”.

Lipps and Madden (1989) compared different assessment systems for their ability to predict yield losses of winter wheat (*Triticum aestivum*) caused by powdery mildew (*Erysiphe graminis*). Correlation between the estimates gained by the different systems was high. Correlation between estimated disease severity and yield loss was good, although r^2 was not the same for all tested cultivars. A 0-10 scale that accounts for the leaf position and the percentage of infected leaf area was found to be most practicable for assessments in field tests at various growth stages. They also compared the area under the disease progress curve (AUDPC) and data from a single assessment and proved that AUDPC does not necessarily improve yield loss prediction compared to a one-time assessment at an optimal growth stage.

O’Brien and van Bruggen (1992) did a similar experiment for corky root (*Rhizomonas suberifaciens*) of lettuce (*Lactuca sativa*). Two of the three compared scales were developed for qualitatively estimating corky root of lettuce: one for seedlings (10 levels), and the other for mature plants (7 levels). The seedling scale was illustrated by line drawings, the mature plant scale just as the third one, a 12 level quantitative Horsfall-Barratt scale for the taproot area, was based on photographs. They did not identify one of these three scales to be the best in all terms. For the particular situation – developmental stage, average disease severity level and question (precision, accuracy or correlation to yield loss) – another scale proved to be the best. The lower precision at moderate disease levels (20-80 %) was not improved when using the Horsfall-Barratt scale compared to the others. Thus they concluded that at best the high variability of the estimates at medium disease levels can partly be explained by the Weber-Fechner law.

3.3.3.3 Correctness of Disease Assessment

The different terms related to correctness of disease assessment used in this thesis are defined as follows:

Accuracy is the measure of the closeness/ the degree of conformity of an estimate to the supposed true value (Read 1982, Everitt 1998, Nutter 2001).

Precision is the relative measure of the reproducibility (reliability and/or repeatability) of disease assessments (Sherwood et al. 1983, Nutter and Schultz 1995, Nutter 2001).

Reproducibility is the closeness of estimates obtained on the same sampling units under changes of methods, raters and so on (Everitt 1998).

Reliability is the extent to which estimates for the same sampling units assessed with the same method by different raters yield similar results (Everitt 1998, Nutter 2001).

Repeatability is the closeness of observations over short intervals of time by the same rater on the same sampling units using the same method (Everitt 1998).

Thus Nutter et al. (1993) and Nutter and Schultz (1995) used the terms **intra-rater repeatability** for the linear relationship between assessments of the same sampling units repeated by the same person, and **inter-rater reliability** between assessments performed by different raters.

Sherwood et al. (1983) were among the first to study accuracy and precision of disease assessment. In their experiment they used the key diagrams of James (1971) for leaf rust (*Puccinia triticina*), stem rust (*P. graminis*) and powdery mildew (*Erysiphe graminis*) of cereals for the assessment of purple leaf spot (*Stagnospora arenaria*) of orchardgrass (*Dactylis glomerata*) as “leaves”. One of their additional questions was, whether there was a difference if raters were used to this scoring scheme or only had experience in disease scoring of other forage crops (two groups). Another question was whether there are differences in scoring errors depending on the size of the symptoms. To know the true value of diseased area, the area of enlarged photographs of the assessed leaves covered with spots was determined by weighing the paper copies. Repeated determinations showed little differences, thus being a good method for estimating true disease severity. With analysis of variance (ANOVA) for testing accuracy they detected significant effects of group, scorer within group, leaf and scorer x leaf effects. In general, disease severity was overestimated. The error was largest at low infection levels. If the diseased area was the same, the score for leaves with more but smaller spots was higher. The mean coefficient of variation (CV) as a measure for precision for each three repeated estimates per leaf per scorer was more than 20 % in this experiment. Significant differences in CV among leaves were not correlated to disease severity, number or size of spots, and there was no effect of the scorer. Correlations between estimated and true disease severity was 0.92-0.97 for the ten participating scorers and 0.85-0.99 between their estimates. Sherwood et al. (1983) referred the differences between groups of scorers to differences in previous disease assessment training. Different scores for leaves with the same disease severity, but different numbers because of different sized spots were due to the different ability of the eye to distinguish areas: If a double amount of diseased area is solely caused by

doubling the area of each lesion, the difference is just a change of 1.4 x diameter compared to a doubling in number of spots if their size remains unchanged. Based on these results, Sherwood et al. (1983) recommend additional diagrams with respect to low infection levels and different spot sizes together with training.

Recalculating the data of Amant (1977 cited in Hau et al. 1989), Hau et al. (1989) got similar results of the value of CV. For higher disease severity levels they detected a negative correlation between CV and disease severity that was not found by Sherwood et al. (1983) due to a lower level of maximum disease severity in their experiment.

The experiments of Forbes and Jeger (1987) went even further. Aware of the results of Amant (1977 cited in Forbes and Jeger 1987) and Sherwood et al. (1983) they extended their experiments to different object characteristics such as plant structures (not only restricted to various leaf shapes) and lesion sizes. Disease assessment was done without the use of standard area diagrams. But the drawings to be assessed were standard area diagrams taken from different assessment keys. Thus there were limited combinations of plant structures, lesion sizes and shapes and disease severities. In general as in the experiment by Sherwood et al. (1983) disease severity was overestimated. Variances of the estimates were largest in the range 25 to 75 % diseased area. This is in accordance with the Weber-Fechner law. Estimation error was calculated as different relations of observed and true severity. Effects of rater, plant structure, and severity on estimation error were tested by means of ANOVA and found to be significant for all methods of error calculation as well as the interactions intensity-by-rater and intensity-by-plant-structure. Whereas overall accuracy (mean value of the average deviation between estimated and true values) was good (3.9 % severity), values of assessors and plant structures varied within a broad range (-1.5 to 16.2 and 0.3 to 16.0 % severity). Precision of raters (measured by the standard deviation of repeated estimates) was more consistent, but even worse (11.2 to 16.7 % severity). Precision and accuracy with regard to disease severity levels were only partly in accordance with the Horsfall-Barratt hypothesis: Severity levels below 25 % were assessed with greatest precision and accuracy, but largest deviations between true and estimated disease severity and biggest differences between repeated assessments occurred at 25 % diseased area and not at 50 %. At the 75 % level, only accuracy was better compared to 50 %. Plant structures with larger symptoms were estimated more accurately. Different transformations of the observed disease severity and the same relations of observed and true severity as in ANOVA were used in simple linear and logarithmic regression analysis. The simplest model (observed severity = $\beta_0 + \beta_1$ true severity) was fitting best ($r^2 = 0.70$). For the logit transformation of the observed severity, the logarithmic model was fitting better than the simple linear one. Correction for observed values (100 minus observed value) if true severity was beyond 50 % did not improve r^2 of the models.

Hau et al. (1989) used the data of Amant (1977) furthermore for testing the influence of leaf shape, leaf color, leaf size, lesion size, and lesion color. They used a weighted regression analysis between the observed differences in disease severity for different leaf shapes etc. vs. the true value of disease severity. The weight was the inverse of the

calculated variances to account for growing observed differences with growing disease levels. Disease severity of an oval apple leaf (*Malus domestica*) was estimated higher than of a linear barley (*Hordeum vulgare*) leaf, with larger differences for growing disease level (intercept and slope about 1). Neither leaf nor lesion color seemed to have an effect on the estimates. On smaller leaves disease severity was estimated higher, but there was little change with severity level (intercept and slope about 0.2). The effect of lesion size was stronger. The same disease level due to smaller lesions was estimated higher compared to bigger lesions. With growing level of disease severity, differences became slighter (intercept -0.74, slope -0.95).

Furthermore Hau et al. (1989) compared the results of assessments with and without the use of standard area diagrams. The diagrams were labeled with roman numbers and depicted true disease severities of 0.5, 2, 4.5, 8, 15, 30 and 50 % infected leaf area. In a first step wheat leaf schemes with simulated *Septoria* infection had to be classified according to the standard area diagrams. Although raters were not experienced only one of 860 classifications differed more than one level from the true class. Estimates of the same leaf schemes without any scoring aid in the second step were more variable. Moreover the mean derived from direct assessments was always higher than the true value. Hau et al. (1989) recommended proving whether their results were also true for experienced assessors.

O'Brien and van Bruggen (1992) did not only test correlation between estimated disease severity and yield loss, but also precision and accuracy of the assessments done with the three different scales. As estimate of accuracy they used the absolute difference between estimated and "correct" score (recorded by the originator of the particular scale). The measure of precision was the standard deviation of the estimates of one assessor for each root and each of the three scales divided by a correction factor depending on the scale. O'Brien and van Bruggen (1992) did not find a general improvement in accuracy and precision using the Horsfall-Barratt scale compared to the others. Experienced raters were able to score more accurately with the Horsfall-Barratt scale than inexperienced ones. They assumed that perhaps for this scale more training would be required.

Godoy et al. (1997) developed diagrammatic scales for four different leaf diseases of bean. They considered minimum and maximum values of disease severity observed in the fields and determined intermediate levels according to the Weber-Fechner law. Specific characteristics of the particular diseases such as chlorotic halo surrounding pustules at higher disease severity levels or necrotic tissue on the veins and borders were taken into account. Using these scales, estimates of all five raters were accurate with intercepts of zero and slopes of one of the linear regression line between actual and estimated disease severity and a coefficient of determination of $r^2 > 0.80$ for 90 % of the assessments. Even for the worst rater the absolute error (actual severity minus estimated severity) was lower than $\pm$ 15 %. Inter-rater reliability measured by the coefficient of determination was different for the diseases. For rust it was about $r^2 = 0.80$, for anthracnose about 0.90 and for angular leaf spot even beyond 0.90. For *Alternaria* spot inter-rater reliability varied between 0.43 – 0.95 due to a low scoring precision of one rater for this particular disease.

Two different assessment methods for estimating *Cercospora* leaf spot (*Cercospora beticola*) on sugar beet (*Beta vulgaris*) were compared by Vereijssen et al. (2003). Accuracy and reproducibility were higher for the whole plant scale with detailed description of the characteristics of the particular severity level than for a single leaf scale with predefined levels of diseased leaf area for each leaf of a plant. Class width was narrowest at low severity levels and broadest in the mid range.

Nita et al. (2003) compared estimates using the Horsfall-Barratt scale with direct visual estimates of percentage of infected leaflet area caused by *Phomopsis* leaf blight (*Phomopsis obscurans*) on strawberry (*Fragaria* x *ananassa*). Four repetitions were done on prepared leaflets in the lab; the fifth was conducted in the field with leaflets still attached to the plants. In all repetitions 25 leaflets were assessed twice (once directly and once using the Horsfall-Barratt scale) by six raters (three with limited and three with substantial experience), in three repetitions two times with both methods. Actual disease severity of each leaflet was estimated by repeated cutting and weighing of the total and the diseased area on photographs. Additionally leaflet and lesion size, lesion number and type were recorded. For determining accuracy, Nita et al. (2003) used Lin's concordance correlation coefficient. This reproducibility index evaluates the agreement between two repeated assessments. It is measuring the variation from a linear regression line with intercept zero and slope one. Thus, it compensates for the detection failures of Pearson's correlation coefficient, paired *t*-test and least square regression (Lin 1989). The concordance correlation coefficient was not generally higher for either direct estimates or assessments using the Horsfall-Barratt scale, the relationship between actual and estimated severity was linear and there was no strong association between estimation error and severity level. Because of the observed linear relationship Nita et al. (2003) tested a new scale with equal intervals of 5 % and the additional class 1 %. Assessing the data from the direct estimation to the appropriate class, results from this scale were better in terms of accuracy and reliability than using the Horsfall-Barratt scale. Thus results of Nita et al. (2003) did not support the principles underlying the Horsfall-Barratt scale; there was little evidence of the validity of the Weber-Fechner law or the Horsfall-Barratt type of stimulus-response relationship. Intra-rater reliability was high for all raters with 89 % of the correlation coefficients greater than r = 0.90 for direct assessment and 81 % when using the Horsfall-Barratt scale. Generally with only a few exceptions, values were higher for direct assessment. Values of inter-rater reliability were a little bit lower. The assessment of plants in the field revealed similar results. Differences between raters with little vs. substantial experience were slight, but both intra- and inter-rater reliability were higher for the latter. With increasing range of disease severity, correlation coefficients were increasing. Using a linear stepwise regression analysis the influence of leaflet size, number of lesions, lesion size and type were determined. Lesion number and leaflet size were significant regression parameters, lesion size and type did not influence severity estimates (Nita et al. 2003).

Nutter and Schultz (1995) gave a good overview on different tests and measures of precision: With analysis of variance (ANOVA) differences between raters and assessment

methods and disease severity x rater interactions can be tested. If the latter are significant, differences between raters cannot be removed by using rater as a factor, because they respond differently to different disease levels. With simple linear regression the degree of error due to raters or assessment methods can be quantified. A slope different from one indicates a systematic bias proportional to the level of disease severity, whereas an intercept different from zero indicates a constant bias. The coefficient of determination (r^2) gives the amount of one assessment explained by another. The product moment correlation coefficient (r) is only a measure of the strength of the linear relationship between two assessments. Neither can it be used for the prediction of the second assessment by the first one nor for estimating systematic and constant bias. The coefficient of variation (CV) calculated as 100 times the square root of the error mean square divided by the grand mean is a measure of the precision of compared assessments (i.e. intra-rater repeatability and intra-rater reliability or different assessment methods). As the experimental error is expressed as a percentage of the mean, values of different assessment methods are normalized and can be compared. The standard error of the estimate for y (SE_y) is another index of precision. Though being a measure similar to the CV, the SE_y provides additional information about the relative precision of the disease estimates predicted by the linear regression. As for the coefficient of variation, the higher the values of the standard error, the lower the precision.
As it is beyond doubt that training improves precision and accuracy of estimates, various computer training programs have been developed, e.g. DISTRAIN (Tomerlin and Howell 1988), Disease.Pro (Nutter and Schultz 1995) or SeverityPro (Nutter et al. 1998). Training with the latter, users can choose between a training mode with feedback after each estimate and a testing mode which calculates parameters for accuracy and precision. Additionally type of disease and lesion size can be selected.
Computer based methods for assessing disease severity have gained importance. Automated disease assessments based on computer assisted image analysis as described by Lindow and Webb (1983) are more accurate than visual estimation, but limited to potted plants or detached leaves. If it is used for determining disease severity of whole field plots, selection of the “average” leaves is restricted to the subjective decision of the rater and likely to be as error prone as direct visual assessment of disease severity.

3.3.4 Mapping Disease Resistance in Wheat

Due to its polyploid genome, hexaploid wheat has a very large genome size: about 16 (Gill and Friebe 2002) to 17 billion base pairs (Brenchley et al. 2012), comprising an estimated number of 164,000 to 334,000 protein-encoding genes, including pseudogenes (Devos et al. 2008). Choulet et al. (2010) found one gene per 87 kb in the distal and one per 184 kb in the proximal contigs. Brenchley et al. 2012 identified about 95,000 genes. Approximately 80 % of the genome is repetitive sequence (Smith and Flavell 1975 cited in Choulet et al. 2010). Thus, a genome-wide study of hexaploid is a challenging task.

3.3.4.1 Molecular Markers

Molecular markers are identifiable DNA sequences. They can be conceived as constant landmarks in a genome, located at specific genome positions and showing Mendelian inheritance (FAO 2003, Griffiths AJF et al. 2012). Thus, molecular markers detect variation in the DNA sequence. The first ones developed were the restriction fragment length polymorphism (RFLP) markers in the 1970s. They belong to the group of markers based on the hybridization between a DNA or RNA probe and genomic DNA. The second group comprises markers based on PCR (polymerase chain reaction) amplification of genomic DNA, e.g. SSR (simple sequence repeats) or AFLP (amplified fragment length polymorphism) markers (Doveri et al. 2008). These authors provide a chronological listing of the different molecular markers.
Already young plants can be characterized with molecular markers and results are independent from environmental influences. Generally, the DNA sequence of a certain trait is unknown and the marker need not be the trait's sequence. But the marker must be located in close proximity to be inherited together with the trait (Becker 2011).
For QTL analysis (3.3.4.3) SSR and AFLP markers are used most frequently (Becker 2011). Another application is marker-assisted selection (3.3.4.4).

Desirable properties of an ideal marker system as listed by Doveri et al. (2008):

- high level of polymorphism and reproducibility,
- co-dominant inheritance (allowing discrimination between homo- and heterozygotes in diploid organisms),
- clear designation of alleles,
- frequent occurrence in the genome,
- even distribution throughout the genome,
- selective neutrality,
- straightforward and cheap development of assay,
- easy/ rapid procedure (amenable to automation if required),
- possibility of exchange between laboratories,
- contained costs in routine analyses (following marker development).

Microsatellites or Simple Sequence Repeats (SSR)

Simple sequence repeats are defined as DNA regions consisting of one to six tandemly repeated nucleotides and a length of 12 to 100 base pairs. It was estimated that microsatellites occur with an average frequency of one every 6-7 kb in the Triticeae (Paux and Sourdille 2009). Genotypes differ in the number of repeats (Becker 2011). Microsatellites are highly polymorphic, loci are discrete and alleles generally co-dominant (Doveri et al. 2008).
Fig. 3 illustrates the principles of SSR markers.
A disadvantage of SSR markers are the high costs for their development as sequencing is necessary. Despite, they are the most widely used marker types and almost four thousand

microsatellites have been published for Triticeae species in the last ten years (Paux and Sourdille 2009). Sequences of primers for WMS (wheat microsatellites), later named GWM (Gatersleben wheat microsatellites), and GDM (Gatersleben D-genome microsatellites) have been developed and published by the Institute for Plant Genetics and Crop Research in Gatersleben, Germany (Korzun et al. 1997, Röder et al. 1998, Pestsova et al. 2000). The Beltsville Agricultural Research Center in Maryland, USA, has developed and published the BARC (Song et al. 2002), the Wheat Microsatellite Consortium the WMC markers (Gupta et al. 2002). Among the others, less frequently used markers are the PSP developed by the John Innes Centre in Norwich, UK (Stephenson et al. 1998) and the CFA, CFD and GPW markers developed by the UMR Amélioration et Santé des plantes in Clermont-Ferrand, France (Sourdille et al. 2001 cited in Sourdille et al. 2004, Guyomarc'h et al. 2002).

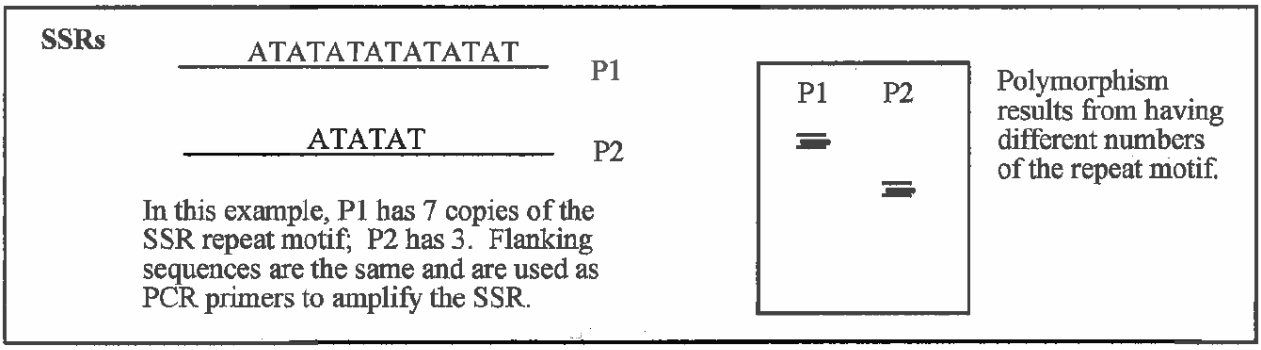

Fig. 3 The principles of SSR markers (Prince and Ogundiwin 2004)

Amplified Fragment Length Polymorphism (AFLP)

In the mid 1990s the AFLP marker technique was developed. It combines restriction fragment length polymorphism with PCR. The genomic DNA is cut using two different enzymes: A so-called rare cutter and a frequent cutter. Then adapters are ligated to the sequences of the restriction sites of the enzymes. The primers for PCR reaction are based on the sequences of the adapters. The number of amplified fragments can be reduced by adding selective nucleotides to the PCR primers. The number of visible fragments can be reduced by labeling the primers amplifying the rare cutter's sequence. Polymorphisms are due to changes in the sequence of the restriction sites or the selective nucleotides of the PCR primer or caused by insertions or deletions in the fragments. AFLP markers are generally dominant, bands are detected as present or absent. In a single reaction several different genetic loci can be screened. Sequence data of the species is not necessary for primer development (Doveri et al. 2008). Thus, costs of development and application of AFLP markers are comparatively low. A disadvantage of AFLP markers is that they lack – in contrast to SSR markers – locus specificity (Somers 2004).
Fig. 4 illustrates the principles of AFLP markers.

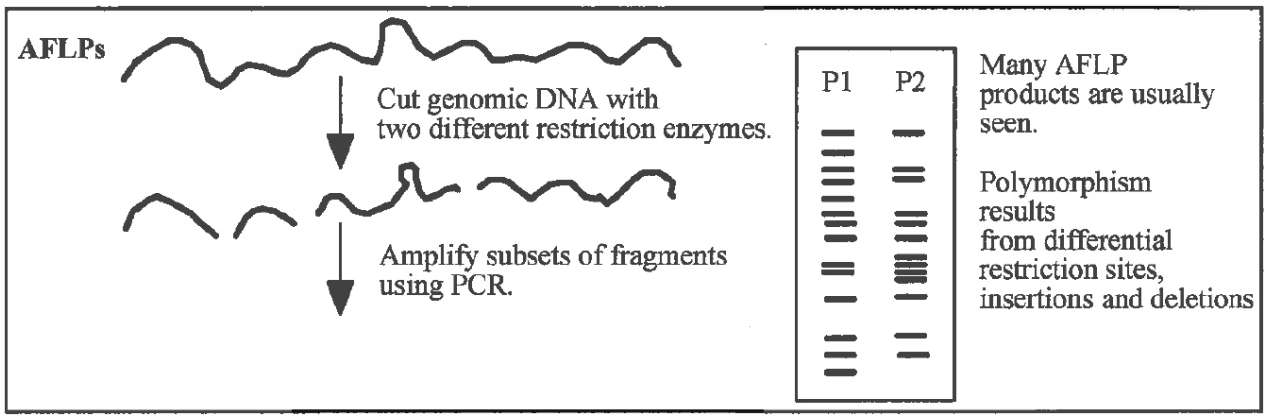

Fig. 4 The principles of AFLP markers (Prince and Ogundiwin 2004)

The map positions of numerous SSR markers have already been published, thus they can be used for identifying the chromosomal position and forming the backbone. To achieve sufficient map coverage, anonymous multi-loci markers, such as AFLP are used (Doveri et al. 2008).

3.3.4.2 Construction of Genetic Linkage Maps

Genetic linkage maps describe the order of loci within linkage groups. Loci can comprise morphological, isozyme and DNA markers. The relative distance between markers is determined by the recombination frequency. Recombination can occur between the alleles at any two loci of the homologous chromosomes. The closer two loci are located on a chromosome, the lower is the likelihood of recombination. The distance between markers is measured in centimorgans (cM). One centimorgan corresponds to a chance of one percent that two loci will be separated by recombination during meiosis. The relationship between recombination frequency and genetic distance is not directly linear. Thus, mapping functions are used to correct the departure from the linear relationship. The two most commonly used are the Haldane (1919) and the Kosambi (1943) function. There is no specific relationship between the physical and the genetic distance of loci. One centimorgan corresponds to a distance of about ten thousand to one million nucleotides (10-1,000 kb). Variation does not only exist between species, but even between regions of a single chromosome. In some regions of the genome recombination occurs more frequently than usual, causing a proportional expansion of these regions of the map. Also regions with the opposite effect are known. Another reason is the so-called interference, the effect of a recombination inhibiting a further recombination in an adjacent region. An area with severely suppressed recombination is the centromeric region.

For the construction of a genetic linkage map several steps are necessary: selection of parents; production of a mapping population; identification of polymorphic markers between the parental lines; genotyping of the population with these polymorphic markers and statistical analysis. The selection of the parental lines for producing the segregating population is a crucial step as they need to exhibit sufficient genetic polymorphism. Suitable mapping populations can, amongst others, consist of F_2 plants, backcrosses,

recombinant inbred or double haploid lines. The latter two are most commonly used for genetic mapping in the Triticeae (Mihovilovich et al. 2008, Lehmensiek et al. 2009, Griffiths AJF et al. 2012).
The construction of genetic linkage maps is facilitated by software such as MAPMAKER/EXP© (Lander et al. 1987, Lincoln et al. 1993a), JoinMap® (Stam 1993), Map Manager QTX© (Manly and Olson 1999, Manly et al. 2001), Carte Blanche© (Buntjer 2002), R/qtl© (Broman et al. 2003), CAR$^{H}_{T}$A GÈNE© (de Givry et al. 2005) and RECORD© (van Os et al. 2005). For hexaploid wheat numerous maps have been published. From the GrainGenes database (USDA 2014) 28 maps with SSR markers, including 18 maps comprising all 21 chromosomes, are available.

3.3.4.3 QTL Analysis

In crop plants several important traits related to yield, quality, efficiency of nutrient uptake, resistance and tolerance to abiotic stress show quantitative variation. There are two possible reasons for a continuous distribution: The trait is influenced by the environment or the trait is controlled by several different genes, termed quantitative trait loci (QTL). Each of these loci has a more or less small effect on the trait, segregates according to Mendel's laws, and can be affected by environmental factors to varying degrees. The genes can act in an additive, dominant or epistatic way. Molecular markers allow to map and identify QTL (Liu 1998, Zeng et al. 2008, Becker 2011, Griffiths AJF et al. 2012).
Studying size differences in beans, Sax (1923) discovered a genetic linkage between a factor for pigmentation and bean size, i.e. between a marker and a quantitative trait. The simplest method to detect QTL is the joint analysis of marker and phenotypic data, the so-called single point or single marker analysis. A genetic linkage map is not required (Lehmensiek et al. 2009). Single point analysis can be based on a simple *t*-test, an analysis of variance (ANOVA), a linear regression or a likelihood approach. Liu (1998) provides an extensive discussion of the statistics behind these methods. Single point analysis suffers from several disadvantages: It cannot be distinguished between a QTL with a small effect and tight linkage, and a QTL with large effect and loose linkage. The effect of QTL not lying at the marker locus may be underestimated and a higher progeny number is required (Lander and Botstein 1989). Therefore, several methods for interval mapping to locate QTL that make use of linkage maps have been developed. Interval mapping considers the joint frequencies of an adjacent marker pair and a putative QTL in between them (Liu 1998). Lander and Botstein (1989) used the maximum likelihood method for interval mapping. The logarithm of odds (LOD) score is the ratio of the probability of observing the data if there is a QTL near the marker locus to the probability without existence of a QTL (Griffiths AJF et al. 2012). Further approaches are nonlinear and linear regression analysis. Liu (1998) also discusses these statistical methods. Zeng (1993, 1994) improved sensitivity and efficiency of QTL mapping by combining interval mapping with multiple regression, known as composite interval mapping. Also other methods such as cross validation (Utz et al. 2000), jack-knifed partial least squares

regression (Bjørnstad et al. 2004), bootstrapping and Bayesian methods (Melchinger et al. 2004), outlier detection (Verbyla et al. 2007), Bayesian interval mapping (Yandell et al. 2007) and machine learning (Bedo et al. 2008) were introduced to improve the power of QTL detection as well as estimated effects and positions.

As an accurate map is one of the most important prerequisites for QTL mapping, Lehmensiek et al. (2005) reported a method for map curation. Other factors influencing the accuracy of QTL detection are the experimental design, the type and size of segregating population, the heritability of the trait, the number and effects of QTL, their interactions and distribution over the genome, the number of markers and their distance in between and the percentage of codominant markers. A recombinant inbred line or double haploid population has – for a given heritability of a quantitative trait – a higher power compared to an F_2 or backcross population and requires a smaller number of lines to be phenotyped, especially if replication is possible (Asíns 2002). The optimal marker density is about one every 10 cM. A further increase has little effect on the power of QTL detection. Piepho (2000) confirmed these simulation results by other authors for interval mapping in a backcross population. Last but not least, the accuracy of QTL mapping depends on the ability of the statistical method to determine the location and to estimate the genetic effect of the QTL (Asíns 2002). Several computer programs have been developed that facilitate QTL mapping, e.g. MAPMAKER/QTL© (Paterson et al. 1988, Lincoln et al. 1993b), QTL Cartographer© (Basten et al. 1994, Wang et al. 2007), PLABQTL© (Utz and Melchinger 1996), QGene™ (Nelson 1997, Joehanes and Nelson 2008), Map Manager QTX© (Manly and Olson 1999, Manly et al. 2001), MapQTL® (van Ooijen 2009), Multimapper© (Silanpää and Arjas 1998, Silanpää 2004), R/qtl© (Broman et al. 2003) and R/qtlbim© (Yandell et al. 2007). Semagn et al. (2010) provide a list of mapping software, Liu (1998) gives a list of the key references for methodology, computer software and experiments for QTL mapping.

3.3.4.4 Marker-assisted Selection (MAS)

Marker-assisted selection is the term for the usage of DNA markers in plant breeding (Collard and Mackill 2008). It is a kind of indirect selection. The big advantage of molecular markers is that they directly show the variability at the DNA level (Becker 2011). Markers have to be reliable; this implies that they need to be tightly linked to the target locus. At best the marker is located within the target gene. To reduce the chance of crossover, two flanking markers or at least markers within a genetic distance of less than 5 cM should be preferred (Collard and Mackill 2008).

Amongst the advantages of marker-assisted selection is the possibility to screen already at the seedling stage. Furthermore some marker types allow distinguishing between hetero- and homozygous plants. Thus, selection can start at an early generation. The results are not biased by environmental conditions. If traits have a low heritability because of environmental interactions, marker-assisted selection is more efficient than phenotypic selection. Marker-assisted selection furthermore enables the screening for resistance to

diseases not present in the region and can also be useful for other traits associated with phenotypes expensive, technically difficult or even impossible to evaluate (Reece and Haribabu 2007, Collard and Mackill 2008, Lübberstedt and Bhattacharyya 2008).

Important applications of marker-assisted selection:

- Marker-assisted evaluation of breeding material: On the one hand it allows getting information about the genetic diversity of the breeding material, on the other hand it enables to confirm the genetic purity of individual plants and thus support the selection of parental lines in breeding programs.
- Marker-assisted backcrossing: If genetic sources from agronomically poorly adapted donors are transferred to elite genotypes, genetic markers can not just help to identify genotypes with the target alleles, but also to reduce the amount of surrounding DNA sequence (brake the “linkage drag”) from the donor, and identify individuals of the backcross population homozygous for a very high percentage of the alleles of the recurrent parent in an early generation.
- Marker-assisted gene pyramiding: If several genes, e.g. conferring rust resistance, are to be combined in a single plant, these genes usually show the same phenotype. DNA markers enable to determine the number of involved resistance genes or confirm the presence of multiple alleles related to a single trait.
- Combined marker-assisted selection: In case of large QTL numbers, combining phenotypic assessment and marker-assisted selection can increase efficiency. Furthermore DNA markers can be used to reduce the number of individuals for phenotypic assessment.

(Holland 2004, Reece and Haribabu 2007, Collard and Mackill 2008, Lightfoot and Iqbal 2008, Kumar et al. 2011)

A potential drawback of marker-assisted selection is the favored usage of genes with high phenotypic effect and tightly linked to molecular markers in breeding and hence a reduction in genetic diversity (Singh 2012). Whether marker-assisted selection is superior to direct selection by than direct selection by phenotyping depends on the inheritance pattern of the trait (mono- to polygenic), the heritability, and the possibility and complexity of phenotypic assessment (Lightfoot and Iqbal 2008, Becker 2011).

4 Materials and Methods

4.1 Plant Material

The tested plant material belonged to recombinant inbred line (RIL) populations (single-seed descents). For the development of the plant material the parental line Capo was used for all crossings:

- Isengrain/Capo: 240 lines, $F_{6:7}$ (341 lines in the field experiments 2004-2006)
- Furore/Capo: 201 lines, $F_{6:7}$
- Arina/Capo: 233 lines, $F_{6:7}$

4.1.1 Parental Lines

4.1.1.1 Capo

Capo is an Austrian high-quality winter wheat cultivar brought to market in 1989. Despite the long time it has been cultivated, it is still the most important high-quality wheat cultivar in the eastern part of Austria with Pannonian climate. Capo is an awned early ripening cultivar of tall plant type that combines yield stability with good bread making quality (BAES 2013b). Out of 5843.94 ha of the certified area for the production of all winter wheat seeds, 984.76 ha (> 16 %) was for the variety Capo only. This is more than two fold the area of the second most important variety Antonius (BAES 2012b). In Lower Austria, the most important region for seeds production, Capo is covering almost a quarter of the total area for production of certified seeds. This is just slightly lower than the total area of Astardo, Element and Energo (BAES 2012c). Also in organic farming Capo is the most important variety with seeds being produced on nearly 30 % of the total area. This is almost the amount of Antonius and Astardo together (BAES 2012a). One reason is Capo's stable adult plant resistance to leaf rust. While it was scored with 2 on a 1 (= absent/ very low) to 9 (= very strong) scale in the year 1989, it was scored with 4 in 2012 – more than 20 years later (BAES 2013b). Therefore Capo's resistance to leaf rust seems to be durable. It does not show an immune reaction, but a typical slow rusting type of resistance. Capo's origin and pedigree is given in Table 3. The parents Pokal and Martin possess good and moderate leaf rust resistance. Capo carries *Lr13* (Winzeler et al. 2000, Mesterházy et al. 2002, Park et al. 2001) which is only effective in a few regions of Europe (Mesterházy et al. 2000, 2002, Błaszczyk et al. 2004, Lind and Gultyaeva 2007, Martínez et al. 2007, Akin et al. 2008, Vida et al. 2009). As Capo was classified as resistant in field tests at all 25 European locations, where it was evaluated over a period of two years at the end of the 1990s together with 71 other European cultivars, it was postulated that it must contain additional yet unknown resistance factors. In more than 80 % of the seedling tests conducted in the course of the same project Capo was rated as susceptible (Winzeler et al. 2000), clearly indicating that Capo possesses adult plant resistance only. Similar results were also obtained by Pathan and Park (2006).

Furthermore Capo is very resistant to yellow rust caused by *Puccinia striiformis* f. sp. *tritici*. To all other diseases tested for the Austrian list of all registered cultivars Capo is moderately susceptible (BAES 2013b). The parents Pokal and Martin are slightly more resistant to *Fusarium spp.* (Bürstmayr et al. 1996).
Capo has been crossed with several leaf rust susceptible cultivars to analyze the leaf rust resistance in the offspring. Table 3 shows the origin and pedigree of all four parents used for developing the testing populations presented in this study.
In a preceding diploma project (Matiasch 2005) the Isengrain/Capo (341 lines), Furore/Capo (201 lines) and five other Capo derived populations from past studies at IFA-Tulln were evaluated for leaf rust severity in the year 2004: SVP72017 – 272 lines (Bürstmayr et al. 2000), Mv17 – 87 lines, Bankuti 1201 – 66 lines, Tiszataj – 17 lines and Korona – 16 lines (Grausgruber-Gröger 2000).

Table 3 Pedigree of the parental lines

line	origin	pedigree and comments
Capo	Probstdorfer Saatzucht®, Austria	Diplomat/Purdue5517//Extrem/HP3517 (= Pokal//Martin)
Isengrain	Florimond Desprez®, France	Apollo/Soissons
Furore	Probstdorfer Saatzucht®, Austria	Carolus//Pokal/Martin (sisterline of Capo)
Arina	Federal Research Station for Agronomy, Switzerland	Moisson/Zenith

4.1.1.2 Isengrain

The awned French cultivar Isengrain was released in 1997 (Martynov et al. 2006). It is very early maturing and combines very high yield and good bread making quality (Elsoms 2008). The National Association of British & Irish Millers (NABIM) classified Isengrain into group 2: varieties that exhibit bread making potential, but are not suited to all kinds of grist (NABIM 2008). It shows good disease resistance, especially to yellow rust, mildew and *Septoria tritici*. Until the beginning of the 2000s it was on the Home-Grown Cereals Authority (HGCA) Recommended List® in the UK (Elsoms 2008).
Isengrain carries *Lr14a* (Błaszczyk et al. 2004, Rimé et al. 2005). The effectiveness of this gene is low, not only in Europe but throughout the world virulence is widespread (Table 93, Table 94, McIntosh et al. 1995, Mesterházy et al. 2002).
Isengrain's parent Soissons, which was already released ten years before Isengrain (Martynov et al. 2006) has been on the HGCA Recommended List® until the season 2009/2010 (HGCA 2009). Similar to Isengrain it combines unique quality characteristics for specialized bread grist and medium to high disease resistance, especially against yellow rust, mildew and *Septoria tritici* (Elsoms 2010).

4.1.1.3 Arina

Arina was released in 1981 (Martynov et al. 2006). It is an awnless Swiss winter wheat variety late in heading (Šíp et al. 2007) and susceptible to leaf rust although it carries *Lr13* (Winzeler et al. 2000, Park et al. 2001, Pathan and Park 2006). It was selected as a parental line in this project as it has been used for various studies before, and there is already plenty of information about the phenotype, physiology and genetics of this cultivar.
A genetic map of a population Arina/Forno had been constructed with SSR and RFLP markers (Paillard et al. 2003) and used for the detection of QTL for *Stagnospora* glume blotch caused by *Stagnospora nodorum*, against which Arina has a highly effective and durable quantitative resistance (Schnurbusch et al. 2003).
As Arina is moderately resistant to *Fusarium* head blight – low disease severity, high yield and relatively low content of the mycotoxin deoxynivalenol (DON) (Jenny et al. 2000) –, extensive studies of the genetic reasons have also been conducted with the same mapping population. Several QTL for the resistance to *Fusarium* head blight inherited from Arina were detected (Paillard et al. 2004).
A detailed study to identify QTL for resistance to *Fusarium* head blight and associated traits such as plant height was also done with a population Arina/Riband with participation of the working group at IFA-Tulln (Draeger et al. 2007). Amongst others, Arina and lines of the populations Arina/Forno and Arina/Riband were evaluated in a multi-environment test for their level and stability of *Fusarium* head blight resistance (Bürstmayr et al. 2008).
Arina was also included as a resistant standard line in various physiological studies about the development of *Fusarium* infection (e.g. Doohan et al. 2000, Kang and Buchenauer 2002, 2003, Browne et al. 2006, Šrobárová et al. 2009), mycotoxin accumulation (e.g. Lemmens et al. 2004, Chrpová et al. 2007, Šíp et al. 2007, 2008) and inheritance and genetic architecture of *Fusarium* resistance (e.g. Bürstmayr et al. 1999, Zwart et al. 2008, Löffler et al. 2009).
In several leaf rust studies Arina was used as the susceptible parent (e.g. Fried and Winzeler 1990, Schachermayr et al. 1994, 1995, Ortelli et al. 1996a, 1996b, 1996c), and the mapping population Arina/Forno was also used to study the genetics of Forno's leaf rust resistance (Schnurbusch et al. 2004) and the development for SSR markers specific for the *Lr34* region (Bossolini et al. 2006). In a study on the suitability of endopeptidase markers for *Lr19* Arina was used as the recurrent parent for developing testing lines (Winzeler et al. 1995).
The population Arina/Forno was furthermore used for mapping seedling and adult plant stem rust resistance. Both parents showed intermediate adult plant stem rust response. (Bansal et al. 2008a). A host-toxin interaction in the wheat-*Stagnospora nodorum* pathosystem was studied in this population with Arina being the susceptible parent (Abeysekara et al. 2009). The relationship between a gene for yellow rust resistance inherited from Forno and for stem rust resistance inherited from Arina was also investigated using this population. The stem rust resistance gene was located on chromosome 2AL and designated *Sr48* (Bansal et al. 2009).

Arina is among the most *Septoria tritici* blotch resistant cultivars (Brown et al. 2001, Chartrain et al. 2004a) and a map based on SSR and AFLP markers was constructed for a population Arina/Riband in order to detect QTL for *Septoria tritici* blotch (Chartrain et al. 2004b). Arina carries the resistance gene *Stb6* (Chartrain et al. 2005). In the population Arina/Forno (Paillard et al. 2003) on 6AS a resistance gene was identified and named *Stb15* and on 7DS a further QTL inherited from Arina (Arraiano et al. 2007).
The very first map containing DArT, AFLP and SSR markers was based on a crossing of Arina with the Norwegian spring wheat breeding line NK93604 (Semagn et al. 2006). QTL for low DON content inherited from Arina were detected in the same mapping population (Semagn et al. 2007).
Furthermore various physiological studies were done on the cultivar Arina about the uptake and distribution of seed treatments (Simmen and Gisi 1996), influences on senescence of leaves (Crafts-Brandner et al. 1998, Herrmann and Feller 1998), or the transport of different metal ions in wheat (Zeller and Feller 1999, 2000, Minder and Feller 2003, Page and Feller 2005, Riesen and Feller 2005).

4.1.1.4 Furore

Furore is a quality winter wheat with good baking and excellent milling quality released in 1998 (BAES 2011). Although it is a relative of Capo (one parent was a sisterline of Capo), it is moderately to heavily susceptible to leaf rust and heavily susceptible to yellow rust. It is only recommended for regions with dry Pannonian climate (BAES 2011).

4.1.2 Thatcher Near Isogenic Lines

In 2007 Thatcher near isogenic lines (NILs) with the genes *Lr1*, *2a*, *2b*, *2c*, *3a*, *3bg*, *3ka*, *9-13*, *14a*, *14b*, *15-26*, *28-30*, *32-35*, *37*, *38*, *44*, *B*, *W=52* and Thatcher were tested at a farmer's field near IFA-Tulln.

4.2 Field Experiments

The three populations Isengrain/Capo, Furore/Capo and Arina/Capo were tested across several years (2004-2009) and different trial sites not only in Austria (Aumühle, Probstdorf, Reichersberg, Schmida/Hausleiten, Tulln), but also in other European countries (Hungary, Romania, Slovakia and Switzerland). Detailed information about all conducted experiments is given in Table 4.
All but eleven experiments had a randomized complete block design with two replications. In 2007 an experiment with only one replication was cut in April to prolong vegetation period in order to have more time for the build up of leaf rust infection and for scoring (FM7 and IM7). IVm5, AT7 and ARe8 had just one replication; the others (IS6, IS7, ARo8, AU8, ASw8, AS8) had two replications, but were not randomized.
About 4 g of seeds were used for sowing double rows, 1 m in length and with a space of 17 cm. With a distance of 29 cm left and right to each testing plot, a mix of lines susceptible to leaf rust (mainly Arina and Ritmo), was grown as a single row (see Fig. 5).

This “spreader row” aimed at provoking an even disease pressure over the whole experimental area. Furthermore this layout offered the opportunity to check uniformity of leaf rust infection across the site (see 5.1.1.3 Analysis of Covariance).
Several standard lines with reported susceptibility or resistance to leaf rust and partly known *Lr* genes were included for comparison in all field experiments.

Fig. 5 Layout of the field plots

Fertilizing and weed control was done as common in the particular growing area. At the Austrian trial sites in the years 2005-2009 some fungicides against *Septoria nodorum* were applied:

- Mirage® 45 EC (Prochloraz; Makhteshim Agan, Cologne, DE): 1.2l /ha, BBCH 59-69,
- Sportak® 45 EC (Prochloraz; BASF SE, Limburgerhof, DE): 1 l/ha, BBCH 59-69 or
- Pugil® 75 WG (Chlorthalonil; Vischim srl, Pero, IT): 1.4 kg/ha, BBCH 51-61

As an example, the experiment in Tulln 2007 is described in detail:

Planting:	2006-10-18	Seed treatment with Rovral® TS (Carbendazim + Iprodion; BASF SE, Limburgerhof, DE)	1 ml/100 g seed
		Rovral® TS: Kwizdaplast® (adhesive, Kwizda Agro GmbH): Water = 1 : 1 : 2	
Fertilizer:			
1st application:	2007-03-04	Vollkorn plus 20.8.8	60 kg N/ha
2nd application:	2007-05-14	NAC (27% N)	30 kg N/ha
Weed control:	2007-04-13	Express® SX (Tribenuron; Du Pont de Nemous GmbH, Neu-Isenburg, DE)	15 g/ha
		Platform® S (Carfentrazone-ethyl; TBH Agrochemie GmbH, Allerheiligen, AT)	0.7 l/ha
Fungicide:	2007-05-16	Mirage® 45 EC (Prochloraz; Makhteshim Agan, Cologne, DE)	1.2 l/ha

Thatcher NILs were only tested in 2007 in Tulln in 1 m^2-plots in one not-randomized replication.
Table 4 lists all experiments from 2004 to 2009 with the RIL populations Isengrain/Capo, Arina/Capo and Furore/Capo: experimental locations, tested populations, the abbreviations of the experiments, the number of leaf rust assessments (LR) and other evaluated traits. If the layout was not a randomized complete block design with two replications (rep.), it is mentioned in the last column “comments”. All evaluated traits are described in

detail in 4.4 and 4.5. “LR spreader” means that the leaf rust infection of the spreader row plants was scored a few days before the first rating of the experimental plots.

Table 4 Overview on all experiments for all three tested populations and all evaluated traits

year	location	population	exp	LR	other traits	comments
2004	Aumühle/AT	Furore	FA4	1	flowering	
2004	Aumühle/AT	Isengrain	IA4	1	flowering, leaf blotch severity	
2004	Tulln/AT	Furore	FT4	2	flowering, plant height, crop density, teliospores	
2004	Tulln/AT	Isengrain	IT4	2	flowering, plant height, crop density, teliospores	
2005	Tulln/AT	Furore	FVm5	0	heading	
2005	Tulln/AT	Isengrain	IVm5	0	heading	1 rep.
2005	Tulln/AT	Isengrain	IVs5	1		
2006	Piešťany/SK	Isengrain	IS6	3	heading	not random.
2006	Probstdorf/AT	Furore	FP6	2	heading, powdery mildew sev., frost heaving sev.	
2006	Probstdorf/AT	Isengrain	IP6	2	heading, powdery mildew sev., frost heaving sev.	
2006	Tulln/AT	Furore	FT6	0	flowering, plant height	
2006	Tulln/AT	Isengrain	IT6	0	flowering, plant height	
2007	Piešťany/SK	Isengrain	IS7	3	heading	not random.
2007	Probstdorf/AT	Furore	FP7	2	heading, lodging severity	
2007	Probstdorf/AT	Isengrain	IP7	2	heading, lodging severity	
2007	Schmida/AT	Isengrain	IH7	3	heading	
2007	Tulln/AT	Arina	AT7	3	heading, plant height, awnedness	1 rep.
2007	Tulln/AT	Furore	FT7	1	heading, plant height	
2007	Tulln/AT	Furore	FM7	1	heading, plant height	1 rep.
2007	Tulln/AT	Isengrain	IM7	1	heading, plant height	1 rep.
2007	Tulln/AT	Isengrain	IT7	0	heading, plant height	
2008	Fundulea/RO	Arina	ARo8	3	heading, infection type, seedling resistance	rep. beside each other
2008	Martonvásár/HU	Arina	AU8	1	infection type	not random.
2008	Nyon/CH	Arina	ASw8	2	heading, powdery mildew severity, *Septoria* leaf blotch severity, yellow rust severity, infection type	not random.
2008	Piešťany/SK	Arina	AS8	3	heading	not random.
2008	Probstdorf/AT	Arina	AP8	1	lodging severity, LR spreader	
2008	Probstdorf/AT	Furore	FP8	0	heading, powdery mildew severity, *Septoria* leaf blotch severity, lodging severity, LR spreader	
2008	Probstdorf/AT	Isengrain	IP8	1	lodging severity, LR spreader	
2008	Reichersberg/AT	Arina	ARe8	1	heading	1 rep.
2008	Rust/AT	Arina	ARu8	1	heading, awnedness, lodging severity, LR spreader	
2008	Rust/AT	Furore	FRu8	0	heading	
2008	Rust/AT	Isengrain	IRu8	1	heading, lodging severity, LR spreader	
2008	Schmida/AT	Arina	AH8	1	heading, awnedness, LR spreader	
2008	Schmida/AT	Furore	FH8	0	heading, LR spreader	
2008	Schmida/AT	Isengrain	IH8	1	heading, LR spreader	
2008	Tulln/AT	Arina	AT8	3	heading, plant height, awnedness, LR spreader	
2008	Tulln/AT	Furore	FT8	3	heading, plant height, LR spreader	
2008	Tulln/AT	Isengrain	IT8	3	heading, plant height, LR spreader	
2009	Rust/AT	Arina	ARu9	5	heading, leaf chlorosis severity, leaf tip necrosis severity, glaucousness	
2009	Rust/AT	Isengrain	IRu9	2	leaf chlorosis severity, leaf tip necrosis severity	
2009	Tulln/AT	Arina	AT9	4	heading, leaf chlorosis sev., leaf tip necrosis sev.	
2009	Tulln/AT	Isengrain	IT9	0	heading, leaf chlorosis sev., leaf tip necrosis sev.	

4.3 Artificial Leaf Rust Inoculation

Collection and storage of spores, inoculum preparation and inoculation techniques were based on the methods described by Browder (1971), McIntosh et al. (1995), Walther (1996), Singh and Huerta-Espino (2003) and Cherukuri et al. (2005).

4.3.1 Collection and Storage of Spores

- Infected leaves were collected at different trial sites and in different experimental units, not only in the leaf rust trials. With a special adaptor for the vacuum cleaner, the spores were sucked directly into 50 ml plastic tubes. Bigger leaf particles were removed with tweezers. Spores with less contamination were harvested from our trousers at the very beginning of the scoring time. Collecting spores by the use of draperies would be a good possibility if this can be done at another location than the experiments to be scored.
- The open tubes were either dried with a lyophilizer for one day or left in an exsiccator with Silica Gel Orange® (2-5 mm, Indicator, Perlform, Carl Roth GmbH + Co. KG, Karlsruhe, DE, #P077.2) for about two weeks.
- Spores were stored in the tubes, additionally packed into a vacuum sealed plastic bag at -80°C.

4.3.2 Inocula Preparation

- To reactivate spores, the tube was put immediately after taking out of the -80°C freezer, in a water bath with 42°C for 5 minutes (modified after McIntosh et al. 1995).
- For a water based suspension (≈ 10 Mio/ml), about 0.2 g spores were suspended in 100 ml dest. H_2O. 75 µl Tween20® (Sigma-Aldrich CHEMIE Gmbh, Steinheim, DE, #P1379) were added to improve moistening and suspending of the spores as described by Cherukuri et al. (2005). The suspension was left on a magnetic stirrer for at least one hour to ensure even concentration.
- For a mineral oil suspension (Light white oil®, Sigma-Aldrich #M-3516), 2.5 g spores were dissolved in 1 l oil.
- Due to the harvesting method, spores were always mixed with small plant particles. Therefore the resulting spore concentration was always checked using a Bürker-Türk counting chamber. As the suspension was – despite mixing well – always rather inhomogeneous, all 16 small squares were counted in both parts of the chamber and it was repeated two times.

4.3.3 Inoculation Techniques

Four different inoculation techniques were applied over the intervening years of the project:

- Planting of infected seedlings
- Inoculation into the leaf sheaths
- Spraying with a hand sprayer and covering over night
- Spraying with Micron® Sprayer
- Brushing with Silica

To control the effectiveness of the inoculum used for spraying the field trials, two multi plates with seedlings were inoculated in the climate chamber (≈ 15 hours, 100% humidity, room temperature). In parallel susceptible wheat plants grown next to the laboratory were inoculated in two replications with the normal, double- and three-fold amount of the inoculum and covered over night with a plastic bag in the same way as the inoculated plants on the trial sites. Doing this it was possible to know which inoculation maybe failed.

Planting of Infected Seedlings

Seedlings at about BBCH 12 – 13 (two to three leaves unfolded) were inoculated with water based inoculum (sprayed several times) and left for about one day in a climate chamber with almost 100% humidity and room temperature. Afterwards they were placed back into the greenhouse until leaf rust symptoms developed. At tillering or latest at about BBCH 30 (beginning of stem elongation) when the weather forecast promised warm and rainy weather one infected seedling was planted in every second spreader row. One or two weeks later planting was repeated for the other spreader rows.

Inoculation into the Leaf Sheaths:

At a rainy day at the beginning of stem elongation 25 µl of water based inoculum were injected with a mechanical Stepper (Handy *Step*®, Brand GmbH + Co KG, Wertheim, DE) directly into the leaf sheaths of the flag leaf of two plants per spreader row.

Spraying with a Hand Sprayer and Covering over Night:

In the afternoon (between about 14 and 17 o'clock) at tillering (BBCH 2) or beginning of stem elongation one plant in every second spreader row was inoculated by means of a hand sprayer with 2 ml of water based inoculum. Then it was covered over night with a plastic bag (see Fig. 6). The next morning between 8:30 and 10 o'clock the bags were removed. The longer the coverage the better, but too much heating of the covered plants should be avoided. About one week later, inoculations were repeated for the other spreader rows. This method seemed to be the most effective one.

Fig. 6 Leaf rust inoculation with hand sprayer and covering over night with plastic bags

Spraying with Micron® Sprayer

In the late afternoon at the end of tillering (BBCH 29) the whole experiment was inoculated with an oil based inoculum. Using ULVA+® (Micron Sprayer Ltd., Bromyard, Herefordshire, UK) with a flow rate of 10 ml/min and a walking speed of 1 plot/sec, each plot was inoculated with about 150 µl.

The little effect of this inoculation method was probably caused by the small amount of spores and the very late application in the growing season (just a few days before heading).

Brushing with Silica

400 mg spores were mixed with 1.5 g silica (Celite® 545, particle size 0.02-0.1 mm, Merck KgaA, Darmstadt, DE, #1.02693.0250). This mixture was diluted 10-fold and 100-fold. Flag leaves were rubbed with one of these three concentrations of spore-silica mixture. The amount of spores per leaf was 5.5 Mio spores for the highest concentration. The leaves were sprayed with aqua dest. immediately afterwards. This was only tried out on some plants at about the end of stem elongation outside the experiments and in the greenhouse.

4.4 Leaf Rust Assessment

Leaf rust severity was visually scored in each plot according to the scale described by Bartels and Backhaus. (2000). On infected leaves brown colored pustules of urediniospores are visible (see Fig. 7). The average percentage of covered area of the upper leaves of all plants in each plot was estimated according to Fig. 8.

Unfortunately some experiments failed to yield data due to late or too weak infection with leaf rust despite artificial inoculation. Table 4 shows, in which experiments and how often leaf rust severity data were collectable. If possible, scoring was repeated several times.

Fig. 7 Symptoms of leaf rust on infected plants

Leaf rust severity measured by **the area under the disease progress curve** (AUDPC) was calculated for each plot according to the modified formula of Shaner and Finney (1977) if leaf rust severity was scorable at least three times for one experiment.

$$AUDPC = \sum_{i=1}^{n-1}[(y_{i+1} + y_i)/2 * (x_{i+1} - x_i)]$$

y_i ..... leaf rust severity (% diseased leaf area) at the i^{th} observation
x_i ..... day of the i^{th} observation
n..... total number of observations

As the period between scorings was different in each experiment, the relative AUDPC was calculated for comparing data:

$$\text{rel. AUDPC} = 100 * \sum_{i=1}^{n-1}[(y_{i+1} + y_i)/2 * (x_{i+1} - x_i)] / (100 * x_n)$$

Its dimension is time in days beginning from the first rating by disease score (% infected leaf area).

The **appearance of teliospores** on the lower leaf surface was noted (0 = no visible teliospores, 1 = teliospores visible).

Infection type was scored by the three project partners in Fundulea, Nyon and Martonvásár in 2008. The scoring schemes used in Fundulea and Martonvásár are different modifications (additional levels) of the scheme described by Roelfs et al. (1992): R = resistance, MR = moderate resistance, MS = moderate susceptibility, S = full susceptibility. In Nyon infection type was scored after McIntosh et al. (1995). The scoring schemes are hardly comparable and these data have not yet been used for any statistical analysis.

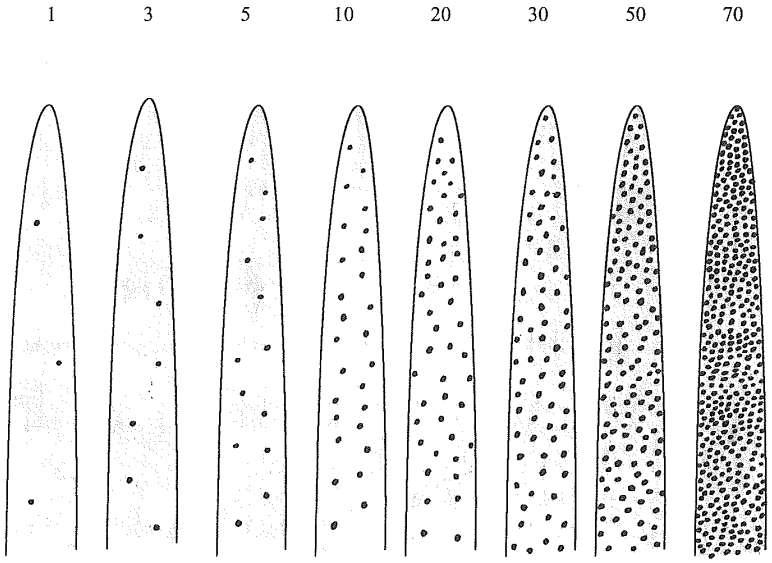

Fig. 8 Scoring aid for estimating the percentage of diseased leaf area (Moll et al. 1996 in Bartels and Backhaus 2000)

Seedling resistance: This experiment was sown and inoculated separately in the greenhouse in one replication. Infection type was scored on a 0 (= immune) to 4 (= susceptible) scale described by Roelfs (1984).

LR infection of spreader row plants was scored a few days before it was possible to score LR infection of the experimental plots, too. The average infection of the whole row was scored on a 1 (= very few pustules) to 5 (= heavily diseased) scale.

4.5 Other Traits

Heading: Generally the day was noted, when about half of the plants were heading or flowering. Plots were checked every second or third day. For further calculations this value was converted to “day of the year”. If **flowering** was scored instead of heading, five days were subtracted. The experiments P6, S6, FT6, H7, S7 and S8, were scored on another scale (e.g. 1 = early to 9 = late), too late or not often enough to be easily comparable with the others. As there were plenty of data for this trait, data of these experiments were not used for further calculations except trait correlations of single experiments.

Plant height was measured by the distance in cm from the ground level to the top of the heads, not taking awns into account, and approximated to ± 5 cm.

Awnedness was scored for the population Arina/Capo with the levels “no” (= all plants awnless), “partly” (= some plants awnless, others awned) and “yes” (= all plants awned).

Crop density was scored on a 1 (= no missing plants) to 5 (= more than half of the plants missing) scale, if there were big differences caused by bad germination, frost heaving or bird damage.

Leaf blotch severity caused among other factors by the fungus *Septoria* sp. was scored on a 1 (= no visible symptoms) to 9 (= completely diseased) scale.

***Septoria* leaf blotch** (*Septoria* sp.) **severity** was scored on a 1 (= no visible symptoms) to 9 (= completely diseased) scale.

Powdery mildew severity caused by the fungus *Erysiphe graminis* was scored on a 1 (= no visible symptoms) to 9 (= completely diseased) scale.

Yellow rust (*Puccinia striiformis* f. sp*. tritici*) **severity** was scored on a 1 (= no visible symptoms) to 9 (= completely diseased) scale.

Glaucousness was scored on a 1 (= no wax) to 5 scale.

Frost heaving severity was scored on a 1 to 9 scale. 1 = no damage, 2 = leaf (tips) slightly damaged, 3 = (outer) leaves partly dead, 4 = single tillers frozen, 5 = definite frostbites of single plants… 9 = all plants frozen.

Lodging severity was scored either on a 1-5 (P7), 0-5 (P8, Ru8) or 1-9 (FP8) scale with 0 or 1 = no lodging and 5 or 9 = complete lodging.

Leaf chlorosis severity was scored on a 0 (= no visible symptoms) to 9 (= extremely chlorotic) scale.

Leaf tip necrosis severity was scored on a 0 (= no visible symptoms) to 9 (= heavy symptoms) scale.

4.5.1 Molecular Markers

For construction of genetic linkage maps and detection of genomic regions linked to phenotypic traits, two different molecular marker techniques were used: microsatellites or simple sequence repeats (SSR) and amplified fragment length polymorphism markers (AFLP). Genotyping was carried out on 240 lines of the population Isengrain/Capo and the whole population Arina/Capo. The 120 lines most susceptible and most resistant to leaf rust were selected based on the phenotypic data of the 2004 and 2005 field experiments, but those exhibiting severe symptoms of other leaf diseases were discarded.

4.5.1.1 DNA Extraction

For isolation of genomic DNA lyophilized young leaves were used, following a modification of the CTAB extraction method described by Hoisington et al. (1994). For details see Appendix A: Lab Instructions and Protocols.

4.5.1.2 SSR Markers

For amplification of directly labeled SSR primers a modification of the PCR protocol described by Röder et al. (1998) was used. In this case the 5' end of the forward primer was directly labeled with a fluorochrome, either IRD700 or IRD800. Alternatively forward primers that had a M13-tail and a modification of the protocol of Steiner et al. (2004) were used. Then a M13-30 oligonucleotide (5' CCC AGT CAC GAC GTT G 3') labeled with the fluorochrome had to be added as a third primer to the reaction mix. Fragments were separated and detected on a LI-COR® 4200 DNA Analyzer (LI-COR® Biosciences GmbH, Bad Homburg, DE). DNA bands were detected with a laser, and a digital image was collected on a PC. On a dual dye machine samples labeled with the two different fluorochrome s could be recorded simultaneously. For details see Appendix A: Lab Instructions and Protocols.

Almost 300 SSR primers were tested for polymorphic markers between the parental lines: 82 BARC (Beltsville Agricultural Research Center, P. Cregan, USDA-ARS, USA), 188 GWM (Gatersleben wheat microsatellite, M.S. Röder, Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany) and GDM (Gatersleben D-genome microsatellite) and 21 WMC (Wheat Microsatellite Consortium, P. Isaac, Agrogene, France). 129 SSR primers were applied on the population Isengrain/Capo, 36 on the population Arina/Capo.

Information about primer sequences was taken from Korzun et al. (1997), Röder et al. (1998), Pestsova et al. (2000), Gupta et al. (2002), Song et al. (2002), and the GrainGenes database (USDA 2014). Unpublished microsatellites were kindly provided by M.S. Röder (IPK Gatersleben), and P. Cregan and Q.J. Song (BARC, USDA-ARS).

If a primer produced more than one polymorphic fragment, they were numbered starting from the shortest fragment. As an example, the pattern of the primer Barc340 is shown in Fig. 9. This primer produces a codominant fragment (*Xbarc340.1*), a polymorphic fragment (but monomorphic for the parents, *Xbarc340.2*) and a dominant fragment for Isengrain

(*Xbarc340.3*). Fragments like *Xbarc340.2* were only used for mapping if the population segregated almost 50 : 50 for the two alleles.

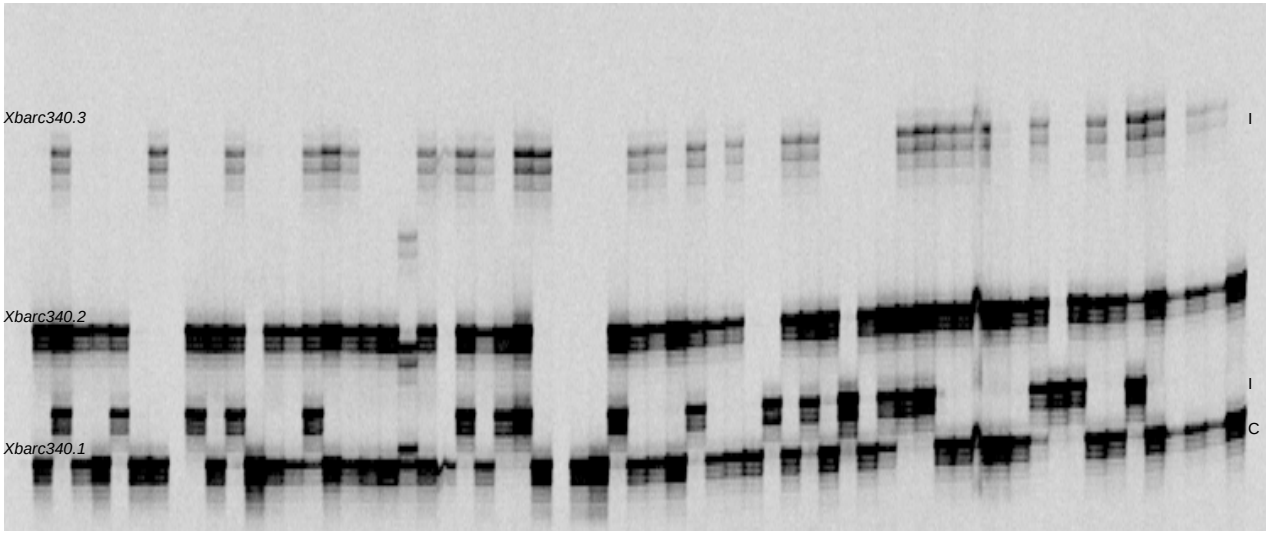

Fig. 9 Polymorphism pattern of the SSR primer Barc340 producing three fragments for 60 lines of the population Isengrain/Capo. I = Isengrain allele, C = Capo allele

4.5.1.3 AFLP Markers

The protocol for AFLP was a modification of the one described by Hartl et al. (1999). Fragments were analyzed on a LI-COR® machine (if labeled with IRD700 or IRD800) or separated on C.B.S.® Vertical Electrophoresis System (Sequencer) (C.B.S. Scientific Company, Del Mar, US) and detected on Typhoon Trio™ Variable Mode Imager (Amersham Biosciences, Buckinghamshire, GB). In the latter case depending on the used dyes up to three samples could be analyzed simultaneously. For details see Appendix A: Lab Instructions and Protocols.

From 42 AFLP primer combinations with two selective nucleotides at the 3' end of both primers tested, 30 were applied on the population Isengrain/Capo. Table 85 on page 121 gives the number of polymorphic loci for each combination. The loci were named depending on the used primer combination according to the list by KeyGene (2010). The numbering of all polymorphic fragments resulting from one primer combination was done beginning with the shortest one. As an example, the pattern of primer combination S11M23 is shown in Fig. 10. C.B.S.® gels were scanned at least two times. The shown picture was collected after four hours, when short fragments were no longer visible because of the shorter time for passing the whole gel.

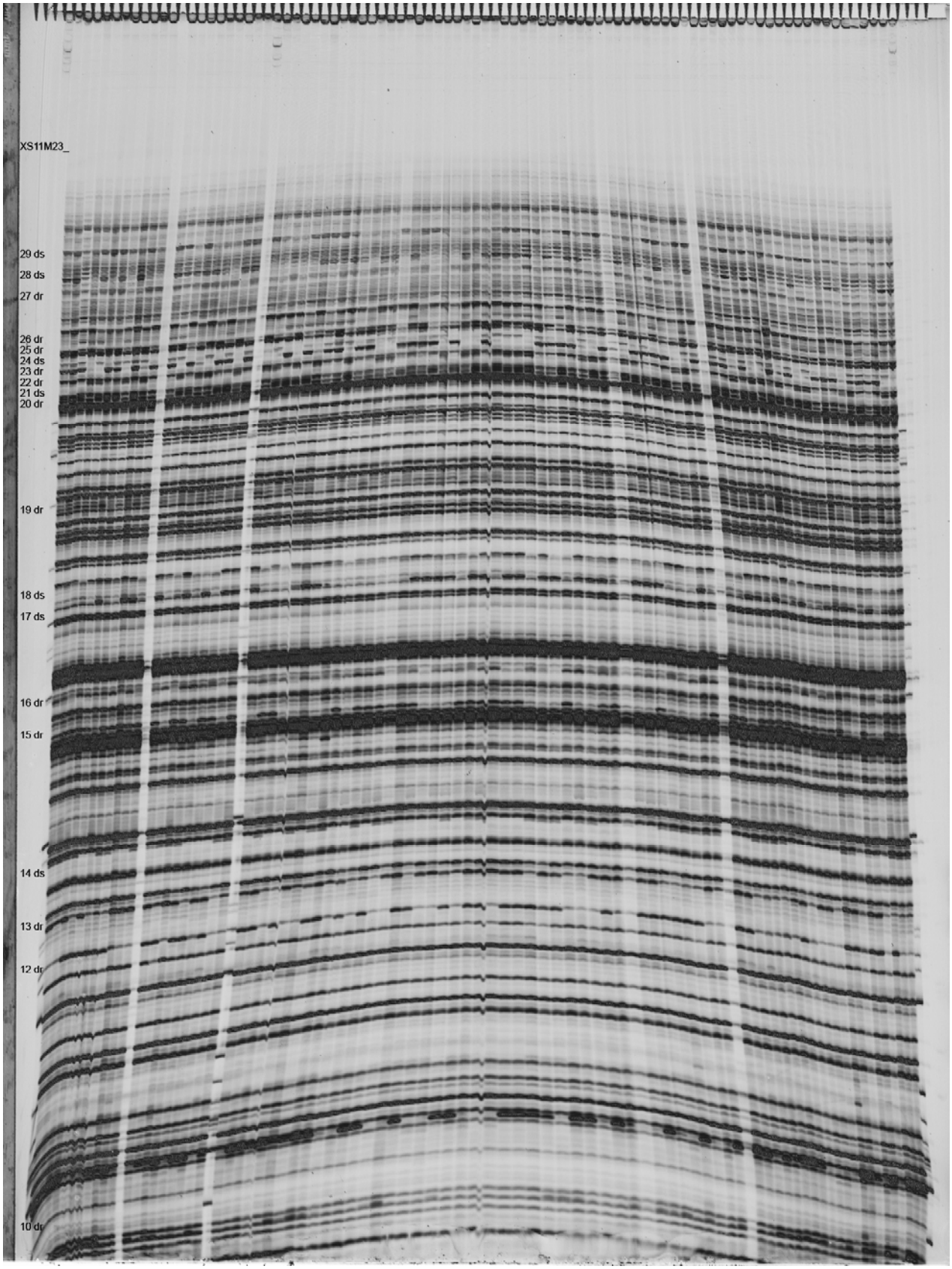

Fig. 10 **Polymorphism pattern of the AFLP primer combination S11M23 for 80 lines of the population Isengrain/Capo. Whether the fragment results from the resistant line Capo or the susceptible line Isengrain is indicated by dr (dominant res.) and ds (d. susceptible)**

4.5.1.4 SSR Marker Haplotype Comparison

In order to test whether the detected QTL for leaf rust resistance (see 5.4 Quantitative Trait Mapping) might correspond to already known *Lr* genes, a haplotype comparison was performed. Capo and Isengrain alleles of SSR markers in these QTL regions were compared to those of standard lines possessing resistance genes previously mapped in or near these regions.

Leaf rust resistance gene *Lr27* has been described for the short arm of chromosome 3B (Singh and McIntosh 1984). Microsatellite markers for the leaf rust resistance gene *Lr27* were chosen according to the map of chromosome 3B (Fig. 45) and the detected QTL (Fig. 86).

Table 5 Microsatellite markers/ primers used for haplotype analysis of the QTL for leaf rust resistance on linkage groups 3B and 7B_2

marker/ primer	linkage group	position (cM)	*Lr14a*	*Lr19*	*Lr27*	references
Xgwm533	3B	1.3				
Xbarc75	3B	4.5				
Xgwm493	3B	23.2			3B	GrainGenes (USDA 2014)
Xwmc273.2	7B_2	54.2	7B			Herrera-Foessel et al. (2008)
Xwmc273.1	7B_2	54.3	7B			Herrera-Foessel et al. (2008)
Xbarc32.2	7B_2	54.7				
Xwmc70	7B_2	58.2				
Xwmc10.2	7B_2	58.5				
Xbarc340.3	7B_2	58.7	7B			Herrera-Foessel et al. (2008)
Xgwm146.1	7B_2	58.8	7B			Dreisigacker (2009), Herrera-Foessel et al. (2008)
Xgwm344.1	7B_2	58.9	7B			Dreisigacker (2009) Herrera-Foessel et al. (2008)
Xgwm132.1	7B_2	59.0				
Xwmc500.3	7B_2	60.2				
Xwmc232	7B_2	60.6				GrainGenes (USDA 2014)
Xbarc182	7B_2	61.1				Xu et al. (2005b+c), Herrera-Foessel et al. (2008)
Xwmc557.1	7B_2	62.8				
Wmc221				7D		Dreisigacker (2009)
Xgwm44	7D	107.8		7D		Li et al. (2006)

For the long arm of chromosome 7B the two resistance genes *Lr14a* (Law and Johnson 1967, Herrera-Foessel et al. 2008) and *Lr19* (Prins et al. 1997, Prins and Marais 1998) have been described. Markers were taken from the map of linkage group 7B_2 (Fig. 57) and the detected QTL for leaf rust severity, AUDPC and the occurrence of teliospores (Fig. 91). In addition probes published for these resistance genes or markers previously mapped to these chromosomal regions were used. Table 5 lists all SSR markers used for haplotype comparison, the resistance genes and chromosomes for which they were described in the specified references. With the exception of *Xbarc340.3*, *Xgwm132.1* and *Xwmc232* they were mapped by Somers et al. (2004) to the same chromosomal regions.

Ten other markers published for the down end of chromosome 7BL by Somers et al. (2004), Herrera-Foessel et al. (2008) or the GrainGenes database (USDA 2014) either produced no fragments, fragments of the same length for Isengrain and Capo or a polymorphic pattern too difficult to be scored for the population and were therefore not considered for haplotype analysis.

For allele comparison 25 varieties and standard lines carrying *Lr14a*, *Lr27* (or both) or *Lr19* were selected. Parents of all three tested populations and the parents of the cultivar Isengrain were included in haplotype analysis. All lines and the described *Lr* genes are given in Table 6. If a line carries two or more *Lr* genes different from *Lr13*, *Lr14a*, *Lr19* or *Lr27*, they are not specified (i.a.).

The cultivars Cranbrook, Shortim, Timgalen, Gatcher, Karl, Kalkee, Rescue, Warigo, Agatha, Agrus, Hand, Kawfars and Transfer found in the Germplasm Resources Information Network (GRIN) database were kindly provided by National Small Grain Collection, Agricultural Research Service, United States Department of Agriculture (NSGC); Apollo by Volker Lindt, PhD; F_4 34/1/1/98, F_4 34-9, F_4 34-10, F_4/8, F_4 1/27/2 by Doc. RNDr. Ján Kraic, PhD (Director of Research Institute of Plant Production Piešťany, Slovakia) and Fundulea-29 by Mariana Ittu, PhD (Senior scientist wheat breeding team, National Agricultural Research and Development Institute Fundulea, Romania).

The cultivar Transfer was confounded with Transfer-12 which carries *Lr19* and included into the analysis although it has only *Lr9*.

Fragments were scored with C (same length as the Capo allele), I (same length as the Isengrain allele), N (neither the length of C or I) or 0 (no visible fragment in the considered size interval).

Table 6 Parental and standard lines with known *Lr* genes used for allele comparison

line	resistance gene	parent of	references
Cranbrook	*Lr27* (i.a.)		Martynov et al. 2006
Shortim	*Lr27+ Lr1*		Martynov et al. 2006
Timgalen	*Lr27* (i.a.)		McIntosh et al. 1995, Martynov et al. 2006
Hope	*Lr14a+ Lr27+ Lr22b*		McIntosh et al. 1995, Martynov et al. 2006
Gatcher	*Lr14a+Lr27* (i.a.)		McIntosh et al. 1995, Park et al. 2002, Martynov et al. 2006
Karl	*Lr14a+Lr27* (i.a.)		Martynov et al. 2006
Kalkee	*Lr14a+Lr27*		Martynov et al. 2006
Rescue	*Lr14a+Lr27*		Martynov et al. 2006
Warigo	*Lr14a+Lr27*		Martynov et al. 2006
Courtot	*Lr14a*		Martynov et al. 2006
Inia-66	*Lr14a*, *Lr13* (i.a.)		McIntosh et al. 1995, Martynov et al. 2006
Récital	*Lr14a*, *Lr13*		Park et al. 2001
Forno	*Lr14a*		Winzeler et al. 2000, Park et al. 2001 Pathan and Park 2006
Renan	*Lr14a+Lr37*		Winzeler et al. 2000, Błaszczyk et al. 2004, Pathan and Park 2006
Soissons	*Lr14a*	Isengrain	Park et al. 2001, Błaszczyk et al. 2004, Rimé et al. 2005
Apollo	*Lr13+Lr26*	Isengrain	Park et al. 2001, Singh D et al. 2001, Pathan and Park 2006
Isengrain	*Lr14a*	the population Isengrain/Capo	Błaszczyk et al. 2004, Rimé et al. 2005
Capo	*Lr13+*	all three tested populations	Winzeler et al. 2000, Park et al. 2001, Pathan and Park 2006
Furore		the population Furore/Capo	
Arina	*Lr13*	the population Arina/Capo	Winzeler et al. 2000, Park et al. 2001, Pathan and Park 2006
F_4 34/1/1/98	*Lr19*		Kraic (2006)
F_4 34-9	*Lr19*		Kraic (2006)
F_4 34-10	*Lr19*		Kraic (2006)
F_4/8	*Lr19*		Kraic (2006)
F_4 1/27/2	*Lr19*		Kraic (2006)
Agatha	*Lr19*		Martynov et al. 2006
Agrus	*Lr19*		Martynov et al. 2006
Hand	*Lr19*		Martynov et al. 2006
Kawfars	*Lr19*		Martynov et al. 2006
Transfer	*Lr9*		Martynov et al. 2006
Fundulea-29	*Lr19* (i.a.)		Martynov et al. 2006

4.6 Statistical Analysis

4.6.1 Reproducibility of Disease Assessment

In the years 2006 and 2007 it was possible that always the same two persons scored the trait leaf rust severity in all experiments in Austria together. Because of more populations and more experiments in 2008, these two persons had to split up populations. Even though using the scoring aid shown in Fig. 8, the standard lines distributed over the three populations at one experimental site were assessed by both raters independently to ensure comparable scores: the first time one part together with the population in which they were planted and in the second run all standards separately. The resulting data should be checked if there were differences between the two scores of one rater and if scores of the two raters fit together.
Additionally data of two ratings for the same plants done by two different persons on the same day from one replication of an experiment in 2007 and one experiment in 2009 ought to be analyzed to see how well these repeated scorings fit.

Comparison of Reproducibility for the Unequally Fine Graded Classes of the Scoring Aid

The scoring aid used for assessing leaf rust severity (% infected leaf area) is finer graded in the area of low leaf rust infection than in the part of higher infection: Between zero and ten percent infected leaf area there are additionally the classes one, three and five percent. Furthermore the classes 20 and 30 percent are shown and 50 and 70 percent. Additionally the classes 40, 60 and above 70 percent in steps of 10 percent were used in assessments.
For each repeated assessment the proportion of lines assigned to the same class in the second scoring/ by the second rater was determined to see, whether reproducibility differs with the range of disease severity, e.g. due to the very fine graded classes beyond ten percent, or in the mid range as would be expected if the Weber-Fechner law was valid.

Test of the Absolute Differences of Repeated Assessments

In the optimal case, all plants get the same score at both times and by both raters. The mean of these absolute differences was tested, null hypothesis H_0: $\mu_{|d|} = 0$ against the one-sided alternative hypothesis H_A: $\mu_{|d|} > 0$.
The required optimal sample size was determined iteratively from

$$n = \left\lceil \left[\frac{\left(t_{n-1;1-\alpha} + t_{n-1;1-\beta}\right) * \sigma}{\delta} \right]^2 \right\rceil$$ (modified after Rasch et al. 1999, pp. 78-79)

with:

n..... sample size (total number of observed plants)
α ..... type I error probability (rejecting a valid null hypothesis)
β..... type II error probability (rejecting a valid alternative hypothesis)
$t_{n-1;\ 1-p}$.... quantile of the *t*-distribution

σ^2 ..... residual variance

δ..... minimal distance to be detected

This is implemented in CADEMO® Modul LIGHT Version 3.27 (BioMath GmbH 16.11.2006): Fallzahl → Einstichprobenproblem → Mittelwert Test…

Precision requirements were set to $\alpha = 0.05$ (risk of the first kind: mistaken rejection of a valid null hypothesis) and $\beta = 0.01$ (risk of the second kind: mistaken acceptance of a wrong null hypothesis and rejection of a valid alternative hypothesis). For the particular problem, the error probability for acceptance of H_0 should be small, whereas error probability for rejection of H_0 can be larger. $s^2 = 100$ was the estimate for σ^2 and a minimal difference of $\delta = 7$ should be detected. An optimal sample size of $n = 35$ would be necessary.

As sample sizes were consistently larger – the number of plants scored two times resulted out of the necessity to have all standard lines scored by one rater and was not based on considerations about precision requirements – and variances differed due to varying maximum disease severity level, the detectable difference d was estimated for each test.

The test statistic was:

$$t = \frac{\bar{\mathbf{x}} - \mu_0}{\mathbf{s}} * \sqrt{n}$$

with:

$\bar{x}$ ..... mean of the absolute differences

μ_0 ..... given value of the mean (= zero)

s..... sample standard deviation of the absolute differences

The null hypothesis H_0: $\mu_{|d|} = 0$ was accepted if $|t| \leq t_{n-1;1-\alpha}$, otherwise it was rejected (modified after Rasch et al. 1999, p. 77).

This test is implemented in R©:

t.test(*variable*, mu=0, alternative="greater", conf.level=0.95)

To account for narrower class width at low disease severity levels, additionally to leaf rust severity measured by the percentage of infected leaf area the absolute class differences were calculated.

Model II Regression Analysis

Regression analysis was used to test whether there was systematic and/or constant bias in the reproducibility of assessments. In the case of no bias the intercept β_0 would be zero and the slope β_1 one in the linear regression model $\mathbf{y}_i = \beta_0 + \beta_1 \mathbf{x}_i$.

These two hypotheses were tested as follows (modified after Rasch et al. 1999, pp. 156-157, 143):

Test of the intercept: H_0: $\beta_0 = 0$ against the two-sided alternative H_A: $\beta_0 \neq 0$

The test statistic was $t_{\beta_0} = (\beta_0 - \beta_{0_0}) / s_{\beta_0}$ and H_0 was rejected if $|t_{\beta_0}| > t_{n-2;\,1-\alpha/2}$ with:

β_{0_0} ..... given value of β_0 (= zero)

n..... sample size (total number of observed plants)

α ..... type I error probability (rejecting a valid null hypothesis)

$t_{n-2;\,1-\alpha/2}$ .. quantile of the *t*-distribution

s_{β_0} ..... standard deviation of β_0, calculated as:

$$s^2_{\beta_0} = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2} * \frac{\sum_{i=1}^{n} x_i^2}{n * \sum_{i=1}^{n}(x_i - \bar{x})^2}$$

with:

y_i ..... score of the i^{th} plant in the second assessment/ by the second person

$\hat{y}_i$ ..... estimated value of the y variable according to the regression model

x_i ..... score of the i^{th} plant in the first assessment/ by the first person

$\bar{x}$ ..... mean value of the x variable

This test is implemented in R©:

model <- lm(*assessment2 ~ assessment1*)
summary(*model*)

Test of the slope: H_0: $\beta_1 = 1$ against the two-sided alternative H_A: $\beta_1 \neq 1$

The test statistic was $t_{\beta_1} = (\beta_1 - \beta_{1_0}) / s_{\beta_1}$ and H_0 was rejected if $|t_{\beta_1}| > t_{n-2;\,1-\alpha/2}$ with

β_{1_0} ..... given value of β_1 (= one)

n..... sample size

α ..... type I error probability (rejecting a valid null hypothesis)

s_{β_1} ..... standard deviation of β_1, calculated as:

$$s^2_{\beta_1} = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2} * \frac{1}{\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

The optimal sample size was determined iteratively from:

$$n = \left\lceil \left[\frac{(t_{n-2;1-\alpha/2} + t_{n-2;1-\beta}) * \sigma}{\delta * s^*} \right]^2 \right\rceil$$ (modified after Rasch et al. 1998, p. 285)

with:

β..... type II error probability (rejecting a valid alternative hypothesis)

σ ..... residual standard deviation

δ..... minimal distance to be detected

s^* ..... $(x_{max} - x_{min}) / 2$

This is implemented in CADEMO® Modul REA Version 3.27 (BioMath GmbH 16.11.2006): Test → Vergleich des Anstiegs mit einer Konstanten

As the error probability for acceptance of H_0 should be small, whereas error probability for rejection of H_0 can be larger, the risk of the first kind α was set to 0.2 and type II error probability β to 0.01. With $\sigma = 10$, $\delta = 0.2$ and $s^* = 30$ the resulting optimal sample size would be $n = 39$. This was beyond the sample size of all but one of the compared assessments.

The coefficient of determination (r^2) is a measure for the variation of the results of the second assessment that are explained by the first ones. It is included in the output of the R© code given above. The underlying formula is given by Rasch et al. 1999 (pp. 171-172).

Test of Correlation Coefficients

Pearson's product moment correlation coefficient is a measure of the linear relationship. Spearman's rho is a rank correlation coefficient and thus a measure of the strength of the monotone relationship of two repeated assessments. The tested null hypothesis was H_0: $0 < |\rho| \leq 0.8$. Type I error probability was $\alpha = 0.05$. In 4.6.2.1 Trait Correlations the details about estimating and testing the correlation coefficient are given. As sample size was lower than required, in case of acceptance of H_0 precision requirements are not met.

4.6.2 Field Data

Because of the different scoring methods, the traits used for further statistical analysis can be divided into the following classes based on their type of scale:

- ordinal scale:
  - appearance of teliospores (no, yes)
  - awnedness (no, partly, yes)seedling resistance (0-4)
  - heading (1-9)
  - crop density (1-5)
  - leaf blotch severity (1-9)
  - *Septoria* leaf blotch severity (1-9)
  - powdery mildew severity (1-9)
  - yellow rust severity (1-9)
  - glaucousness (1-5)
  - frost heaving severity (1-9)
  - lodging severity (0-5, 1-5, 1-9)
  - leaf chlorosis severity (0-9)
  - leaf tip necrosis severity (0-9)
- ratio/ interval scale:
  - leaf rust severity (% infected leaf area, AUDPC)
  - heading (day of the year)
  - plant height (cm)

Means over the two replications of an experiment were calculated for all traits except appearance of teliospores. If the scoring scheme was the same for different experiments, the mean over experiments was calculated too, but only for lines assessable in all experiments. Thus sample size of mean values can be below those of single experiments. The trait awnedness was treated similarly: If scores were different for different replications or experiments, the level "partly" was applied as it can be explained by genetic segregation in the population. In the histograms always the means are shown. They are the basis for most of the further statistical analysis.

Not all scorings were usable for all further descriptive statistics and statistical tests due to large numbers of missing lines, or scoring schemes that were not comparable.
R© version 2.8.0 (R© Development Core Team 2008) was used for drawing diagrams, and for calculating correlation coefficients. For the fast generation of histograms of the main traits (leaf rust severity, heading and plant height) of all experiments, an R© function was developed (see Appendix B: R© Functions and SAS® Codes).
Contingency tables (chi-squared tests of independence) were analyzed with SPSS© 15.0.1 für Windows Version 15.0.1 (SPSS Inc., 1989-2006).
The software package SAS® for Windows version 9.2 TS Level 1M0 was used for calculating analysis of variance (ANOVA) tables and estimating variance components. The procedure therefore was GLM (general linear model) with type III sum of squares. Due to different experimental locations in different years, the factor "experiment" was introduced for year–location combinations.

4.6.2.1 Trait Correlations

For estimating and testing trait correlations between proportionally scaled traits, Pearson's product moment correlation coefficient was calculated and tested as a measure of the linear relationship. In SAS®, SPSS© and R© only the test of the null hypothesis H_0: $\rho = 0$ against the two-sided alternative hypothesis H_A: $\rho \neq 0$ is implemented. As the information whether a correlation coefficient is significantly different from zero or not is of few practical relevance (Kubinger et al. 2007), the composite null hypothesis H_0: $0 < |\rho| \leq |\rho_0|$ was tested against the one sided alternative hypothesis H_A: $|\rho| > |\rho_0|$. The square root of the coefficient of determination ρ^2 explaining a relevant amount of variance for the particular question was chosen as the value of ρ_0. The correlation coefficients calculated with R©

cor.test(*assessment1*, *assessment2*, method = c("pearson"))

were tested according to Rasch et al. (1998, p. 326). For testing the null hypothesis H_0: $\rho = \rho_0$ ($\rho_0 \neq 0$), **r** had to be transformed as follows:

$$\mathbf{z} = \frac{1}{2} \ln \frac{1+\mathbf{r}}{1-\mathbf{r}}$$

The testing value ρ_0 had to be transformed in the same way, therefore it would not be possible to test H_0: $\rho = 1$:

$$z_0 = \frac{1}{2} \ln \frac{1+\rho_0}{1-\rho_0}$$

The test statistic was

$$u = (\mathbf{z} - z_0)\sqrt{n-3}\,.$$

The null hypothesis was rejected if $u > u_{1-\alpha}$ with:

r ..... estimated correlation coefficient
ρ_0 ..... testing value
n..... sample size
$u_{1-\alpha}$ ..... quantile of the normal distribution
α ..... type I error probability (rejecting a valid null hypothesis)

The optimal sample size was determined from

$$n = \left\lceil \left[(u_{1-\alpha} + u_{1-\beta}) / (z_1 - z_0) \right]^2 \right\rceil + 3$$ (modified after Rasch et al. 1998, p. 325)

with:

z_1..... transformation as above with $\rho_1 = \rho_0 + \delta$

δ..... minimal distance to be detected

β..... type II error probability (rejecting a valid alternative hypothesis)

This is implemented in CADEMO® Modul LIGHT Version 3.27 (BioMath GmbH 16.11.2006): Fallzahl → Einstichprobenproblem → Korrelationskoeffizient Test…

Additionally Spearman's rho was calculated and tested. It is a measure of the strength of the monotone dependence of the traits. It corresponds to Pearson's product moment correlation coefficient calculated with the ranks (Rasch et al. 1996, pp. 598-600). This correlation coefficient can be used for ordinally scaled traits too. Spearman's rho can be tested in the same way as Pearson's product moment coefficient if the sample size exceeds $n \geq 30$ (Kubinger 1990). Spearman's rho was calculated using R©:

cor.test(*trait1*, *trait2*, alternative = c("two.sided"), method = c("spearman"))

The interdependency between appearance of teliospores, awnedness and other traits was analyzed with contingency tables (chi-squared tests of independence). The null hypothesis is that both traits are independent. H_0: The probability of a certain trait combination is the product of the single trait occurrences. The alternative hypothesis is that this is not valid H_A: $p_{ij} \neq p_{i.}\, p_{.j}$ for at least one pair (i, j). The underlying formulas and test statistics are given in Rasch et al. (1998, pp. 199-200). R© code for chi-squared test of independence:

chisq.test(*traitcombination*)

chisq.test(*traitcombination*)$expected

gives the expected observation for every combination. It should be beyond 5 as the chi-squared distribution is only valid asymptotically.

In addition Cramér's V was calculated as a measure of dependence according to the formula

$$V = \sqrt{\frac{X^2}{n * \min(a-1; b-1)}}$$ (Rasch et al. 1998, pp. 193-194) with:

X^2..... chi-squared test statistics (calculated with R©)

n..... sample size

a..... number of rows (number of levels of trait A)

b..... number of columns (number of levels of trait B)

Correlation of Traits Assessed in the Same Experiment

In the case of proportionally scaled traits, Pearson's product moment correlation coefficient and Spearman's rho were estimated and tested, in the case of ordinally scaled traits only the latter.

As correlations with a coefficient of determination r^2 less 0.35 – meaning that less than 35 % of the variability of the y-variable can be explained by the x-variable – are of no practical relevance, the tested null hypothesis was $H_0: 0 < |\rho| \leq 0.59$. Type I error probability was $\alpha = 0.05$. Due to population sizes of n = 201 for Furore/Capo and larger for Isengrain/Capo and Arina/Capo, the other precision requirements could be set to $\beta = 0.1$ (type II error probability) and $\delta = 0.12$ (minimal effect of relevance) requiring an optimal sample size of n = 199.

To test correlations of the ordinally scaled traits appearance of teliospores of *P. triticina* or awnedness with the other traits by means of contingency tables, the proportionally scaled traits were categorized as follows: The classes of leaf rust severity measured by the percentage of infected leaf area were 0-10, > 10-20, > 20-30 and > 30-50. As no teliospores can be produced if there is no infection with leaf rust (no visible urediniospores), all plants without symptoms were in this case excluded from analysis. Data of the trait heading were categorized to groups spanning two days, plant height to groups of ten centimeters. In some cases classes at the end of the range had to be merged in order to meet the assumption that the frequencies expected under validity of the null hypothesis are not less than two and for at least 50 % of the cells not less than five. Due to the small differences between the RILs of the population Furore/Capo, data of the trait heading were categorized to groups spanning only one day, plant height to groups of five centimeters.

These tests were performed for all traits within one experiment and also for the means over experiments.

Correlation Between Experiments

To obtain estimates of the reproducibility of the assessments, Spearman's rho between the same trait assessed in different experiments was estimated and tested. In this case the tested null hypothesis was $H_0: 0 < |\rho| \leq 0.71$. The precision requirements for these tests were set to $\alpha = 0.05$ (type I error probability), $\beta = 0.05$ (type II error probability) and $\delta = 0.1$ (minimal effect of relevance), requiring an optimal sample size of n = 192.

4.6.2.2 Estimating the Coefficient of Broad Sense Heritability

As a further measure of the reproducibility of evaluations the coefficient of broad sense heritability was estimated according to Hallauer and Miranda (1981 cited in Klahr et al. 2007), Nyquist (1991) and Becker (2011). The notation is following Rasch et al. (1999).

The heritability h^2 is defined as the proportion of genotypic (g) variance of the total phenotypic (p) variance:

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

The variance of the phenotype comprises the variance of the genotype and the environment (experiment) and the error variance:

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma^2}{er}$$

These variance components were calculated from the ANOVA table after Nyquist (1991):

source of variation	**df**	**MS**	**expected mean square**
experiments (E)	e-1	$\mathbf{MS}_E$	
replications (R) within E	e(r-1)	$\mathbf{MS}_R$	
genotypes (G)	g-1	$\mathbf{MS}_G$	$\sigma^2 + r\sigma_{ge}^2 + er\sigma_g^2$
interaction G x E	(g-1)(e-1)	$\mathbf{MS}_{GE}$	$\sigma^2 + r\sigma_{ge}^2$
experimental error (ε)	e(r-1)(g-1)	$\mathbf{MS}_\varepsilon$	σ^2
total (T)	erg-1	$\mathbf{MS}_T$	

e ..... number of experiments

r ..... number of replications

g ..... number of genotypes

σ_g^2 ..... variance of genotypic values of genotypes in the population (total genotypic var.)

σ_{ge}^2 ..... variance of interaction effects between genotypes and experiments

σ^2 ..... variance of total effect(s) of experimental unit(s) within a replication

As Nyquist (1991) demonstrated for the case of several experiments with a randomized complete block design and the same number of replications that the exact formular for estimating the coefficient of heritability

$$\hat{h}^2 = \frac{s_g^2}{s_p^2}$$

can be simplified:

$$\hat{h}^2 = 1 - \frac{MS_{GE}}{MS_G}$$

because

$$s_g^2 = \frac{MS_G - MS_{GE}}{er}$$

$$s_p^2 = s_g^2 + \frac{s_{ge}^2}{e} + \frac{s^2}{er} = \frac{MS_G - MS_{GE}}{er} + \frac{MS_{GE} - MS_\varepsilon}{er} + \frac{MS_\varepsilon}{er} = \frac{MS_G}{er}.$$

ANOVA tables were calculated with SAS®. The main effects of the levels of E, R and G and the interaction G x E were considered random:

```
proc GLM data=… outsat=…;
class R G E;
model trait = R(E) G E G*E / SS3;
random R(E) G E G*E / test;
run;
```

As the number of replications was varying between experiments or data were otherwise missing, the presumptions were not met and the coefficient r (averaged number of

replications) was different for each variance component. Therefore estimates of the coefficient of heritability were calculated exactly on the basis of variance components and in addition after the simplified formula despite missing data to compare differences in the results.

As the effects of the main factors and the interaction were considered random, the F-values are calculated by dividing the mean squares (MS) of the factor (or the interaction) by the MS of the particular error.

Another question was whether information about the leaf rust infection of the spreader rows next to each plot can help improving data and results. Therefore ANOVA were calculated for the two populations Isengrain/Capo and Arina/Capo for the four experiments in Probstdorf, Rust, Schmida and Tulln in 2008. In a second step the mean value of the spreader rows next to each plot was included in an analysis of covariance. Coefficients of heritability were estimated for each population with the exact and the simplified method. For covariance analysis in SAS® the covariate simply has to be added to the code line for the model:

model LR = R(E) G E G*E mSpreader / SS3;

4.6.2.3 Least Significant Difference (LSD)

If the difference between the mean values of lines is lower than the LSD, they are said to be equal. The type I error probability α was set to 0.05. The values of $LSD_{5\%}$ were estimated according to the formula

$$LSD_{5\%} = \sqrt{\frac{2 * MS_{\varepsilon}}{r}} * t_{df_{\varepsilon},0.975}$$

with

MS_{ε} ..... mean square error

r ..... number of replications (two)

$t_{df,\gamma}$ ..... quantile of the *t*-distribution ($\alpha = 0.05$, $\gamma = 0.975$)

df_{ε}..... degrees of freedom for the error

In SAS® the model was specified as

model *trait* = R G / SS3;

4.6.3 Marker Data

For constructing linkage maps JoinMap® 4 (van Ooijen 2006) with evaluation license was used. Default values of all parameters were not changed with the exception of using Haldane's mapping function instead of Kosambi's. The resulting linkage groups were checked with MAPMAKER/EXP© 3.0b (Lincoln et al. 1993a).

With JoinMap® 4 genotype frequencies for all loci were tested whether segregation is different from the Mendelian ratio, considering the particular classification (dominant for the Capo allele, codominant or dominant for the allele of the other parent). The underlying test is a chi-squared test of independence.

To preclude wrong marker data, particularly AFLP markers often were not scored for the following reasons:

- very weak
- badly separated from other fragments
- pattern just "weak" – "strong"
- seemingly varying fragment sizes due to different neighboring bands for the different parts of the population
- not polymorphic between the parental lines and segregation vastly diverging from the Mendelian ratio

In some cases marker data were only available for a part of the population due to scoring difficulties. If these markers were mapped to different linkage groups, whether JoinMap® 4 or MAPMAKER/EXP© 3.0b was used, they were retrospectively excluded from linkage analysis.

Maps were drawn using MapChart© 2.1 (Voorrips 2002).

To assign linkage maps to certain chromosomes or chromosomal arms, they were compared to microsatellite maps of wheat published by Röder et al. (1998), Pestsova et al. (2000), Gupta et al. (2002), and the genetic markers information and the following maps available from the GrainGenes database (USDA 2014):

- Wheat, Consensus SSR, 2004 (Somers et al. 2004)
- Wheat, Composite, 2004
- *T. boeoticum* x *monococcum* [modern grouping: *T. monococcum* ssp. *aegilopoides* x *T. monococcum* ssp. *monococcum*] (Singh et al. 2007)
- Wheat, Arina x Forno (Paillard et al. 2003)
- Wheat, Chinese Spring x SQ1 (Quarrie et al. 2005)
- Wheat, Physical, SSR (Sourdille et al. 2004)
- Wheat, Synthetic x Opata, BARC (Song et al. 2005)
- *T. turgidum* [modern grouping: *T. turgidum* ssp. *turgidum*], Langdon x G18-16 (Peleg et al. 2008)
- *T. turgidum* [modern grouping: *T. turgidum* ssp. *turgidum*], Messapia x *dicoccoides*, SSR (Korzun et al. 1999)

4.6.4 QTL Analysis

4.6.4.1 Interval Mapping

For the populations Isengrain/Capo and Arina/Capo LOD values (logarithm of odds) were calculated with QTL Cartographer© Version 2.5 (Basten et al. 1994, Brown 2005, Wang et al. 2007) using “Interval Mapping” and 1.0 cM as “Walk speed”. The threshold for each trait was estimated by permutations (1000 permutation times, significance level 0.05). All QTL with a likelihood ratio value ≥ the threshold were said to be significant (LOD = 0.217 ∗ likelihood ratio, Basten et al. 2005). In order to detect solely additive effects as only homozygous lines are valuable in plant breeding, all heterozygous marker data were treated as “missing values” in QTL analysis. Results were checked with PLABQTL© Version 1.2 (Utz and Melchinger 1996, 2006).
QTL analysis was done for the following traits:

- **leaf rust severity:** population Isengrain/Capo and Arina/Capo
- **relative AUDPC (leaf rust severity):** population Isengrain/Capo and Arina/Capo
- **appearance of teliospores:** population Isengrain/Capo
- **seedling resistance:** population Arina/Capo
- **heading:** population Isengrain/Capo and Arina/Capo
- **plant height:** population Isengrain/Capo and Arina/Capo
- **awnedness:** population Arina/Capo
- **crop density:** population Isengrain/Capo
- **leaf blotch severity:** population Isengrain/Capo
- ***Septoria* leaf blotch severity:** population Arina/Capo
- **powdery mildew severity:** population Isengrain/Capo and Arina/Capo
- **yellow rust severity:** population Arina/Capo
- **glaucousness:** population Arina/Capo
- **frost heaving severity:** population Isengrain/Capo
- **lodging severity:** population Isengrain/Capo and Arina/Capo
- **leaf chlorosis severity:** population Isengrain/Capo and Arina/Capo
- **leaf tip necrosis severity:** population Isengrain/Capo and Arina/Capo

4.6.4.2 Single Point ANOVA

Analysis of variance (ANOVA) for all single markers (not only those in linkage groups) was done for all traits assessed in the population Arina/Capo. As markers with significant LOD values detected by interval mapping (see 4.6.4.1 Interval Mapping) had a p-value less than 0.01 in the single point ANOVA, this value was used for the type I error probability α. ANOVA was calculated with the SAS® procedure GLM. The program for the trait mheading (heading, mean over experiments) is given as an example in Appendix B: R© Functions and SAS® Codes.

4.6.4.3 Additive Effects

Additive effects were calculated as half of the difference of the mean values of the homozygous lines for the corresponding marker:

$$additive\ effect = \frac{m_C - m_I}{2}$$

with

m_c ..... mean over all lines with the same allele as the parental line Capo
m_I ..... mean over all lines with the same allele as the parental line Isengrain

4.6.4.4 Epistatic Interactions

If resistance genes are combined in cultivars, they can either act additively or epistatic interactions can occur. In quantitative genetics epistasis is defined as the interaction of genes with the exception of additive interaction. Epistatic interactions can produce a particularly favorable or unfavorable effect, e.g. with regard to disease resistance (Kolmer 1992a, Becker 2011).

For calculation of epistatic interactions with the SAS® program written by Holland and Ingle (2001) a framework map of the population Isengrain/Capo with not more than one locus every 2 to 3 cM was used. As the number of heterozygous plants in a F_6 population is almost negligible, this group is prone to be the outlier. Therefore – if it was possible because of more than one locus within 2 to 3 cM – codominant loci and also those markers that were scorable on just a small part of the population were removed. To avoid type I error (mistaken detection of epistatic interactions), the risk of the first kind was set to $\alpha = 0.0001$. Mean over three, six and eleven experiments (see Table 26), all single assessments of leaf rust severity measured by the percentage of infected leaf area, leaf rust severity measured by AUDPC (single experiments and the mean over them) and the appearance of teliospores were used as traits.

To ensure finding all epistatic interactions between Capo and Isengrain QTL calculation was repeated with the original map for the relevant linkage groups assigned to the chromosomes 3B, 7B and all possibly assigned to 2B, where *Lr13* described for Capo is located.

A further ANOVA (SAS® procedure GLM) was calculated without and with the marker1*marker2 interaction term. Data of the eleven leaf rust assessments (see Table 26), the mean over these eleven as well as over three and six experiments and AUDPC in the experiments Piešťany 2006 and Tulln 2008 and the mean over these two were used as traits. The risk of the first kind was set to $\alpha = 0.05$. In the case of significant interactions the improvement of the model measured by R^2 was determined.

5 Results

5.1 Field Experiments

5.1.1 Leaf Rust Assessment

First the results of the reproducibility of disease assessment are presented as reproducibility is a prerequisite for reliable data analyses.

5.1.1.1 Inter-rater and Intra-rater Reproducibility

For assessing leaf rust severity measured by the percentage of infected leaf area, all raters were using the scoring aid given in Fig. 8. In addition to the classes shown, all raters used the scores 40, 60 and 80 %. In a few cases two raters used scores even finer than these classes. For evaluating reproducibility based on the classes, these values were randomly assigned to one of the neighboring classes. On the other hand, one rater did not use the score three percent.

Comparison of Reproducibility for the Unequally Fine Graded Classes of the Scoring Aid

In Table 7 to Table 17 the results of the comparisons of leaf rust severity scoring (% infected leaf area) repeated by one rater or by different raters are given. The number of lines assigned to the same or a deviating class are shown. Additionally the percentage of identically scored plants was calculated for each class separately, for all classes i.e. for all lines, for all but the zero percent class, for all classes lower than ten percent without and with the class zero percent, and the classes ten percent and beyond.

In the repeated ratings of standard lines in 2008 by rater one 56-58 % of lines got the same score. Reproducibility was almost 10 % lower for rater two. The amount of the same scores given by rater one and two was smallest on the third day of assessment (30 %) and highest (68 %) on the second day. On this day 52 lines were scored in the morning by the two raters together to compare and adjust rating again after one day (about 500 to 1000 plots) of separate assessment. On the third day of scoring the overall reproducibility of the scores of rater one by rater two was only 30 %, whereas for the classes < 10 % it was over 60 % that is higher than on the first two days. If scores for the class with zero infection were not taken into account, intra-rater and inter-rater reproducibility differed much: values ranged from 23 to 66 % and were with no exception lower compared to the results including the zero scores. The difference of overall reproducibility was slightest for the repeated scoring by person two. In five out of the nine comparisons of assessments in 2008, all lines with no detected infection in the first scoring got the same score in the second rating. The lowest level of identically scored plants in the class zero infection were 2 out of 3 lines scored by rater one again in the second rating and 9 out of 13 scored by rater one compared to the assessment of rater one and two together (both on the second day of assessment). On the first and second day reproducibility for the range below 10 % (not taking the zero class into account) was well (more than 20 %) below the reproducibility for the classes 10 % and more. Only on the third day of assessment, the difference was below 10 %; for rater two it was even reverse.

For single classes there was neither a clear trend towards higher or lower scores in the second assessment or by a specific rater, nor was there a range that was assessed with a definitely higher or lower reproducibility.
In most of the compared assessments well beyond 90 % of the lines were assessed to the same or a neighboring class in the second rating. Only on the third day assessments repeated by rater two showed larger deviations. Scores differing by three classes occurred rarely with the exception of these two repeated assessments. The detailed results are given in Table 21.
As counts for each disease level were rather low, overall sums for the experiments in 2008 were calculated and are given in Table 18 to Table 20. The amount of identically scored plots by rater one was higher than for rater two. In the assessment of rater one repeated by rater two, the percentage of identical scored plants was well below 50 %.
In the leaf rust assessment by rater two and three together in 2007 and repeated by rater three alone, values of percentage of identically scored lines were 48 % for the range below ten percent infected leaf area, and 34 % for the range ten percent and higher. The values of these ranges were 36 % and 33 % in the comparison of rater three and four in 2009. This is in contrast to the compared assessments by rater one and two in 2008.
Table 22 lists the differences in classes (percentage of all scores) for the comparisons rater one first scoring vs. rater one second scoring, rater one vs. rater two, rater two first scoring vs. rater two second scoring (mean over all disease assessments in 2008), rater two and three together vs. rater three alone and rater three vs. rater four. Table 23 gives the percentage of identically rated plots for these comparisons.

Table 7 Results of the repeated scoring on the first day of leaf rust assessment in 2008 by rater one

lr class 1st scoring	deviation in lr classes 2nd scoring								
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	% identical
0				**11**	3			14	79
1			4	**1**	8	1		14	7
3			1	**8**	2			11	73
5		1		**1**				2	50
10			4	**3**	1			8	38
20	1			**8**	7			16	50
30		1	1	**18**	4			24	75
40				**1**				1	100
50									
60									
70									
80									
90									
100									
total 0 ≤ x ≤ 100 %	1	2	10	**51**	25	1		90	57
classes 0 < x ≤ 100 %	1	2	10	**40**	22			76	53
classes 0 < x < 10 %		1	5	**10**	10	1		27	37
classes x < 10 %		1	5	**21**	13	1		41	51
classes x ≥ 10 %	1		5	**30**	12			49	61

Table 8 **Results of the repeated scoring on the first day of leaf rust assessment in 2008 by rater one and two**

lr class rater 1	deviation in lr classes rater 2								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**14**	1			15	93
1			3	**2**	1			6	33
3			7	**5**	4	1		17	29
5			2	**2**	2	2		8	25
10			1	**1**	2			4	25
20			1	**5**	4			10	50
30			5	**13**	6	1		25	52
40		1	1	**3**				5	60
50									
60									
70									
80									
90									
100									
total $0 \leq x \leq 100$ %		1	20	**45**	20	4		90	50
classes $0 < x \leq 100$ %		1	20	**34**	19	4		75	45
classes $0 < x < 10$ %			12	**9**	7	3		31	29
classes $x < 10$ %			12	**23**	8	3		46	50
classes $x \geq 10$ %		1	8	**22**	12	1		44	50

Table 9 **Results of the repeated scoring on the second day of leaf rust assessment in 2008 by rater one**

lr class 1st scoring	deviation in lr classes 2nd scoring								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**2**	1			3	67
1									
3					1			1	0
5		1		**1**				2	50
10				**3**				3	100
20			2	**1**				3	33
30		2	2	**1**	1			6	17
40				**3**	3			6	50
50			1	**9**	2			12	75
60				**2**				2	100
70									
80									
90									
100									
total $0 \leq x \leq 100$ %		3	5	**22**	8			38	58
classes $0 < x \leq 100$ %		3	5	**20**	7			35	57
classes $0 < x < 10$ %		1		**1**	1			3	33
classes $x < 10$ %		1		**3**	2			6	50
classes $x \geq 10$ %		2	5	**19**	6			32	59

Table 10 Results of the repeated scoring on the second day of leaf rust assessment in 2008 by rater one and two

lr class rater 1	deviation in lr classes rater 2								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**4**				4	100
1				**3**			1	4	75
3					1			1	0
5			1	**3**	4			8	38
10				**5**	5			10	50
20			2	**3**	1			6	50
30				**10**	1			11	91
40			3	**4**	1			8	50
50				**22**	6			28	79
60				**3**	1			4	75
70									
80									
90									
100									
total 0 ≤ x ≤ 100 %			6	**57**	20		1	84	68
classes 0 < x ≤ 100 %			6	**53**	20		1	80	66
classes 0 < x < 10 %			1	**6**	5		1	13	46
classes x < 10 %			1	**10**	5		1	17	59
classes x ≥ 10 %			5	**47**	15			67	70

Table 11 Results of the repeated scoring on the second day of leaf rust assessment in 2008 by rater two

lr class 1st scoring	deviation in lr classes 2nd scoring								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**1**				1	100
1			1	**1**				2	50
3					1			1	0
5		1		**1**	2			4	25
10		1	1	**3**	1			6	50
20		1	1		2			4	0
30			1	**7**	1			9	78
40			1	**2**	6			9	22
50				**8**				8	100
60			2					2	0
70									
80									
90									
100									
total 0 ≤ x ≤ 100 %		3	7	**23**	13			46	50
classes 0 < x ≤ 100 %		3	7	**22**	13			45	49
classes 0 < x < 10 %		1	1	**2**	3			7	29
classes x < 10 %		1	1	**3**	3			8	38
classes x ≥ 10 %		2	6	**20**	10			38	53

Table 12 Results of the repeated scoring on the second day of leaf rust assessment in 2008 by rater one and two together and rater one alone

lr class rater 1+2	deviation in lr classes rater 1								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**9**	4			13	69
1			1					1	0
3					1			1	0
5						1		1	0
10			1	**1**	1	1		4	25
20			2	**2**	2			6	33
30				**6**	1			7	86
40			2	**1**				3	33
50				**7**	2			9	78
60			5	**2**				7	29
70									
80									
90									
100									
total $0 \leq x \leq 100$ %			11	**28**	11	2		52	54
classes $0 < x \leq 100$ %			11	**19**	7	2		39	49
classes $0 < x < 10$ %			1	**0**	1	1		3	0
classes $x < 10$ %			1	**9**	5	1		16	56
classes $x \geq 10$ %			10	**19**	6	1		36	53

Table 13 Results of the repeated scoring on the third day of leaf rust assessment in 2008 by rater one

lr class 1st scoring	deviation in lr classes 2nd scoring								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**8**				8	100
1			4	**4**				8	50
3									
5		1						1	0
10			1					1	0
20			1	**3**	3			7	43
30			2	**1**	1			4	25
40		1	9	**7**	1			18	39
50		1	2	**8**	3			14	57
60			4	**15**	4			23	65
70			2	**4**				6	67
80									
90									
100									
total $0 \leq x \leq 100$ %		3	25	**50**	12			90	56
classes $0 < x \leq 100$ %		3	25	**42**	12			82	51
classes $0 < x < 10$ %		1	4	**4**				9	44
classes $x < 10$ %		1	4	**12**				17	71
classes $x \geq 10$ %		2	21	**38**	12			73	52

Table 14 Results of the repeated scoring on the third day of leaf rust assessment in 2008 by rater one and two

lr class rater 1	deviation in lr classes rater 2								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**12**				12	100
1			5	**2**				7	29
3		1			1			2	0
5		1	1					2	0
10				**1**			1	2	50
20			2	**2**	1	2		7	29
30		1	6	**9**	5	1		22	41
40			3	**7**	12			22	32
50				**5**	8	7	5	25	20
60				**2**	9	16		27	7
70				**1**	7			8	13
80									
90									
100									
total 0 ≤ x ≤ 100 %		3	17	**41**	43	26	6	136	30
classes 0 < x ≤ 100 %		3	17	**29**	43	26	6	124	23
classes 0 < x < 10 %		2	6	**2**	1			11	18
classes x < 10 %		2	6	**14**	1			23	61
classes x ≥ 10 %		1	11	**27**	42	26	6	113	24

Table 15 Results of the repeated scoring on the third day of leaf rust assessment in 2008 by rater two

lr class 1st scoring	deviation in lr classes 2nd scoring								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**1**				1	100
1				**2**				2	100
3									
5	1				1			2	0
10									
20		1	2	**1**				4	25
30			3	**2**				5	40
40			1	**5**	1			7	71
50	1	1	2	**4**	2			10	40
60				**3**	3	2		8	38
70				**4**	2			6	67
80			1					1	0
90									
100									
total 0 ≤ x ≤ 100 %	2	2	9	**22**	9	2		46	48
classes 0 < x ≤ 100 %	2	2	9	**21**	9	2		45	47
classes 0 < x < 10 %	1			**2**	1			4	50
classes x < 10 %	1			**3**	1			5	60
classes x ≥ 10 %	1	2	9	**19**	8	2		41	46

Table 16 Results of the repeated leaf rust assessment in 2007 by rater two and three together and rater three alone

lr class rater 2+3	deviation in lr classes rater 3								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**2**				2	100
1				**8**	18	10		36	22
3			5	**20**	9			34	59
5		1	28	**40**	5			74	54
10		3	28	**19**	4			54	35
20		1	14	**12**	10			37	32
30			8	**4**	3	1		16	25
40			4	**2**	4			10	20
50			1	**6**	1			8	75
60		2	1	**1**				4	25
70			1					1	0
80		1						1	0
90									
100									
total 0 ≤ x ≤ 100 %		8	90	**114**	54	11		277	41
classes 0 < x ≤ 100 %		8	90	**112**	54	11		275	41
classes 0 < x < 10 %		1	33	**68**	32	10		144	47
classes x < 10 %		1	33	**70**	32	10		146	48
classes x ≥ 10 %		7	57	**44**	22	1		131	34

Table 17 Results of the repeated leaf rust assessment in 2009 by rater three and four

lr class rater 3	deviation in lr classes rater 4											%
(% infected leaf area)	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	total	identical
0					**2**						2	100
1					**9**	8	5		1		23	39
3												
5			30	26	**49**	29	7	1		1	143	34
10	1	5	2	27	**39**	32	8	3			117	33
20			3	11	**37**	30	7				88	42
30		1	2	8	**24**	18	20	1	7		81	30
40				3	**8**	14	1	6	2		34	24
50			1		**2**		1	4			8	25
60							1				1	0
70												
80												
90												
100												
total 0 ≤ x ≤ 100 %	1	6	38	75	**170**	131	50	15	10	1	497	34
classes 0 < x ≤ 100 %	1	6	38	75	**168**	131	50	15	10	1	495	34
classes 0 < x < 10 %			30	26	**58**	37	12	1	1	1	166	35
classes x < 10 %			30	26	**60**	37	12	1	1	1	168	36
classes x ≥ 10 %	1	6	8	49	**110**	94	38	14	9		9	33

Table 18 Overall results of the repeated scoring of leaf rust assessment in 2008 by rater one

lr class 1st scoring	deviation in lr classes 2nd scoring								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**21**	4			25	84
1			8	**5**	8	1		22	23
3			1	**8**	3			12	67
5		3		**2**				5	40
10			5	**6**	1			12	50
20	1		3	**12**	10			26	46
30		3	5	**20**	6			34	59
40		1	9	**11**	4			25	44
50		1	3	**17**	5			26	65
60			4	**17**	4			25	68
70			2	**4**				6	67
80									
90									
100									
total 0 ≤ x ≤ 100 %	1	8	40	**123**	45	1		218	56
classes 0 < x ≤ 100 %	1	8	40	**102**	41	1		193	53
classes 0 < x < 10 %		3	9	**15**	11	1		39	38
classes x < 10 %		3	9	**36**	15	1		64	56
classes x ≥ 10 %	1	5	31	**87**	30			154	56

Table 19 Overall results of the repeated scoring of leaf rust assessment in 2008 by rater one and two

lr class rater 1	deviation in lr classes rater 2								%
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	identical
0				**30**	1			31	97
1			8	**7**	1		1	17	41
3		1	7	**5**	6	1		20	25
5		1	4	**5**	6	2		18	28
10			1	**7**	7		1	16	44
20			5	**10**	6	2		23	43
30		1	11	**32**	12	2		58	55
40		1	7	**14**	13			35	40
50				**27**	14	7	5	53	51
60				**5**	10	16		31	16
70				**1**	7			8	13
80									
90									
100									
total 0 ≤ x ≤ 100 %		4	43	**143**	83	30	7	310	46
classes 0 < x ≤ 100 %		4	43	**113**	82	30	7	279	41
classes 0 < x < 10 %		2	19	**17**	13	3	1	55	31
classes x < 10 %		2	19	**47**	14	3	1	86	55
classes x ≥ 10 %		2	24	**96**	69	27	6	224	43

Table 20 Overall results of the repeated scoring of leaf rust assessment in 2008 by rater two

lr class 1st scoring	deviation in lr classes 2nd scoring								
(% infected leaf area)	-3	-2	-1	0	+1	+2	+3	total	% identical
0				**2**				2	100
1			1	**3**				4	75
3					1			1	0
5	1	1		**1**	3			6	17
10		1	1	**3**	1			6	50
20		2	3	**1**	2			8	13
30			4	**9**	1			14	64
40			2	**7**	7			16	44
50	1	1	2	**12**	2			18	67
60			2	**3**	3	2		10	30
70				**4**	2			6	67
80			1					1	0
90									
100									
total 0 ≤ x ≤ 100 %	2	5	16	**45**	22	2		92	49
classes 0 < x ≤ 100 %	2	5	16	**43**	22	2		90	48
classes 0 < x < 10 %	1	1	1	**4**	4			11	36
classes x < 10 %	1	1	1	**6**	4			13	46
classes x ≥ 10 %	1	4	15	**39**	18	2		79	49

Table 21 Differences in classes between compared assessments in 2008 (number of scored lines)

day	1	2	3	1	2	3	2	3	over all	over all	over all
comparison	1-1	1-1	1-1	1-2	1-2	1-2	2-2	2-2	1-1	1-2	2-2
-3	1							2	1		2
-2	2	3	3	1		3	3	2	8	4	5
-1	10	5	25	20	6	17	7	9	40	43	16
0	51	22	50	45	57	41	23	22	123	143	45
+1	25	8	12	20	20	43	13	9	45	83	22
+2	1			4		26		2	1	30	2
+3					1	6				7	
total	90	38	90	90	84	136	46	46	218	310	92

Table 22 Differences in classes between all compared assessments (percentage of scored lines)

comparison	1-1	1-2	2-2	1+2-1	2+3-3	3-4
-5	-	-	-	-	-	-
-4	-	-	-	-	-	< 1
-3	< 1	-	2	-	-	1
-2	4	1	5	-	3	8
-1	18	14	17	21	32	15
0	56	46	49	54	41	34
+1	21	27	24	21	19	26
+2	< 1	10	2	4	4	10
+3	-	2	-	-	-	3
+4	-	-	-	-	-	2
+5	-	-	-	-	-	< 1

Table 23 Results of all repeated leaf rust assessments (percentage of identically scored lines)

lr score	percentage of identically scored lines					
(% infected leaf area)	rater 1-1	rater 1-2	rater 2-2	rater 1+2-1	rater 2+3-3	rater 3-4
0	84	97	100	69	100	100
1	23	41	75	0	22	39
3	67	25	0	0	59	
5	40	28	17	0	54	34
10	50	44	50	25	35	33
20	46	43	13	33	32	42
30	59	55	69	86	25	30
40	44	40	50	33	20	24
50	65	51	65	78	75	25
60	68	16	30	29	25	0
70	67	13	67		0	
80			0		0	
90						
100						
x = 0	84	97	100	69	100	100
10 ≤ x ≤ 30	53	51	46	53	33	35
40 ≤ x ≤ 60	59	39	50	58	41	23
70 ≤ x ≤ 90	67	13	57		0	
x = 100						
1 ≤ x ≤ 30	48	43	44	45	41	35
0 ≤ x ≤ 100	56	46	49	54	41	34
0 < x < 10	38	31	36	0	47	35
x < 10	56	55	46	56	48	36
x ≥ 10	56	40	49	53	34	33

Test of the Absolute Difference of Repeated Assessments

Leaf rust severity of the standards compared in 2008 was increasing between the first and the third day of scoring. The number of lines scored by different raters or two times by the same rater was varying. Compared to rater one, rater two assigned the most diseased plants on the second and third day to a higher disease severity class.

The mean of the absolute differences of repeated assessments by rater one was lowest at the first day of scoring (2.5 %) and increased to 4.1 % with increasing leaf rust severity. The coefficient of variation (CV) of the absolute differences was decreasing from 171.1 % on the first to 127.9 % on the third day. Rater two did no repeat assessment on the first day. Mean value was slightly larger compared to rater one and also increasing (from 4.0 to 6.2 %), CV was lower and did not change much. CV for the repeated assessments by rater one and rater two was declining from 143.7 % on the first day to 91.8 % on the third day and thus below the values of rater one. From the first to the second day the mean absolute difference was dropping from 3.5 to 3.1 % and was highest on the last day (9.3 %) like for the other comparisons.

Modus (most frequent value) for the absolute difference was 0 % except for the repeated scoring for rater one and two on the third assessment day in 2008 (10 %). In half of the comparisons, including all repeated assessments by rater one, the median of the absolute

differences was 0 %, indicating that at least 50 % of the plots were rated identically in the two assessments. For the repeated assessment by rater one and two on the first day, median was 0.5 % and on the third day 10 %. In the repeated assessments by rater two, median was 1.5 % on the second and 5 % on the third day of assessment.
Maximum value of the difference of two scorings for the same plot was in most of the comparisons 20 %. It was higher (30 %) for the repeated assessments by rater two on the third day and lower (15 %) on the second day. The lowest value was 10 % for the repeated assessments by rater one and two on the second day.
Sample size for the repeated assessments of RILs and standard lines by rater two and three together and by rater three alone was a multiple of that in 2008. Therefore the risk of the second kind is much smaller. Values of mean and CV of absolute differences were similar: 3.4 % and 123.7 %, the median was 2 %.
Mean value of the comparison of rater three and four was 7.2 %, CV 120.8 %, and median 5 %. Sample size was almost doubled.
The null hypothesis of no difference between the two repeated assessments was rejected in all cases. The detailed results are given in Table 25. In Table 24 the statistic measures for the class differences are shown.

Table 24 Statistics for absolute class differences of all repeated assessments of leaf rust severity

day in 2008	1	2	3	1	2	3	2	3	2	2007	2009
comparison	1-1	1-1	1-1	1-2	1-2	1-2	2-2	2-2	1+2-1	2+3-3	3-4
sample size	90	38	90	90	84	136	46	46	52	277	497
mean value (class)	0.5	0.5	0.5	0.6	0.3	1.0	0.6	0.7	0.5	0.7	1.0
median (class)	0	0	0	0.5	0	1	0.5	1	0	1	1
modus (class)	0	0	0	0	0	1	0	0	0	1	1
standard dev. (class)	0.6	0.6	0.6	0.6	0.5	0.8	0.6	0.8	0.6	0.6	1.0
variance	0.4	0.4	0.3	0.4	0.3	0.7	0.4	0.6	0.3	0.4	0.9
coefficient of variation	127.3	129.4	118.3	108.3	158.9	83.4	109.7	113.0	115.5	91.8	96.6
minimum value (class)	0	0		0	0		0	0	0	0	0
maximum value (cl.)	3	2	2	2	3	3	2	3	2	2	5

Intra-rater and inter-rater reproducibility: model II regression analysis

Scatterplots of all repeated assessments of leaf rust severity (% infected leaf area) either by the same or by a different rater are shown in Fig. 11a-k. The diameters of the circles are directly proportional to the number of lines with the same scores. To see better whether there is a trend to higher or lower scores in the second rating, a line with slope 1 and intercept 0 is given too.
Values of the regression parameters are given in Table 25. Acceptance of the null hypothesis proves a good reproducibility of the disease assessment. Rejection of the null hypothesis for beta 0 (intercept) indicates a constant bias, for beta 1 (slope) a systematic bias proportional to the level of disease severity.
The value of the coefficient of determination (r^2) was between 0.72 and 0.94.

Test of Correlation Coefficients

The linear relationship, tested by Pearson's product moment correlation coefficient, and the monotonous relationship, tested by Spearman's rho, was in all cases significantly beyond 0.8 (Table 25).

Table 25 Statistics for absolute differences (% infected leaf area), including regression and correlation parameters and test results of all repeated assessments of leaf rust severity

day in 2008	**1**	**2**	**3**	**1**	**2**	**3**	**2**	**3**	**2**	**2007**	**2009**
comparison	**1-1**	**1-1**	**1-1**	**1-2**	**1-2**	**1-2**	**2-2**	**2-2**	**1+2-1**	**2+3-3**	**3-4**
sample size	90	38	90	90	84	136	46	46	52	277	497
mean value	2.5	4.1	4.1	3.5	3.1	9.3	4.0	6.2	3.8	3.4	7.2
median	0	0	0	0.5	0	10	1.5	5	0	2	5
modus	0	0	0	0	0	10	0	0	0	0	0
standard deviation	4.3	5.9	5.3	5.0	4.3	8.5	4.6	7.2	5.3	4.3	8.7
variance	18.1	34.4	28.1	25.2	18.3	72.4	20.8	51.3	27.7	18.1	76.1
coefficient of variation	171.1	141.9	127.9	143.7	136.5	91.8	113.9	115.6	138.9	123.7	120.8
minimum value	0	0	0	0	0	0	0	0	0	0	0
maximum value	20	20	20	20	10	20	15	30	20	20	45
t-test H_0	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.
min. detectable diff.	2.0	3.3	2.0	2.0	1.1	1.6	2.2	4.4	2.7	1.1	1.9
beta 0	0.48	-2.81	-0.89	1.37	2.10	-2.08	1.13	-5.64	2.46	0.42	-0.23
H_0	acc.	acc.	acc.	rej.	rej.	rej.	acc.	rej.	rej.	acc.	acc.
beta 1	1.02	1.06	0.98	0.97	0.99	1.24	0.98	1.11	0.91	0.90	1.28
H_0	acc.	acc.	acc.	acc.	acc.	rej.	acc.	rej.	rej.	rej.	rej.
r^2	0.86	0.89	0.92	0.83	0.94	0.90	0.89	0.87	0.92	0.86	0.72
Pearson's corr. coeff.	0.93	0.94	0.96	0.91	0.97	0.95	0.95	0.93	0.96	0.93	0.85
H_0	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.
Spearman's rho	0.93	0.94	0.95	0.93	0.97	0.94	0.94	0.94	0.96	0.85	0.85
H_0	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.	rej.

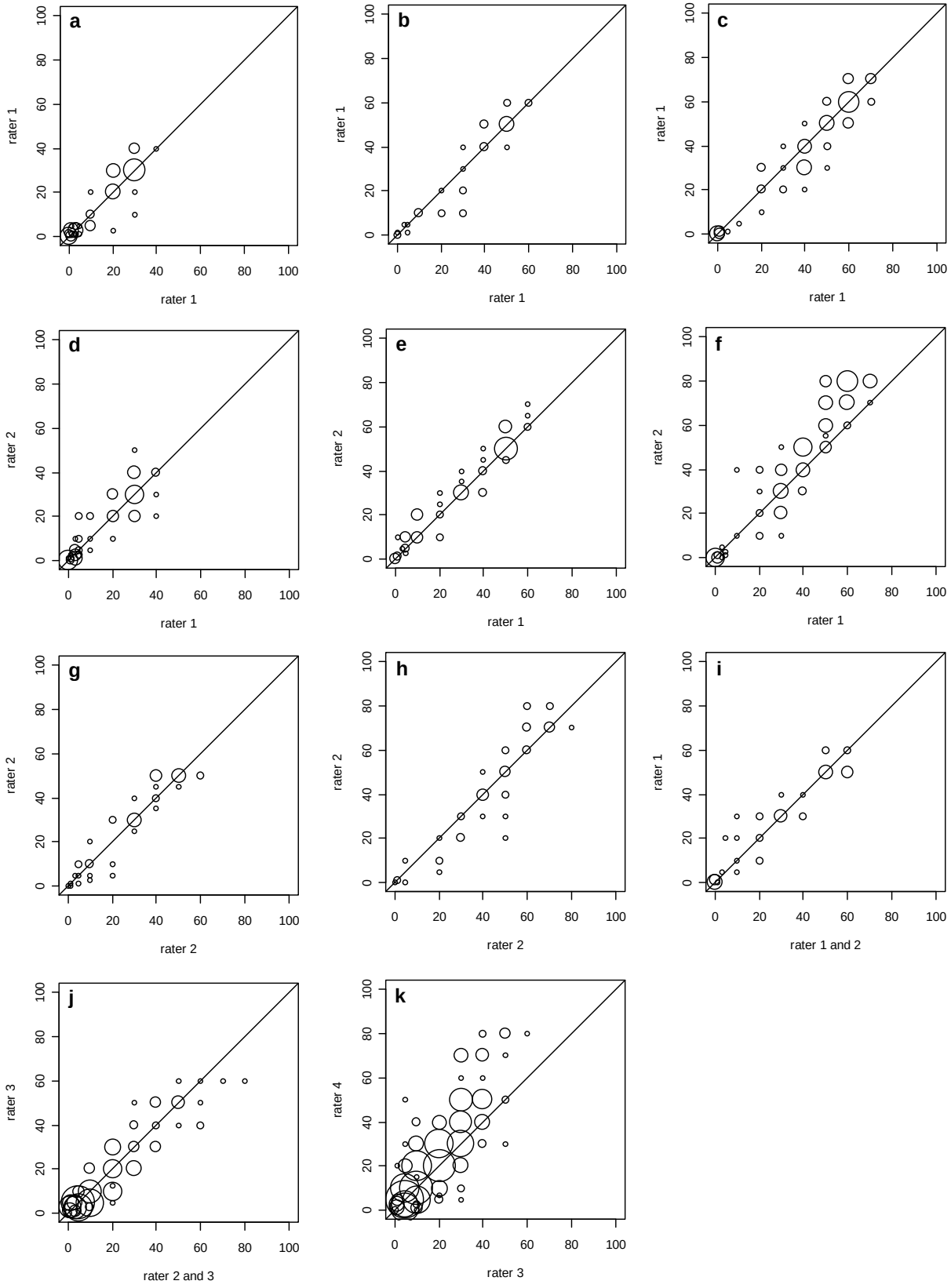

Fig. 11a-k Scatterplots of all repeated assessments of leaf rust severity (% infected leaf area). a-c: rater one on day one (a), two (b) and three (c) in 2008; d-f: rater one and two on day one (d), two (e) and three (f) in 2008; g-h: rater two on day two (g) and three (h) in 2008; i: rater one and two together and rater one alone on day two in 2008; j: rater 2 and 3 together and rater 3 alone in 2007; k: rater 3 and rater 4 in 2009. The diameters of the circles are directly proportional to the number of lines with the same scores.

5.1.1.2 Leaf Rust Infection

For the population **Isengrain/Capo** the trait leaf rust severity measured by the percentage of infected leaf area was assessed in 14 experiments, in some of them several times. Data resulting from eleven of these experiments were used for testing correlations between experiments and estimating the coefficient of broad sense heritability (h^2). The detailed results of the parental lines Isengrain (susceptible) and Capo (resistant) and the population are given in Table 26.

Table 26 Leaf rust severity (% infected leaf area): scoring day, mean values of the parents Isengrain and Capo and the population Isengrain/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		sc. day	Isengrain	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Tulln/AT	2004[1,2,3)]	1	17.3	3.9	12.6 ± 11.6	0.0 – 45.0	12.3	234
Piešťany/SK	2006[1,2)]	3	25.0	20.0	18.8 ± 7.4	5.0 – 50.0	8.5	240
Probstdorf/AT	2006[1,2,3)]	1	45.0	27.5	38.1 ± 20.1	0.0 – 80.0	23.3	239
Probstdorf/AT	2007[1)]	2	38.8	37.8	31.6 ± 16.9	0.0 – 65.0	36.9	232
Schmida/AT	2007[1,3)]	2	40.0	45.0	37.4 ± 14.5	4.0 – 70.0	16.5	234
Tulln/AT (M)	2007[1)]	1	40.0	20.0	26.7 ± 17.5	1.0 – 70.0	1 rep.	213
Probstdorf/AT	2008[1)]	1	52.5	47.5	47.0 ± 7.7	1.5 – 60.0	9.5	213
Rust/AT	2008[1,3)]	1	25.0	28.5	29.1 ± 15.1	1.0 – 50.0	19.9	234
Schmida/AT	2008[1,3)]	1	21.9	9.5	18.6 ± 13.8	0.5 – 50.0	20.1	240
Tulln/AT	2008[1,3)]	3	45.0	32.5	33.3 ± 15.5	0.0 – 65.0	13.4	240
Rust/AT	2009[1)]	2	40.0	21.9	32.9 ± 21.2	0.0 – 80.0	26.3	192
mean over 11 experiments[1)]			35.5	26.7	29.9 ± 10.9	3.7 – 52.3	5.6	158
mean over 3 experiments[2)]			29.1	17.1	23.2 ± 11.1	2.5 – 48.3	9.7	233
mean over 6 experiments[3)]			32.4	24.5	28.4 ± 12.8	2.1 – 55.8	7.5	224

1) used for calculating “mean over 11 experiments”, h^2: 0.90 (simpl. formula), 0.91 (variance comp.)
2) used for calculating “mean over 3 experiments”, h^2: 0.70 (simpl. formula), 0.70 (variance comp.)
3) used for calculating “mean over 6 experiments”, h^2: 0.91 (simpl. formula), 0.91 (variance comp.)

The recombinant inbred line (RIL) population showed continuous variation for leaf rust severity. In Fig. 12a-l the frequency distributions of these eleven experiments and the mean over them are illustrated.

In seven out of the eleven experiments Capo was clearly less infected with leaf rust than Isengrain, as would be expected. In the two experiments Probstdorf 2007 and Probstdorf 2008 there were minor differences in leaf rust severity between the two parental lines with Capo being the less susceptible one. But in the two experiments Schmida 2007 and Rust 2008 Capo was more diseased than Isengrain. Hence positive alleles for leaf rust resistance inherited from both parents (5.4.1.1 QTL for Leaf Rust Resistance) seemed to be more effective in different experiments. Therefore these eleven experiments were divided into those three in which resistance inherited from Capo was seen best and those six in which resistance inherited from Isengrain was detectable. Partly these experiments overlap.

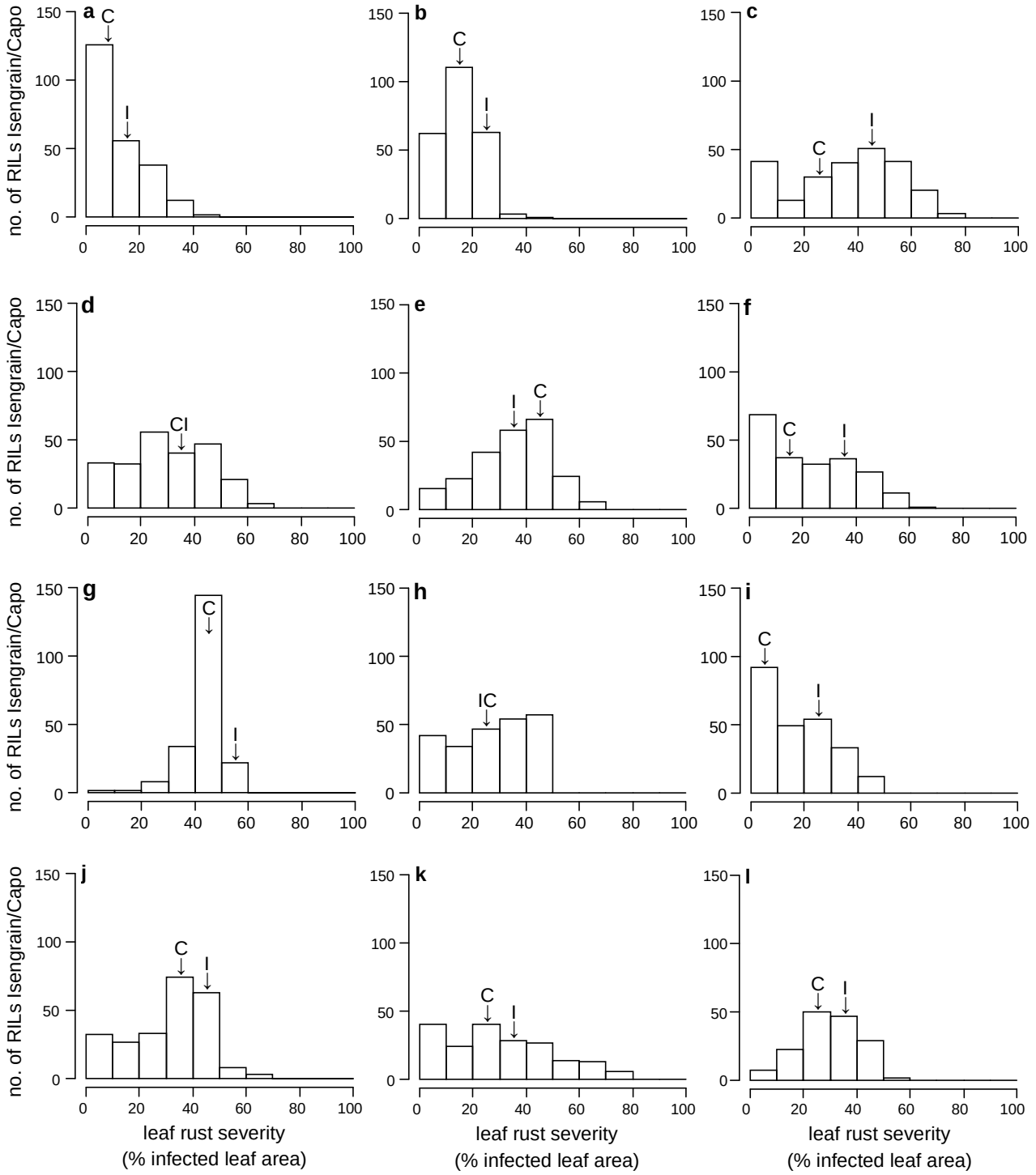

Fig. 12a-l Frequency distributions of leaf rust severity (% infected leaf area) of the RIL population Isengrain/Capo in the experiments 2004 Tulln (a), 2006 Piešťany (b), 2006 Probstdorf (c), 2007 Probstdorf (d), 2007 Schmida (e), 2007 Tulln (M) (f), 2008 Probstdorf (g), 2008 Rust (h), 2008 Schmida (i), 2008 Tulln (j), 2009 Rust (k) and the mean over these eleven experiments (l). Values of the parental lines are indicated by arrows, number of lines according to Table 26.

Table 27 Sample size (n) for Spearman's rho (r) between the eleven assessments of leaf rust severity (% infected leaf area) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$, not t. = not tested

		IS6	IP6	IP7	IH7	IM7	IP8	IRu8	IH8	IT8	IRu9
IT4	n	234	233	227	228	208	211	230	234	234	189
	r	0.51	0.63	0.32	0.52	0.40	0.33	0.59	0.59	0.69	0.42
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	not t.
IS6	n		239	232	234	213	213	234	240	240	192
	r		0.53	0.35	0.37	0.35	0.29	0.42	0.50	0.53	0.45
	H_0		acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
IP6	n			231	233	213	213	234	239	239	192
	r			0.51	0.70	0.50	0.48	0.65	0.67	0.76	0.60
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	rej.	acc.
IP7	n				228	207	206	226	232	232	187
	r				0.43	0.26	0.36	0.44	0.44	0.47	0.43
	H_0				acc.	acc.	acc.	acc.	acc.	acc.	not t.
IH7	n					211	208	228	234	234	189
	r					0.56	0.49	0.65	0.69	0.76	0.51
	H_0					acc.	acc.	acc.	acc.	acc.	not t.
IM7	n						191	209	213	213	176
	r						0.32	0.43	0.50	0.58	0.39
	H_0						not t.	acc.	acc.	acc.	not t.
IP8	n							212	213	213	182
	r							0.44	0.39	0.50	0.56
	H_0							acc.	acc.	acc.	not t.
IRu8	n								234	234	190
	r								0.67	0.71	0.47
	H_0								acc.	acc.	not t.
IH8	n									240	192
	r									0.78	0.54
	H_0									rej.	acc.
IT8	n										192
	r										0.66
	H_0										acc.

A comparative view of the frequency distributions of the population Isengrain/Capo for the calculated means over eleven, three and six experiments are shown in Fig. 63.

The values of Spearman's rho between these eleven experiments and whether the null hypothesis H_0: $0 < \rho \leq 0.71$ was accepted (acc.) or rejected (rej.) are presented in Table 27. If sample size (n) was below 192, the correlation coefficient was not tested (not t.). The lowest value of r was 0.26 (between the experiments Probstdorf and Tulln (M) 2007), the highest 0.78 (Schmida and Tulln 2008). Scatterplots of these two are shown in Fig. 13.

Solely the correlation coefficients between the experiments Probstdorf 2006 and Tulln 2008 plus Schmida and Tulln 2008 were significantly beyond 0.71.

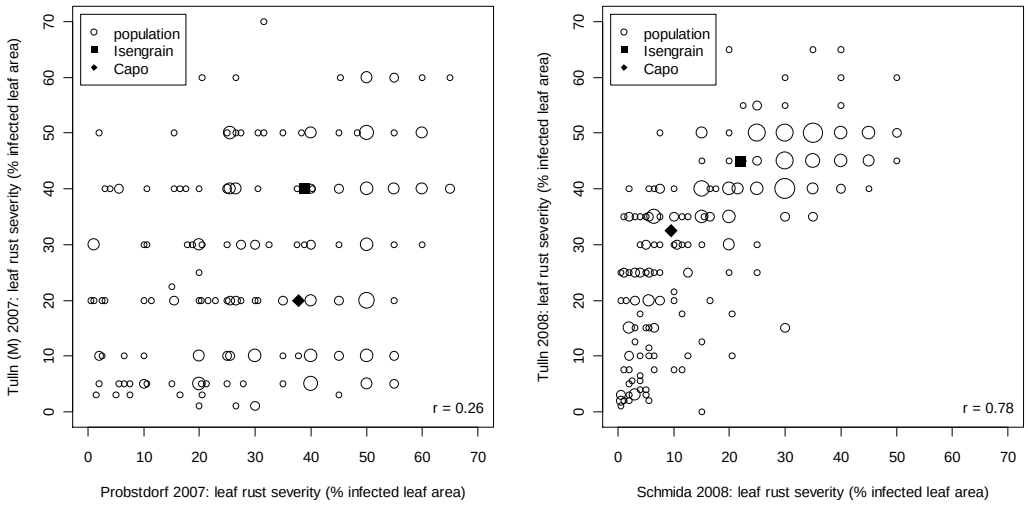

Fig. 13 Scatterplots for the correlation of leaf rust severity between the experiments Probstdorf and Tulln (M) 2007 (left) and Schmida and Tulln 2008 (right). The diameters of the circles are directly proportional to the number of lines with the same scores.

Analysis of variance (ANOVA) with experiments, replications within experiments, genotypes as main factors and the interaction term genotype x experiment revealed statistically significant effects for all these four random factors on leaf rust severity. Table 28 gives the degrees of freedom (df), the mean squares (MS), the F-values and the decisions about the null hypotheses based on a type I error rate of $\alpha = 0.05$. Additionally the compositions of the mean squares are shown for the estimation of the coefficient of heritability (h^2) by means of variance components. The value of the coefficient of broad sense heritability over all eleven experiments was 0.90 (calculated using the simplified formula) and 0.91 (calculated by means of variance components). The estimated coefficients of heritability were for both calculation methods 0.70 for the three experiments in which the QTL inherited from Capo was seen best and 0.91 for the six experiments in which the QTL inherited from Isengrain was seen best.

Table 28 Analysis of variance for leaf rust severity (% infected leaf area) of the population Isengrain/Capo across eleven experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	10	36484.2	13.75	significant ($p < 0.0001$)
replications (R) within E	10	2538.3	24.18	significant ($p < 0.0001$)
genotypes (G)	239	2091.0	10.84	significant ($p < 0.0001$)
interaction G x E	2261	199.1	1.90	significant ($p < 0.0001$)
experimental error (ε)	1979	105.0		

$MS_{GE} = s^2 + 1.782 * s^2_{ge}$

$MS_G = s^2 + 1.666 * s^2_{ge} + 17.434 * s^2_g$

h^2: 0.90 (using the simplified formula), 0.91 (using variance components)

For the population **Arina/Capo** leaf rust severity (measured by the percentage of infected leaf area) was assessed in twelve experiments, in some of them several times. Data from all but the experiment in Reichersberg 2008 (infection too low) were used for further statistical analysis. The detailed results of the parental lines Arina (susceptible) and Capo (resistant) and the population are presented in Table 29. The frequency distributions of the single experiments and the mean over these eleven experiments showing continuous variation are illustrated in Fig. 14a-l. In all experiments Capo was the less susceptible parent, as would be expected. Population mean and maximum were higher compared to the population Isengrain/Capo. As for the population Isengrain/Capo ANOVA (results presented in Table 30) revealed statistically significant effects for all three main factors and the interaction term on leaf rust severity. The estimated coefficient of broad sense heritability was 0.89 (calculated using the simplified formula) and 0.90 (calculated by means of variance components) – slightly lower than for Isengrain/Capo.

Table 29 Leaf rust severity (% infected leaf area): scoring day, mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		sc. day	Arina	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Tulln/AT	2007	2	70.0	56.7	68.0 ± 10.0	30.0 - 90.0	1 rep.	230
Fundulea/RO	2008	3	90.0	3.0	50.7 ± 22.5	0.0 – 100.0	0.0*)	230
Martonvásár/HU	2008	1	90.0	20.0	60.1 ± 25.3	0.0 – 100.0	26.0	228
Nyon/CH	2008	2	50.0	5.0	21.8 ± 16.5	0.0 - 80.0	26.9	226
Piešťany/SK	2008	3	30.0	10.0	25.6 ± 10.8	4.0 - 50.0	8.1	229
Probstdorf/AT	2008	1	58.8	47.5	51.4 ± 4.3	30.0 - 65.0	9.0	231
Rust/AT	2008	1	60.0	16.9	45.3 ± 14.2	5.0 - 70.0	21.0	226
Schmida/AT	2008	1	53.8	17.3	31.8 ± 14.4	0.0 - 60.0	25.1	233
Tulln/AT	2008	2	63.8	37.5	36.8 ± 11.4	1.0 - 60.0	12.2	233
Rust/AT	2009	4	82.5	21.3	46.7 ± 23.6	2.0 - 90.0	26.1	227
Tulln/AT	2009	4	80.0	21.9	44.7 ± 24.2	3.0 - 90.0	23.5	231
mean over 11 experiments			66.3	23.4	44.3 ± 12.1	9.1 - 68.2	5.5	206

*) identical scores in both replications

h^2: 0.89

Table 30 Analysis of variance for leaf rust severity (% infected leaf area) of the population Arina/Capo across eleven experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	10	66815.3	72.70	significant (p < 0.0001)
replications (R) within E	10	711.8	7.54	significant (p < 0.0001)
genotypes (G)	232	2755.7	9.92	significant (p < 0.0001)
interaction G x E	2281	290.4	3.08	significant (p < 0.0001)
experimental error (ε)	2014	94.4		

$MS_{GE} = s^2 + 1.793 * s^2_{ge}$

$MS_G = s^2 + 1.677 * s^2_{ge} + 18.181 * s^2_g$

h^2: 0.89 (using the simplified formula), 0.90 (using variance components)

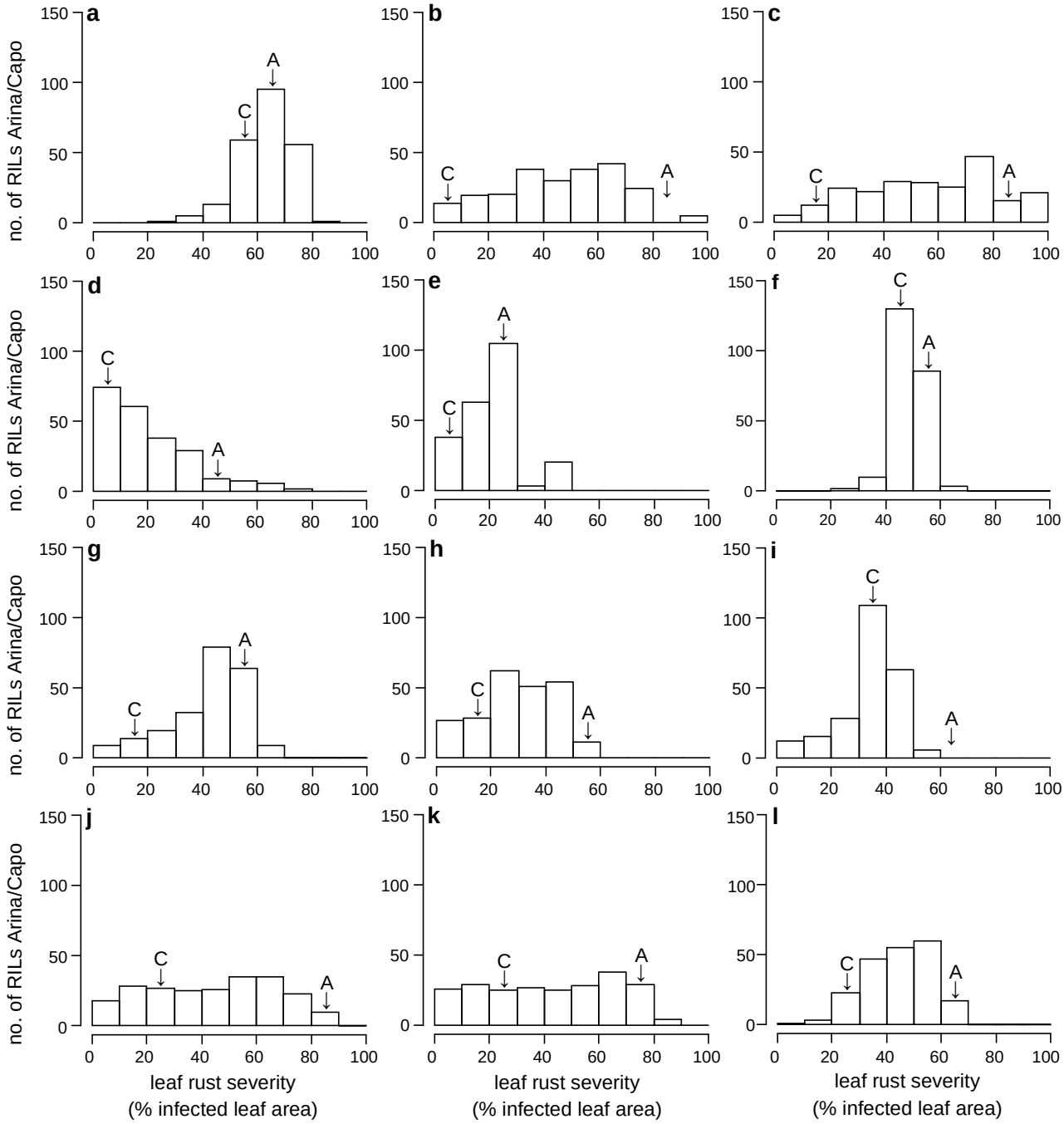

Fig. 14a-l Frequency distributions of leaf rust severity (% infected leaf area) of the RIL population Arina/Capo in the experiments 2007 Tulln (a), 2008 Fundulea (b), 2008 Martonvásár (c), 2008 Nyon (d), 2008 Piešťany (e), 2008 Probstdorf (f), 2008 Rust (g), 2008 Schmida (h), 2008 Tulln (i), 2009 Rust (j), 2009 Tulln (k) and the mean over these eleven experiments (l). Values of the parental lines are indicated by arrows, number of lines according to Table 29.

The values of Spearman's rho ranged from 0.19 (between the experiments Tulln 2007 and Probstdorf 2008) to 0.78 (Rust and Tulln 2009) with the latter being the only one significantly beyond 0.71. Values and sample sizes of all pairwise correlation coefficients are presented in Table 31.

Table 31 Sample size (n) for Spearman's rho (r) between the eleven assessments of leaf rust severity (% infected leaf area) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		ARo8	AU8	ASw8	AS8	AP8	ARu8	AH8	AT8	ARu9	AT9
AT7	n	227	225	223	226	228	223	230	230	224	228
	r	0.35	0.37	0.34	0.32	0.19	0.43	0.42	0.43	0.39	0.41
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
ARo8	n		228	226	228	230	225	230	230	224	228
	r		0.57	0.47	0.33	0.39	0.54	0.52	0.56	0.60	0.61
	H_0		acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
AU8	n			224	226	228	223	228	228	222	226
	r			0.39	0.37	0.46	0.59	0.55	0.62	0.63	0.61
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
ASw8	n				224	226	221	226	226	220	224
	r				0.26	0.35	0.41	0.35	0.45	0.53	0.51
	H_0				acc.	acc.	acc.	acc.	acc.	acc.	acc.
AS8	n					229	224	229	229	223	227
	r					0.25	0.26	0.33	0.43	0.34	0.36
	H_0					acc.	acc.	acc.	acc.	acc.	acc.
AP8	n						226	231	231	225	229
	r						0.43	0.53	0.56	0.61	0.58
	H_0						acc.	acc.	acc.	acc.	acc.
ARu8	n							226	226	220	224
	r							0.56	0.61	0.67	0.69
	H_0							acc.	acc.	acc.	acc.
AH8	n								233	227	231
	r								0.69	0.68	0.64
	H_0								acc.	acc.	acc.
AT8	n									227	231
	r									0.74	0.73
	H_0									acc.	acc.
ARu9	n										225
	r										0.78
	H_0										rej.

Data from five out of the seven experiments of the population **Furore/Capo** in which leaf rust severity (measured by the percentage of infected leaf area) was assessed were used for further statistical analysis. In all experiments except Probstdorf 2007 Capo was less infected than Furore. The frequency distributions of the five experiments showing continuous variation and the mean values over them are illustrated in Fig. 15a-g. The detailed results of the parental lines Furore (susceptible) and Capo (resistant) and the population are given in Table 32. Due to small sample sizes it was not possible to test all

Table 32 Leaf rust severity (% infected leaf area): scoring day, mean values of the parents Furore and Capo and the population Furore/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		sc. day	Furore	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Tulln/AT	2004	1	33.3	7.0	22.0 ± 10.5	1.0 – 45.0	15.2	200
Probstdorf/AT	2006	1	57.5	47.5	53.5 ± 12.6	4.0 – 80.0	12.4	201
Probstdorf/AT	2007	2	23.6	50.0	41.7 ± 15.2	0.0 – 70.0	33.7	189
Tulln/AT	2007	1	27.5	25.0	24.2 ± 9.8	5.0 – 50.0	22.3	201
Tulln/AT (M)	2007	1	22.5	10.0	17.3 ± 11.5	1.0 – 50.0	1 rep.	194
Tulln/AT	2008	3	47.5	27.5	38.8 ± 11.9	4.0 – 60.0	16.1	201
mean over 6 experiments			35.3	27.8	33.0 ± 8.5	9.8 – 51.7	9.3	181

h^2: 0.79

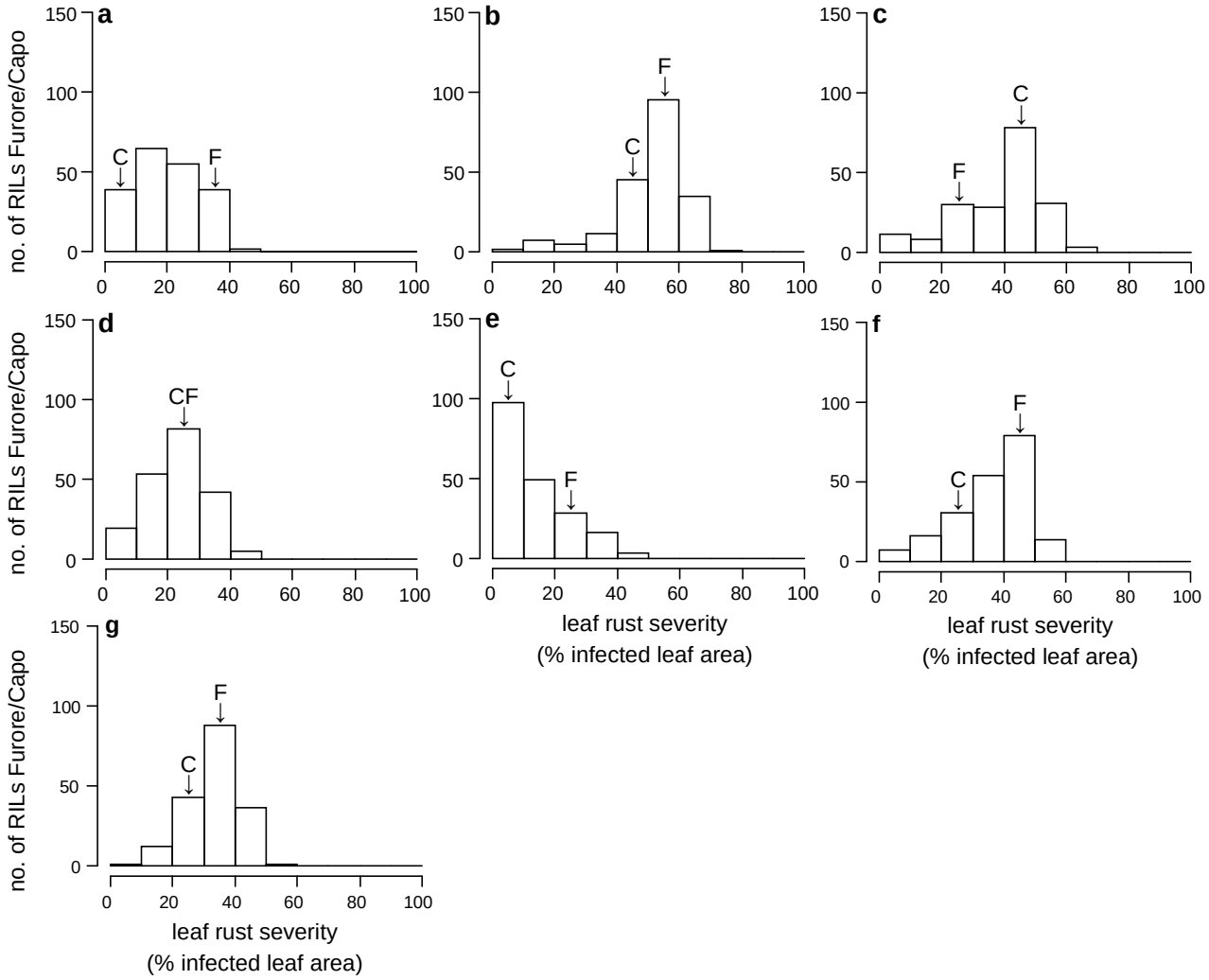

Fig. 15a-g Frequency distributions of leaf rust severity (% infected leaf area) of the RIL population Furore/Capo in the experiments 2004 Tulln (a), 2006 Probstdorf (b), 2007 Probstdorf (c), 2007 Tulln (d), 2007 Tulln (M) (e), 2008 Tulln (f) and the mean over these six experiments (g). Values of the parental lines are indicated by arrows, number of lines according to Table 32.

correlations between these five experiments. The values of Spearman's rho ranged from 0.17 (between Probstdorf and Tulln 2007) to 0.71 (Probstdorf 2006 and Tulln 2008), none being statistically significantly beyond 0.71. All values and the corresponding sample sizes are presented in Table 33. In Table 34 results of the ANOVA are given. In contrast to the populations Isengrain/Capo and Arina/Capo no significant effect of the factor experiment on leaf rust severity was detected. The estimated coefficient of broad sense heritability was 0.79 (calculated using the simplified formula) and 0.80 (calculated by means of variance components), thus being lower than for the two other populations.

Table 33 Sample size (n) for Spearman's rho (r) between the five assessments of leaf rust severity (% infected leaf area) of the population Furore/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$, not t. = not tested

		FP6	FP7	FT7	FM7	FT8
FT4	n	200	188	200	193	200
	r	0.68	0.34	0.40	0.38	0.63
	H_0	acc.	not t.	acc.	acc.	acc.
FP6	n		189	200	194	200
	r		0.40	0.48	0.41	0.71
	H_0		not t.	acc.	acc.	acc.
FP7	n			189	181	189
	r			0.17	0.19	0.41
	H_0			not t.	not t.	not t.
FT7	n				193	200
	r				0.26	0.43
	H_0				acc.	acc.
FM7	n					194
	r					0.37
	H_0					acc.

Table 34 Analysis of variance for leaf rust severity (% infected leaf area) of the population Furore/Capo across six experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	5	64868.7	4.23	not significant (p = 0.0694)
replications (R) within E	5	15040.8	145.37	significant (p < 0.0001)
genotypes (G)	200	692.1	4.90	significant (p < 0.0001)
interaction G x E	980	143.8	1.39	significant (p < 0.0001)
experimental error (ε)	914	103.5		

$MS_{GE} = s^2 + 1.755 * s^2_{ge}$

$MS_G = s^2 + 1.644 * s^2_{ge} + 9.684 * s^2_g$

h^2: 0.79 (using the simplified formula), 0.80 (using variance components)

In several experiments it was possible to assess leaf rust severity measured by the percentage of infected leaf area three times of (almost) all lines: Piešťany 2006, Fundulea 2008, Piešťany 2008 and Tulln 2008. Scoring in Piešťany (2006) was done the second time 10 days and the third time 17 days after the first assessment. In Fundulea (2008) leaf rust assessment was repeated after 20 and 36 days, in Piešťany (2008) after 6 and 18 days and in Tulln (2008) after 4 and 6 days. To make results better comparable, AUDPC was calculated as relative AUDPC (percentage of AUDPC compared to the highest possible AUDPC in the experiment).

Results of the population Isengrain/Capo (Piešťany 2006 and Tulln 2008) are presented in Table 35 (population characteristics and values of the parents), Fig. 16a-b (frequency distributions), Table 36 (Spearman's rho) and Table 37 (results of the ANOVA). In both experiments all lines were assessed three times. For the three experiments of the population Arina/Capo (Fundulea, Piešťany and Tulln 2008), the corresponding results are shown in Table 38, Fig. 17a-c, Table 39 and Table 40. The detailed results of the population Furore/Capo (Tulln 2008) are presented in Table 41 and illustrated in Fig. 18.

Values of AUDPC differed more between different locations and years than between the three populations in Tulln 2008. Considering the population Isengrain/Capo, all main factors and the interaction term had statistically significant effects on AUDPC; in Arina/Capo replications were not significant. Spearman's rho was 0.57 between the two experiments of the population Isengrain/Capo and ranged from 0.31 to 0.53 between the three experiments of the population Arina/Capo. Coefficients of broad sense heritability estimated for Isengrain/Capo were 0.55 for both calculation methods, and for Arina/Capo 0.61 (calculated using the simplified formula) and 0.62 (calculated by means of variance components).

In Fig. 67 disease progress curves are illustrated for the different alleles of the two markers most tightly associated with leaf rust severity of the RILs and the parental lines.

Table 35 Leaf rust severity (relative AUDPC): mean values of the parents Isengrain and Capo and the population Isengrain/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Isengrain	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Piešťany/SK	2006	13.0	7.0	9.6 ± 5.1	1.0 – 27.9	6.4	240
Tulln/AT	2008	30.2	15.0	18.5 ± 12.0	0.0 – 47.0	8.2	240

h^2: 0.55

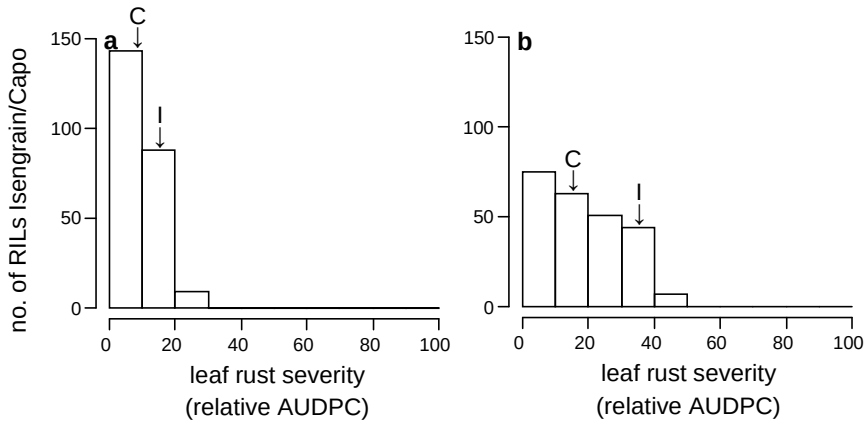

Fig. 16a-b Frequency distributions of leaf rust severity (relative AUDPC) of the RIL population Isengrain/Capo in the experiments 2006 Piešťany (a) and 2008 Tulln (b). Values of the parental lines are indicated by arrows, number of lines according to Table 35.

Table 36 Sample size (n) for Spearman's rho (r) between the two assessments of leaf rust severity (relative AUDPC) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IT8
IS6	n	240
	r	0.57
	H_0	acc.

Table 37 Analysis of variance for leaf rust severity (relative AUDPC) of the population Isengrain/Capo across two experiments

source of variation	**df**	**MS**	**F-value**	**$\alpha = 0.05$**
experiments (E)	1	19091.52	49.22	significant (p = 0.0039)
replications (R) within E	2	297.59	20.58	significant (p < 0.0001)
genotypes (G)	239	234.36	2.24	significant (p < 0.0001)
interaction G x E	239	104.81	7.25	significant (p < 0.0001)
experimental error (ε)	477	14.46		

$MS_{GE} = s^2 + 1.997 * s^2_{ge}$

$MS_G = s^2 + 1.997 * s^2_{ge} + 3.994 * s^2_g$

h^2: 0.55 (using the simplified formula), 0.55 (using variance components)

Table 38 Leaf rust severity (relative AUDPC): mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		**Arina**	**Capo**	**pop. $\bar{x} \pm s$**	**pop. range**	**$LSD_{5\%}$**	**n**
Fundulea/RO	2008	46.5	1.6	27.1 ± 14.3	0.8 – 54.6	0.5	229
Piešťany/SK	2008	14.9	4.3	11.5 ± 5.3	1.6 – 31.8	4.4	229
Tulln/AT	2008	47.0	17.8	33.6 ± 10.0	1.0 – 56.0	9.8	233

h^2: 0.61

Table 39 Sample size (n) for Spearman's rho (r) between the three assessments of leaf rust severity (relative AUDPC) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		AS8	AT8
ARo8	n	228	230
	r	0.31	0.53
	H_0	acc.	acc.
AS8	n		229
	r		0.38
	H_0		acc.

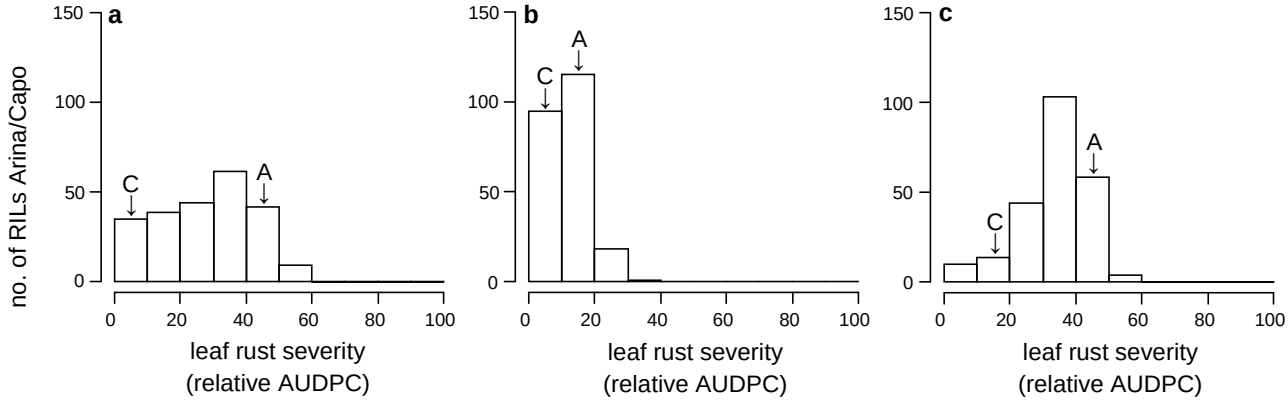

Fig. 17a-c Frequency distributions of leaf rust severity (relative AUDPC) of the RIL population Arina/Capo in the experiments 2008 Fundulea (a), 2008 Piešťany (b) and 2008 Tulln (c). Values of the parental lines are indicated by arrows, number of lines according to Table 38.

Table 40 Analysis of variance for leaf rust severity (relative AUDPC) of the population Arina/Capo across three experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	2	59654.05	438.77	significant ($p < 0.0001$)
replications (R) within E	3	0.81	0.08	not significant ($p = 0.9704$)
genotypes (G)	232	376.07	2.59	significant ($p < 0.0001$)
interaction G x E	456	145.14	14.45	significant ($p < 0.0001$)
experimental error (ε)	688	10.05		

$MS_{GE} = s^2 + 2 * s^2_{ge}$

$MS_G = s^2 + 2 * s^2_{ge} + 5.931 * s^2_g$

h^2: 0.61 (using the simplified formula), 0.62 (using variance components)

Table 41 Leaf rust severity (relative AUDPC): mean values of the parents Furore and Capo and the population Furore/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Furore	Capo	pop. $\bar{x} \pm s$	pop. range	$LSD_{5\%}$	n
Tulln/AT	2008	26.4	10.0	18.0 ± 7.8	1.9 – 40.5	9.6	201

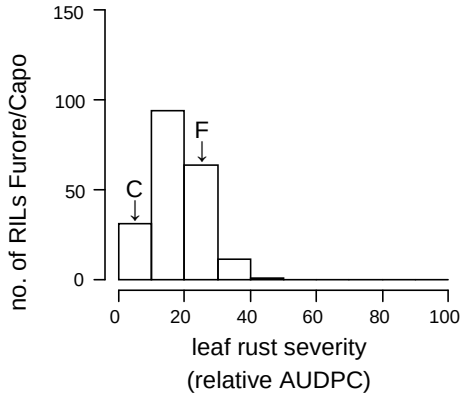

Fig. 18 Frequency distribution of leaf rust severity (relative AUDPC) of the 201 RILs of the population Furore/Capo in the experiment 2008 Tulln. Values of the parental lines are indicated by arrows.

Seedling resistance (measured on a 0 to 4 scale) was only assessed for the population Arina/Capo in a single replicated greenhouse experiment in Fundulea in the year 2008. The mean value 2.7 was the same for both parents and the population. The frequency distribution showing continuous variation is illustrated in Fig. 19, the corresponding values are presented in Table 42.

Table 42 Seedling resistance (0-4): mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Arina	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Fundulea/RO	2008	2.7	2.7	2.7 ± 0.4	2.0 – 4.0	1 rep.	220

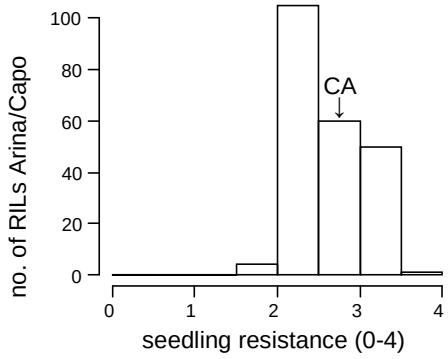

Fig. 19 Frequency distribution of seedling resistance (0-4) of the 220 RILs of the population Arina/Capo in the experiment 2008 Fundulea. Values of the parental lines are indicated by arrows.

5.1.1.3 Analysis of Covariance

Plants of the spreader rows were artificially inoculated to provoke leaf rust infection. From these infection points the disease spread over the whole area. At the optimal scoring date, leaf rust severity of the plants of a particular line is not dependent on the proximity to initially inoculated spreader row plants (or other environmental influences) but solely on their susceptibility. It was tested, whether data of leaf rust infection of the spreader row plants next to each plot assessed a few days earlier improved results in terms of the estimated coefficient of broad sense heritability. These tests were conducted in 2008 in the four experiments Probstdorf, Rust, Schmida and Tulln of the populations Isengrain/Capo and Arina/Capo and for standard lines distributed among the experiments of all three populations. Spreader row data were included as a covariate in an analysis of covariance. Results of Isengrain/Capo are presented in Table 43, of Arina/Capo in Table 45 and of the standard lines in Table 47. For comparison of estimated coefficients of heritability ANOVA were calculated for the same experiments. Results are presented in Table 44, Table 46 and Table 48. The value of the population Isengrain/Capo increased by 1.2 % if spreader row data were included calculated after the simplified formula and by 1.4 % for the value based on variance components. The increase of the population Arina/Capo was 0.4 % for both procedures. In the case of the standard lines the value decreased by 0.4 and 0.2 %.

Table 43 Analysis of covariance for leaf rust severity (% infected leaf area) of the population Isengrain/Capo across four experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	3	32665.94	83.18	significant (p < 0.0001)
replications (R) within E	4	453.64	6.44	significant (p < 0.0001)
genotypes (G)	239	877.23	6.63	significant (p < 0.0001)
interaction G x E	661	134.18	1.90	significant (p < 0.0001)
covariate (S)	1	4635.08	65.78	significant (p < 0.0001)
experimental error (ε)	766	70.46		

$MS_{GE} = s^2 + 1.828 * s^2_{ge}$

$MS_G = s^2 + 1.776 * s^2_{ge} + 6.659 * s^2_g$

h^2: 0.85 (using the simplified formula), 0.85 (using variance components)

Table 44 Analysis of variance for leaf rust severity (% infected leaf area) of the population Isengrain/Capo across four experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	3	44016.17	70.04	significant (p = 0.0002)
replications (R) within E	4	577.18	7.70	significant (p < 0.0001)
genotypes (G)	239	862.22	6.21	significant (p < 0.0001)
interaction G x E	684	140.67	1.88	significant (p < 0.0001)
experimental error (ε)	798	74.96		

$MS_{GE} = s^2 + 1.845 * s^2_{ge}$

$MS_G = s^2 + 1.793 * s^2_{ge} + 6.913 * s^2_g$

h^2: 0.84 (using the simplified formula), 0.84 (using variance components)

Table 45 Analysis of covariance for leaf rust severity (% infected leaf area) of the population Arina/Capo across four experiments

source of variation	df	MS	F-value	α = 0.05
experiments (E)	3	34353.80	64.00	significant (p = 0.0003)
replications (R) within E	4	672.32	8.69	significant (p < 0.0001)
genotypes (G)	232	676.71	6.09	significant (p < 0.0001)
interaction G x E	687	111.62	1.44	significant (p < 0.0001)
covariate (S)	1	4143.35	53.57	significant (p < 0.0001)
experimental error (ε)	846	77.35		

$MS_{GE} = s^2 + 1.910 * s^2_{ge}$

$MS_G = s^2 + 1.878 * s^2_{ge} + 7.436 * s^2_g$

h^2: 0.84 (using the simplified formula), 0.84 (using variance components)

Table 46 Analysis of variance for leaf rust severity (% infected leaf area) of the population Arina/Capo across four experiments

source of variation	df	MS	F-value	α = 0.05
experiments (E)	3	34584.47	52.71	significant (p = 0.0006)
replications (R) within E	4	619.15	7.58	significant (p < 0.0001)
genotypes (G)	232	703.59	5.98	significant (p < 0.0001)
interaction G x E	687	118.13	1.45	significant (p < 0.0001)
experimental error (ε)	864	81.72		

$MS_{GE} = s^2 + 1.932 * s^2_{ge}$

$MS_G = s^2 + 1.907 * s^2_{ge} + 7.551 * s^2_g$

h^2: 0.83 (using the simplified formula), 0.83 (using variance components)

Table 47 Analysis of covariance for leaf rust severity (% infected leaf area) of the standard lines across four experiments

source of variation	df	MS	F-value	α = 0.05
experiments (E)	3	3056.67	17.64	significant (p < 0.0001)
replications (R) within E	4	148.87	2.06	not significant (p = 0.0852)
genotypes (G)	26	4546.66	21.31	significant (p < 0.0001)
interaction G x E	48	253.28	3.51	significant (p < 0.0001)
covariate (S)	1	745.77	10.34	significant (p = 0.0014)
experimental error (ε)	337	72.13		

$MS_{GE} = s^2 + 4.882 * s^2_{ge}$

$MS_G = s^2 + 3.806 * s^2_{ge} + 12.577 * s^2_g$

h^2: 0.94 (using the simplified formula), 0.96 (using variance components)

Table 48 Analysis of variance for leaf rust severity (% infected leaf area) of the standard lines across four experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	3	3406.08	18.20	significant ($p < 0.0001$)
replications (R) within E	4	150.74	2.05	not significant ($p = 0.0856$)
genotypes (G)	26	4710.68	22.43	significant ($p < 0.0001$)
interaction G x E	49	243.51	3.32	significant ($p < 0.0001$)
experimental error (ε)	345	73.39		

$MS_{GE} = s^2 + 4.945 * s^2_{ge}$

$MS_G = s^2 + 3.971 * s^2_{ge} + 13.244 * s^2_g$

h^2: 0.95 (using the simplified formula), 0.96 (using variance components)

5.1.2 Heading

For the population **Isengrain/Capo** the trait heading was assessed in 15 experiments. In the experiment Schmida 2007 the scoring period did not cover the whole heading period, therefore it was impossible to use these data for any further statistical analysis. In the experiments Probstdorf 2006 instead of monitoring the day of the year a 1 to 9 scale was used for heading. In Piešťany those plants that had already started heading were determined on three days. Thus, these data were not useable for comparisons with the other experiments. If flowering was assessed instead of heading, data were converted by subtracting five days.

Table 49 gives the detailed results of the parental lines and the population. With the exception of the experiment Tulln 2006 Capo was heading one to five days later than Isengrain. The population showed continuous variation as illustrated in Fig. 20a-l.

Table 49 Heading (day of the year): mean values of the parents Isengrain and Capo and the population Isengrain/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Isengrain	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Aumühle/AT	2004	154.9	155.9	153.8 ± 3.2	148.0 – 159.5	2.5	240
Tulln/AT	2004	156.4	157.3	154.5 ± 3.2	149.5 – 162.5	2.7	240
Tulln/AT (Vm)	2005	148.5	150.5	150.3 ± 2.5	144.0 – 155.0	1 rep.	240
Tulln/AT	2006	163.0	162.3	162.0 ± 2.6	156.0 – 167.0	2.3	240
Probstdorf/AT	2007	139.0	142.0	140.1 ± 3.4	131.0 – 145.0	2.8	239
Tulln/AT (M)	2007	137.0	142.0	139.5 ± 2.8	134.0 – 146.0	1 rep.	227
Tulln/AT	2007	134.8	138.0	136.5 ± 3.6	128.5 – 143.0	2.6	240
Rust/AT	2008	148.0	151.4	150.1 ± 2.8	144.0 – 156.0	1.9	240
Schmida/AT	2008	150.4	152.9	152.0 ± 2.1	148.0 – 156.5	1.6	216
Tulln/AT	2008	148.1	150.0	149.4 ± 2.2	144.5 – 154.0	1.4	240
Tulln/AT	2009	141.9	144.3	143.9 ± 2.9	137.0 – 150.0	1.9	240
mean over 11 experiments		147.5	149.7	148.8 ± 2.3	143.6 – 153.3	0.7	204

h^2: 0.98

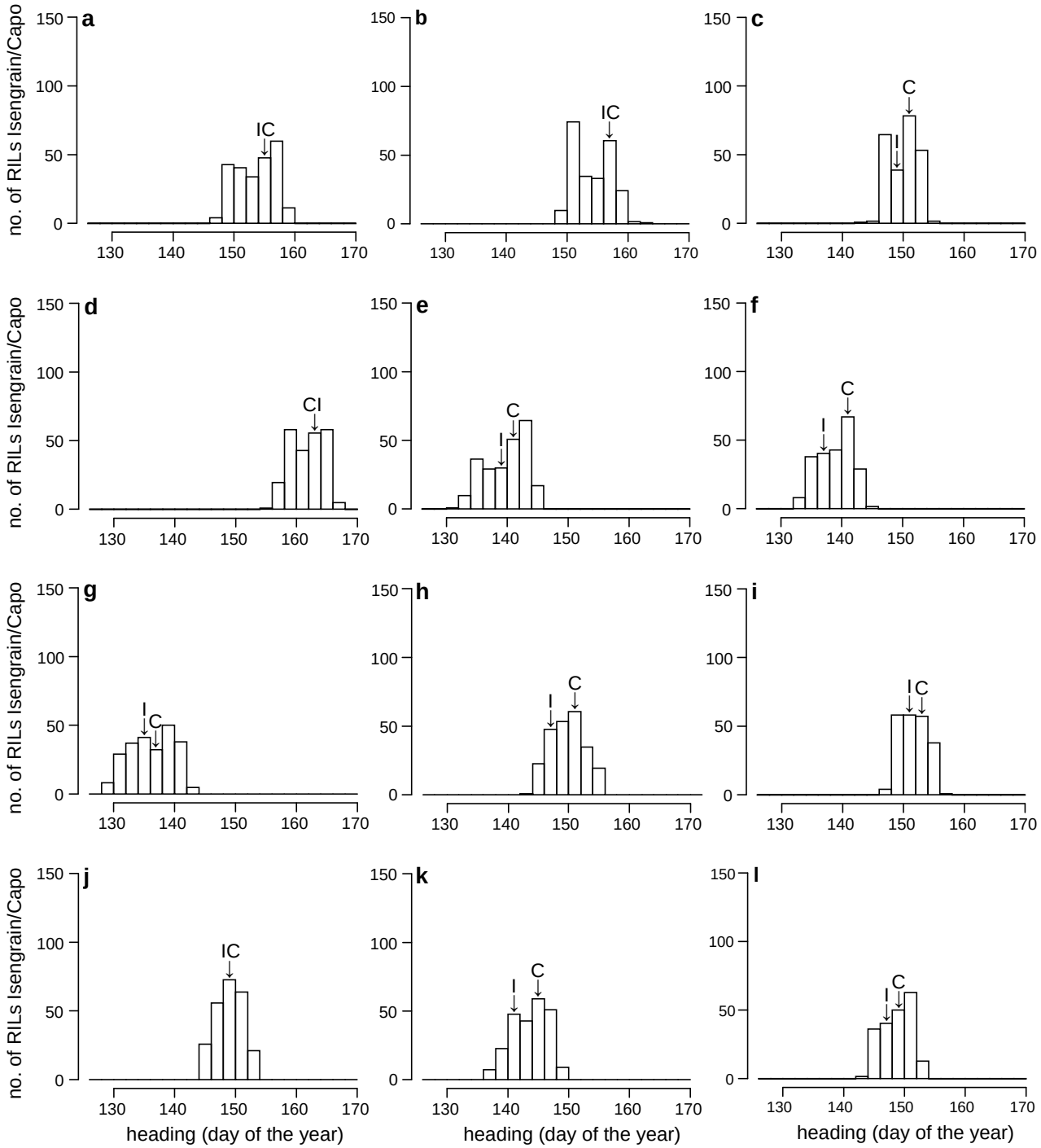

Fig. 20a-l Frequency distributions of heading (day of the year) of the RIL population Isengrain/Capo in the experiments 2004 Aumühle (a), 2004 Tulln (b), 2005 Tulln (Vm) (c), 2006 Tulln (d), 2007 Probstdorf (e), 2007 Tulln (M) (f), 2007 Tulln (g), 2008 Rust (h), 2008 Schmida (i), 2008 Tulln (j), 2009 Tulln (k) and the mean over these eleven experiments (l). Values of the parental lines are indicated by arrows, number of lines according to Table 49.

The values of Spearman's rho were statistically significantly beyond 0.71 for most of these eleven comparisons. Exceptions were the comparisons between Tulln 2004 and all other experiments except Aumühle 2004. Four out of ten comparisons with Aumühle 2004 and the comparison Tulln (Vm) 2005 and Schmida 2008 were neither significant. The

estimated correlation coefficients and whether the null hypothesis H_0: $0 < \rho \leq 0.71$ was accepted (acc.) or rejected (rej.) are presented in Table 50.

Table 50 Sample size (n) for Spearman's rho (r) between the eleven assessments of heading (day of the year) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IT4	IVm5	IT6	IP7	IM7	IT7	IRu8	IH8	IT8	IT9
IA4	n	240	240	240	239	227	240	240	216	240	240
	r	0.92	0.73	0.71	0.76	0.76	0.79	0.77	0.72	0.76	0.77
	H_0	rej.	acc.	acc.	rej.	acc.	rej.	rej.	acc.	rej.	rej.
IT4	n		240	240	239	227	240	240	216	240	240
	r		0.68	0.68	0.72	0.71	0.75	0.73	0.68	0.74	0.73
	H_0		acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
IVm5	n			240	239	227	240	240	216	240	240
	r			0.80	0.81	0.77	0.78	0.79	0.75	0.80	0.81
	H_0			rej.	rej.	rej.	rej.	rej.	acc.	rej.	rej.
IT6	n				239	227	240	240	216	240	240
	r				0.83	0.84	0.84	0.84	0.80	0.87	0.85
	H_0				rej.	rej.	rej.	rej.	rej.	rej.	rej.
IP7	n					227	239	239	215	239	239
	r					0.89	0.91	0.91	0.90	0.90	0.90
	H_0					rej.	rej.	rej.	rej.	rej.	rej.
IM7	n						227	227	204	227	227
	r						0.94	0.90	0.88	0.93	0.92
	H_0						rej.	rej.	rej.	rej.	rej.
IT7	n							240	216	240	240
	r							0.91	0.87	0.92	0.92
	H_0							rej.	rej.	rej.	rej.
IRu8	n								216	240	240
	r								0.91	0.93	0.92
	H_0								rej.	rej.	rej.
IH8	n									216	216
	r									0.92	0.91
	H_0									rej.	rej.
IT8	n										240
	r										0.94
	H_0										rej.

ANOVA with experiments, replications within experiments, genotypes as main factors and the interaction term genotype x experiment revealed statistically significant effects for all these four random factors on the date of heading. Table 51 gives the degrees of freedom (df), the mean squares (MS), the F-values and the decisions about the null hypotheses

based on a type I error rate of $\alpha = 0.05$. Additionally the compositions of the mean squares are shown for the estimation of the coefficient of heritability by the means of variance components. The value of the coefficient of broad sense heritability (h^2) over all eleven experiments was 0.98 for both calculation methods.

Table 51 Analysis of variance for heading (day of the year) of the population Isengrain/Capo across eleven experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	10	25166.528	4447.81	significant ($p < 0.0001$)
replications (R) within E	9	4.052	3.11	significant ($p = 0.0010$)
genotypes (G)	239	122.586	43.54	significant ($p < 0.0001$)
interaction G x E	2352	2.925	2.25	significant ($p < 0.0001$)
experimental error (ε)	2080	1.303		

$MS_{GE} = s^2 + 1.794 * s^2_{ge}$

$MS_G = s^2 + 1.673 * s^2_{ge} + 18.133 * s^2_g$

h^2: 0.98 (using the simplified formula), 0.98 (using variance components)

For the population **Arina/Capo** data of all heading assessments except from the experiment Piešťany 2008 were used for further statistical analysis. The detailed results of the parental lines and the population are given in Table 52. The corresponding frequency distributions showing continuous variation are illustrated in Fig. 21a-j. In all experiments Capo was heading a few days earlier than Arina.

Table 52 Heading (day of the year): mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Arina	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Tulln/AT	2007	139.3	137.7	138.0 ± 2.4	130.0 – 144.0	1 rep.	232
Fundulea/RO	2008	143.8	142.8	142.0 ± 1.9	139.0 – 149.0	1 rep.	230
Nyon/CH	2008	147.5	145.5	146.6 ± 1.8	142.5 – 150.5	1.2	228
Reichersberg/AT	2008	154.0	151.0	152.1 ± 1.9	149.0 – 161.0	1 rep.	230
Rust/AT	2008	152.9	150.8	152.0 ± 1.6	148.5 – 155.5	1.5	230
Schmida/AT	2008	153.5	151.4	152.3 ± 1.5	149.5 – 156.0	1.6	232
Tulln/AT	2008	150.4	149.9	150.5 ± 1.2	148.0 – 156.0	1.4	233
Rust/AT	2009	147.3	145.0	146.7 ± 2.2	142.5 – 154.0	2.1	232
Tulln/AT	2009	146.8	145.0	146.4 ± 2.0	142.0 – 157.5	1.8	233
mean over 9 experiments		148.4	146.6	147.4 ± 1.6	143.9 – 151.4	0.6	228

h^2: 0.96

The values of Spearman's rho (Table 53) between these nine experiments ranged from 0.54 (between the experiments Tulln 2007 and Reichersberg 2008) to 0.89 (Nyon and Rust 2008). Two third of the correlations had a coefficient statistically significantly beyond 0.71. Mainly correlations with the experiment Tulln 2007 were lower. As for the population Isengrain/Capo ANOVA (results presented in Table 54) revealed statistically

Table 53 **Sample size (n) for Spearman's rho (r) between the nine assessments of heading (day of the year) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$**

		ARo8	ASw8	ARe8	ARu8	AH8	AT8	ARu9	AT9
AT7	n	229	228	229	230	232	232	232	232
	r	0.58	0.78	0.54	0.74	0.71	0.73	0.73	0.72
	H_0	acc.	rej.	acc.	acc.	acc.	acc.	acc.	acc.
ARo8	n		228	230	229	229	230	229	230
	r		0.76	0.81	0.76	0.75	0.73	0.79	0.72
	H_0		rej.	rej.	rej.	acc.	acc.	rej.	acc.
ASw8	n			228	228	228	228	228	228
	r			0.76	0.89	0.85	0.82	0.85	0.79
	H_0			rej.	rej.	rej.	rej.	rej.	rej.
ARe8	n				229	229	230	229	230
	r				0.79	0.76	0.71	0.79	0.72
	H_0				rej.	rej.	acc.	rej.	acc.
ARu8	n					230	230	230	230
	r					0.85	0.81	0.86	0.80
	H_0					rej.	rej.	rej.	rej.
AH8	n						231	231	231
	r						0.80	0.83	0.77
	H_0						rej.	rej.	rej.
AT8	n							231	232
	r							0.81	0.80
	H_0							rej.	rej.
ARu9	n								232
	r								0.82
	H_0								rej.

Table 54 **Analysis of variance for heading (day of the year) of the population Arina/Capo across nine experiments**

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	8	6073.550	259.96	significant ($p < 0.0001$)
replications (R) within E	6	25.682	37.31	significant ($p < 0.0001$)
genotypes (G)	232	35.406	26.37	significant ($p < 0.0001$)
interaction G x E	1838	1.408	2.04	significant ($p < 0.0001$)
experimental error (ε)	1382	0.688		

$MS_{GE} = s^2 + 1.651 * s^2_{ge}$

$MS_G = s^2 + 1.502 * s^2_{ge} + 13.395 * s^2_g$

h^2: 0.96 (using the simplified formula), 0.96 (using variance components)

significant effects for all main factors (experiments, replications and genotypes) and the interaction term experiment x genotype on the date of heading. The estimated coefficient of broad sense heritability (h^2) was for both calculation methods 0.96, thus being slightly lower than for Isengrain/Capo.

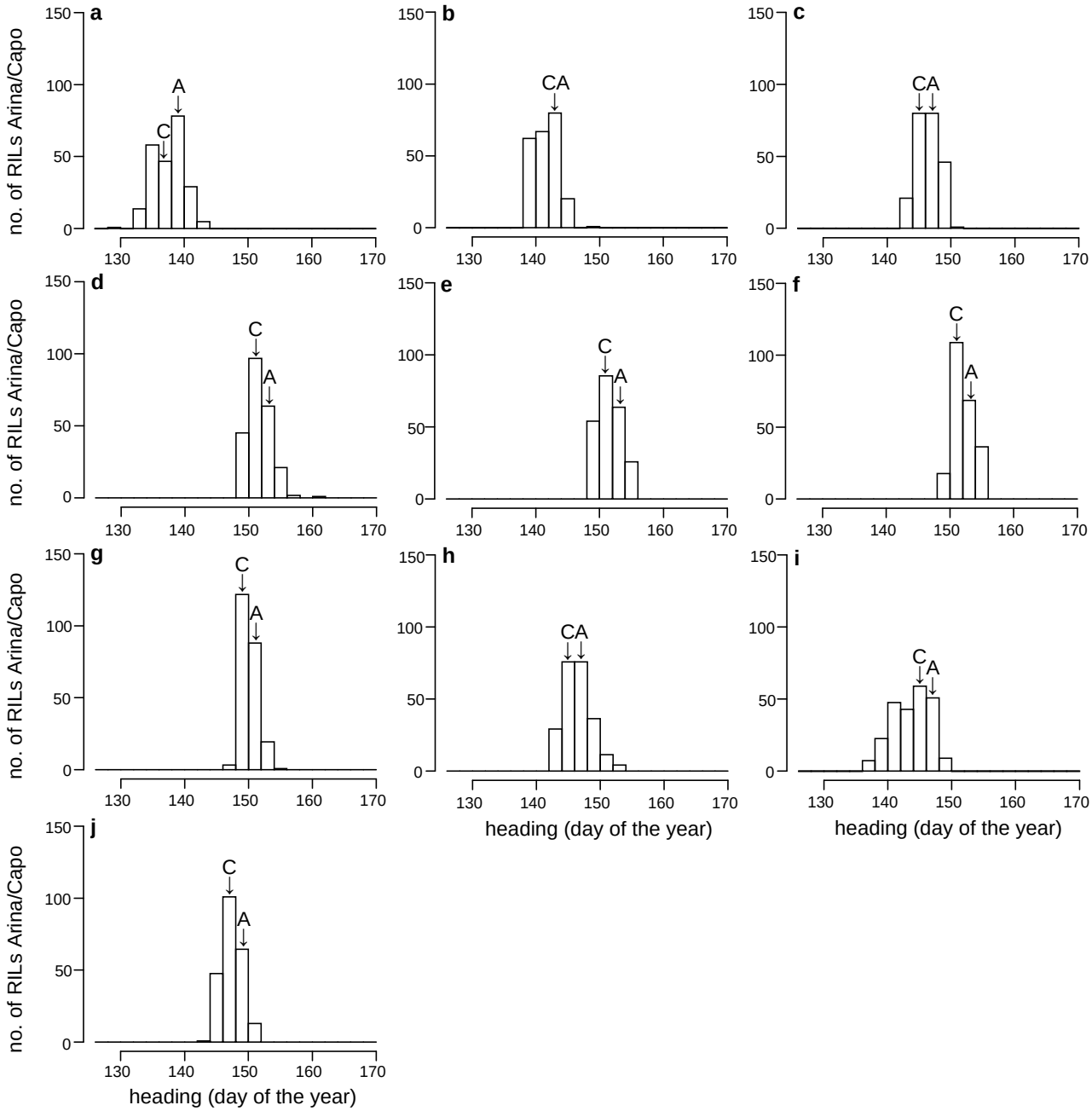

Fig. 21a-j Frequency distributions of heading (day of the year) of the RIL population Arina/Capo in the experiments 2007 Tulln (a), 2008 Fundulea (b), 2008 Nyon (c), 2008 Reichersberg (d), 2008 Rust (e), 2008 Schmida (f), 2008 Tulln (g), 2009 Rust (h), 2009 Tulln (i) and the mean over these nine experiments (j). Values of the parental lines are indicated by arrows, number of lines according to Table 52.

For the population **Furore/Capo** the trait heading was assessed of all lines in twelve experiments of which two had only one replication. In the experiments Probstdorf 2006 and 2008 heading was scored on a 1 to 9 scale, thus data were not comparable with those of the other experiments. In Tulln 2006 the majority of RILs were flowering within a period of a few days between two scoring dates, thus data fit badly to those of other experiments. If flowering was assessed instead of heading, data were converted by subtracting five days. The detailed results of the parental lines and the population are given in Table 55. The corresponding frequency distributions showing continuous variation are illustrated in Fig. 22a-j.

The values of Spearman's rho between these nine assessments of heading ranged from 0.25 (between the experiments Aumühle 2004 and Tulln (Vm) 2005) to 0.67 (Probstdorf and Tulln (M) 2007), none being statistically significantly beyond 0.71. These results are presented in Table 57.

Table 55 Heading (day of the year): mean values of the parents Furore and Capo and the population Furore/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Furore	Capo	pop. $\bar{x} \pm s$	pop. range	$LSD_{5\%}$	n
Aumühle/AT	2004	156.3	157.0	156.0 ± 1.0	153.0 – 158.0	2.4	201
Tulln/AT	2004	157.5	156.3	156.7 ± 1.0	151.0 – 158.5	1.6	201
Tulln/AT (Vm)	2005	150.5	150.5	151.3 ± 0.9	147.5 – 155.0	1 rep.	201
Probstdorf/AT	2007	140.0	142.2	141.1 ± 1.3	137.5 – 144.0	1.7	201
Tulln/AT	2007	135.0	138.5	136.6 ± 1.6	134.0 – 141.0	2.9	201
Tulln/AT (M)	2007	139.5	140.0	140.2 ± 1.0	138.0 – 142.0	1 rep.	201
Rust/AT	2008	150.8	151.6	151.6 ± 1.0	149.5 – 154.5	1.7	201
Schmida/AT	2008	152.1	152.3	152.4 ± 0.9	150.0 – 155.5	1.6	201
Tulln/AT	2008	150.1	150.0	150.3 ± 0.6	149.0 – 153.0	1.1	201
mean over 9 experiments		148.0	148.7	148.5 ± 0.8	146.8 – 150.6	0.7	201

h^2: 0.86

Table 56 Analysis of variance for heading (day of the year) of the population Furore/Capo across nine experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	8	19321.582	2746.67	significant (p < 0.0001)
replications (R) within E	7	6.848	7.06	significant (p < 0.0001)
genotypes (G)	200	8.436	7.41	significant (p < 0.0001)
interaction G x E	1600	1.152	1.19	significant (p = 0.0005)
experimental error (ε)	1395	0.970		

$MS_{GE} = s^2 + 1.763 * s^2_{ge}$

$MS_G = s^2 + 1.633 * s^2_{ge} + 14.697 * s^2_g$

h^2: 0.86 (using the simplified formula), 0.87 (using variance components)

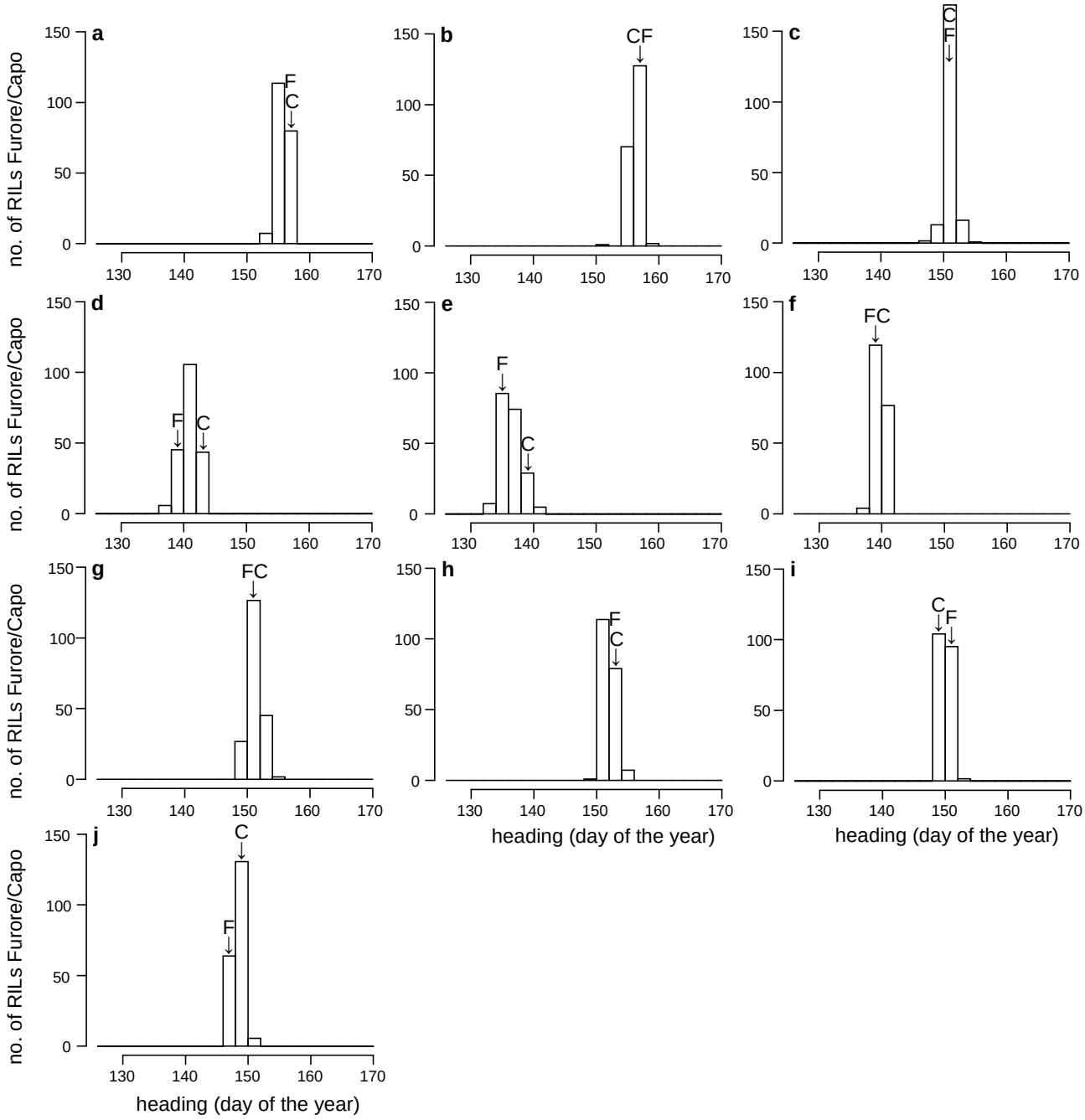

Fig. 22a-j Frequency distributions of heading (day of the year) of the 201 RILs of the population Furore/Capo in the experiments 2004 Aumühle (a), 2004 Tulln (b), 2005 Tulln (Vm) (c), 2007 Probstdorf (d), 2007 Tulln (e), 2007 Tulln (M) (f), 2008 Rust (g), 2008 Schmida (h), 2008 Tulln (i) and the mean over these nine experiments (j). Values of the parental lines are indicated by arrows.

As for the two other populations ANOVA revealed statistically significant effects for all main factors and the interaction term on the date of heading. The estimated coefficient of broad sense heritability (h^2) was 0.86 (calculated using the simplified formula) and 0.87 (calculated by means of variance components), thus being clearly lower than for the two other populations.

Table 57 Sample size (n) for Spearman's rho (r) between the nine assessments of heading (day of the year) of the population Furore/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		FT4	FVm5	FP7	FT7	FM7	FRu8	FH8	FT8
FA4	n	201	201	201	201	201	201	201	201
	r	0.50	0.25	0.46	0.34	0.36	0.41	0.37	0.32
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
FT4	n		201	201	201	201	201	201	201
	r		0.34	0.40	0.35	0.39	0.34	0.41	0.38
	H_0		acc.	acc.	acc.	acc.	acc.	acc.	acc.
FVm5	n			201	201	201	201	201	201
	r			0.35	0.34	0.37	0.39	0.39	0.32
	H_0			acc.	acc.	acc.	acc.	acc.	acc.
FP7	n				201	201	201	201	201
	r				0.66	0.67	0.59	0.60	0.49
	H_0				acc.	acc.	acc.	acc.	acc.
FT7	n					201	201	201	201
	r					0.57	0.58	0.61	0.48
	H_0					acc.	acc.	acc.	acc.
FM7	n						201	201	201
	r						0.56	0.55	0.57
	H_0						acc.	acc.	acc.
FRu8	n							201	201
	r							0.65	0.61
	H_0							acc.	acc.
FH8	n								201
	r								0.60
	H_0								acc.

5.1.3 Plant Height

Table 58 Plant height (cm): mean values of the parents Isengrain and Capo and the population Isengrain/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Isengrain	Capo	pop. $\bar{x} \pm s$	pop. range	$LSD_{5\%}$	n
Tulln/AT	2004	71.9	100.6	88.3 ± 9.5	65.0 – 110.0	6.8	240
Tulln/AT	2006	70.0	93.8	86.2 ± 9.0	65.0 – 110.0	9.7	240
Tulln/AT (M)	2007	80.0	107.5	94.5 ± 8.7	75.0 – 115.0	1 rep.	227
Tulln/AT	2007	81.3	106.3	101.8 ± 9.4	75.0 – 122.5	9.0	240
Tulln/AT	2008	71.9	90.6	86.3 ± 8.1	67.5 – 107.5	8.5	240
mean over 5 experiments		75.0	99.8	91.7 ± 8.0	71.0 – 108.0	4.0	227

h^2: 0.95

For the population **Isengrain/Capo** the trait plant height measured in cm was assessed in five experiments. In the non replicated experiment Tulln (M) 2007 only 227 lines were tested. All data were used for testing correlations between experiments and estimating the coefficient of broad sense heritability (h^2). The detailed results of the parental lines and the population are given in Table 58. In Fig. 23a-f the frequency distributions of these five experiments showing continuous variation and the mean over them are illustrated.

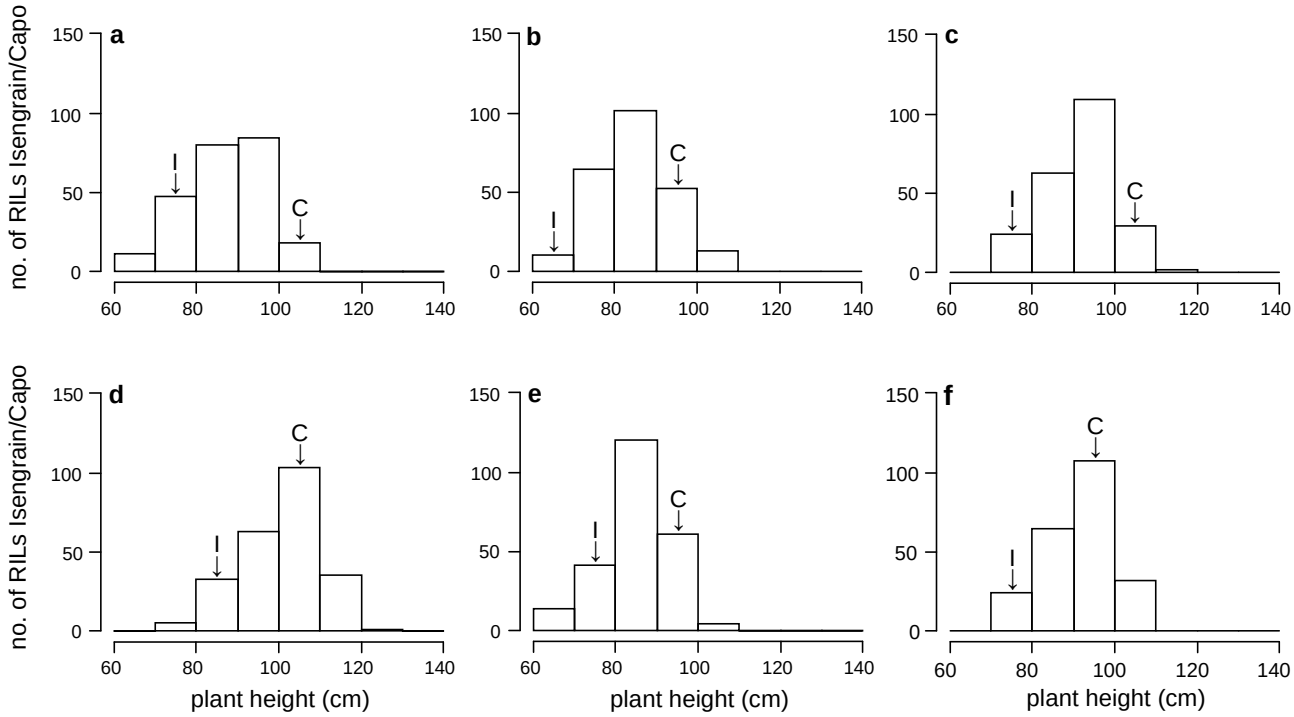

Fig. 23a-f Frequency distributions of plant height (cm) of the RIL population Isengrain/Capo in the experiments 2004 Tulln (a), 2006 Tulln (b), 2007 Tulln (M) (c), 2007 Tulln (d), 2008 Tulln (e) and the mean over these five experiments (f). Values of the parental lines are indicated by arrows, number of lines according to Table 58.

Table 59 Analysis of variance for plant height (cm) of the population Isengrain/Capo across five experiments

source of variation	df	MS	F-value	α = 0.05
experiments (E)	4	20563.44	169.10	significant (p < 0.0001)
replications (R) within E	4	111.50	5.89	significant (p = 0.0001)
genotypes (G)	239	553.96	18.75	significant (p < 0.0001)
interaction G x E	943	30.21	1.59	significant (p < 0.0001)
experimental error (ε)	947	18.94		

$MS_{GE} = s^2 + 1.779 * s^2_{ge}$

$MS_G = s^2 + 1.673 * s^2_{ge} + 8.264 * s^2_g$

h^2: 0.95 (using the simplified formula), 0.95 (using variance components)

ANOVA with experiments, replications within experiments, genotypes as main factors and the interaction term genotype x experiment revealed statistically significant effects of all these four random factors on plant height. Table 59 gives the degrees of freedom (df), the

mean squares (MS), the F-values and the decisions about the null hypotheses based on a type I error rate of $\alpha = 0.05$. Additionally the compositions of the mean squares are shown for the estimation of the coefficient of heritability by the means of variance components. The value of the coefficient of broad sense heritability over all five experiments was 0.95 for both calculation methods.

The values of Spearman's rho between these five experiments and whether the null hypothesis H_0: $0 < \rho \leq 0.71$ was accepted (acc.) or rejected (rej.) are presented in Table 60. Values of r ranged from 0.68 (between the experiments Tulln 2006 and 2008), to 0.84 (Tulln 2006 and 2007 (M)). Correlations with the experiment Tulln 2008 were consistently lower and not statistically significantly beyond 0.71.

Table 60 Sample size (n) for Spearman's rho (r) between the five assessments of plant height (cm) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IT6	IM7	IT7	IT8
IT4	n	240	227	240	240
	r	0.83	0.81	0.77	0.70
	H_0	rej.	rej.	rej.	acc.
IT6	n		227	240	240
	r		0.84	0.78	0.68
	H_0		rej.	rej.	acc.
IM7	n			227	227
	r			0.80	0.75
	H_0			rej.	acc.
IT7	n				240
	r				0.76
	H_0				acc.

For all lines of the population **Arina/Capo** plant height was assessed in two experiments (the first with only one replication). The detailed results of the parental lines and the population are given in Table 61, the corresponding frequency distributions are illustrated in Fig. 24a-c.

Table 61 Plant height (cm): mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Arina	Capo	pop. $\bar{x} \pm s$	pop. range	$LSD_{5\%}$	n
Tulln/AT	2007	106.7	111.7	110.7 ± 9.4	75.0 – 130.0	1 rep.	233
Tulln/AT	2008	95.6	92.5	93.0 ± 7.9	72.5 – 112.5	9.8	233
mean over 2 experiments		101.2	102.1	101.9 ± 8.0	73.8 – 121.3	9.4	233

h^2: 0.83

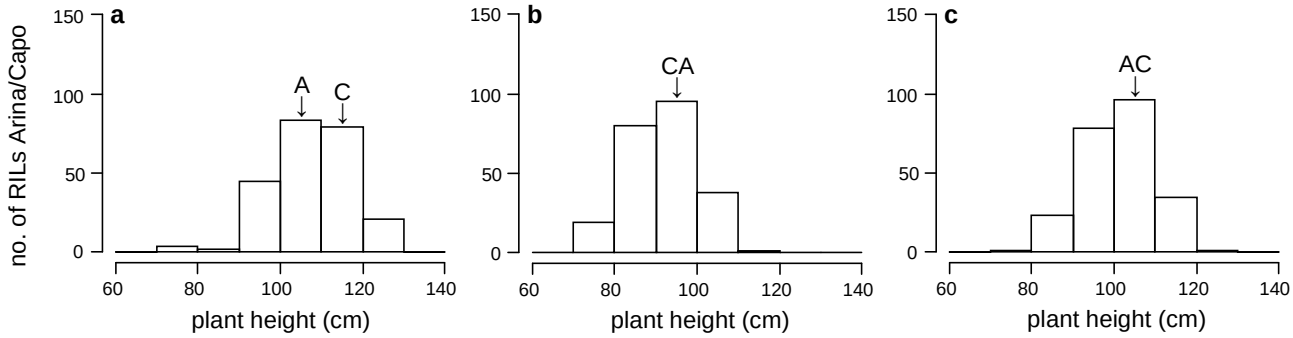

Fig. 24a-c Frequency distributions of plant height (cm) of the 233 RILs of the population Arina/Capo in the experiments 2007 Tulln (a), 2008 Tulln (b) and the mean over these two experiments (c). Values of the parental lines are indicated by arrows.

Table 62 Sample size (n) for Spearman's rho (r) between the two assessments of plant height (cm) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: 0 < ρ ≤ 0.71 is given. α = β = 0.05

		AT8
AT7	n	233
	r	0.73
	H_0	acc.

Table 63 Analysis of variance for plant height (cm) of the population Arina/Capo across two experiments

source of variation	**df**	**MS**	**F-value**	**α = 0.05**
experiments (E)	1	48567.67	6567.54	significant (p < 0.0001)
replications (R) within E	1	3.43	0.14	not significant (p = 0.7088)
genotypes (G)	232	172.35	6.04	significant (p < 0.0001)
interaction G x E	232	28.52	1.16	not significant (p = 0.1277)
experimental error (ε)	232	24.55		

$MS_{GE} = s^2 + 1.333 * s^2_{ge}$

$MS_G = s^2 + 1.333 * s^2_{ge} + 2.667 * s^2_g$

h^2: 0.83 (using the simplified formula), 0.83 (using variance components)

The value of Spearman's rho between the two experiments was 0.73 (Table 62). In contrast to the population Isengrain/Capo ANOVA revealed no significant effects of the factor replication and there were no significant interactions between genotypes and experiments. The detailed results are given in Table 63. The estimated coefficient of broad sense heritability was for both calculation methods 0.83, thus clearly lower than of the population Isengrain/Capo.

For the population **Furore/Capo** the trait plant height measured in cm was assessed of all lines in five experiments in the same locations as for the population Isengrain/Capo. The detailed results of the parental lines and the population are given in Table 64, the corresponding frequency distributions showing continuous variation are illustrated in Fig. 25a-f.

Table 64 Plant height (cm): mean values of the parents Furore and Capo and the population Furore/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$) and number of assessed lines

experiment		Furore	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	n
Tulln/AT	2004	92.5	105.0	97.2 ± 5.4	85.0 – 110.0	5.4	201
Tulln/AT	2006	86.7	96.3	93.0 ± 4.8	80.0 – 105.0	6.4	201
Tulln/AT	2007	102.5	113.8	109.3 ± 5.0	97.5 – 120.0	8.6	201
Tulln/AT (M)	2007	92.5	100.0	96.8 ± 4.0	85.0 – 105.0	1 rep.	201
Tulln/AT	2008	86.9	91.9	89.3 ± 4.5	77.5 – 100.0	8.5	201
mean over 5 experiments		92.2	101.4	97.1 ± 3.8	87.5 – 106.0	3.3	201

h^2: 0.84

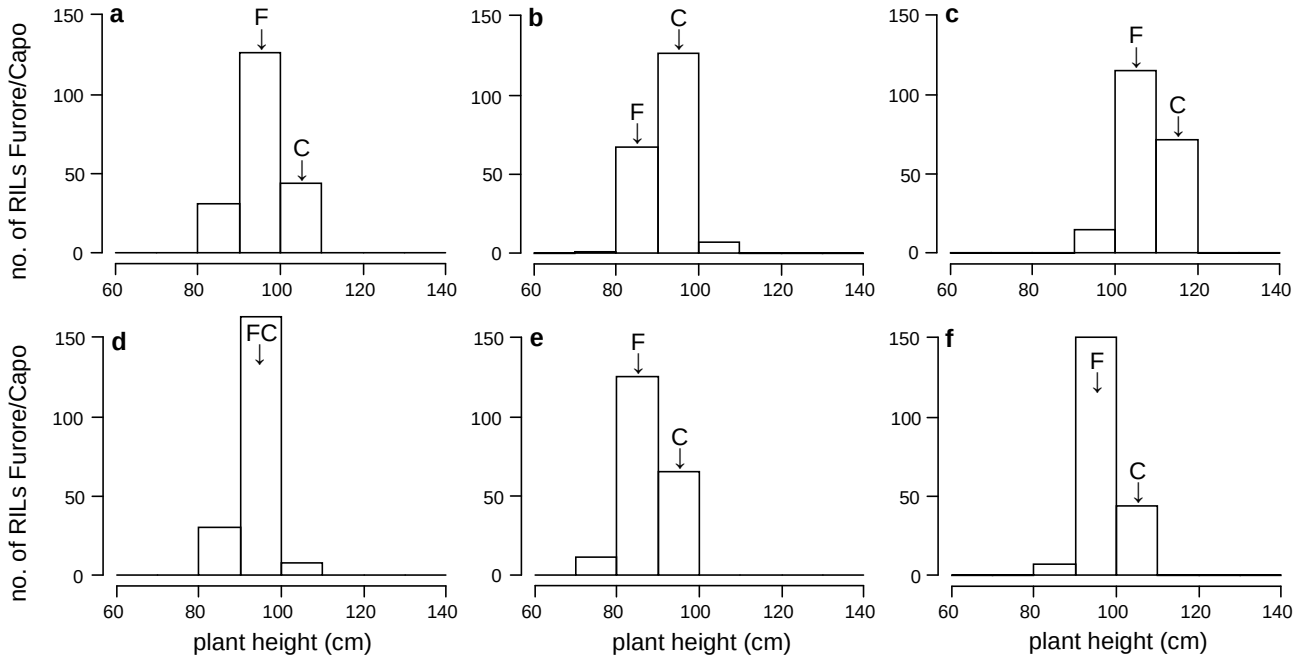

Fig. 25a-f Frequency distributions of plant height (cm) of the 201 RILs of the population Furore/Capo in the experiments 2004 Tulln (a), 2006 Tulln (b), 2007 Tulln (c), 2007 Tulln (M) (d), 2008 Tulln (e) and the mean over these five experiments (f). Values of the parental lines are indicated by arrows.

The values of Spearman's rho between these five experiments ranged from 0.46 (between the experiments Tulln 2004 and 2008) to 0.66 (Tulln 2004 and 2006), thus the same correlations being lowest and highest as for the population Isengrain/Capo. But in contrast, for the Furore/Capo no single coefficient was statistically significantly beyond 0.71.

Results of the ANOVA (presented in Table 66) were similar to the population Isengrain/Capo: All main factors and the interaction term had significant effects on plant height. The estimated coefficient of broad sense heritability was for both calculation methods 0.84, thus being clearly below the values of the population Isengrain/Capo, but slightly beyond those of Arina/Capo.

Table 65 Sample size (n) for Spearman's rho (r) between the five assessments of plant height (cm) of the population Furore/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		FT6	FT7	FM7	FT8
FT4	n	201	201	201	201
	r	0.66	0.60	0.54	0.46
	H_0	acc.	acc.	acc.	acc.
FT6	n		201	201	201
	r		0.58	0.62	0.47
	H_0		acc.	acc.	acc.
FT7	n			201	201
	r			0.56	0.48
	H_0			acc.	acc.
FM7	n				201
	r				0.49
	H_0				acc.

Table 66 Analysis of variance for plant height (cm) of the population Furore/Capo across five experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	4	22870.29	224.17	significant ($p < 0.0001$)
replications (R) within E	4	96.72	6.97	significant ($p < 0.0001$)
genotypes (G)	200	120.90	6.41	significant ($p < 0.0001$)
interaction G x E	800	19.18	1.38	significant ($p < 0.0001$)
experimental error (ε)	800	13.88		

$MS_{GE} = s^2 + 1.778 * s^2_{ge}$

$MS_G = s^2 + 1.667 * s^2_{ge} + 8.333 * s^2_g$

h^2: 0.84 (using the simplified formula), 0.84 (using variance components)

5.1.4 Other Traits

In addition to the traits leaf rust severity, heading and plant height, several other traits were assessed in some experiments. The detailed results of the parental lines and the populations are given in Table 67 (Isengrain/Capo), Table 68 (Arina/Capo) and Table 69 (Furore/Capo).

As Capo is an awned cultivar, whereas Arina awnless, the trait awnedness was assessed in the population Arina/Capo in five experiments. If some plants of a particular line were awned, whereas others were not, they were classified as "partly". No single line was classified "awned" in one and "awnless" in another experiment. In Fig. 26a-e bar charts illustrate the distributions of awned, partly awned and awnless lines in these five experiments and the mean over them.

Table 67 Mean values of the parents Isengrain and Capo and the population Isengrain/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$), heritability (h^2) and number of assessed lines for all other traits

trait experiment	Isengrain	Capo	pop. $\bar{x} \pm s$	pop. range	$LSD_{5\%}$	h^2	n
leaf blotch severity (1-9) Aumühle/AT 2004	8.4	5.1	6.1 ± 1.3	4.0 – 9.0	1 rep.		239
powdery mildew sev. (1-9) Probstdorf/AT 2006	3.3	3.3	3.1 ± 1.0	2.0 – 6.5	1.3		240
frost heaving severity (1-9) Probstdorf/AT 2006	4.3	2.8	3.7 ± 1.2	1.0 – 7.0	3.0		240
lodging severity (1-5) Probstdorf/AT 2007	1.0	2.5	1.8 ± 1.0	1.0 – 5.0	1.9		239
lodging severity (0-5) Probstdorf/AT 2008	0.5	3.1	1.9 ± 1.8	0.0 – 5.0	3.0		240
lodging severity (0-5) Rust/AT 2008	0.0	0.5	0.2 ± 0.5	0.0 – 2.5	1.5		240
lodging severity (0-5) Rust/AT 2008	0.0	1.0	0.5 ± 1.0	0.0 – 4.5	2.1		240
leaf chlorosis severity (0-9) Rust/AT 2009	0.0	0.5	1.1 ± 1.7	0.0 – 8.0	1.3		240
leaf chlorosis severity (0-9) Tulln/AT 2009	0.3	0.4	1.3 ± 1.6	0.0 – 7.0	1.8		240
leaf chlorosis severity (0-9) mean over 2 experiments	0.2	0.5	1.2 ± 1.6	0.0 – 7.5	1.1	0.93	240
leaf tip necrosis sev. (0-9) Rust/AT 2009	2.1	1.0	2.5 ± 1.7	0.0 – 8.0	1.7		240
leaf tip necrosis sev. (0-9) Tulln/AT 2009	2.0	0.5	2.6 ± 1.9	0.0 – 8.5	2.0		240
leaf tip necrosis sev. (0-9) mean over 2 experiments	2.1	0.8	2.6 ± 1.7	0.0 – 8.3	1.3	0.92	240

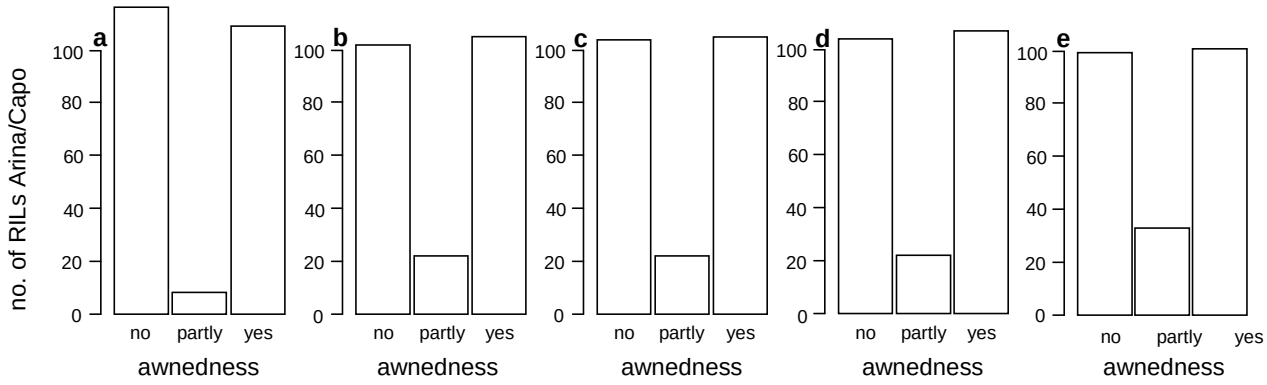

Fig. 26a-e Bar charts of the 233 RILs of the population Arina/Capo for awnedness in the experiments 2007 Tulln (a), 2008 Rust (b), 2008 Schmida (c), 2008 Tulln (d) and the mean over these four experiments (e). Capo: awned, Arina: awnless.

Table 68 Mean values of the parents Arina and Capo and the population Arina/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$), heritability (h^2) and number of assessed lines for all other traits

trait experiment	Arina	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	h^2	n
Septoria leaf blotch s. (1-9) Nyon/CH 2008	2.5	2.0	2.9 ± 0.4	2.0 – 4.5	1.0		229
powdery mildew sev. (1-9) Nyon/CH 2008	2.5	1.5	2.5 ± 0.7	1.5 – 5.0	1.6		229
yellow rust severity (1-9) Nyon/CH 2008	2.5	1.5	1.7 ± 0.9	1.0 – 5.0	1.1		229
glaucousness (1-5) Rust/AT 2009	3.6	1.0	2.5 ± 1.3	1.0 – 5.0	1.4		233
lodging severity (0-5) Probstdorf/AT 2008	0.9	0.5	1.2 ± 1.5	0.0 – 5.0	2.8		231
lodging severity (0-5) Rust/AT 2008	0.8	0.6	0.8 ± 1.2	0.0 – 4.5	2.7		231
leaf chlorosis severity (0-9) Rust/AT 2009	0.0	1.4	1.4 ± 1.9	0.0 – 8.5	1.2		233
leaf chlorosis severity (0-9) Tulln/AT 2009	0.0	0.8	1.0 ± 1.5	0.0 – 7.0	1.2		233
leaf chlorosis severity (0-9) mean over 2 experiments	0.0	1.1	1.2 ± 1.7	0.0 – 7.3	0.9	0.92	233
leaf tip necrosis sev. (0-9) Rust/AT 2009	1.3	1.1	2.2 ± 1.5	0.0 – 7.0	1.8		233
leaf tip necrosis sev. (0-9) Tulln/AT 2009	0.8	0.6	1.8 ± 1.6	0.0 – 7.5	1.6		233
leaf tip necrosis sev. (0-9) mean over 2 experiments	1.1	0.9	2.0 ± 1.5	0.0 – 6.8	1.1	0.91	233

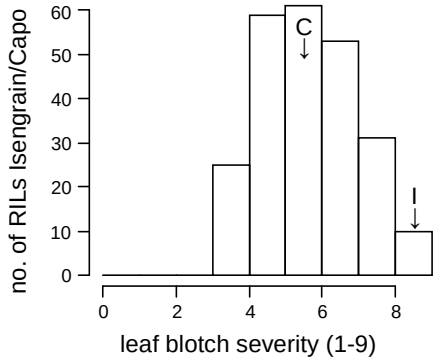

Fig. 27 Frequency distribution of leaf blotch severity (1-9) of the 239 RILs of the population Isengrain/Capo in the experiment 2004 Aumühle. Values of the parental lines are indicated by arrows.

Leaf blotch severity rated on a 1 to 9 scale was assessed only for the population Isengrain/Capo in the experiment Aumühle 2004. The frequency distribution showing continuous variation is illustrated in Fig. 27.

Table 69 Mean values of the parents Furore and Capo and the population Furore/Capo, population standard deviation, population range, least significant difference ($LSD_{5\%}$), heritability (h^2) and number of assessed lines for all other traits

trait experiment	Furore	Capo	pop. $\bar{x}$ ± s	pop. range	$LSD_{5\%}$	h^2	n
Septoria leaf blotch s. (1-9) Probstdorf/AT 2008	5.4	5.0	4.7 ± 0.5	3.5 – 6.0	1.4		201
powdery mildew sev. (1-9) Probstdorf/AT 2006	3.8	3.5	3.3 ± 0.7	2.0 – 5.0	1.5		201
powdery mildew sev. (1-9) Probstdorf/AT 2008	4.8	4.0	3.8 ± 0.6	2.5 – 5.5	1.6		201
powdery mildew sev. (1-9) mean over 2 experiments	4.3	3.8	3.6 ± 0.5	2.5 – 5.3	1.1	0.02	201
frost heaving severity (1-9) Probstdorf/AT 2006	2.8	3.0	3.0 ± 0.8	1.0 – 5.5	2.0		201
lodging severity (1-5) Probstdorf/AT 2007	1.2	4.3	2.4 ± 1.1	1.0 – 5.0	2.8		201
lodging severity (0-5) Probstdorf/AT 2008	0.5	1.3	1.0 ± 1.3	0.0 – 5.0	2.8		201
lodging severity (1-9) Probstdorf/AT 2008	1.0	1.0	2.7 ± 1.4	1.0 – 7.0	4.9		201

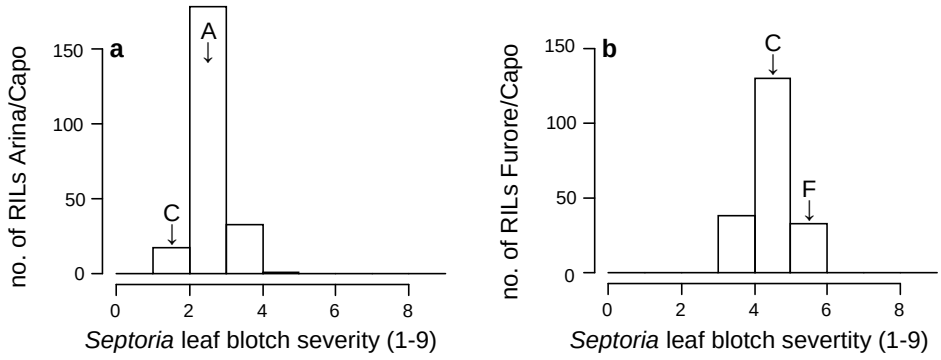

Fig. 28a-b Frequency distributions of *Septoria* leaf blotch severity (1-9) of the RIL populations. 229 lines Arina/Capo 2008 Nyon (a), 201 lines Furore/Capo 2008 Probstdorf (b). Values of the parental lines are indicated by arrows.

Infection with *Septoria* leaf blotch rated on a 1 to 9 scale was assessed for the population Arina/Capo in the experiment Nyon 2008 and for Furore/Capo in Probstdorf 2008. The frequency distributions showing continuous variation are illustrated in Fig. 28a-b. Infection was more severe in the latter experiment than in the first.

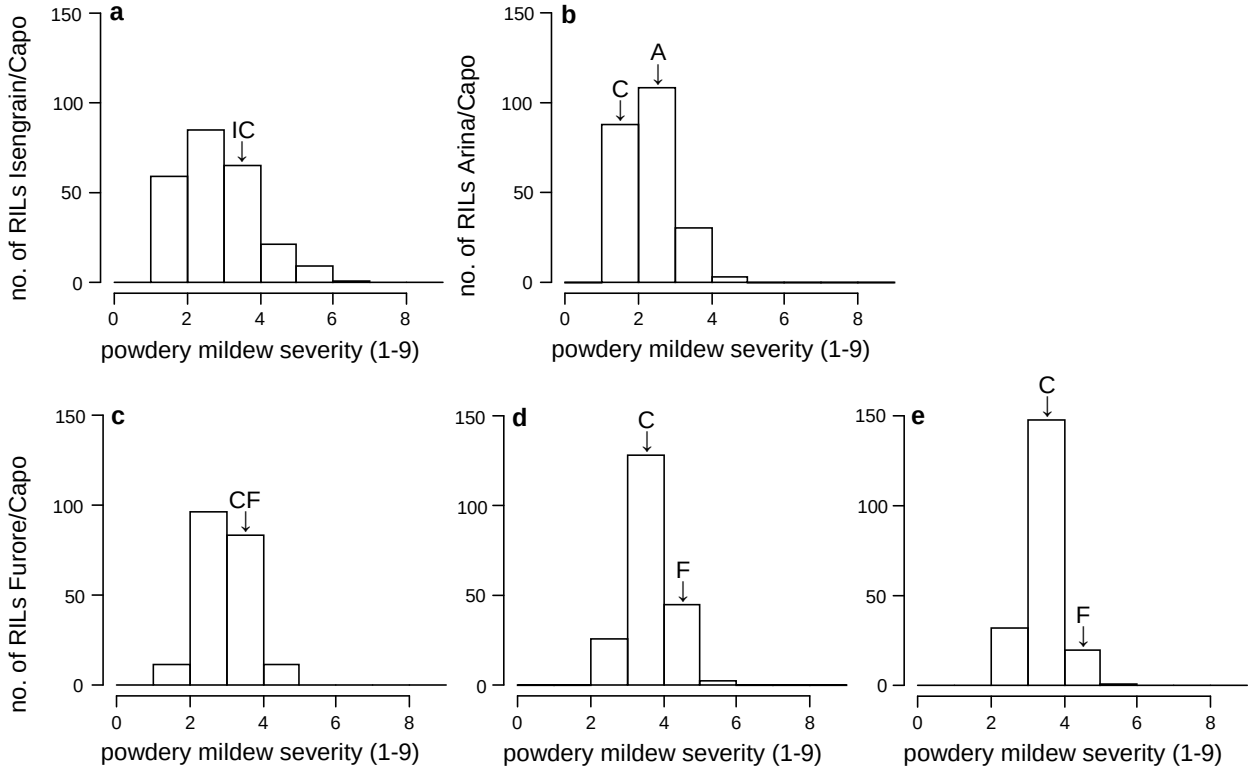

Fig. 29a-e Frequency distributions of powdery mildew severity (1-9) of the RIL populations. 240 lines Isengrain/Capo 2006 Probstdorf (a), 229 lines Arina/Capo 2008 Nyon (b); 201 lines Furore/Capo: 2006 Probstdorf (c), 2008 Probstdorf (d) and the mean over these two experiments (e). Values of the parental lines are indicated by arrows.

Table 70 Sample size (n) for Spearman's rho (r) between the two assessments of powdery mildew severity (1-9) of the population Furore/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		FP8
FP6	n	201
	r	0.02
	H_0	acc.

Table 71 Analysis of variance for powdery mildew severity (1-9) of the population Furore/Capo across two experiments

source of variation	**df**	**MS**	**F-value**	**α = 0.05**
experiments (E)	1	64.0908	24.55	significant (p = 0.0306)
replications (R) within E	2	2.4689	4.07	significant (p = 0.0178)
genotypes (G)	200	0.7613	1.02	not significant (p = 0.4516)
interaction G x E	200	0.7483	1.23	significant (p = 0.0403)
experimental error (ε)	400	0.6064		

$MS_{GE} = s^2 + 2 * s^2_{ge}$

$MS_G = s^2 + 2 * s^2_{ge} + 4 * s^2_g$

h^2: 0.02 (using the simplified formula), 0.02 (using variance components)

Powdery mildew severity was assessed on a 1 to 9 scale in the experiments Probstdorf 2006 (Isengrain/Capo and Furore/Capo), Nyon 2008 (Arina/Capo) and Probstdorf 2008 (Furore/Capo). The frequency distributions of the single experiments and the mean values over the Furore/Capo experiments showing continuous variation are illustrated in Fig. 29a-e. The value of Spearman's rho between the two experiments of the population Furore/Capo (Table 70) was very low. ANOVA (Table 71) revealed no significant effect of the factor genotypes on powdery mildew infection. As all lines were rated in both replications of these experiments, the coefficient of broad sense heritability (h^2) 0.02 was identical for both calculation methods. The difference between the two Furore/Capo experiments was larger than the difference between the two populations Isengrain/Capo and Furore/Capo in the experiment Probstdorf 2006.

Yellow rust severity rated on a 1 to 9 scale was assessed only for the population Arina/Capo in the experiment Nyon 2008. The frequency distribution showing continuous variation is illustrated in Fig. 30.

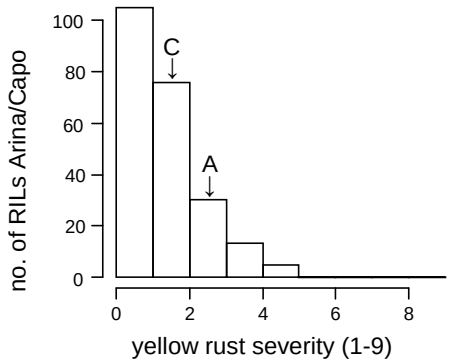

Fig. 30 Frequency distribution of yellow rust severity (1-9) of the 229 RILs of the population Arina/Capo in the experiment 2008 Nyon. Values of the parental lines are indicated by arrows.

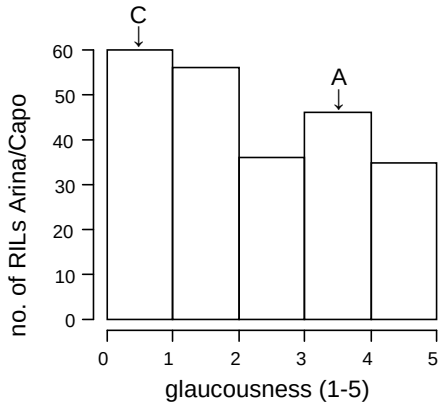

Fig. 31 Frequency distribution of glaucousness (1-5) of the 233 RILs of the population Arina/Capo in the experiment 2009 Rust. Values of the parental lines are indicated by arrows.

Glaucousness rated on a 1 to 5 scale was assessed only for the population Arina/Capo in the experiment Rust 2009. The frequency distribution showing continuous variation is illustrated in Fig. 31.

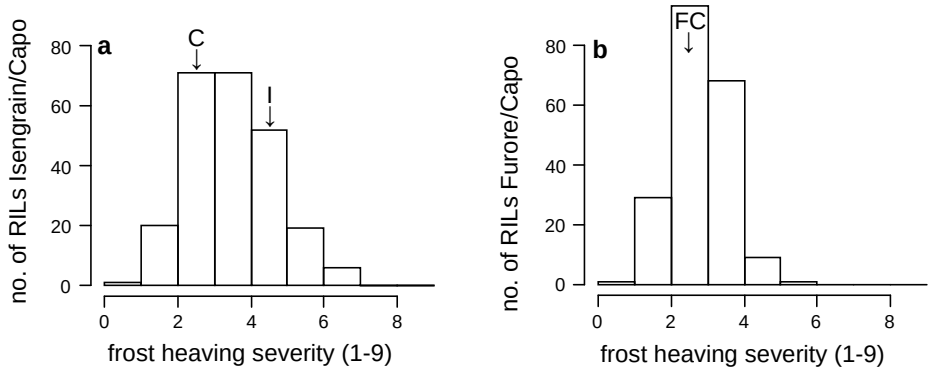

Fig. 32a-b Frequency distributions of frost heaving severity (1-9) of the RIL populations in the experiment 2006 Probstdorf: 240 lines Isengrain/Capo (a), 201 lines Furore/Capo (b). Values of the parental lines are indicated by arrows.

Frost heaving severity measured on a 1 to 9 scale was assessed in the experiment Probstdorf 2006. The frequency distribution of the population Isengrain/Capo is illustrated in Fig. 32a, of the population Furore/Capo in Fig. 32b. Both showed continuous variation. Furore and Capo were almost equally susceptible; Isengrain was clearly more prone to frost heaving. Thus, mean value, range and LSD were higher for the population Isengrain/Capo than for the population Furore/Capo.

Lodging severity rated on different scales was assessed in three experiments of the population Isengrain/Capo, two of Arina/Capo and three of Furore/Capo. In the experiment Rust 2008 lodging severity of the population Isengrain/Capo was assessed twice. Due to the different scales, results are hardly comparable. It seems that variability between experiments was equal to variability between different populations at the same trial site. The values of Spearman's rho ranged from 0.18 to 0.59 for the population Isengrain/Capo, from 0.04 to 0.39 for Furore/Capo and was 0.32 for Arina/Capo, none being significantly beyond 0.71. The detailed results are presented in Table 72, Table 73 and Table 74.

Table 72 Sample size (n) for Spearman's rho (r) between the four assessments of lodging severity (1-5 or 0-5) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IP8	IRu8_1	IRu8_2
IP7	n	239	239	239
	r	0.52	0.18	0.41
	H_0	acc.	acc.	acc.
IP8	n		240	240
	r		0.22	0.43
	H_0		acc.	acc.
IRu8_1	n			240
	r			0.59
	H_0			acc.

Table 73 Sample size (n) for Spearman's rho (r) between the two assessments of lodging severity (0-5) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		ARu8
AP8	n	231
	r	0.32
	H_0	acc.

Table 74 Sample size (n) for Spearman's rho (r) between the three assessments of lodging severity (1-5 or 0-5) of the population Furore/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		FP8_1	FP8_2
FP7	n	201	201
	r	0.39	0.05
	H_0	acc.	acc.
FP8_1	n		201
	r		0.04
	H_0		acc.

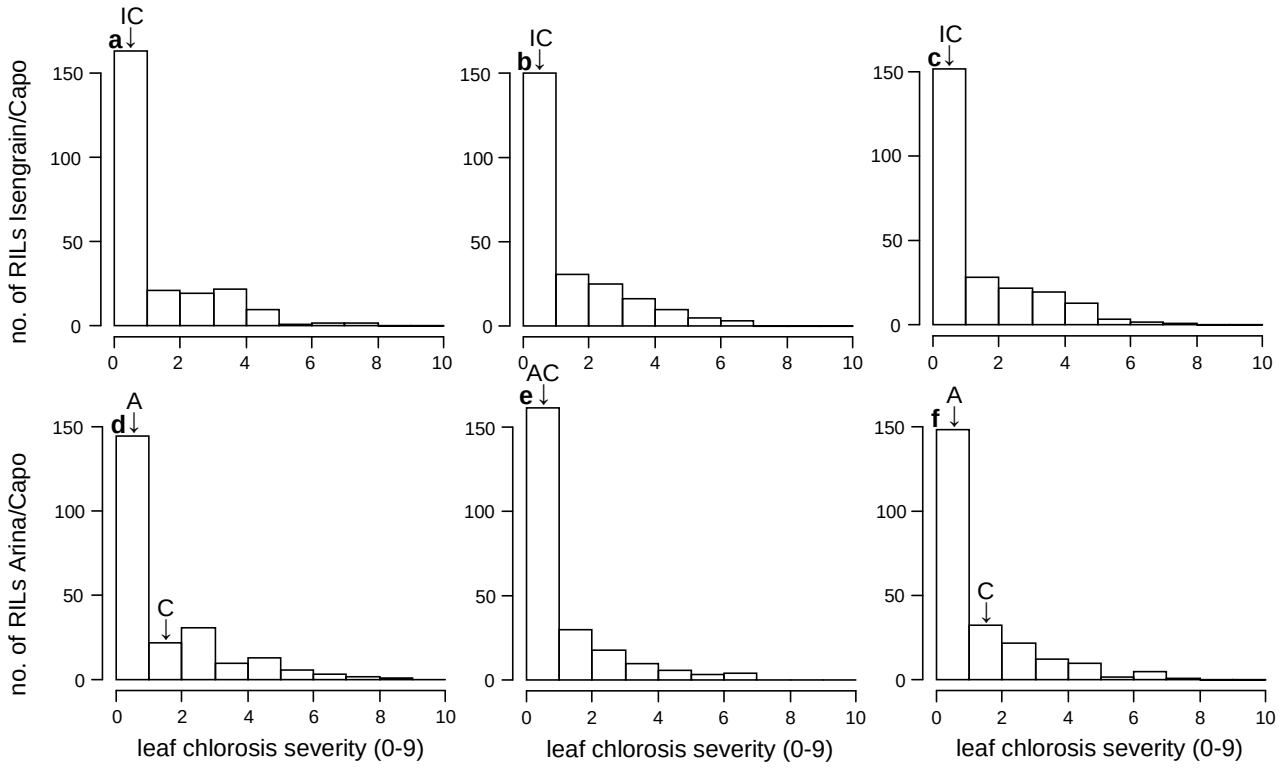

Fig. 33a-f Frequency distributions of leaf chlorosis severity (0-9) of the RIL populations. 240 lines Isengrain/Capo 2009 Rust (a), 2009 Tulln (b) and the mean over these two experiments (c); 233 lines Arina/Capo 2009 Rust (d), 2009 Tulln (e) and the mean over these two experiments (f). Values of the parental lines are indicated by arrows.

Symptoms of leaf chlorosis rated on a 0 to 9 scale were assessed in the experiments Rust and Tulln 2009 for the populations Isengrain/Capo and Arina/Capo. The frequency distributions of the single experiments and the mean over them showing continuous variation are illustrated in Fig. 33a-f. In the experiment Rust 2009 Isengrain and Arina showed no symptoms, in Tulln 2009 Isengrain was about as susceptible as Capo. Both populations showed similar variation. Due to the very slight differences there was no clear tendency which population was more or less susceptible and in which experiment symptoms were more severe. Capo was rated highest in the experiment Rust 2009 of the population Arina/Capo. The values of Spearman's rho of both populations were statistically significantly beyond 0.71. The detailed results are presented in Table 75 and Table 77. For both populations ANOVA revealed no significant effect of the experiments on leaf chlorosis severity. In the case of Isengrain/Capo there were no significant interactions either (Table 76 and Table 78). The values of the coefficient of broad sense heritability were 0.93 for Isengrain/Capo and 0.92 for Arina/Capo. Despite missing values in the first population, results of both populations were independent from the calculation method.

Table 75 Sample size (n) for Spearman's rho (r) between the two assessments of leaf chlorosis severity (0-9) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IT9
IRu9	n	240
	r	0.81
	H_0	rej.

Table 76 Analysis of variance for leaf chlorosis severity (0-9) of the population Isengrain/Capo across two experiments

source of variation	**df**	**MS**	**F-value**	**$\alpha = 0.05$**
experiments (E)	1	5.6916	0.82	not significant (p = 0.4583)
replications (R) within E	2	6.8354	11.15	significant (p < 0.0001)
genotypes (G)	239	10.2727	14.29	significant (p < 0.0001)
interaction G x E	239	0.7188	1.17	not significant (p = 0.0741)
experimental error (ε)	477	0.6128		

$MS_{GE} = s^2 + 1.997 * s^2_{ge}$

$MS_G = s^2 + 1.997 * s^2_{ge} + 3.994 * s^2_g$

h^2: 0.93 (using the simplified formula), 0.93 (using variance components)

Table 77 Sample size (n) for Spearman's rho (r) between the two assessments of leaf chlorosis severity (0-9) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		AT9
ARu9	n	233
	r	0.84
	H_0	rej.

Table 78 Analysis of variance for leaf chlorosis severity (0-9) of the population Arina/Capo across two experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	1	25.7779	7.37	not significant (p = 0.0806)
replications (R) within E	2	2.9882	8.18	significant (p = 0.0003)
genotypes (G)	232	10.9366	12.53	significant (p < 0.0001)
interaction G x E	232	0.8727	2.39	significant (p < 0.0001)
experimental error (ε)	464	0.3654		

$MS_{GE} = s^2 + 2 * s^2_{ge}$

$MS_G = s^2 + 2 * s^2_{ge} + 4 * s^2_g$

h^2: 0.92 (using the simplified formula), 0.92 (using variance components)

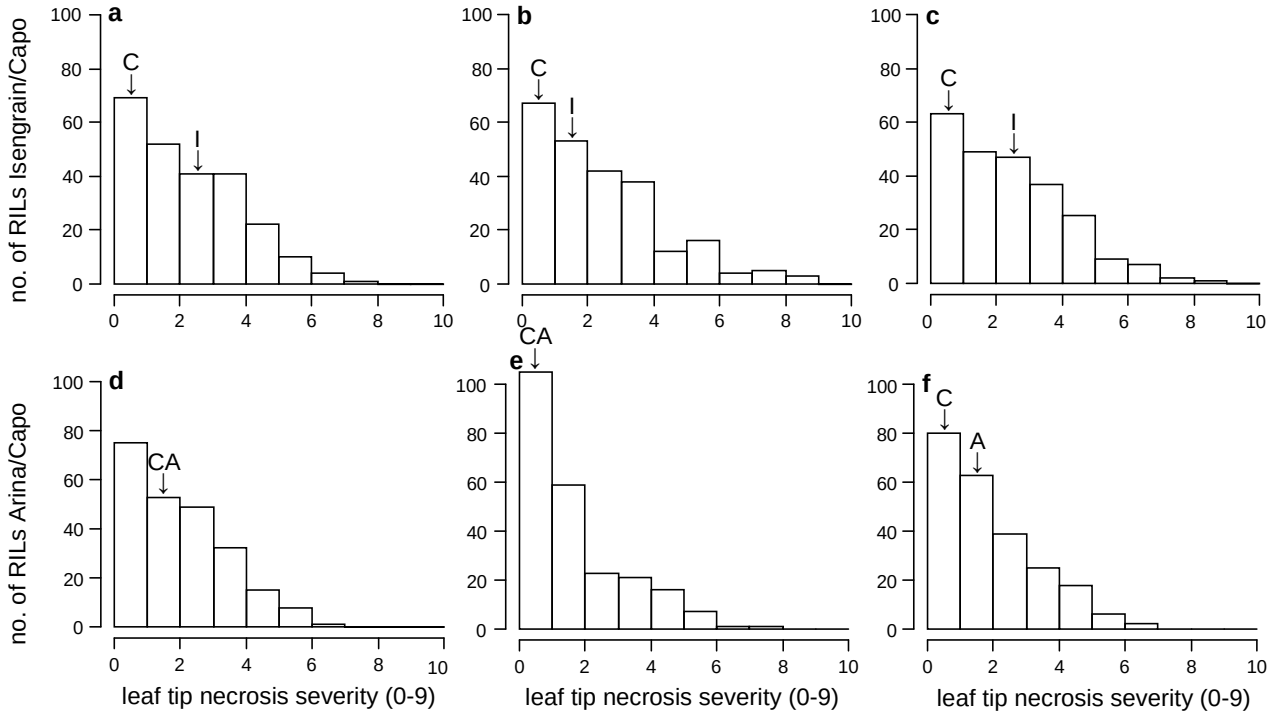

Fig. 34a-f Frequency distributions of leaf tip necrosis severity (0-9) of the RIL populations. 240 lines Isengrain/Capo 2009 Rust (a), 2009 Tulln (b) and the mean over these two experiments (c); 233 lines Arina/Capo 2009 Rust (d), 2009 Tulln (e) and the mean over these two experiments (f). Values of the parental lines are indicated by arrows.

Leaf tip necrosis severity rated on a 0 to 9 scale was assessed in the same experiments as leaf chlorosis severity. The frequency distributions of the single experiments and the mean over them showing continuous variation are illustrated in Fig. 34a-f. Arina showed more severe leaf tip necrosis than Capo, but clearly less than Isengrain. Thus, mean value, standard deviation and maximum value of the population Isengrain/Capo was higher than of the population Arina/Capo. For both populations differences between the two experiments were low. The values of Spearman's rho of both populations were about the same as for leaf chlorosis severity and statistically significantly beyond 0.71. The detailed results are presented in Table 79 and Table 81. For both populations ANOVA

revealed no significant effect of the experiments on leaf tip necrosis severity and no significant interactions (Table 80 and Table 82). The values of the coefficient of broad sense heritability were 0.92 for Isengrain/Capo and 0.91 for Arina/Capo, thus being a little bit lower than for leaf chlorosis severity.

Table 79 Sample size (n) for Spearman's rho (r) between the two assessments of leaf tip necrosis severity (0-9) of the population Isengrain/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		IT9
IRu9	n	240
	r	0.86
	H_0	rej.

Table 80 Analysis of variance for leaf tip necrosis severity (0-9) of the population Isengrain/Capo across two experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	1	7.6722	2.05	not significant (p = 0.2805)
replications (R) within E	2	3.6092	4.16	significant (p = 0.0161)
genotypes (G)	239	12.1634	12.24	significant (p < 0.0001)
interaction G x E	239	0.9939	1.15	not significant (p = 0.1080)
experimental error (ε)	476	0.8672		

$MS_{GE} = s^2 + 1.994 * s^2_{ge}$

$MS_G = s^2 + 1.994 * s^2_{ge} + 3.989 * s^2_g$

h^2: 0.92 (using the simplified formula), 0.92 (using variance components)

Table 81 Sample size (n) for Spearman's rho (r) between the two assessments of leaf tip necrosis severity (0-9) of the population Arina/Capo. Additionally acceptance (acc.) or rejection (rej.) of the H_0: $0 < \rho \leq 0.71$ is given. $\alpha = \beta = 0.05$

		AT9
ARu9	n	233
	r	0.81
	H_0	rej.

Table 82 Analysis of variance for leaf tip necrosis severity (0-9) of the population Arina/Capo across two experiments

source of variation	df	MS	F-value	$\alpha = 0.05$
experiments (E)	1	33.0940	4.88	not significant (p = 0.1578)
replications (R) within E	2	6.7840	8.65	significant (p = 0.0002)
genotypes (G)	232	8.8519	11.26	significant (p < 0.0001)
interaction G x E	232	0.7859	1.00	not significant (p = 0.4885)
experimental error (ε)	462	0.7845		

$MS_{GE} = s^2 + 1.994 * s^2_{ge}$

$MS_G = s^2 + 1.994 * s^2_{ge} + 3.989 * s^2_g$

h^2: 0.91 (using the simplified formula), 0.91 (using variance components)

5.1.5 Trait Correlations

Pearson's product moment coefficient and/or Spearman's rho were calculated and tested to find possible correlations between leaf rust severity, heading, plant height and other proportionally or ordinally scaled traits. If possible, these tests were not only done for a particular experiment but also for the mean over experiments. All results (number of lines n, correlation coefficient r and whether the null hypothesis H_0: $0 < |\rho| \leq 0.59$ was accepted or rejected are given in Table 9 – 47 in Appendix C.

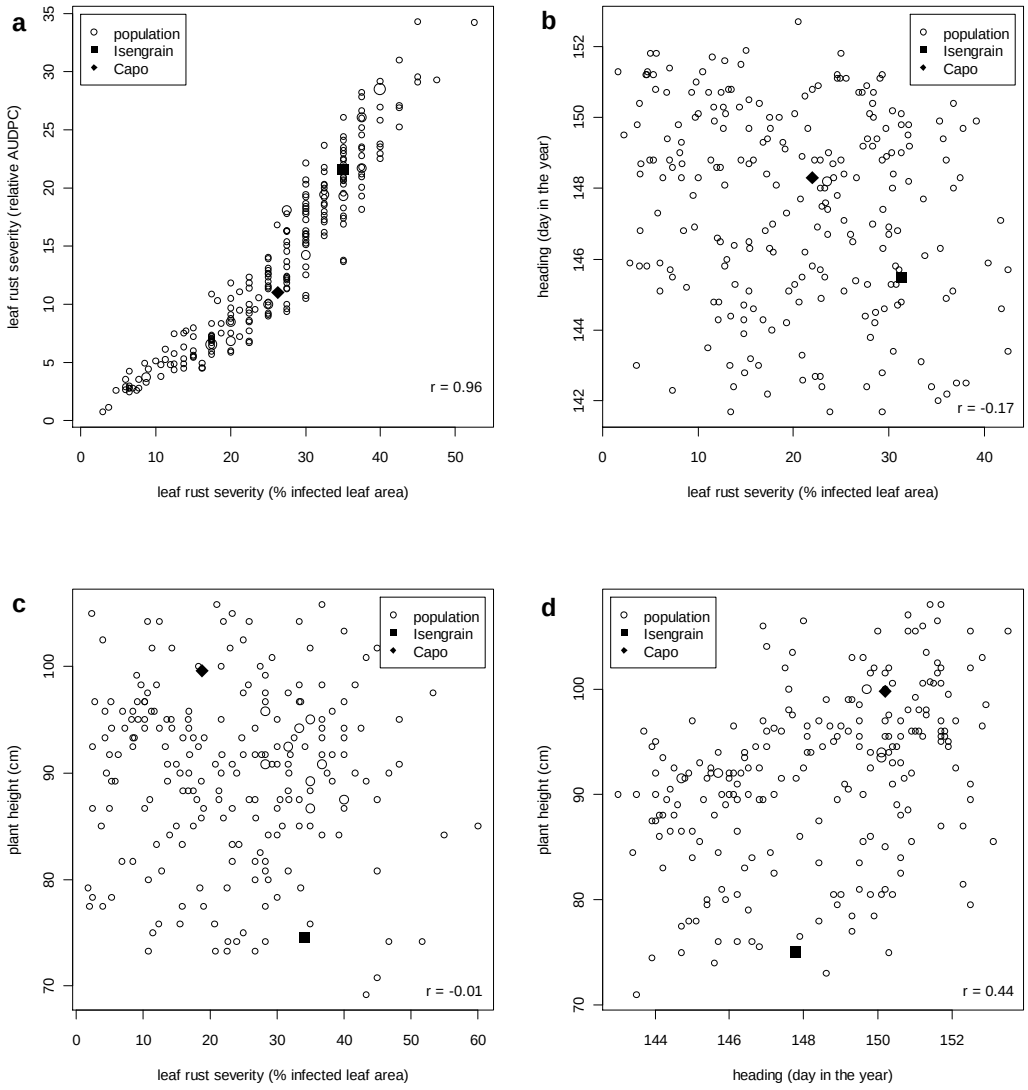

Fig. 35a-d Scatterplots of the traits (a) leaf rust severity measured by the percentage of infected leaf area versus rel. AUDPC (mean over two experiments), (b) leaf rust severity versus heading (mean over six experiments), (c) leaf rust severity versus plant height (mean over three experiments) and (d) heading versus plant height (mean over five experiments) for the RIL population Isengrain/Capo. The positions of the parental lines are indicated. The diameters of the circles are directly proportional to the number of lines with the same scores.

In none of the three populations Isengrain/Capo, Arina/Capo or Furore/Capo a relevant correlation ($r^2 > 0.35$ or $r > |0.59|$) between different traits was detected. For an example, the scatterplots of the population Isengrain/Capo and the parental lines for the trait leaf rust severity versus heading and plant height are shown in Fig. 35b-d. The value of Spearman's rho is also given there. Means were calculated over those experiments in which both traits were assessed. The diameters of the circles are directly proportional to the number of observations.

The only exceptions of correlations with coefficients $r > |0.59|$ were detected between the repeated assessments of leaf rust severity (measured by the percentage of infected leaf area and the relative AUDPC). Exemplarily the scatterplots of the population Isengrain/ Capo of the experiment Tulln 2008 are presented in Fig. 37a-f. In Fig. 35a the scatterplot of leaf rust severity measured by the percentage of infected leaf area versus relative AUDPC for the mean over the experiments Piešťany 2006 and Tulln 2008 is shown.

Interdependencies between the traits appearance of teliospores and awnedness were analyzed by means of contingency tables and chi-squared tests of independence. The appearance of teliospores was significantly correlated with the leaf area exhibiting symptoms of leaf rust. In the population Isengrain/Capo as well as Furore/Capo the proportion of plants with teliospores increased with leaf rust severity. The bar charts are presented in Fig. 36. Correlations with the traits heading and plant height were not significant, though lines with later heading dates were less likely to have teliospores. Values of Cramér's V are given in Table 83.

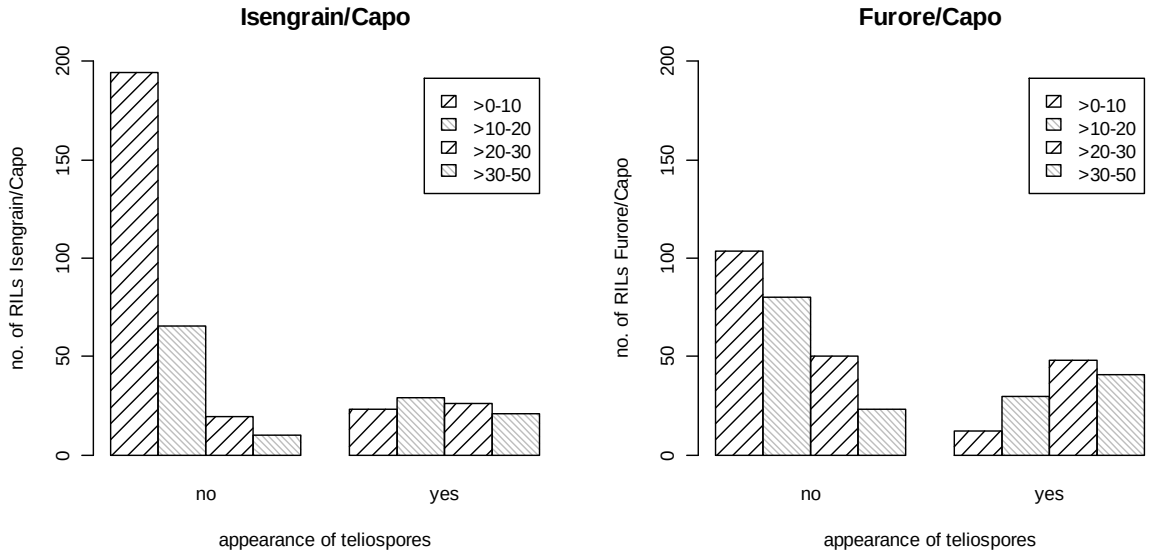

Fig. 36 Bar charts of the RIL populations Isengrain/Capo (left) and Furore/Capo (right) for the different classes of leaf rust severity comparing the plants without and with teliospores

Table 83 Cramér's V for the correlation of the appearance of teliospores and the other traits assessed in the experiments in Tulln 2004

population	heading	plant height	leaf rust severity
Isengrain/Capo	0.09	0.10	0.45
Furore/Capo	0.10	0.10	0.42

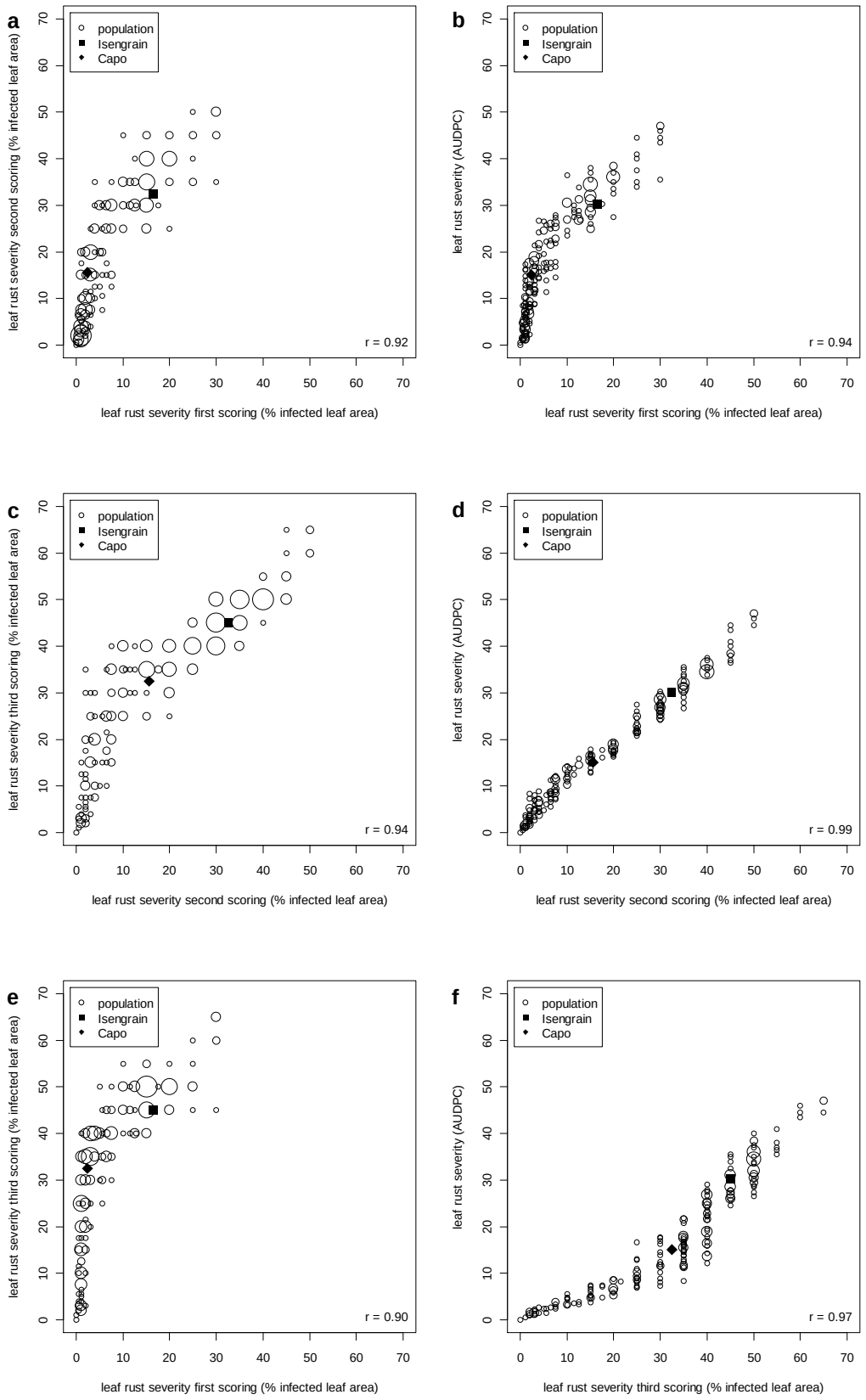

Fig. 37a-f Scatterplots depicting the correlation of the three single assessments of the trait leaf rust severity in 2008 Tulln and the resulting relative AUDPC for the RIL population Isengrain/ Capo. The positions of the parental lines are indicated. The diameters of the circles are directly proportional to the number of lines with the same scores.

Table 84 Cramér's V for the correlation of awnedness and the other traits in the population Arina/Capo assessed in four experiments and for mean values

experiment	heading	plant height	leaf rust sev.	lodging sev.	AUDPC
Tulln 2007	0.10	0.11	0.10		
Rust 2008	0.01		0.17	0.15	
Schmida 2008	0.15		0.14		
Tulln 2008	0.08	0.15	0.07		0.08
mean	0.08	0.13	0.10		

No significant correlation was detected between awnedness and any other trait in the population Arina/Capo. The values of Cramér's V are presented in Table 84.

5.2 Genetic Map Population Isengrain/Capo

5.2.1 Microsatellite (SSR) Markers

290 SSR primer pairs were analyzed on the two parental lines Isengrain and Capo. Exactly half of them revealed clearly polymorphic bands, 91 (31 %) produced solely monomorphic bands and for 10 % no fragments were detectable. Bands of the remaining 25 primer pairs (9 %) were either very difficult to score because of small fragment size differences or might be dominant.

129 SSR primer pairs were applied to the population Isengrain/Capo, generating 165 different polymorphic marker bands. As an example the polymorphism pattern of the SSR primer *Barc340* is given in Fig. 9 on page 48. The primer pair producing maximum polymorphic bands was *Wmc500* with five distinct markers that were assigned to four different chromosomes of the B-genome. 17 markers were excluded from map calculation.

5.2.2 Amplified Fragment Length Polymorphism (AFLP) Markers

30 AFLP primer combinations were applied to the population Isengrain/Capo. Out of the 673 generated polymorphic bands, 39 were excluded from mapping (15 of them being monomorphic for the parents) and 98 polymorphisms were not be scorable at all. Therefore some numbered AFLP loci are missing in the map.

The list of the used selective nucleotides, the name according to the list by KeyGene (2010), the number of detected polymorphic fragments and the number of mapped loci are given in Table 85. As an example the polymorphism pattern of the AFLP primer combination S11M23 is given in Fig. 10 on page 49.

Table 85 AFLP primer combinations applied

sel. nucleotides *Sse8387I-Mse*I	name	polym. fragments	mapped loci	sel. nucleotides *Sse8387I-Mse*I	name	polym. fragments	mapped loci
AA-AT	S11M14	23	18	GA-TA	S19M23	29	20
AA-CG	S11M17	15	12	GC-AA	S20M11	14	12
AA-CT	S11M18	10	7	GC-AT	S20M14	15	4
AA-TA	S11M23	29	25	GC-TA	S20M23	42	24
AA-TT	S11M26	7	6	GT-AG	S22M13	27	24
AC-AG	S12M13	38	28	GT-CG	S22M17	28	25
AC-CT	S12M18	23	17	GT-GC	S22M20	11	8
AG-GC	S13M20	13	11	TA-AG	S23M13	36	27
AT-AC	S14M12	28	15	TA-AT	S23M14	41	24
AT-CT	S14M18	26	15	TA-CA	S23M15	30	23
CC-CC	S16M16	23	19	TA-CG	S23M17	20	15
CG-CT	S17M18	12	12	TC-GA	S24M19	3	2
CT-AT	S18M14	14	12	TT-AT	S26M14	28	19
CT-GC	S18M20	15	8	TT-CT	S26M18	27	16
GA-CG	S19M17	14	9	TT-GG	S26M21	32	25

5.2.3 Linkage Map

In total data of 684 molecular markers were used for map construction in the population Isengrain/Capo. 83 % of these markers segregated according to the Mendelian ratio ($\alpha = 0.05$). 619 markers were mapped into 49 linkage groups covering a total genetic distance of 2,150 cM: 137 SSR markers (34 BARC, 86 GWM and 17 WMC) and 482 AFLP markers with an average marker distance of 3.5 cM.

33 linkage groups were unambiguously assigned to a chromosome based on the SSR markers with known chromosomal position, covering a total genetic distance of 1,873 cM. To all 21 chromosomes of the hexaploid wheat genome at least one linkage group was assigned. The obtained genetic linkage maps of the population Isengrain x Capo are presented in Fig. 38 to Fig. 58 with their short arm at the top. On the left-hand side the genetic distance in cM is indicated, on the right-hand side the corresponding marker used for map construction (AFLP – black, SSR in color: Xbarc – blue, Xgwm – green, Xwmc – red). On the very left the wheat consensus reference map of Somers et al. (2004) is shown, in the right part linkage groups detected in the population Arina x Capo and the Arina x Forno reference map of Paillard et al. (2003). In the reference maps only SSR markers assigned to comparable linkage groups in at least one mapping population are highlighted in the same colors and connected by a straight line. All distances are cM (Haldane) with the exception of the wheat consensus map (Kosambi).

Markers clustered at certain chromosomal regions and were not evenly distributed across the whole genome. Therefore, marker coverage varied between the A, B and D genome and within as well as between chromosomes: 146 loci were mapped to the A genome (covering 652 cM), 302 to the B genome (796 cM) and 78 to the D genome (405 cM).

Eight linkage groups (covering 189 cM) that were not definitely assigned to a certain chromosome are shown in Fig. 59. Further eight linkage groups (covering 108 cM) comprised solely of AFLP markers (presented in Fig. 60), 11 SSR and 54 AFLP markers remained yet unlinked.

5.3 Genetic Map Population Arina/Capo

32 SSR primer pairs were applied to the population Arina/Capo, generating 36 different polymorphic marker bands, out of which 22 were mapped to nine linkage groups. Comparing the Capo alleles with those detected in the population Isengrain/Capo, all linkage groups were assigned to seven different chromosomes, covering a total genetic distance of 140 cM. All linkage groups and single markers that matched to loci mapped in the population Isengrain/Capo by comparing Capo alleles are presented in Fig. 38 to Fig. 58 in comparison to the Isengrain/Capo map and two reference maps as described in 5.2.3 Linkage Map.

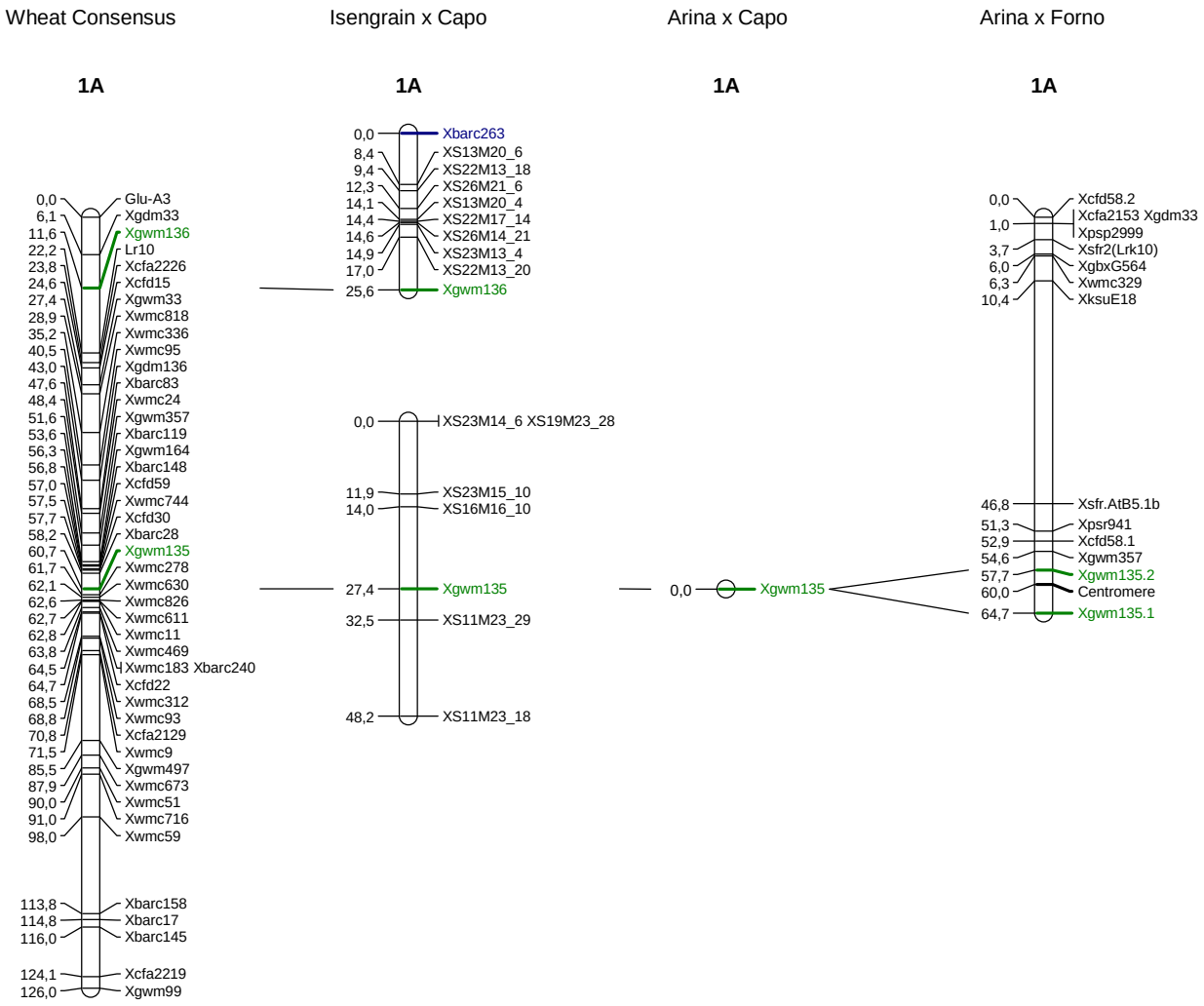

Fig. 38 Linkage groups assigned to chromosome 1A

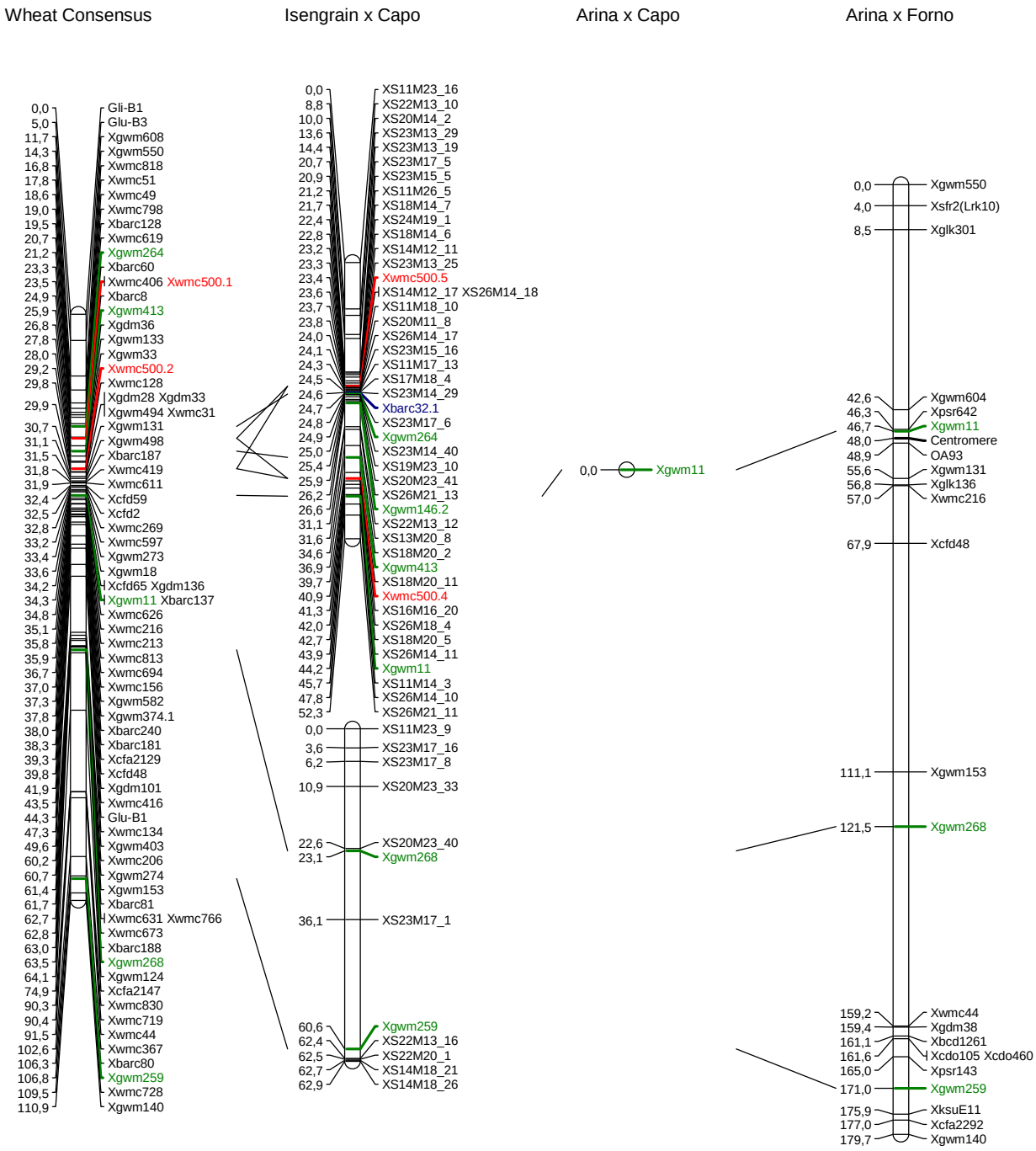

Fig. 39 Linkage groups assigned to chromosome 1B

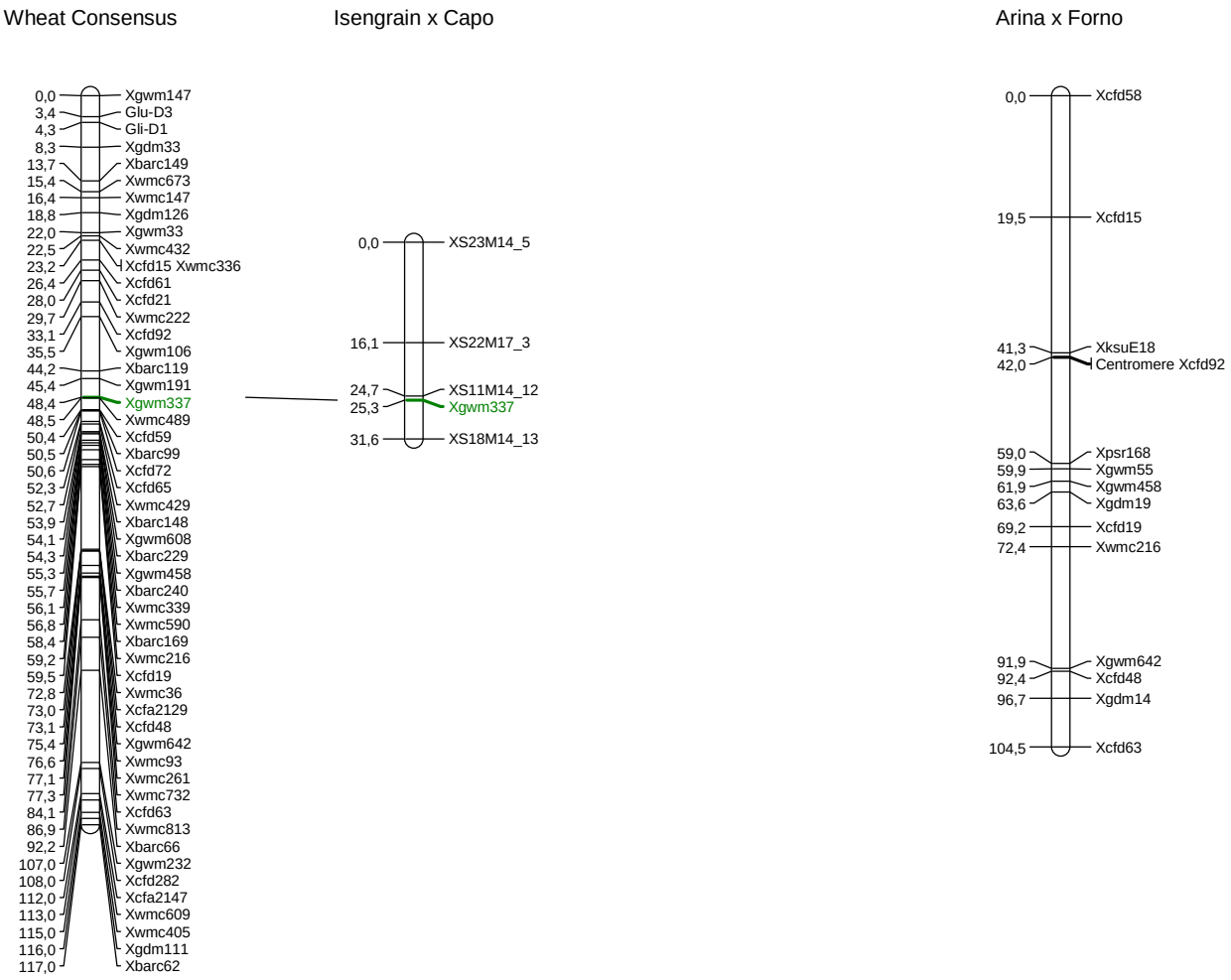

Fig. 40 Linkage groups assigned to chromosome 1D

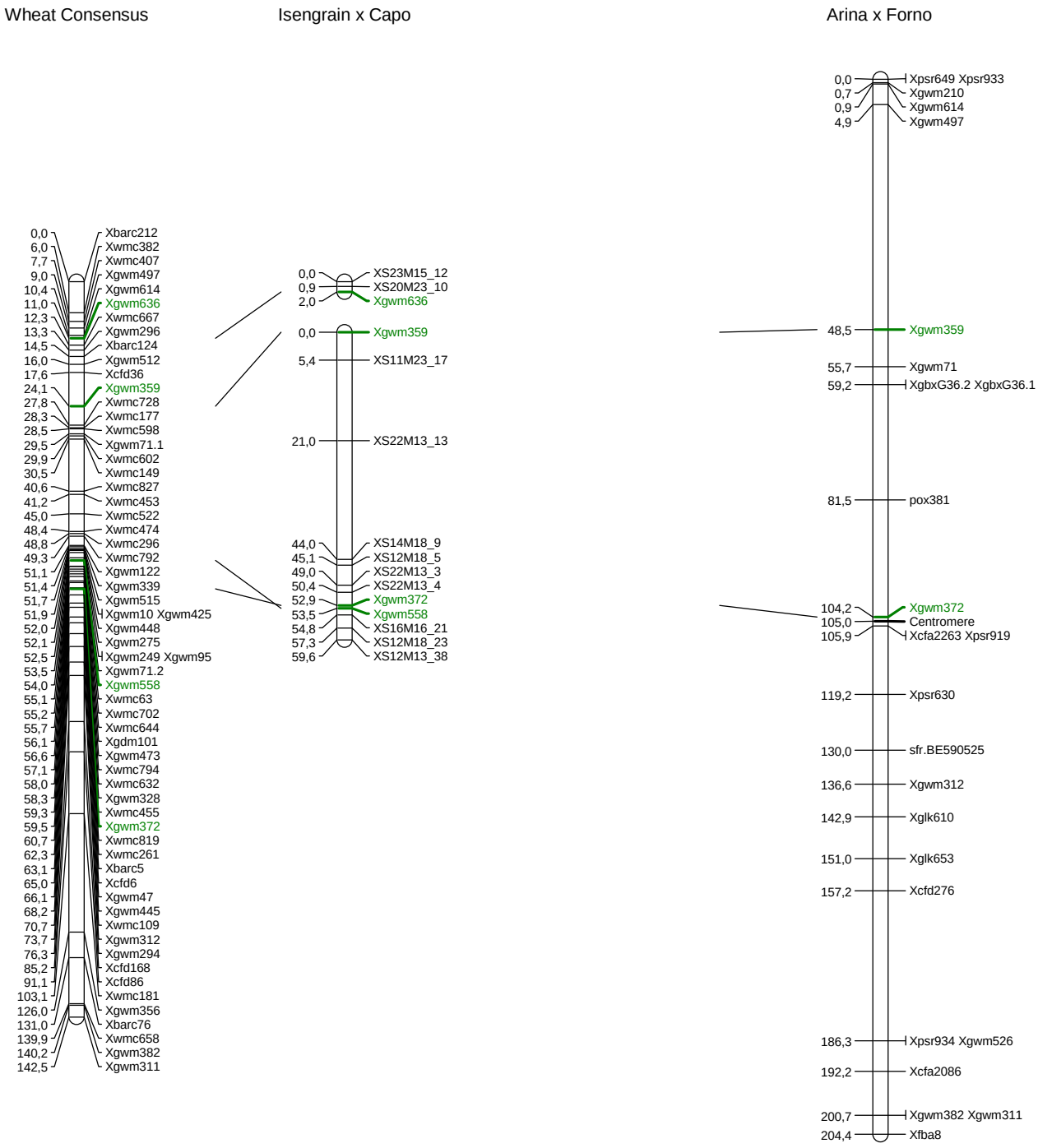

Fig. 41 Linkage groups assigned to chromosome 2A

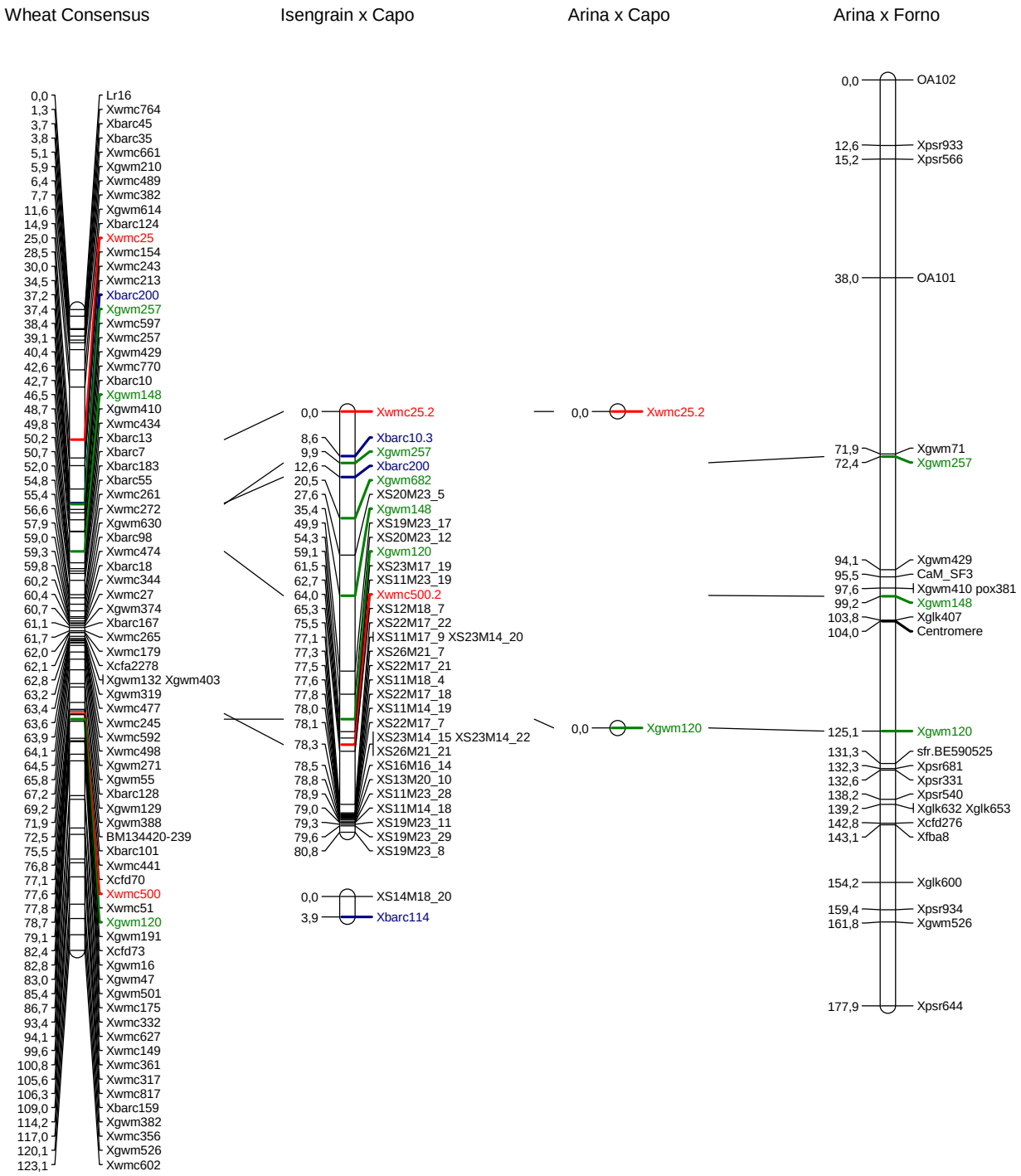

Fig. 42 Linkage groups assigned to chromosome 2B

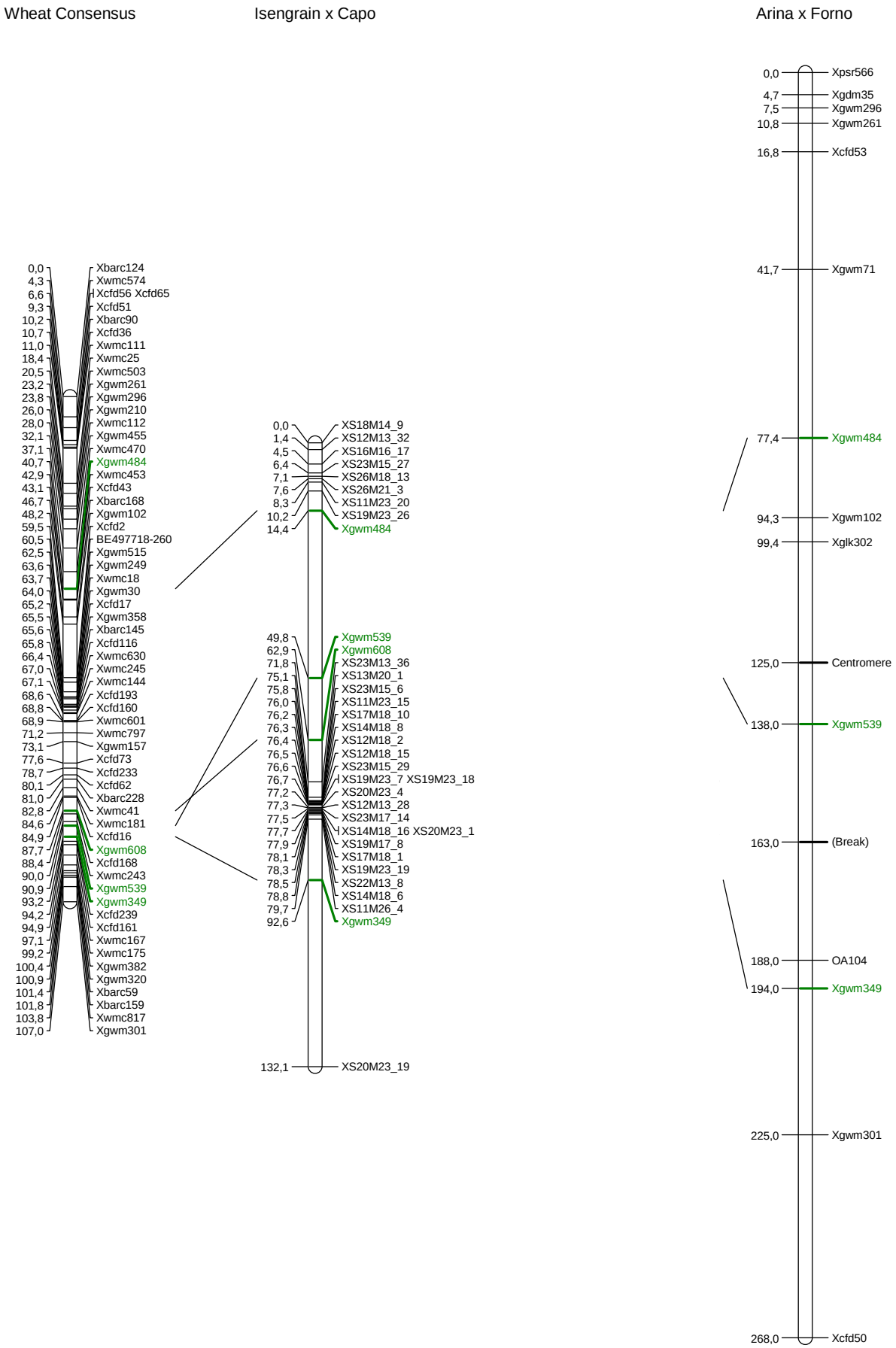

Fig. 43 Linkage groups assigned to chromosome 2D

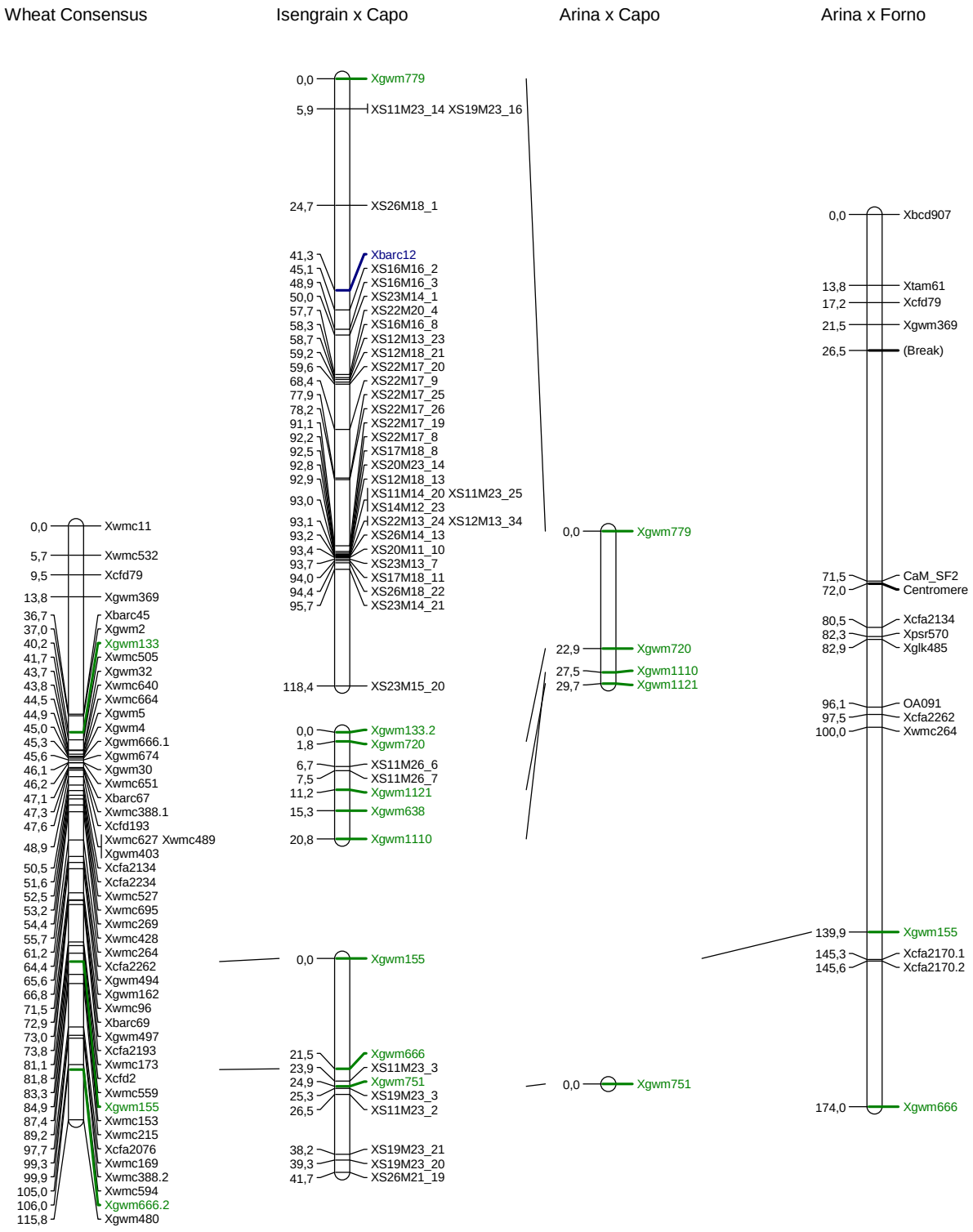

Fig. 44 Linkage groups assigned to chromosome 3A

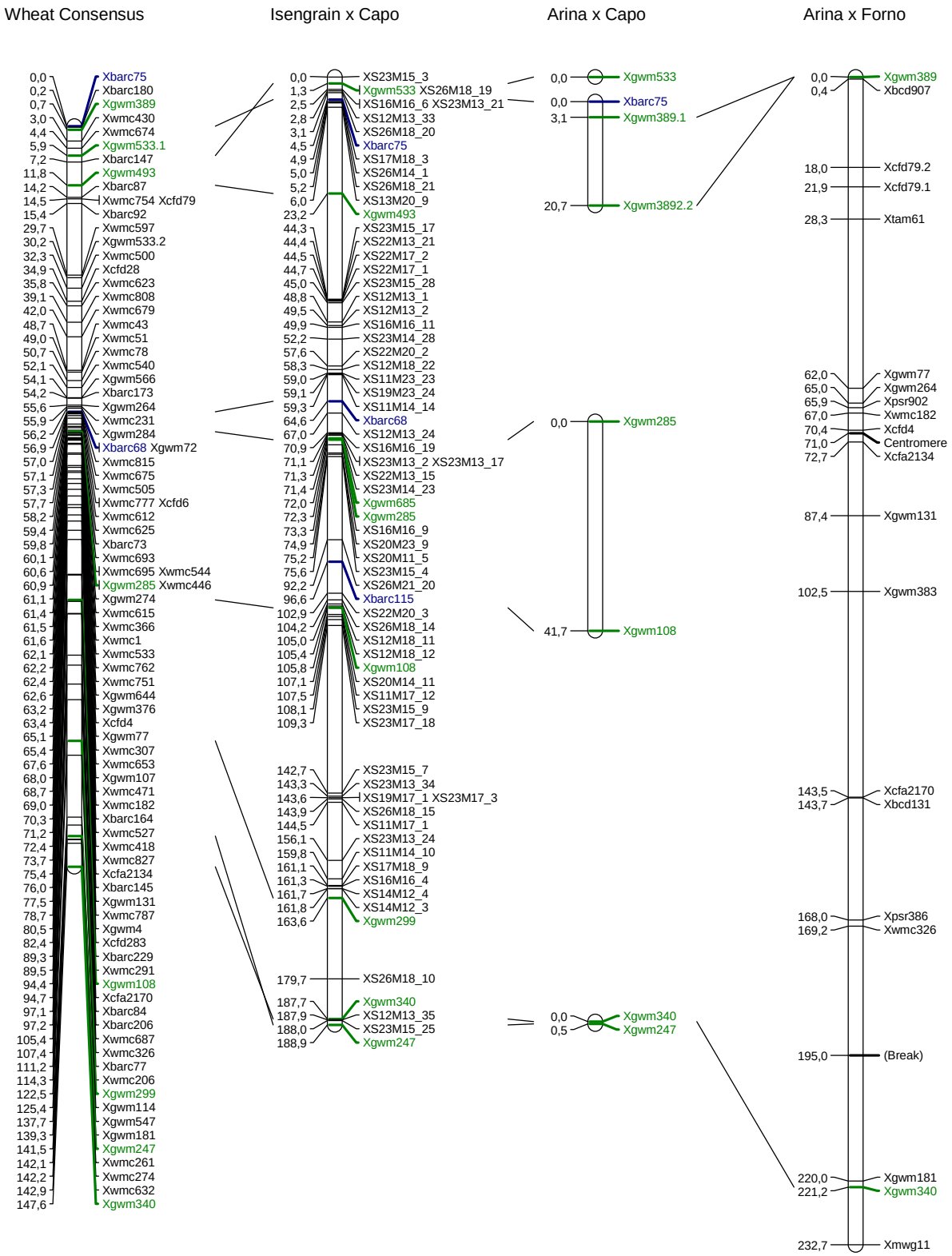

Fig. 45 Linkage groups assigned to chromosome 3B

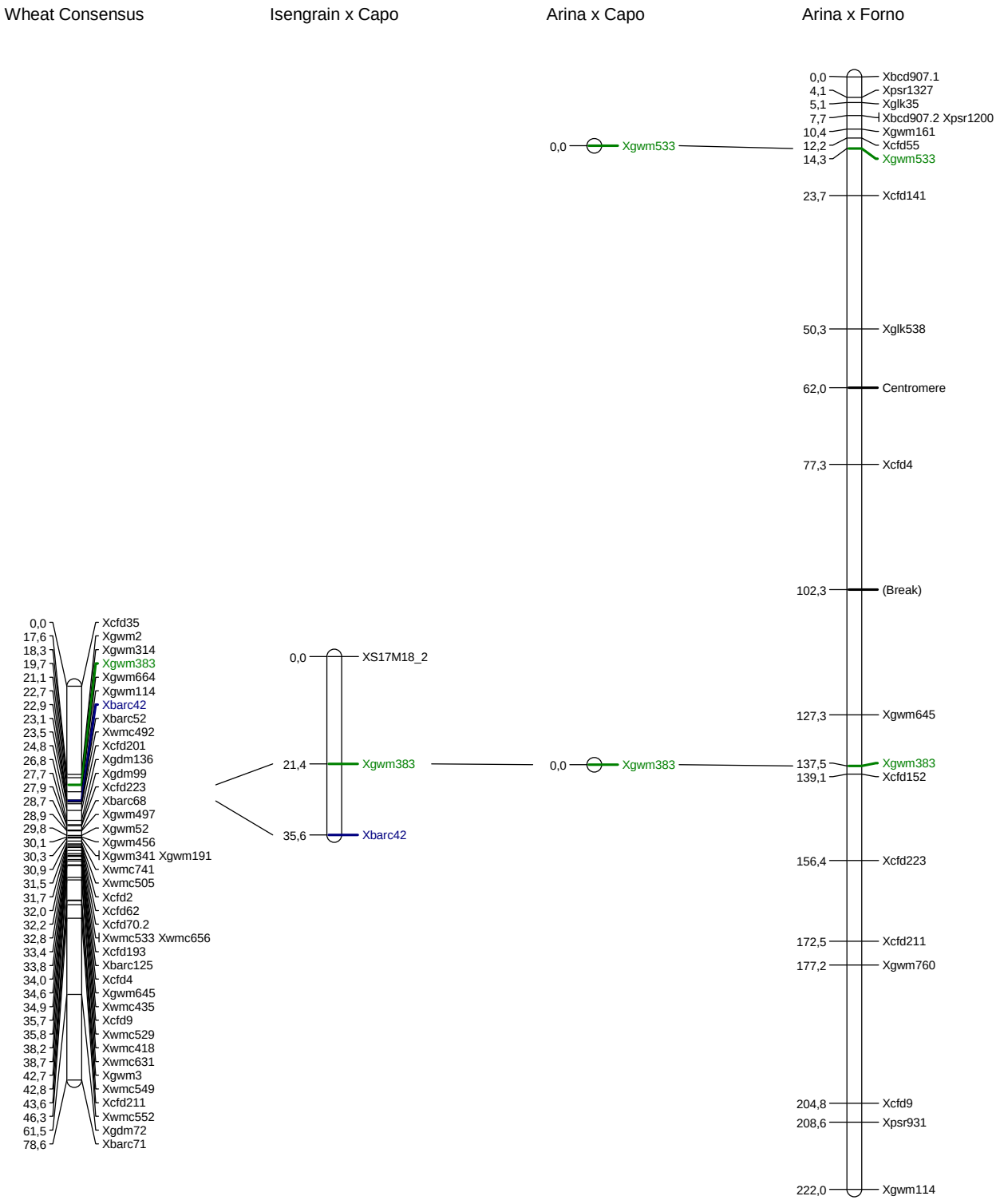

Fig. 46 Linkage groups assigned to chromosome 3D

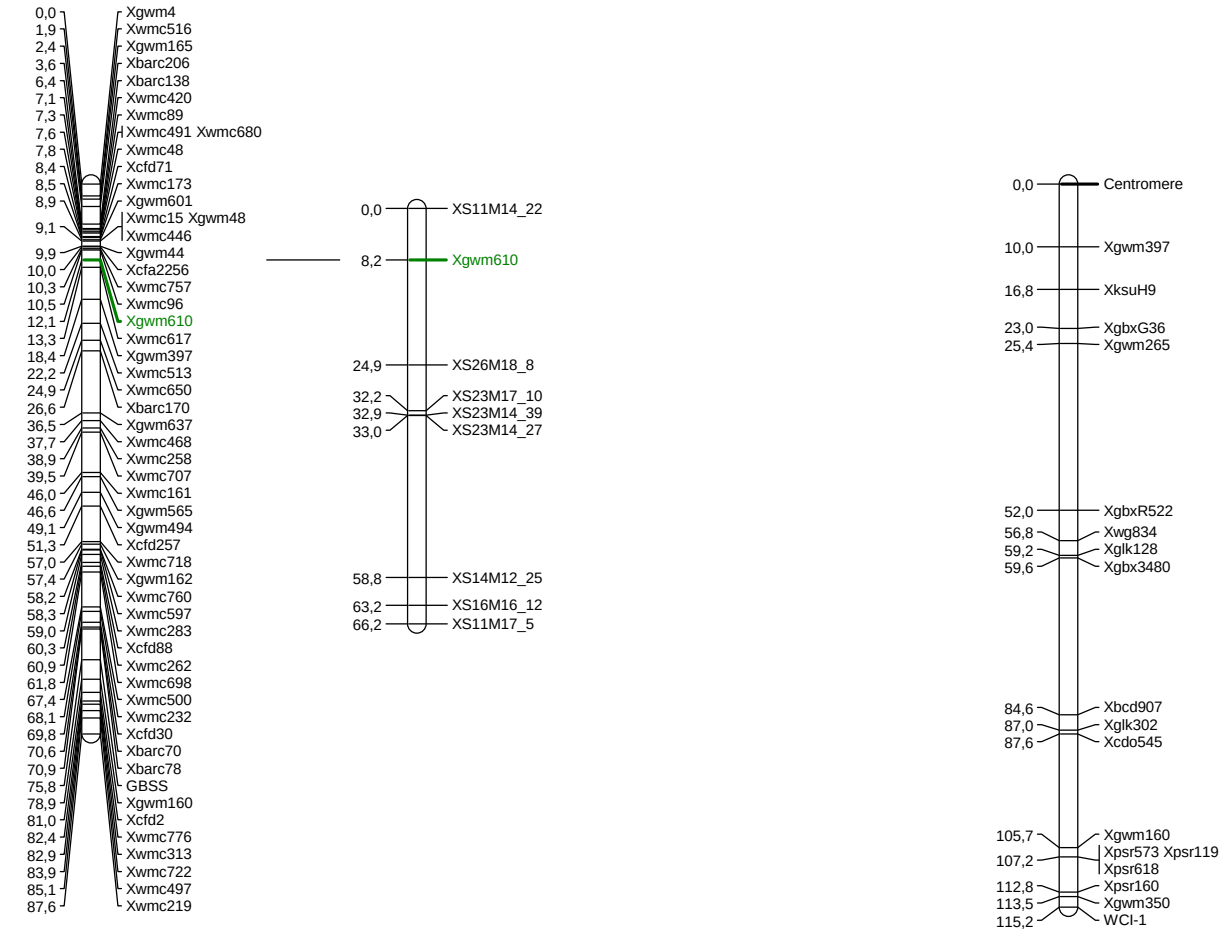

Fig. 47 Linkage groups assigned to chromosome 4A

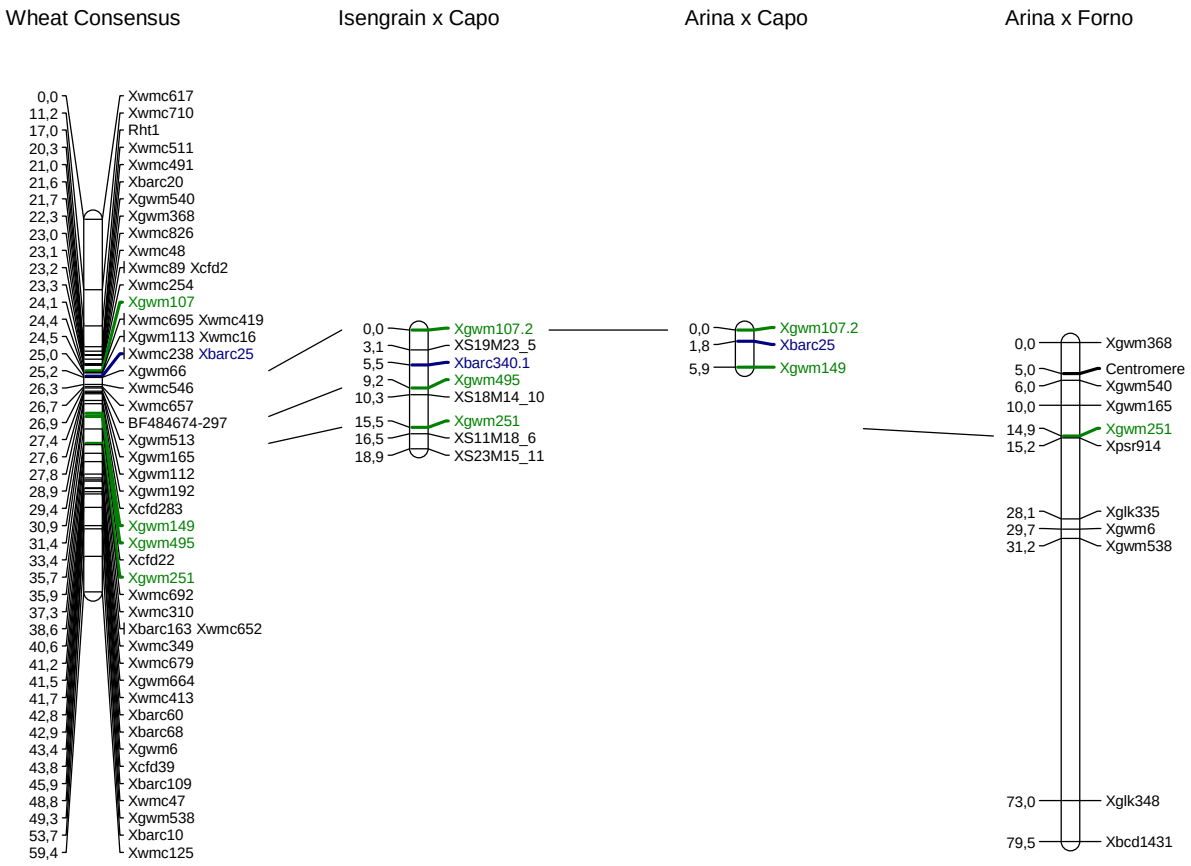

Fig. 48 Linkage groups assigned to chromosome 4B

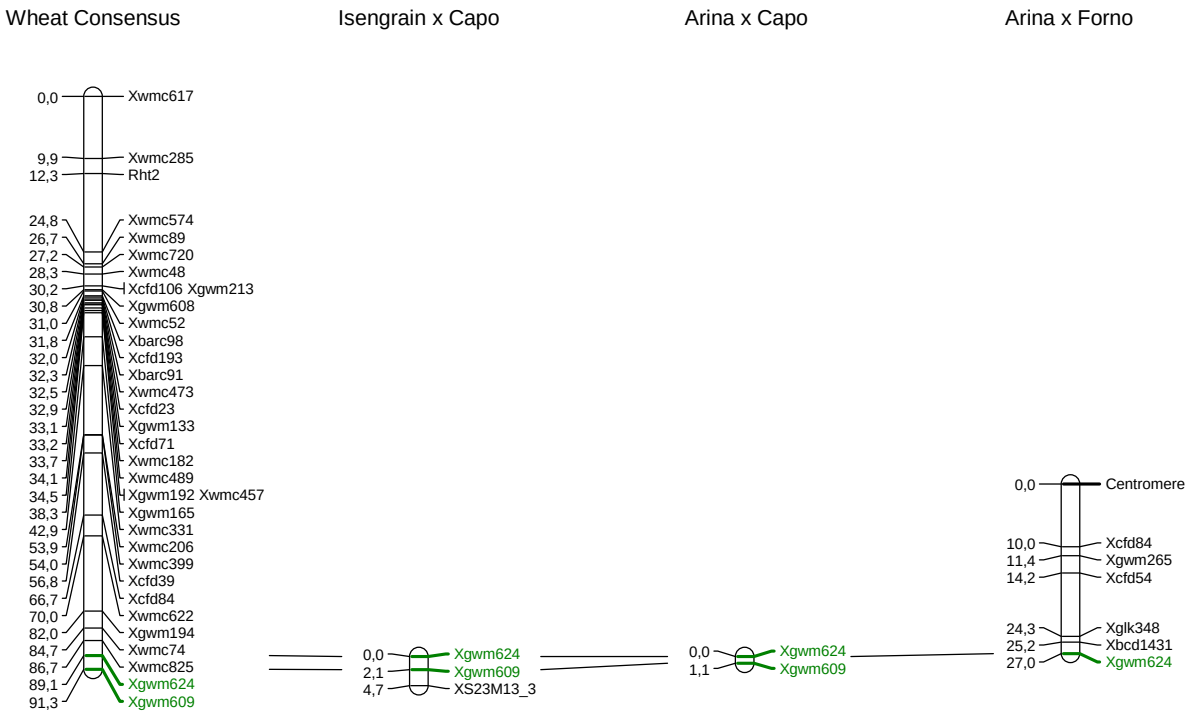

Fig. 49 Linkage groups assigned to chromosome 4D

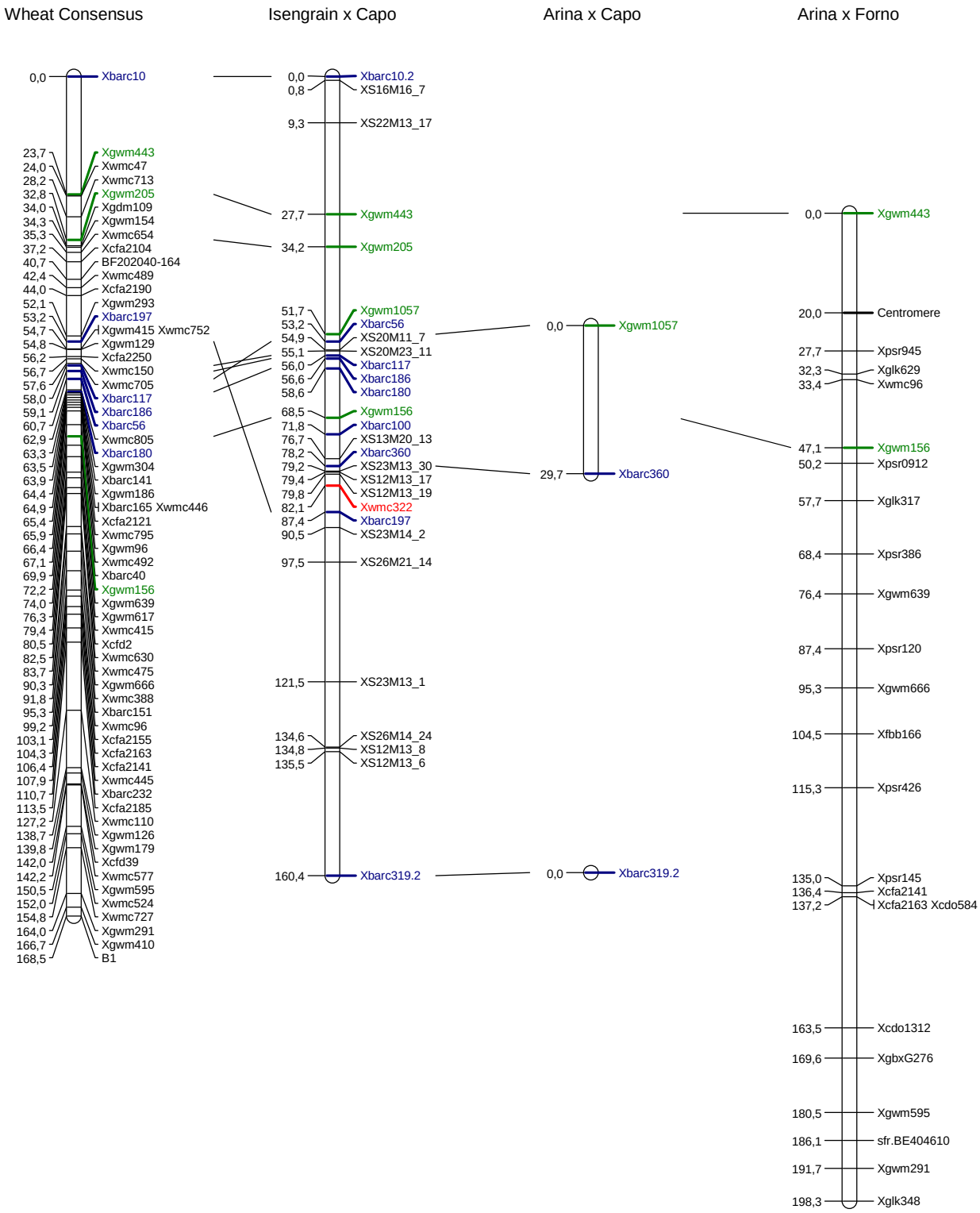

Fig. 50 Linkage groups assigned to chromosome 5A

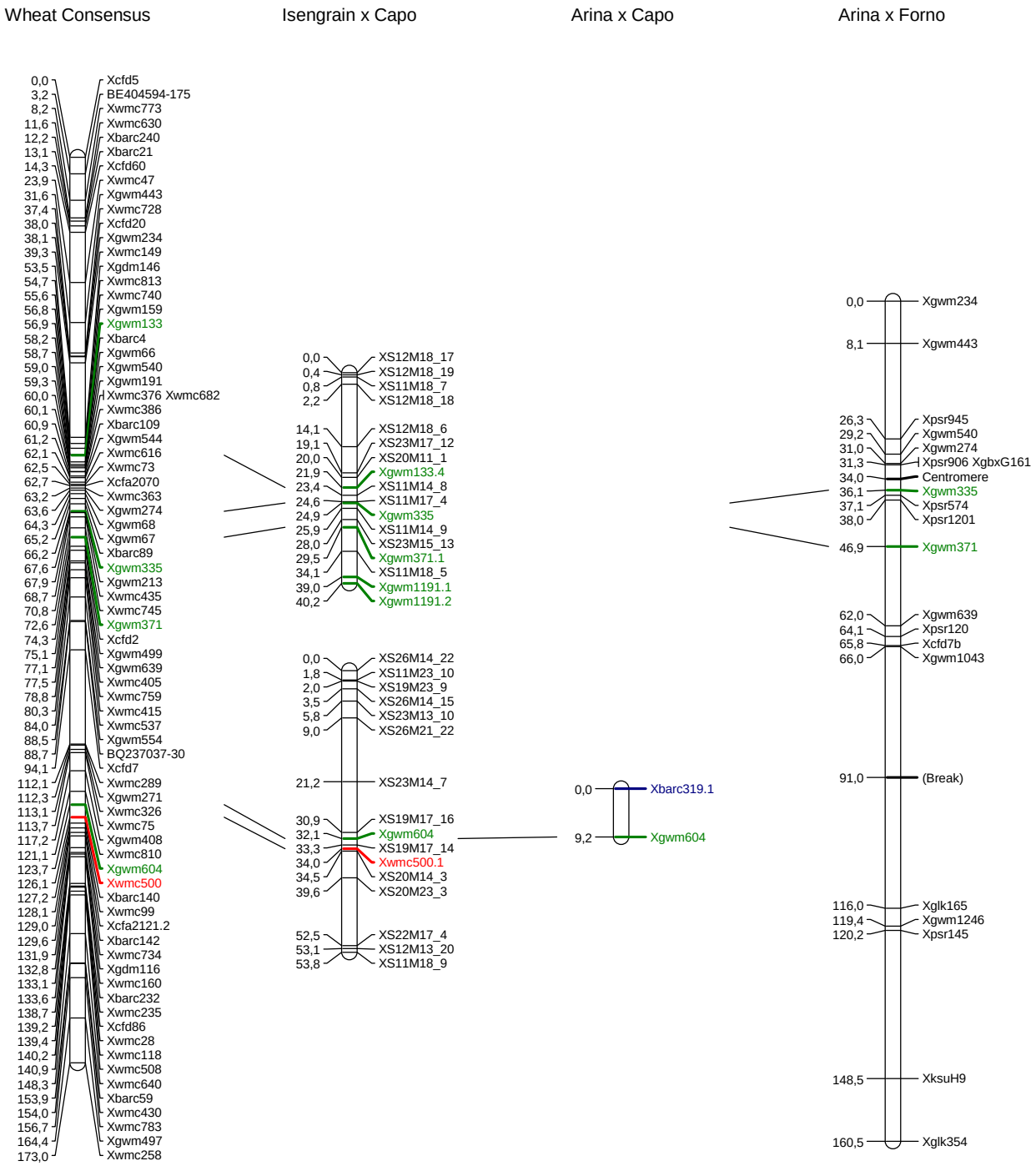

Fig. 51 Linkage groups assigned to chromosome 5B

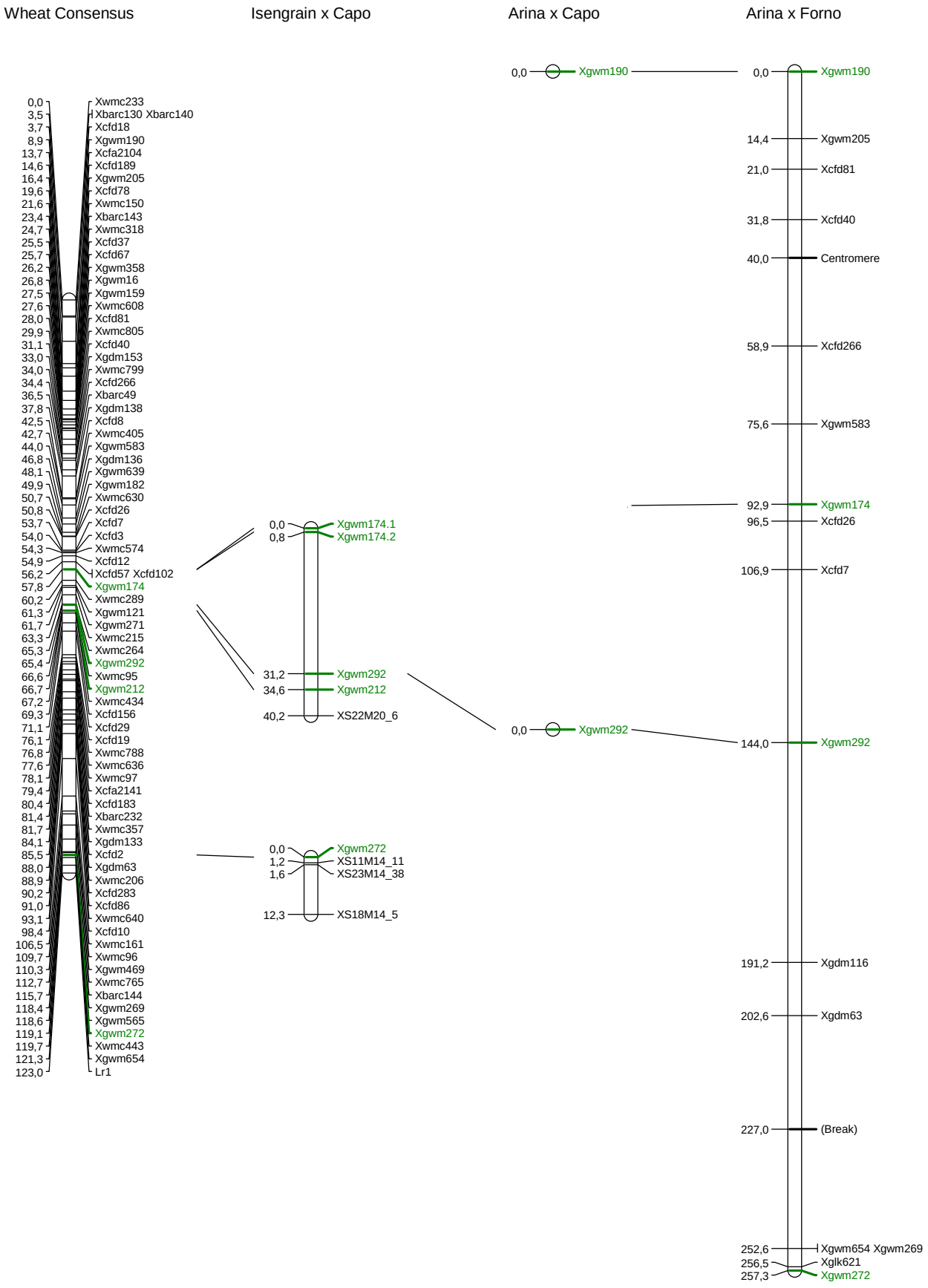

Fig. 52 Linkage groups assigned to chromosome 5D

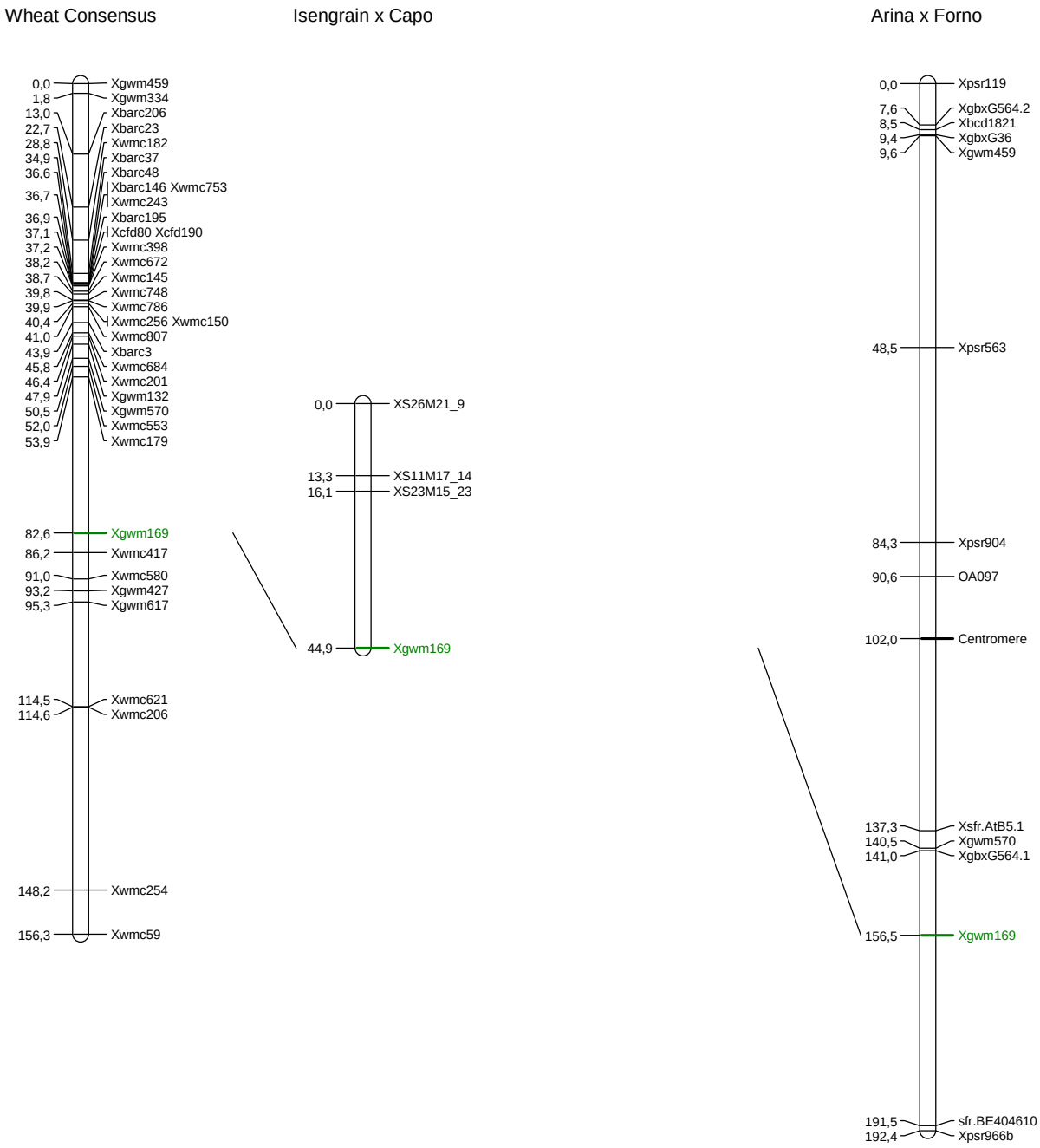

Fig. 53 Linkage groups assigned to chromosome 6A

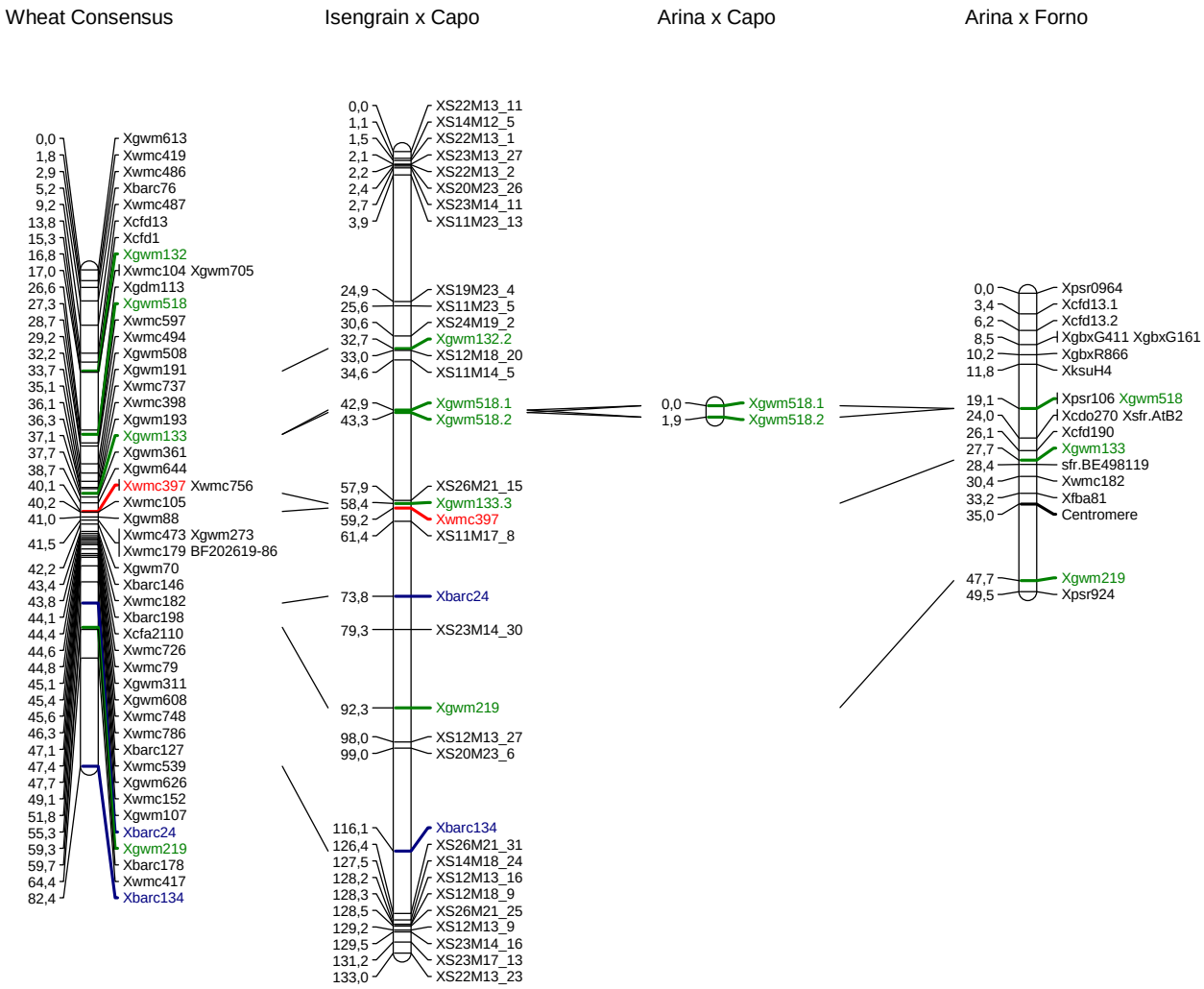

Fig. 54 Linkage groups assigned to chromosome 6B

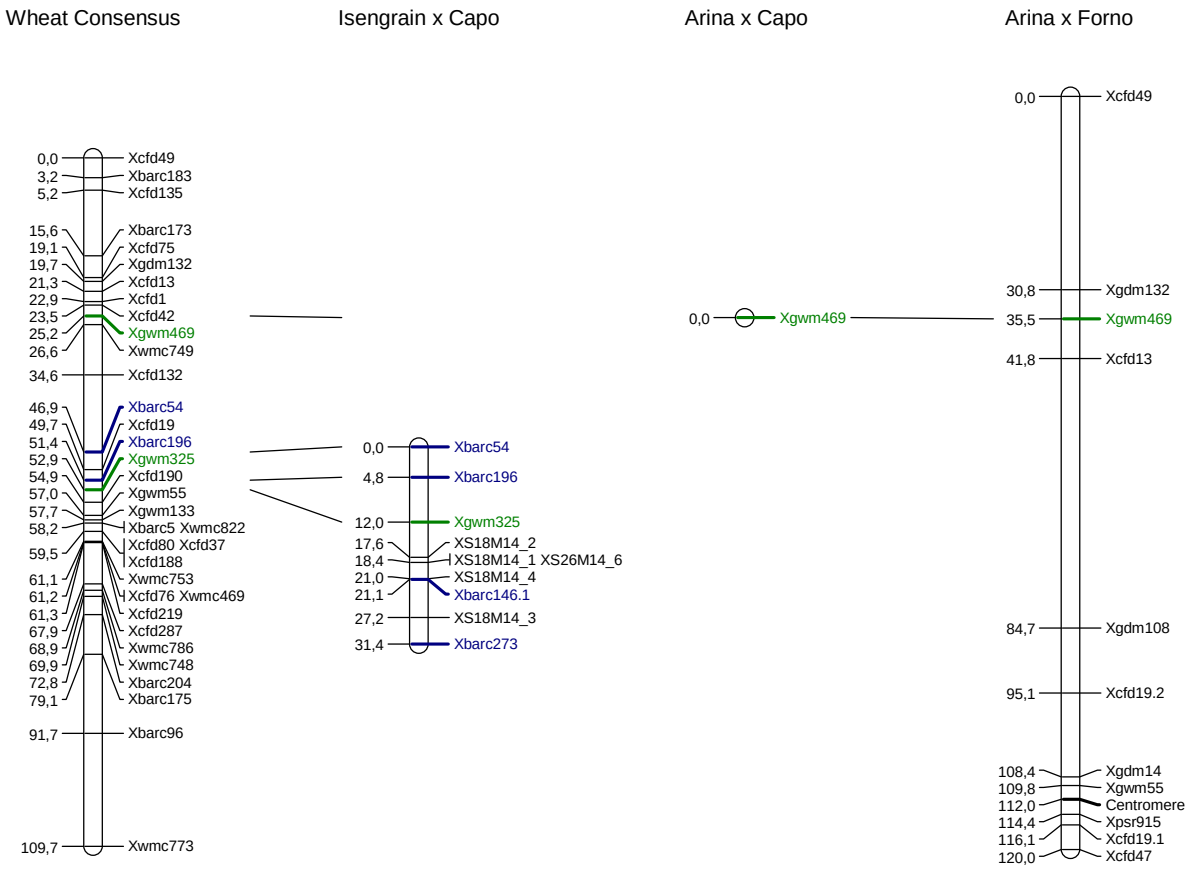

Fig. 55 Linkage groups assigned to chromosome 6D

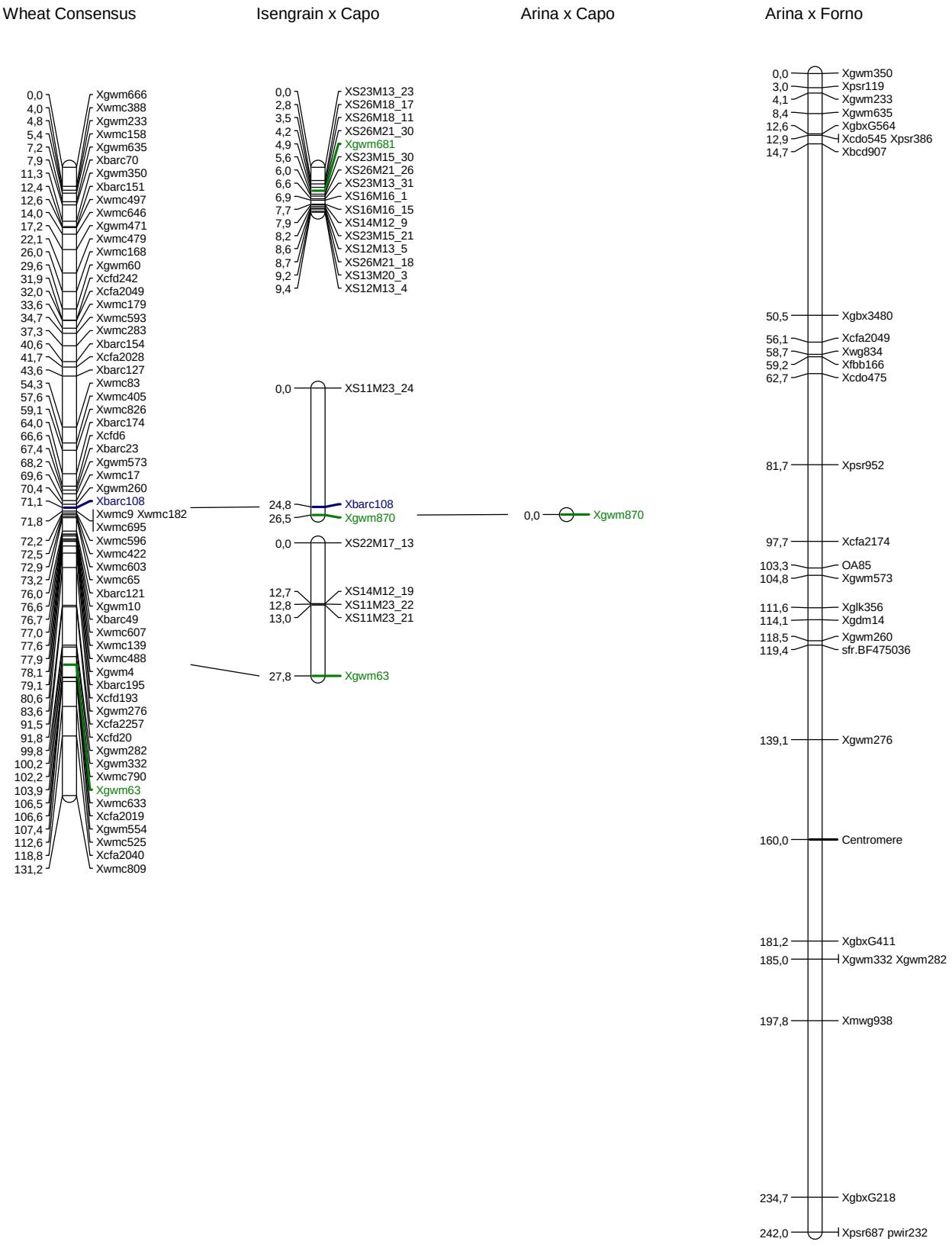

Fig. 56 Linkage groups assigned to chromosome 7A

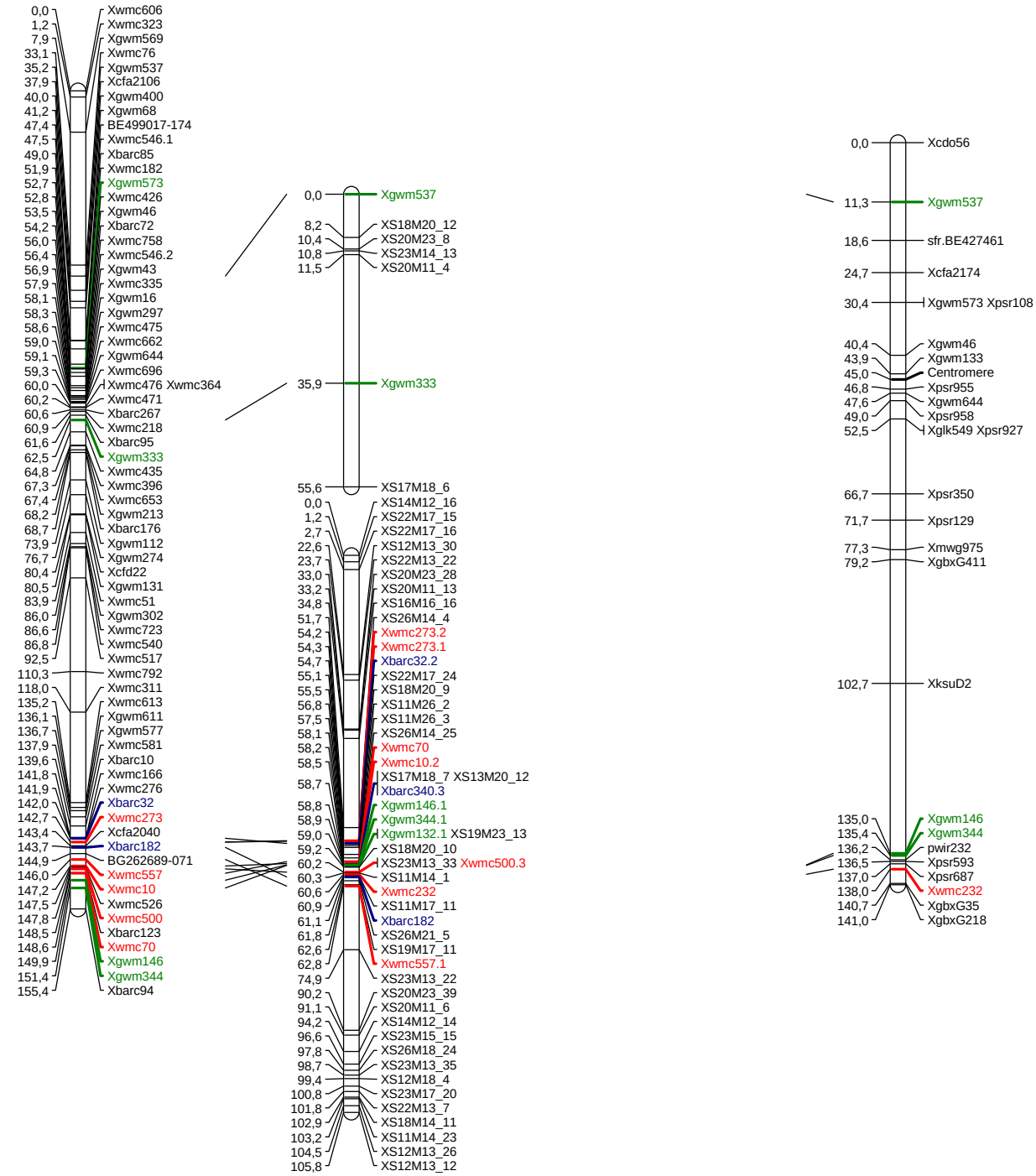

Fig. 57 Linkage groups assigned to chromosome 7B

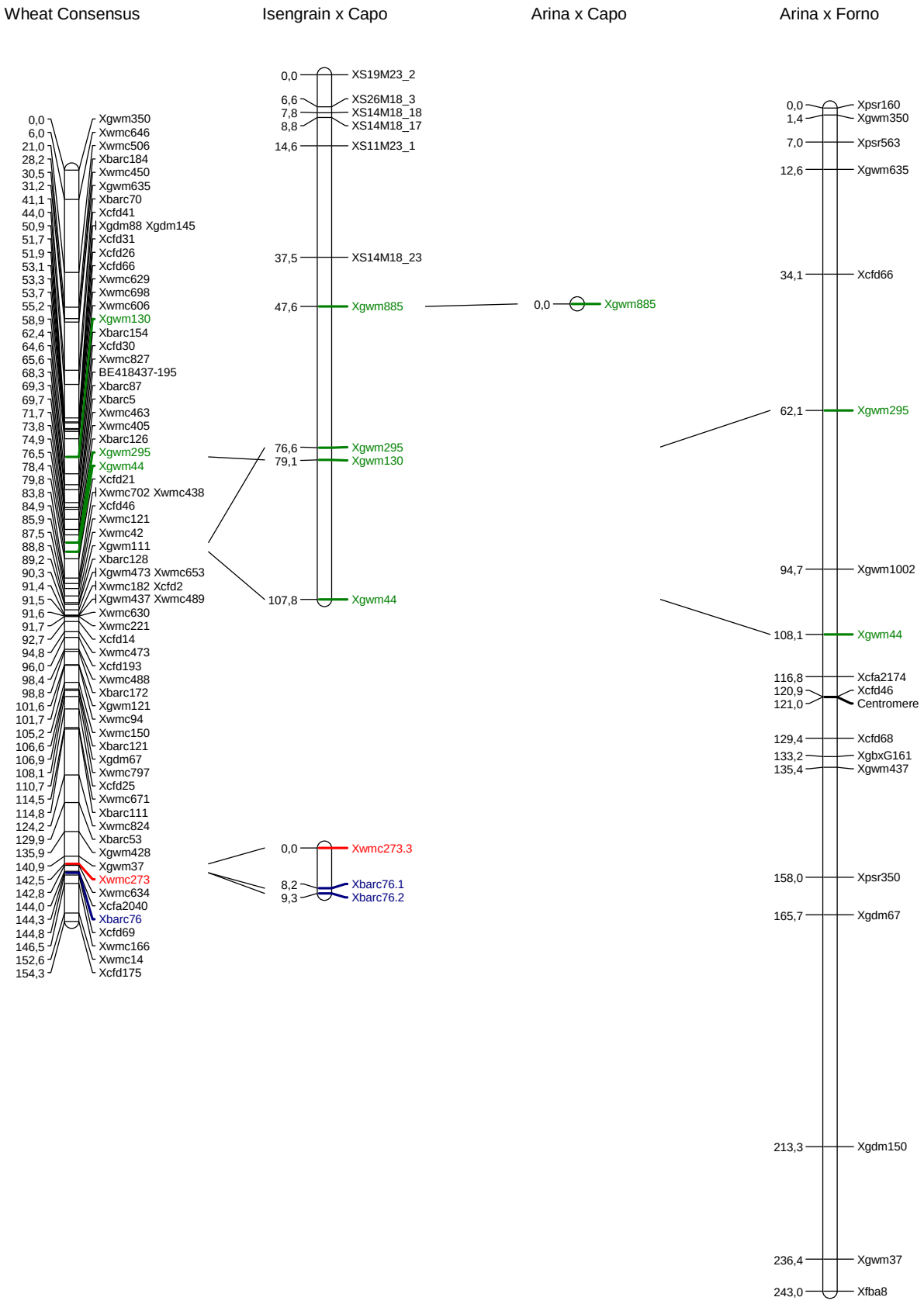

Fig. 58 Linkage groups assigned to chromosome 7D

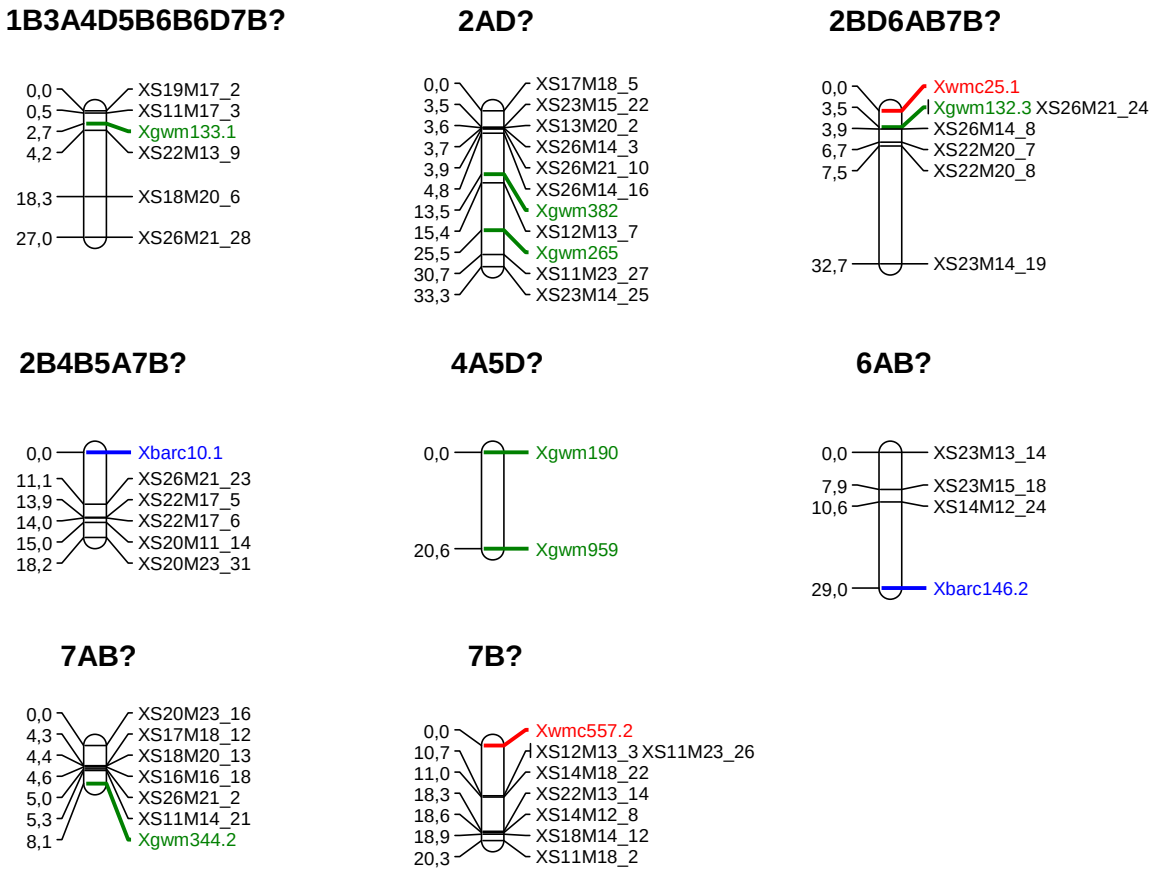

Fig. 59 Linkage groups of the population Isengrain/Capo not definitely assigned to a certain chromosome

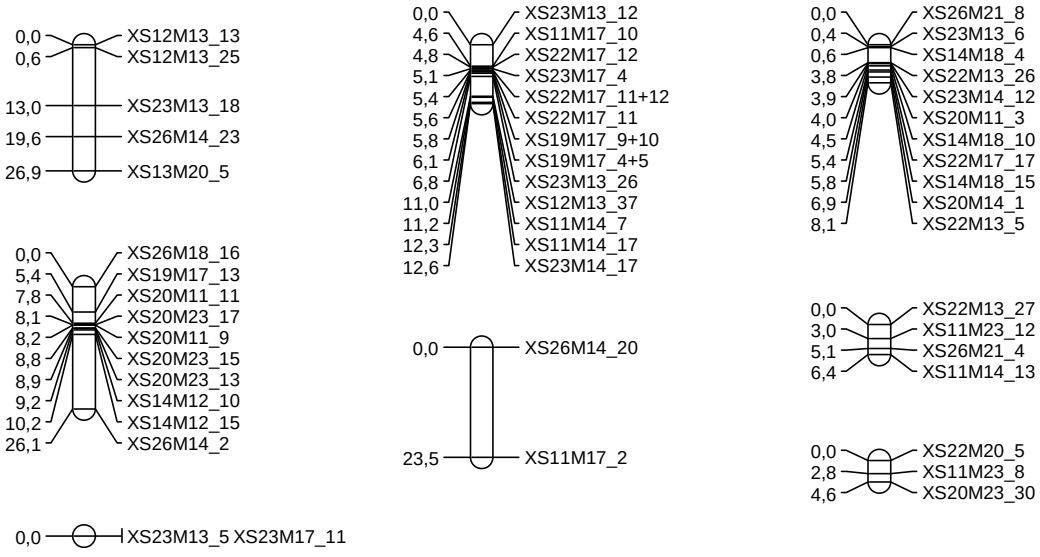

Fig. 60 Unassigned linkage groups of the population Isengrain/Capo

5.4 Quantitative Trait Mapping

5.4.1 Population Isengrain/Capo

By interval mapping several quantitative trait loci (QTL) were detected in the population Isengrain/Capo (Table 86). For each chromosome the LOD values of the detected QTL are given in the first line, the corresponding R^2 in the second line, the additive effect in the third line and the marker next to the QTL peak in the fourth line. In addition interval mapping was also done for all single experiments. All QTL with a maximum LOD ≥ threshold are listed in Table 87 in black, not significant effects in grey color. Experiments are abbreviated as described in Table 4 on page 40. Means were calculated over the same experiments as in chapter 5.1. For all QTL the parental allele conferring a lower scoring value is given too. In the two experiments not listed (IVs5 and IS7) no QTL were identified. Low values for leaf blotch severity were conferred by alleles originating from Capo, for the appearance of teliospores, heading, plant height, powdery mildew severity and lodging severity from Isengrain. Alleles of both parents contributed to resistance to leaf rust (measured by the percentage of infected leaf area and the relative AUDPC), leaf chlorosis severity and leaf tip necrosis severity. The positions of all detected QTL are shown in Fig. 83 to Fig. 94. In Fig. 65, Fig. 66 and Fig. 68 to Fig. 73 boxplots are shown for these traits. The groups are separated according to the alleles of the marker next to the QTL peak. The alleles are named after the parental line from which they originated: C = Capo, I = Isengrain. Below the boxplots the number of RILs (n), their mean value (m) and their standard deviation (s) are given.

5.4.1.1 QTL for Leaf Rust Resistance

In the population Isengrain/Capo five QTL for leaf rust resistance (measured by the percentage of infected leaf area) were detected (Table 87). They were located in linkage groups corresponding to parts of the chromosomes 1B, 2B, 3B, 7B and one yet not definitely assigned group. Only the two QTL located on the short arm of chromosome 3B (Fig. 45, Fig. 86) and the long arm of 7B (Fig. 57, Fig. 91) were consistent over several experiments and the mean over experiments (Table 86 and Table 87). Both were classified as major QTL as they explained up to 15 % and 50 % of the phenotypic variance. The allele associated with increased leaf rust resistance on chromosome 3B was inherited from Capo, whereas in the case of 7B it originated from Isengrain. Both resistance mechanisms seem to work in a different way as the effectiveness of these two QTL differed within experiments:

The QTL on 3B was most effective in the experiments Piešťany 2006, Probstdorf 2006 and Tulln 2004, accounting for 15-8 % of the phenotypic variance, and furthermore slightly beyond the threshold in the experiments Schmida 2007, Rust 2009 and Probstdorf 2007.

The QTL mapped to chromosome 7B was most effective in the experiments Rust 2008, Tulln 2008, Schmida 2008, Schmida 2007, Tulln 2004 and Probstdorf 2006, explaining 46-22 % of the phenotypic variance. Furthermore it was detected in the experiments Tulln

(M) 2007, Probstdorf 2007, Piešťany 2006, Aumühle 2004, Rust 2009 and Probstdorf 2008 (15-6 %).

Table 86 Detected QTL, LOD thresholds, chromosomes, LOD values (1st line), R^2 (2nd line), additive effects (3rd line) and nearest marker (4th line) in the population Isengrain/Capo (interval mapping)

QTL	2A	2BD6AB7E	2B4B5A7B	2D	3B	4B	5B	6AB	6B	7B
leaf rust severity 3.13 (11B)					3.06 0.085 -2.62 *S12M13_33*					17.43 0.399 6.39 *Xgwm132.1*
leaf rust severity 3.22 (3B)					8.48 0.154 -4.30 *S12M13_33*					18.08 0.300 5.78 *Xgwm132.1*
leaf rust severity 3.30 (6B)					4.05 0.080 -3.38 *S12M13_33*					33.84 0.501 8.66 *Xgwm132.1*
relative AUDPC 3.08					4.21 0.077 -1.78 *S12M13_33*					24.89 0.381 4.57 *Xgwm132.1*
appearance of teliospores 3.20										8.25 0.150 *S19M23_13*
heading 3.20		4.14 0.089 0.84 *Xgwm132.3*		13.97 0.700 1.11 *Xgwm484*						
plant height 3.20			3.87 0.075 2.28 *S22M17_5*			12.36 0.223 4.72 *Xgwm107.2*		4.52 0.150 2.02 *Xbarc146.2*		
leaf blotch severity 3.36						9.92 0.187 -0.66 *Xgwm107.2*				
powdery mildew severity 3.24						6.48 0.119 0.37 *Xgwm107.2*				
lodging severity 3.13									3.78 0.093 0.18 *S26M21_15*	
leaf chlorosis severity 3.12				5.58 0.124 -0.55 *Xgwm539*		3.85 0.073 -0.43 *Xgwm107.2*				3.46 0.065 0.46 *S26M18_24*
leaf tip necrosis severity 3.09	3.67 0.073 0.51 *S16M16_21*					4.96 0.095 -0.57 *S11M18_6*	3.65 0.087 -0.49 *S23M14_7*			

Table 87 **Traits, chromosomes, maximum LOD values/corresponding R^2 (1^{st} line) and nearest marker (2^{nd} line) for all detected QTL. For the abbreviations of the experiments see Table 4, for the calculation of means see 5.1 Field Experiments; Capo: lines with Capo allele lower score than lines with Isengrain allele, Isengrain: vice versa; LOD value below threshold; na: not assessed (or not scored on a sufficient number of lines); nc: not calculated; 1) first scoring, 2) second scoring, 3) third scoring, 4) mean over three experiments, 5) mean over six experiments**

trait	chromosome	mean	IA4	IT4	IVm5	IS6	IP6	IT6	IP7	IH7	IM7	IT7	IP8	IRu8	IH8	IT8	IRu9	IT9
leaf rust severity	1B_1	1.47[4]/0.03	0.32[1]/0.01	1.10[1]/0.02	na	5.42[2]/0.10	2.39[2]/0.08	na	0.46[1]/0.01	0.35[1]/0.01	0.53[1]/0.01	na	0.26[1]/0.01	0.27[1]/0.01	0.14[1]/0.00	0.67[1]/0.01	0.54[2]/0.01	na
	Capo	*Xgwm413*	*Xgwm146.2*	*Xgwm413*		*Xgwm413*	*XS22M13_12*		*XS11M23_16*	*XS22M13_12*	*XS23M14_40*		*XS26M21_13*	*Xgwm146.2*	*XS23M13_19*	*XS23M13_19*	*XS23M17_5*	
leaf rust severity	2B_1	1.26[4]/0.03	0.31[1]/0.01	0.89[1]/0.02	na	1.51[3]/0.03	2.06[1]/0.04	na	0.63[1]/0.02	3.48[1]/0.08	0.64[1]/0.01	na	0.77[1]/0.02	0.39[1]/0.01	0.29[1]/0.01	0.49[3]/0.01	1.05[2]/0.03	na
	Isengrain	*Xwmc25.2*	*Xwmc25.2*	*XS23M17_19*		*Xgwm682*	*Xwmc25.2*		*Xgwm148*	*Xgwm148*	*Xwmc25.2*		*XS11M14_18*	*XS23M17_19*	*Xgwm148*	*Xwmc25.2*	*XS22M17_22*	
leaf rust severity	2BD6AB7B	0.87[4]/0.02	0.14[1]/0.00	0.47[1]/0.01	na	3.67[1]/0.08	0.25[1]/0.01	na	0.47[1]/0.01	0.80[1]/0.02	1.58[1]/0,04	na	0.32[1]/0.01	0.58[1]/0.02	0.40[1]/0.01	1.57[1]/0.03	3.60[1]/0.07	na
	Capo	*XS22M20_8*	*XS23M14_19*	*XS22M20_8*		*XS22M20_8*	*XS22M20_8*		*XS22M20_8*	*XS22M20_8*	*Xwmc25.1*		*XS22M20_8*	*XS23M14_19*	*XS23M14_19*	*Xwmc25.1*	*Xwmc25.1*	
leaf rust severity	3B	8.48[4]/0.15	2.63[1]/0.06	4.12[1]/0.08	na	8.27[3]/0.15	6.74[1]/0.12	na	2.83[1]/0.05	3.17[1]/0.06	1.96[1]/0.04	na	1.29[1]/0.03	1.74[1]/0.03	3.15[1]/0.06	3.16[1]/0.06	3.16[2]/0.07	na
	Capo	*XS12M13_33*	*XS12M13_33*	*XS12M13_33*		*XS12M13_33*	*XS12M13_33*		*XS23M15_3*	*XS12M13_33*	*XS23M15_3*		*Xgwm533*	*XS12M13_33*	*XS12M13_33*	*XS12M13_33*	*XS23M15_3*	
leaf rust severity	7B_2	33.84[5]/0.50	3.94[1]/0.09	18.81[1]/0.31	na	4.38[3]/0.08	13.14[1]/0.22	na	6.17[2]/0.12	21.18[1]/0.34	7.39[1]/0.15	na	2.92[1]/0.06	31.85[1]/0.46	25.89[1]/0.39	31.07[2]/0.45	3.78[2]/0.09	na
	Isengrain	*Xgwm132.1*	*XS26M14_4*	*Xgwm132.1*		*XS11M26_2*	*Xgwm132.1*		*XS11M26_2*	*Xgwm132.1*	*Xgwm132.1*		*Xgwm132.1*	*Xgwm132.1*	*Xgwm132.1*	*Xgwm132.1*	*XS22M17_24*	
relative AUDPC	1B_1	1.54/0.03	na	na	na	4.80/0.09	na	na	na	na	na	na	na	na	na	0.47/0.01	na	na
	Capo	*XS26M14_10*				*XS26M14_10*										*XS26M14_10*		
relative AUDPC	2BD6AB7B	1.63/0.03	na	na	na	3.38/0.07	na	na	na	na	na	na	na	na	na	0.78/0.02	na	na
	Capo	*Xwmc25.1*				*Xwmc25.1*										*Xwmc25.1*		
relative AUDPC	3B	4.21/0.08	na	na	na	4.98/0.09	na	na	na	na	na	na	na	na	na	2.88/0.05	na	na
	Capo	*XS12M13_33*				*XS12M13_33*										*XS12M13_33*		
relative AUDPC	7B_2	24.89/0.38	na	na	na	3.61/0.07	na	na	na	na	na	na	na	na	na	32.87/0.47	na	na
	Isengrain	*Xgwm132.1*				*Xgwm132.1*										*Xgwm132.1*		
app. teliospores	7B_2	nc	na	8.25/0.15	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Isengrain			*XS19M23_13*														
heading	2BD6AB7B	4.14/0.09	7.45/0.14	6.87/0.12	5.26/0.10	5.28/0.10	6.43/0.12	6.34/0.11	5.57/0.10	na	4.99/0.10	5.22/0.10	na	5.66/0.10	4.15/0.08	7.33/0.13	na	7.20/0.13
	Isengrain	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*		*Xgwm132.3*	*Xgwm132.3*		*Xgwm132.3*	*Xgwm132.3*	*Xgwm132.3*		*Xgwm132.3*
heading	2D	13.97/0.70	14.74/0.61	18.81/0.77	11.22/0.60	75.41/0.94	20.54/0.67	16.53/0.69	22.78/0.75	na	16.40/0.63	15.10/0.57	na	14.15/0.52	13.26/0.54	16.01/0.52	na	14.70/0.53
	Isengrain	*Xgwm484*	*Xgwm484*	*Xgwm484*	*Xgwm484*	*Xgwm484*	*Xgwm484*	*Xgwm484*	*Xgwm484*		*Xgwm484*	*Xgwm484*		*Xgwm484*	*Xgwm484*	*Xgwm484*		*Xgwm484*
plant height	2B_2	2.21/0.05	na	1.62/0.03	na	na	na	2.04/0.04	na	na	2.47/0.05	4.90/0.09	na	na	na	2.75/0.06	na	na
	Isengrain	*XS14M18_20*		*Xbarc114*				*XS14M18_20*			*XS14M18_20*	*XS14M18_20*				*XS14M18_20*		
plant height	2B4B5A7B	3.87/0.08	na	2.73/0.05	na	na	na	2.24/0.04	na	na	2.25/0.04	5.14/0.09	na	na	na	5.08/0.09	na	na
	Isengrain	*XS22M17_5*		*XS22M17_5*				*XS22M17_5*			*XS22M17_5*	*XS22M17_5*				*XS22M17_5*		
plant height	4B	12.36/0.22	na	11.26/0.20	na	na	na	7.71/0.14	na	na	9.44/0.18	15.05/0.25	na	na	na	16.01/0.27	na	na
	Isengrain	*Xgwm107.2*		*Xgwm107.2*				*Xgwm107.2*			*Xgwm107.2*	*Xgwm107.2*				*Xgwm107.2*		
plant height	6AB	4.52/0.15	na	4.23/0.13	na	na	na	4.06/0.12	na	na	3.77/0.12	3.42/0.11	na	na	na	4.77/0.14	na	na
	Isengrain	*Xbarc146.2*		*Xbarc146.2*				*Xbarc146.2*			*Xbarc146.2*	*Xbarc146.2*				*Xbarc146.2*		
leaf blotch severity	4B	nc	9.92/0.19	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Capo		*Xgwm107.2*															
powdery mildew	4B	nc	na	na	na	na	6.48/0.12	na	na	na	na	na	na	na	na	na	na	na
	Isengrain						*Xgwm107.2*											
lodging severity	6AB	2.31/0.05	na	na	na	na	na	na	0.38/0.01	na	na	na	4.29/0.09	0.79[1]/0.02	na	na	na	na
	Isengrain	*Xbarc146.2*							*Xbarc146.2*				*Xbar146.2*	*Xbarc146.2*				
lodging severity	6B	3.78/0.09	na	na	na	na	na	na	1.59/0.04	na	na	na	3.86/0.10	1.71[2]/0.03	na	na	na	na
	Isengrain	*XS26M21_15*							*Xbarc24*				*XS26M21_15*	*XS11M17_8*				
leaf chlorosis severity	2D	5.58/0.12	na	na	na	na	na	na	na	na	na	na	na	na	na	na	4.70/0.11	5.02/0.11
	Capo	*Xgwm539*															*Xgwm539*	*Xgwm539*
leaf chlorosis severity	4B	3.85/0.07	na	na	na	na	na	na	na	na	na	na	na	na	na	na	3.22/0.06	3.71/0.07
	Capo	*Xgwm107.2*															*Xgwm107.2*	*Xgwm107.2*
leaf chlorosis severity	7B_2	3.46/0.07	na	na	na	na	na	na	na	na	na	na	na	na	na	na	4.47/0.08	2.55/0.05
	Isengrain	*XS26M18_24*															*XS26M18_24*	*XS26M18_24*
leaf tip necrosis sev.	2A_2	3.67/0.07	na	na	na	na	na	na	na	na	na	na	na	na	na	na	4.21/0.08	2.57/0.05
	Isengrain	*XS16M16_21*															*XS16M16_21*	*Xgwm372*
leaf tip necrosis sev.	4A	2.27/0.05	na	na	na	na	na	na	na	na	na	na	na	na	na	na	3.27/0.07	1.71/0.03
	Isengrain	*XS26M18_8*															*XS26M18_8*	*XS26M18_8*
leaf tip necrosis sev.	4B	4.96/0.10	na	na	na	na	na	na	na	na	na	na	na	na	na	na	3.17/0.06	6.32/0.12
	Capo	*XS11M18_6*															*XS11M18_6*	*XS11M18_6*
leaf tip necrosis sev.	5B_2	3.65/0.09	na	na	na	na	na	na	na	na	na	na	na	na	na	na	3.66/0.09	3.49/0.08
	Capo	*XS23M14_7*															*XS23M14_7*	*XS23M14_7*

Table 88 **Traits, chromosomes, maximum LOD values/corresponding R^2 (1^{st} line) and nearest marker (2^{nd} line) for all detected QTL. For the abbreviations of the experiments see Table 4, for the calculation of means see 5.1 Field Experiments; Capo: lines with Capo allele lower score than lines with Arina allele, Arina: vice versa; LOD value below threshold; na: not assessed (or not scored on a sufficient number of lines); nc: not calculated; [1)] first scoring, [2)] second scoring, [3)] third scoring [4)] fourth scoring, [5)] fifth scoring**

trait	chromosome	mean	AT7	ARo8	AU8	ASw8	AP8	ARe8	ARu8	AH8	AT8	ARu9	AT9
leaf rust severity	3A	0.27/0.01	0.36[2)]/0.01	0.44[2)]/0.01	0.03/0.00	0.22/0.00	0.01/0.00	2.15/0.05	0.56/0.02	0.70/0.01	0.58[1)]/0.02	0.59[1)]/0.01	0.27[2)]/0.01
	Arina	*Xgwm720*	*Xgwm779*	*Xgwm779*	*Xgwm720*	*Xgwm779*	*Xgwm1110*	*Xgwm720*	*Xgwm720*	*Xgwm702*	*Xgwm779*	*Xgwm779*	*Xgwm779*
leaf rust severity	3B_1	3.69/0.08	2.35[3)]/0.06	3.18[3)]/0.07	5.14/0.11	0.11/0.00	1.79/0.04	0.41/0.01	6.04/0.12	2.75/0.05	3.20[1)]/0.07	2.66[5)]/0.05	0.80[2)]/0.02
	Capo	*Xgwm389.1*	*Xbarc75*	*Xgwm389.1*	*Xbarc75*	*Xgwm389.2*	*Xgwm389*	*Xgwm389.1*	*Xgwm389.1*	*Xgwm389.1*	*Xgwm389.1*	*Xgwm389.1*	*Xgwm389.1*
leaf rust severity	5A	0.78/0.03	0.41[2)]/0.01	0.14[3)]/0.00	0.27/0.01	1.76[2)]/0.05	1.16/0.04	0.63/0.01	0.20(0.00	1.65/0.06	1.02[2)]/0.04	1.14[4)]/0.03	2.49[3)]/0.09
	Capo	*Xgwm1075*	*Xbarc360*	*Xgwm1075*	*Xbarc360*	*Xgwm1075*	*Xgwm1075*	*Xgwm1075*	*Xbarc360*	*Xgwm1075*	*Xgwm1057*	*Xgwm1075*	*Xgwm1057*
relative AUDPC	3B_1	3.92/0.08	na	3.02/0.06	na	na	na	na	na	na	2.48/0.05	na	na
	Capo	*Xgwm389.1*		*Xgwm389.1*							*Xgwm389.1*		
plant height	5A	2.19/0.08	2.43/0.09	na	na	na	na	na	na	na	1.46/0.05	na	na
	Capo	*Xgwm1057*	*Xgwm1057*								*Xgwm1057*		
plant height	6B	2.26/0.05	1.29/0.03	na	na	na	na	na	na	na	2.96/0.06	na	na
	Capo	*Xgwm518.1*	*Xgwn518.1*								*Xgwm518.1*		
awnedness	4D	1.81/0.04	1.57/0.03	na	na	na	na	na	1.46/0.04	1.77/0.04	1.92/0.04	na	na
	Capo	*Xgwm624*	*Xgwm624*						*Xgwm624*	*Xgwm624*	*Xgwm624*		
yellow rust severity	3B_1	nc	na	na	na	10.90/0.20	na	na	na	na	na	na	na
	Capo					*Xbarc75*							
glaucousness	3A	nc	na	na	na	na	na	na	na	na	na	12.61/0.43	na
	Capo											*Xgwm720*	
glaucousness	4B	nc	na	na	na	na	na	na	na	na	na	2.47/0.05	na
	Arina											*Xgwm149*	
lodging severity	5A	2.09/0.09	na	na	na	na	2.17/0.06	na	2.07/0.07	na	na	na	na
	Capo	*Xgwm1057*					*Xgwm1057*		*Xgwm1057*				
leaf chlorosis severity	3A	2.61/0.05	na	na	na	na	na	na	na	na	na	2.33/0.05	2.73/0.05
	Arina	*Xgwm1110*										*Xgwm1110*	*Xgwm1110*
leaf tip necrosis severity	3B_2	2.43/0.11	na	na	na	na	na	na	na	na	na	1.19/0.06	2.22/0.08
	Capo	*Xgwm108*										*Xgwm108*	*Xgwm108*
leaf tip necrosis severity	5A	2.12/0.09	na	na	na	na	na	na	na	na	na	2.11/0.09	2.28/0.10
	Arina	*Xgwm1057*										*Xgwm1057*	*Xgwm1057*
leaf tip necrosis severity	5B	4.18/0.09	na	na	na	na	na	na	na	na	na	4.25/0.09	3.35/0.07
	Capo	*Xgwm604*										*Xgwm604*	*Xgwm604*

Table 89 **Markers not assigned to linkage groups with significant effects on traits detected in single point ANOVA in the population Arina/Capo. Alleles as in Table 88.**

trait	positive allele	marker	chromosome Isengr./Capo	detected in Isengr./Capo	additive effect	experiments
leaf rust severity	Capo	*Xwmc25.1*	2BD6AB7B	IS6, IRu9	-3.08	ARo8, ASw8, ARe8, ARu9, AT9
relative AUDPC	Capo	*Xwmc25.1*	2BD6AB7B	IS6	-2.78	ARo8
Septoria leaf blotch severity	Arina	*Xgwm870*	7A_2	not analyzed	0.09	ASw8
glaucousness	Capo	*Xgwm120*	2B_1	not analyzed	-0.33	ARu9
lodging severity	Arina	*Xgwm120*	2B_1	no	0.28	AP8, ARu8
leaf tip necrosis severity	Capo	*Xgwm135*	1A_2	no	-0.26	ARu9
leaf tip necrosis severity	Arina	*Xgwm870*	7A_2	no	0.34	ARu9, AT9

Whereas the LOD value of the experiment Piešťany 2006 was highest for 3B, it was relatively low for 7B. On the other hand, the LOD value of the experiment Rust 2008 was highest for the QTL on 7B, but was not even beyond the threshold for 3B. The LOD curves for both chromosomes are presented in Fig. 61. Boxplots of the allele groups of the two markers next to these QTL for the results of the experiments in Piešťany 2006 and Rust 2008 are illustrated in Fig. 62a-b (chromosome 3B) and e-f (7B). Based on the results of the single experiments three different mean values were calculated, comprising results of different experiments: For “mean over 3 experiments” results of those three in which the QTL on 3B was most effective were used, for “mean over 6 experiments” data of those six in which LOD values of 7B were highest. As an overall mean, including most of the informative experiments, the “mean over 11 experiments” was calculated as described in Table 26 and used for various statistical analyses presented in 5.1.1.2 and 5.1.5. The frequency distributions of the single experiments are illustrated in Fig. 12a-l, for the different mean values in Fig. 63, boxplots of the different alleles of the QTL markers are

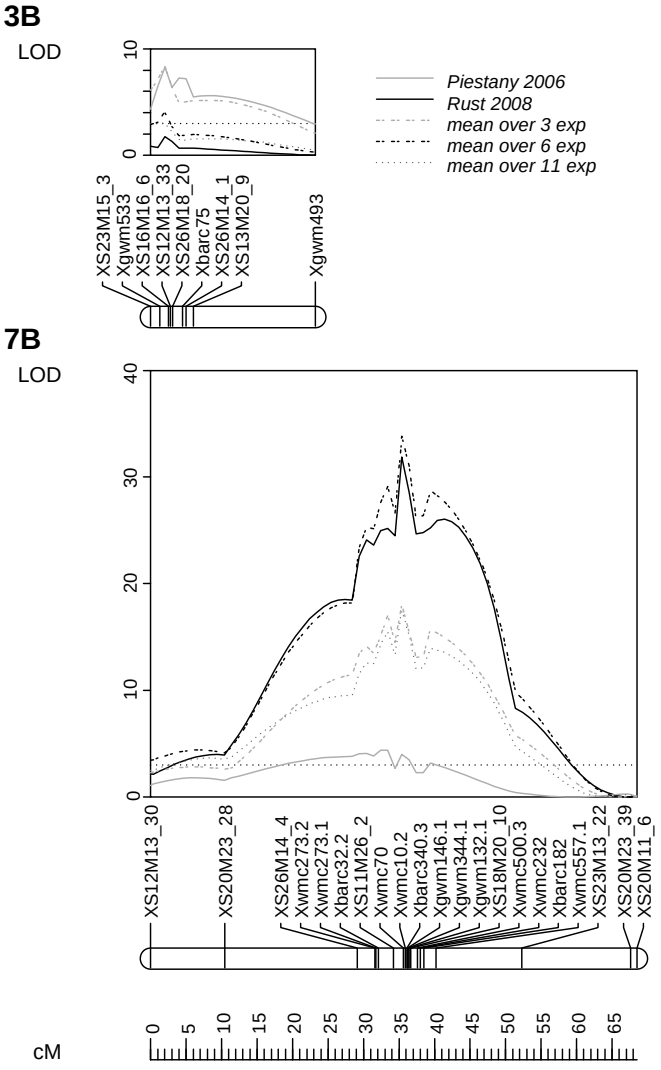

Fig. 61 Comparative diagram of the leaf rust severity QTL on chromosomes 3B and 7B. LOD curves for the experiments Piešťany 2006, Rust 2008, mean over three experiments, mean over six experiments and mean over eleven experiments are shown. The experiments used for calculating the means are presented in Table 26.

shown in Fig. 62a-d (chromosome 3B), Fig. 62e-h (7B) and in Fig. 65 (mean over eleven experiments, both chromosomes). In Fig. 64 LOD curves are illustrated for the eleven experiments and the mean over them. In the case of chromosome 3B they are shown in comparison to the mean over three experiments, the corresponding LOD curves of which are drawn in black color. For 7B LOD curves of the six experiments in which the QTL was most effective are shown in black color and the mean over eleven experiments is compared with the mean over these six experiments. In both cases the LOD value of the mean over the experiments with the highest LOD values was even beyond them.

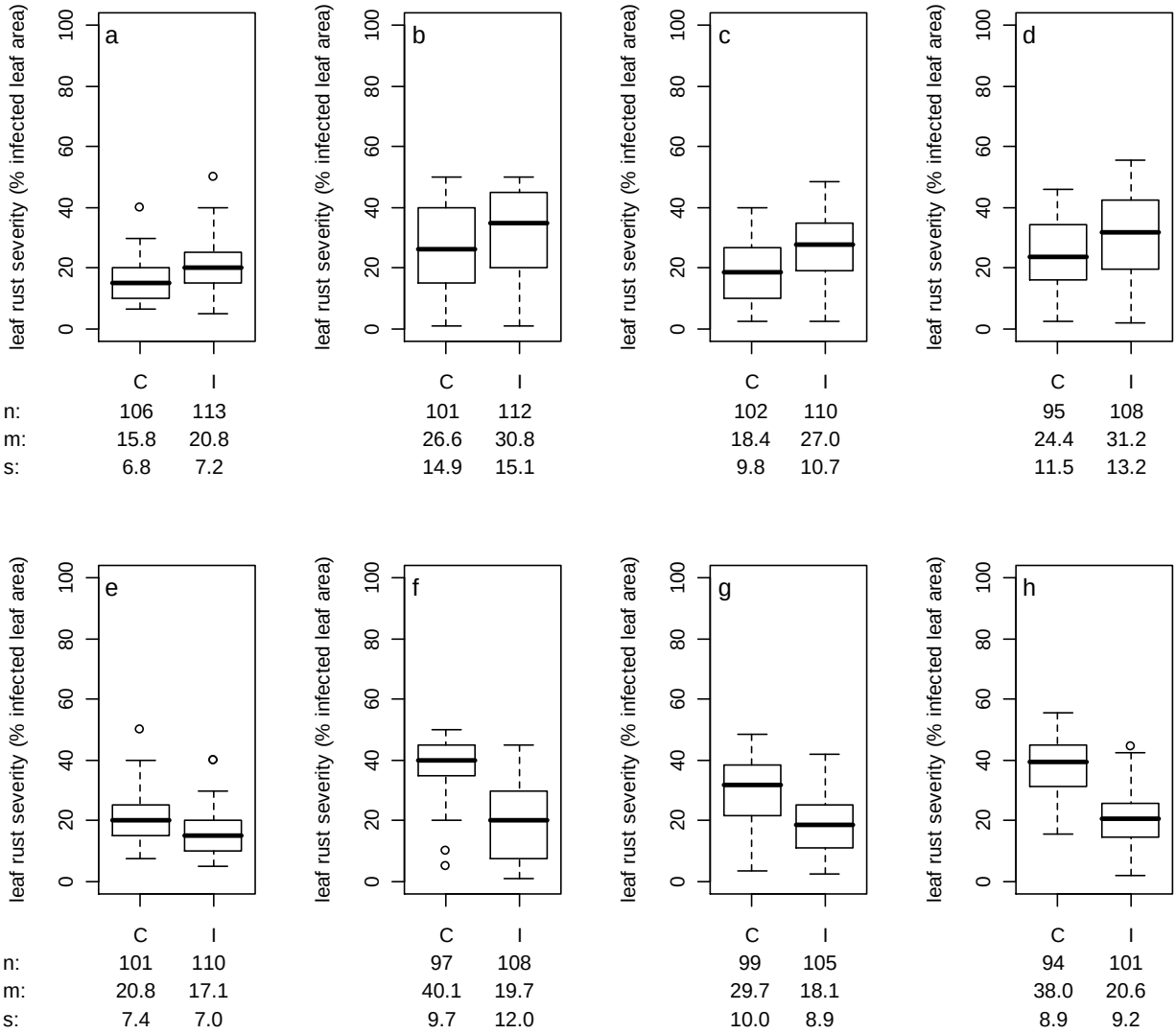

Fig. 62a-h Leaf rust severity: Boxplots of the different allele groups of the QTL markers *XS12M13_33* on chromosome 3B (a-d) and *Xgwm132.1* on chromosome 7B (e-h). Results of the experiments Piešťany 2006 (a+e), Rust 2008 (b+f), mean over three experiments (c+g) and mean over six experiments (d+h). Capo: 20.0 (Piešťany 2006), 28.5 (Rust 2008), 17.1 (three exp.), 24.5 (six exp.), Isengrain: 25.0 (Piešťany 2006), 25.0 (Rust 2008), 29.1 (three exp.), 32.4 (six exp.)

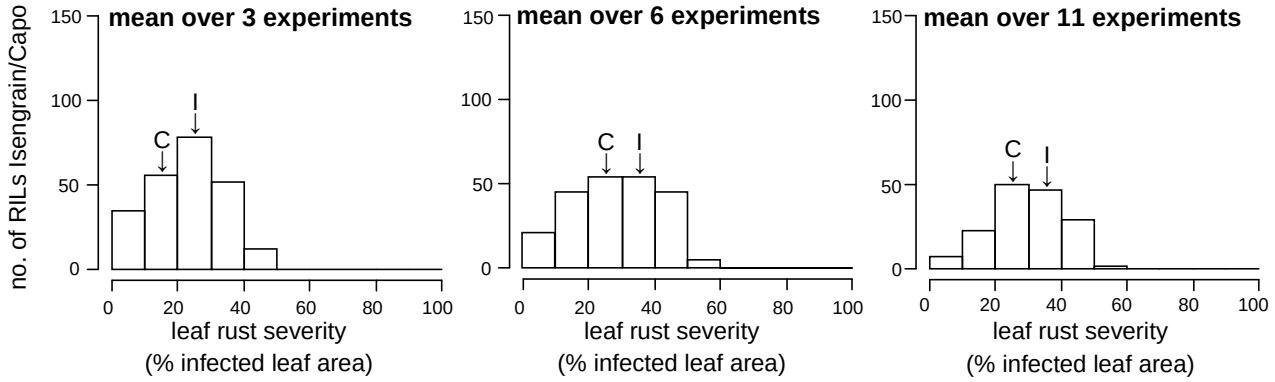

Fig. 63 **Frequency distributions of leaf rust severity (% infected leaf area) of the population Isengrain/Capo (calculated mean over three, six and eleven experiments). Values of the parental lines are indicated by arrows. The single experiments including number of lines are shown in Table 26 and Fig. 12.**

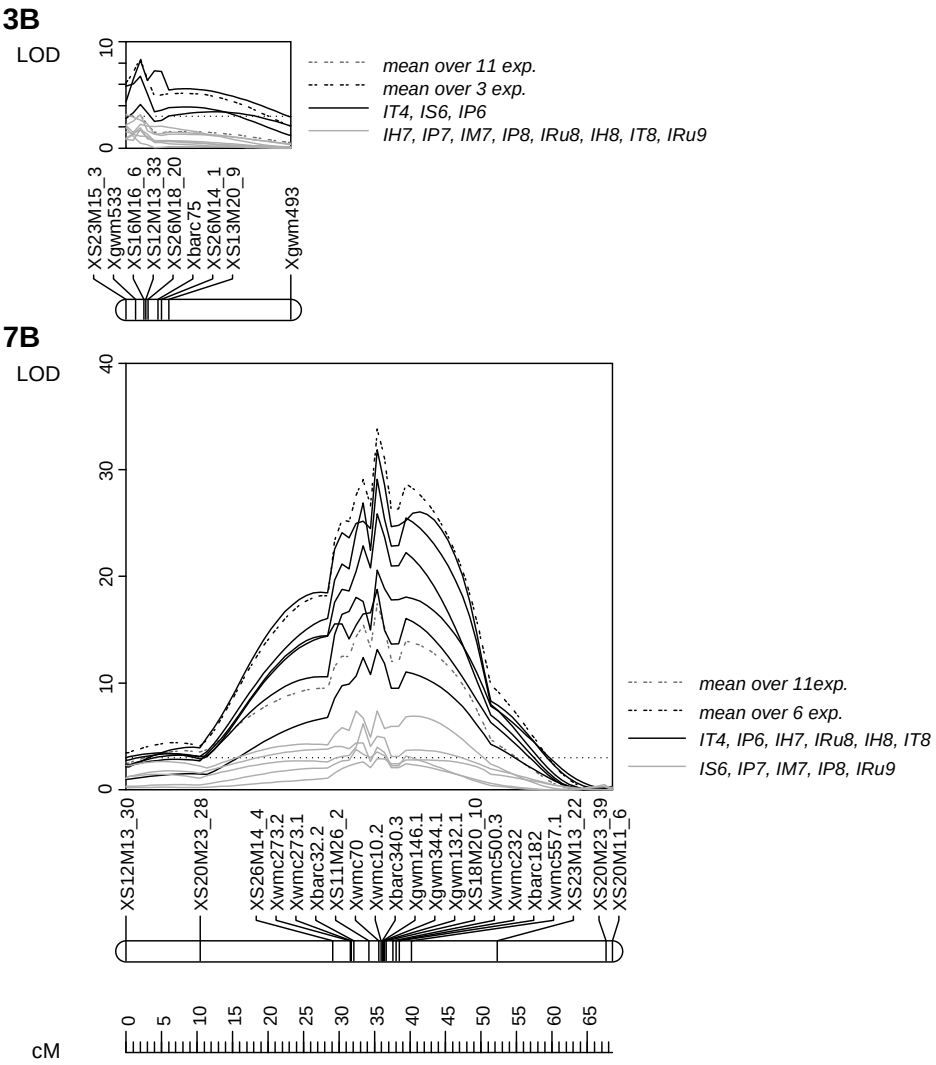

Fig. 64 **Comparative diagram of the leaf rust severity QTL on chromosomes 3B and 7B. LOD curves for the mean over eleven experiments, mean over three experiments and mean over six experiments and the particular experiments used for calculating these means.**

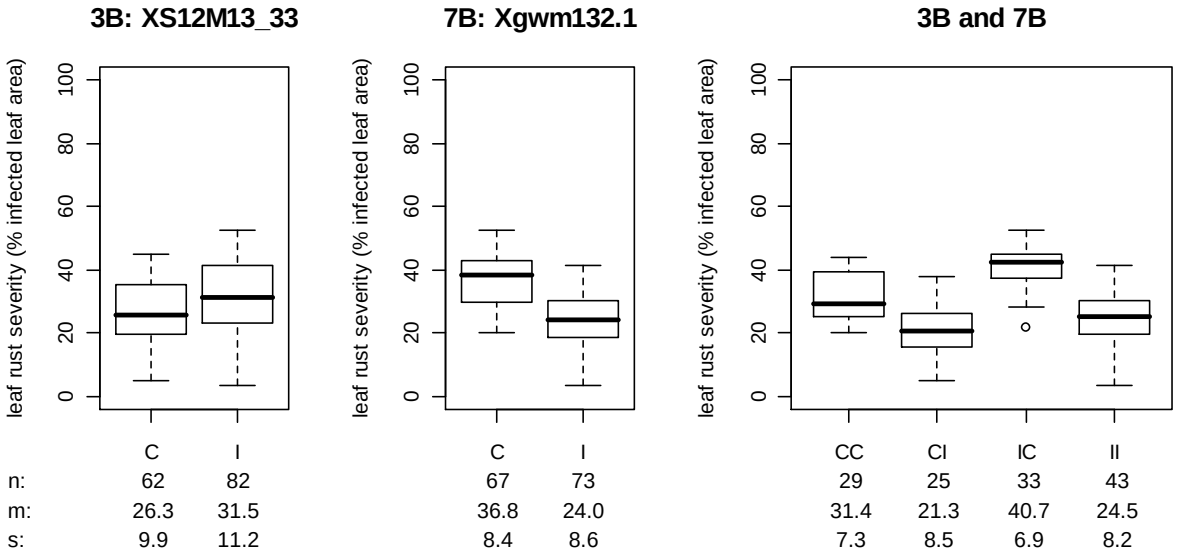

Fig. 65 Leaf rust severity, mean over eleven experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 26.7, Isengrain: 35.5

From Fig. 65 it can be seen that the average infection with leaf rust was lowest for the lines possessing both positive alleles and by far highest for those with the adverse allele combination. Standard deviation was reduced when regarding the allele combination compared to both single loci. As the Isengrain QTL on 7B was in most of the experiments much more effective than the Capo allele, lines possessing the Isengrain allele at both loci were on average just slightly more infected than those with the ideal allele combination.

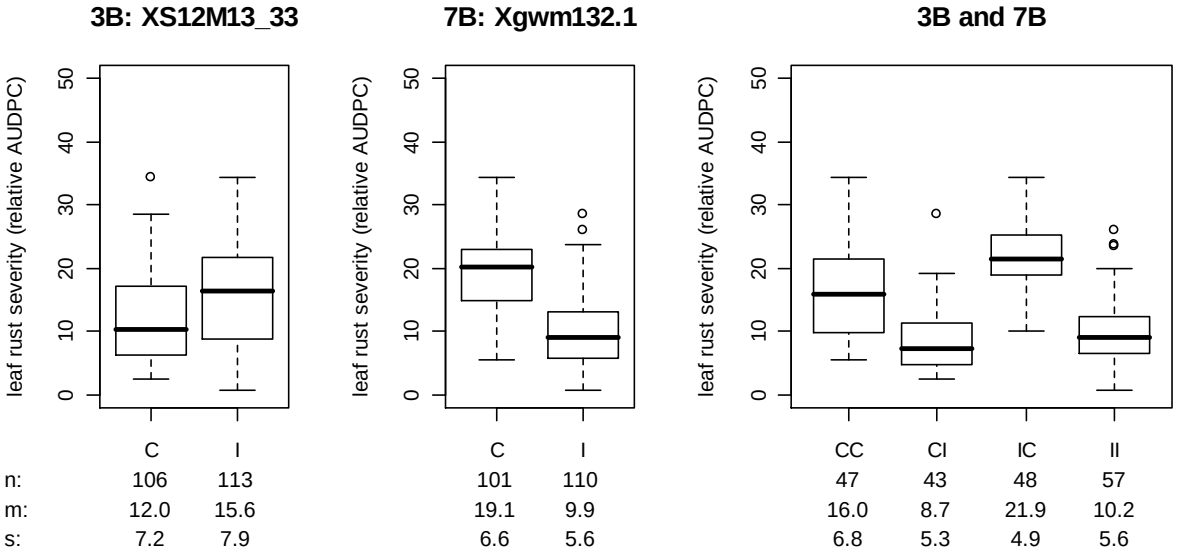

Fig. 66 Leaf rust severity, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 11.0, Isengrain: 30.2

In addition to QTL analysis for single assessments of leaf rust severity, interval mapping was also done for the relative AUDPC in the experiments Piešťany 2006 and Tulln 2008 (Table 35). The effects detected near the centromere of chromosome 1B, in the linkage group 2BD6AB7B, on the short arm of 3B and on the long arm of 7B coincided with the

QTL for single assessments. Only the last one was consistent over both experiments and the mean over them. The QTL on chromosome 3B was identified in the experiment Piešťany 2006 and the mean, the other two only in Piešťany 2006. Not only in the case of single assessments, but also for the relative AUDPC the QTL on the long arm of chromosome 7B was much more effective, similar to single assessments. It was classified as a major QTL, explaining about 40 % of the phenotypic variance, whereas the effect on 3B explained less than 10 % (Table 86 and Table 87). Boxplots of the allele groups of both peak markers and all allele combinations are illustrated in Fig. 66. As for the QTL detected for single assessments, lines possessing both positive alleles had on average the lowest value of relative AUDPC and lines with the adverse alleles by far the highest. As the QTL inherited from Isengrain was more effective, the lines possessing the Isengrain allele at both loci had on average a relative AUDPC just slightly higher than those with the optimum allele combination.

In Fig. 67a-b the disease progress curves are plotted. Means of the parental genotypes Isengrain and Capo are shown in comparison to the RIL population. RILs are divided into four groups acccording to the alleles of the two markers for leaf rust resistance QTL. The first marker is the AFLP marker *XS12M13_33* on chromosome 3B (QTL inherited from Capo). The second marker is SSR marker *Xgwm132.1* (QTL inherited from Isengrain). In the left part (Fig. 67a) curves are shown for the experiment Piešťany 2006, in the right part (Fig. 67b) for the experiment Tulln 2008. On the first day of disease assessment in 2006, all groups did not differ much. Until the end of the scoring period the difference between the groups with lowest and highest leaf rust severity was about 10 % infected leaf area. In the experiment Tulln 2008 the difference increased from 15 % at the beginning to more than 20 % at the second scoring and declined again a little bit towards the end due to a linear increase in leaf rust severity of the lines with the allele combination IC and a progressing increase of the three others. Whereas the increase in infected leaf area was almost linear for the parental line Isengrain, it was lower for Capo between first and second scoring, but faster than Isengrain between second and third assessment.

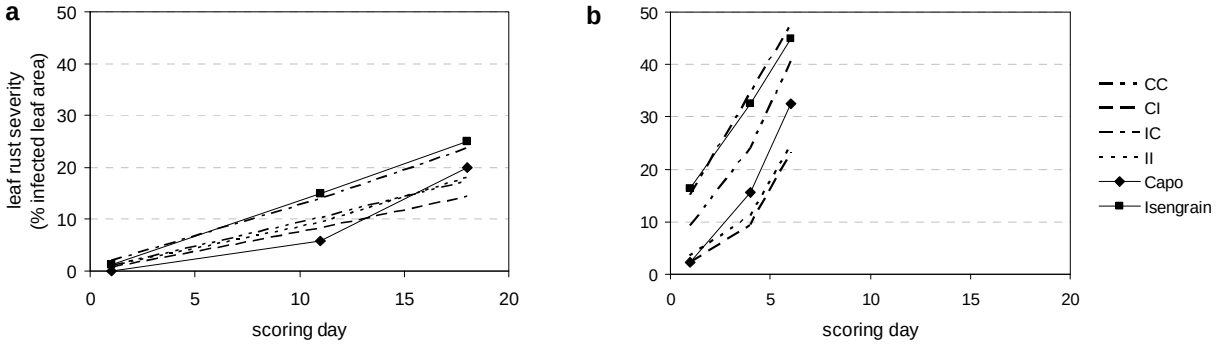

Fig. 67a-b Disease progress curves for leaf rust severity of the population Isengrain/Capo in the experiments Piešťany 2006 (a) and Tulln 2008 (b). Means of the parental genotypes Capo and Isengrain are shown in comparison to the four RIL group means of the parental allele combinations for the QTL markers *XS12M13_33* (chromosome 3B) and *Xgwm132.1* (7B)

In the experiment Tulln 2004 the appearance of teliospores of *P. triticina* was recorded on a yes/no scale (Fig. 36). An effect associated with this trait was detected on the long arm of chromosome 7B coinciding with the QTL for leaf rust severity of single assessments and AUDPC in a distance of less than 1 cM from the marker *Xgwm132.1* (Table 86, Fig. 57, Fig. 91). Explaining 15 % of the phenotypic variance it was classified as a major QTL.

5.4.1.2 QTL for Other Traits

Although the parental line Capo was in all experiments **heading** on the same or just a few days later than Isengrain (Table 49), two QTL for heading were identified in the RIL population (Table 86). Both effects were consistent over all experiments in which heading was assessed except Piešťany 2007 and the mean over them (Table 87). The explained phenotypic variance was 9 % (8-14 % in the single experiments) and 70 % (52-94 %). At both loci earliness was associated with the Isengrain allele. Boxplots of the different allele groups of the markers *Xgwm132.3* and *Xgwm484* and all possible allele combinations are illustrated in Fig. 68. The average difference between those lines carrying at both loci the Capo allele and those with the Isengrain allele was slightly bigger than for the single loci, the standard deviation was reduced. This suggests that the loci in the linkage group assigned to the short arm of chromosome 2D (Fig. 43, Fig. 84) and in the yet not definitely assignable linkage group 2BD6AB7B (Fig. 59, Fig. 92) are different.

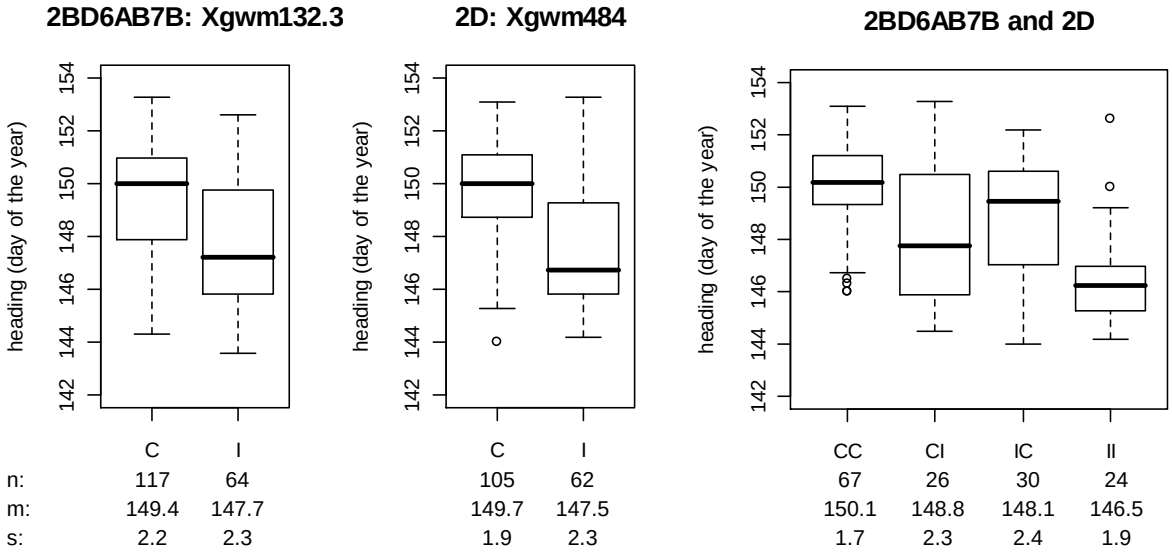

Fig. 68 Heading, mean over eleven experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 149.7, Isengrain: 147.5

For the trait **plant height** QTL analysis revealed four effects in the population Isengrain/Capo (Table 86 and Table 87). The QTL located in a linkage group assigned to the long arm of chromosome 2B (Fig. 42) was only detected in the experiment Tulln 2007 and thus not considered in further analysis. Only the two QTL located near the centromere of chromosome 4B (Fig. 48, Fig. 87) and the yet not definitely assigned linkage group 6AB (Fig. 59, Fig. 94) were consistent over all five experiments in which plant height was

assessed (Table 58). These two each explained 22 % (14-27 % in the single experiments) and 15 % (11-14 %) of the phenotypic variance and were thus classified as major QTL. A further but less strong effect was detected in the linkage group 2B4B5A7B (Fig. 59, Fig. 93), but only in two experiments and the mean over experiments. Boxplots of the different allele groups of the markers *XS22M17_5*, *Xgwm107.2* and *Xbarc146.2*, associated with the three most effective QTL for plant height, and all possible allele combinations are illustrated in Fig. 69. At all loci the allele for taller plants originated from Capo. The average difference between plants carrying the Capo allele at all three loci and those with the Isengrain alleles was larger than for the single loci, the standard deviation was reduced. The parental line Isengrain was slightly taller than the shortest plants with the allele for reduced plant height at all three loci, but below the average height of these plants. Lines carrying the Capo allele at all three loci were on average shorter than Capo

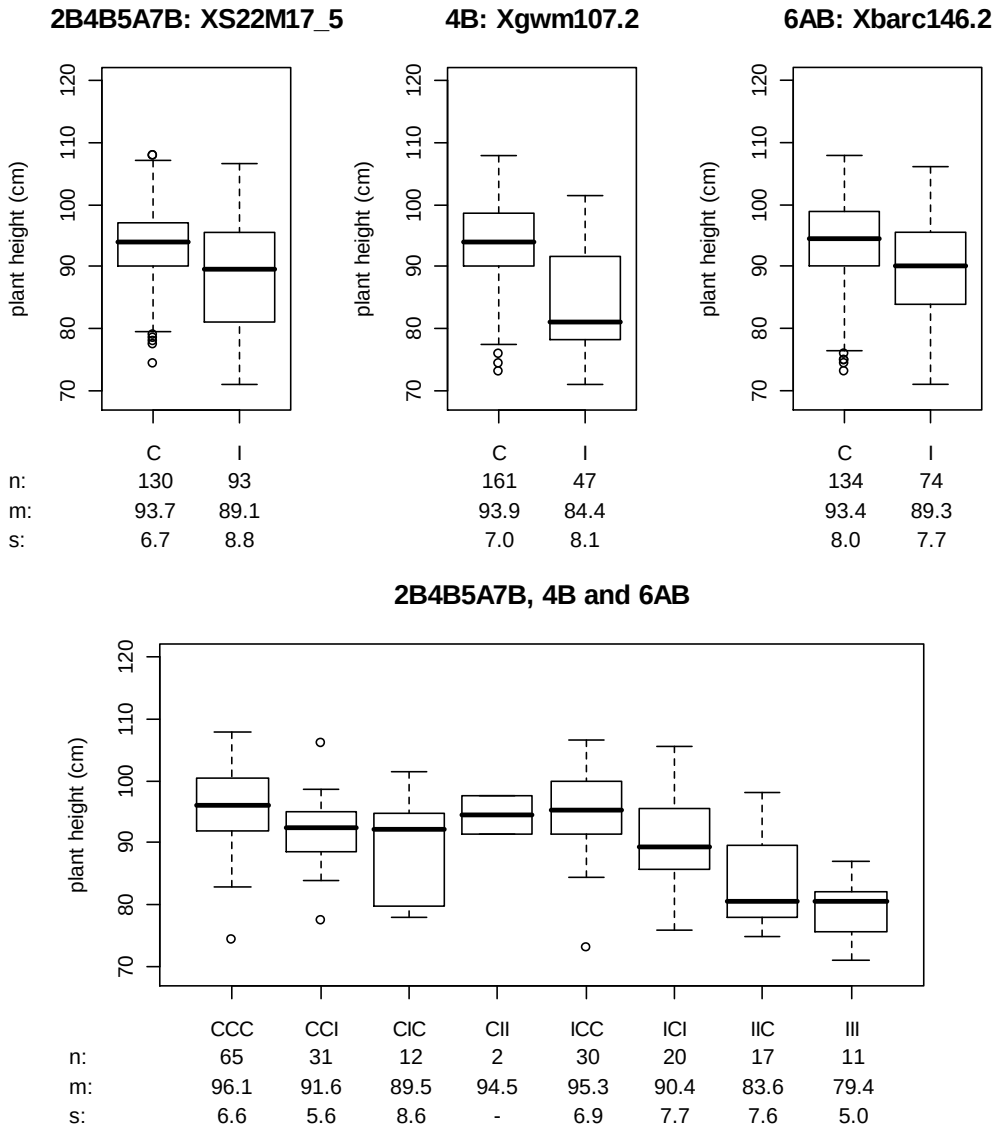

Fig. 69 Plant height, mean over five experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 99.8, Isengrain: 75.0

itself, but several plants of some allele combinations were taller. It is not clear whether all three loci are independent.

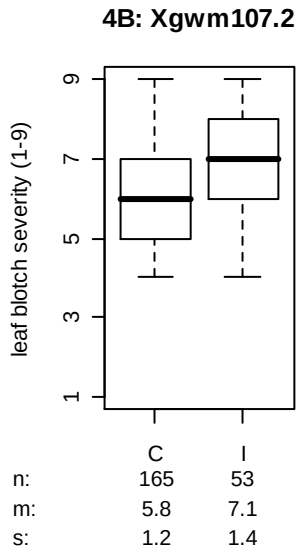

Fig. 70 Leaf blotch severity: Boxplots of the different allele groups of marker *Xgwm107.2* linked to a QTL. Capo: 5.1, Isengrain: 8.4

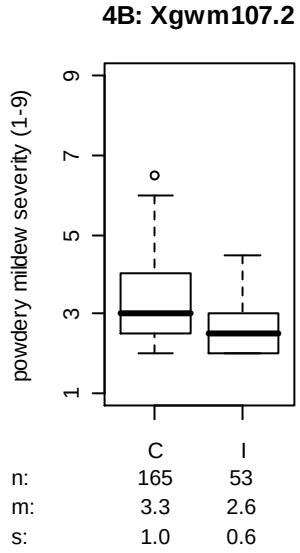

Fig. 71 Powdery mildew severity: Boxplots of the different allele groups of marker *Xgwm107.2* linked to a QTL. Capo: 3.3, Isengrain: 3.3

QTL detection for the traits **leaf blotch severity** and **powdery mildew severity** assessed on a 1 (= no visible symptoms) to 9 (= completely diseased) scale revealed effects located near the centromere of chromosome 4B (Fig. 48, Fig. 87) coinciding with the QTL for plant height and leaf chlorosis severity (Table 86, Table 87). For both traits results are only based on single experiments (Aumühle 2004 for leaf blotch severity and Probstdorf 2006 for powdery mildew severity, Table 67). The QTL for leaf blotch severity accounted for 19 % of the phenotypic variance. The Capo allele was associated with reduced susceptibility; lines carrying this allele were on average slightly more diseased than Capo itself. The mean of the plants with the Isengrain allele was clearly below the parental line.

The boxplots are illustrated in Fig. 70. Despite no difference in powdery mildew infection between the two parental lines, the detected effect was classified as a major QTL explaining 12 % of the phenotypic variance. The allele from Isengrain conferred reduced disease severity. In Fig. 71 the boxplots are illustrated.

Despite that **lodging severity** was assessed in the experiment Probstdorf 2007 on a 1 (= no lodging) to 5 (= complete lodging) and in the experiments in 2008 on a 0 (= no lodging) to 5 (= complete lodging) scale (Table 67), a mean over the assessments was calculated for QTL analysis. Minor effects were identified on the short arm of chromosome 6B (Fig. 54, Fig. 90) and in the yet not definitely assigned linkage group 6AB (Fig. 59), the latter solely in the experiment Probstdorf 2008. The QTL on chromosome 6B accounted for 9 % of the variance and was detected in the experiment Probstdorf 2008 and the mean (Table 86, Table 87). Lodging severity of Capo was well beyond the mean of the lines with its allele, lodging severity of Isengrain well below the lines carrying the other.

QTL analysis of **leaf chlorosis severity** assessed on a 0 (= no visible symptoms) to 9 (= extremely chlorotic) scale in the two experiments Rust and Tulln 2009 presented in Table 67 revealed effects on the long arm of chromosome 2D (Fig. 43, Fig. 84), near the centromere of 4B (Fig. 48, Fig. 87) and on the long arm of 7B (Fig. 57, Fig. 91). The effect on chromosome of 4B coincided with the QTL for plant height, leaf blotch severity and powdery mildew severity. Solely the effects on 2D and 4B were consistent over both experiments, the one on 7B was not identified in Tulln 2009 (Table 86, Table 87). The QTL on 2D seemed to be located between the markers *Xgwm484* and *Xgwm539*, a region around the centromere with no single marker within a distance of more than 35 cM, in another chromosomal part than heading and accounted for 12 % of the phenotypic variance, the others for 7 %. Boxplots of the different allele groups of the single markers and all possible allele combinations are illustrated in Fig. 72. The alleles on 2D and 4B conferring reduced leaf chlorosis severity originated from Capo, on 7B from Isengrain. The mean difference between the lines with the ideal and the adverse allele combination was clearly larger than for the single loci, the standard deviation decreased or increased.

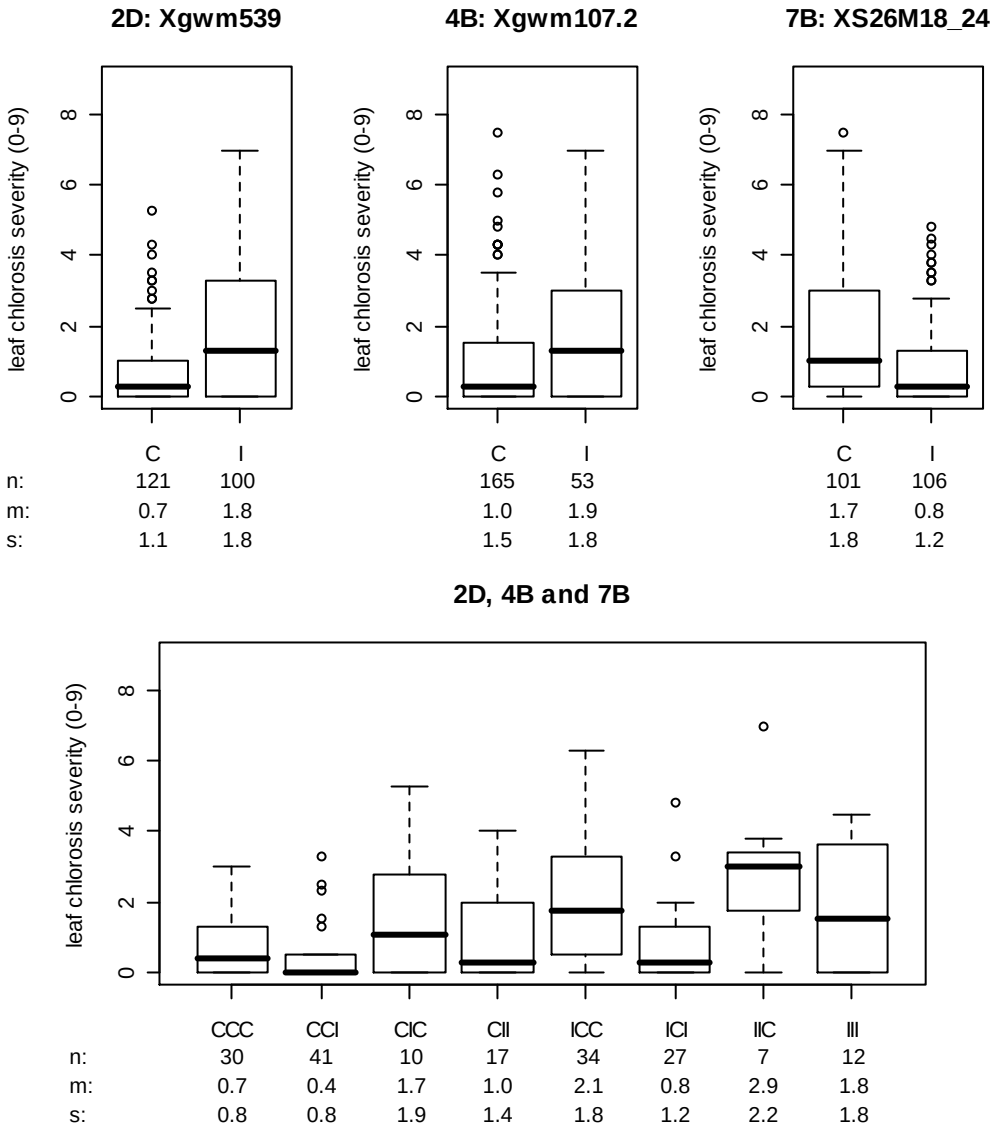

Fig. 72 Leaf chlorosis severity, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 0.5, Isengrain: 0.2

Leaf tip necrosis severity was assessed in the same two experiments as leaf chlorosis severity on a 0 (= no visible symptoms) to 9 (= heavy symptoms) scale (Table 67). By interval mapping effects associated with leaf tip necrosis severity were identified near the centromere of chromosome 2A (Fig. 41, Fig. 83), on the long arm of the chromosomes 4A (Fig. 47), 4B (Fig. 48, Fig. 87) and 5B (Fig. 51, Fig. 89). Only the QTL on chromosome 5B was consistent over both experiments and the mean (Table 86, Table 87). In the single experiments it explained 8-9 % of the phenotypic variance, and 9 % for the mean. The QTL on 4B was located at a distance of 16.5 cM from the QTL for plant height, leaf blotch severity, powdery mildew severity and leaf chlorosis severity and accounted for 12 % (Tulln 2009) and 10 % (mean over experiments) of the phenotypic variation, the QTL on 2A 8 % (Rust 2009) and 7 % (mean) and the QTL on 4A only 7 % (Rust 2009). In the case of 2A and 4A the allele for reduced leaf tip necrosis severity originated from Isengrain, in

the others Capo transmitted the positive allele. Boxplots of the allele groups of the markers *XS16M16_21*, *XS11M18_6* and *XS23M14_7* and all possible allele combinations are illustrated in Fig. 73. The lines with the ideal allele combination exhibited well more symptoms than Capo, but less than Isengrain. The average difference between these lines and those with the adverse allele combination was clearly larger than for the single loci, the standard deviation was reduced only marginally.

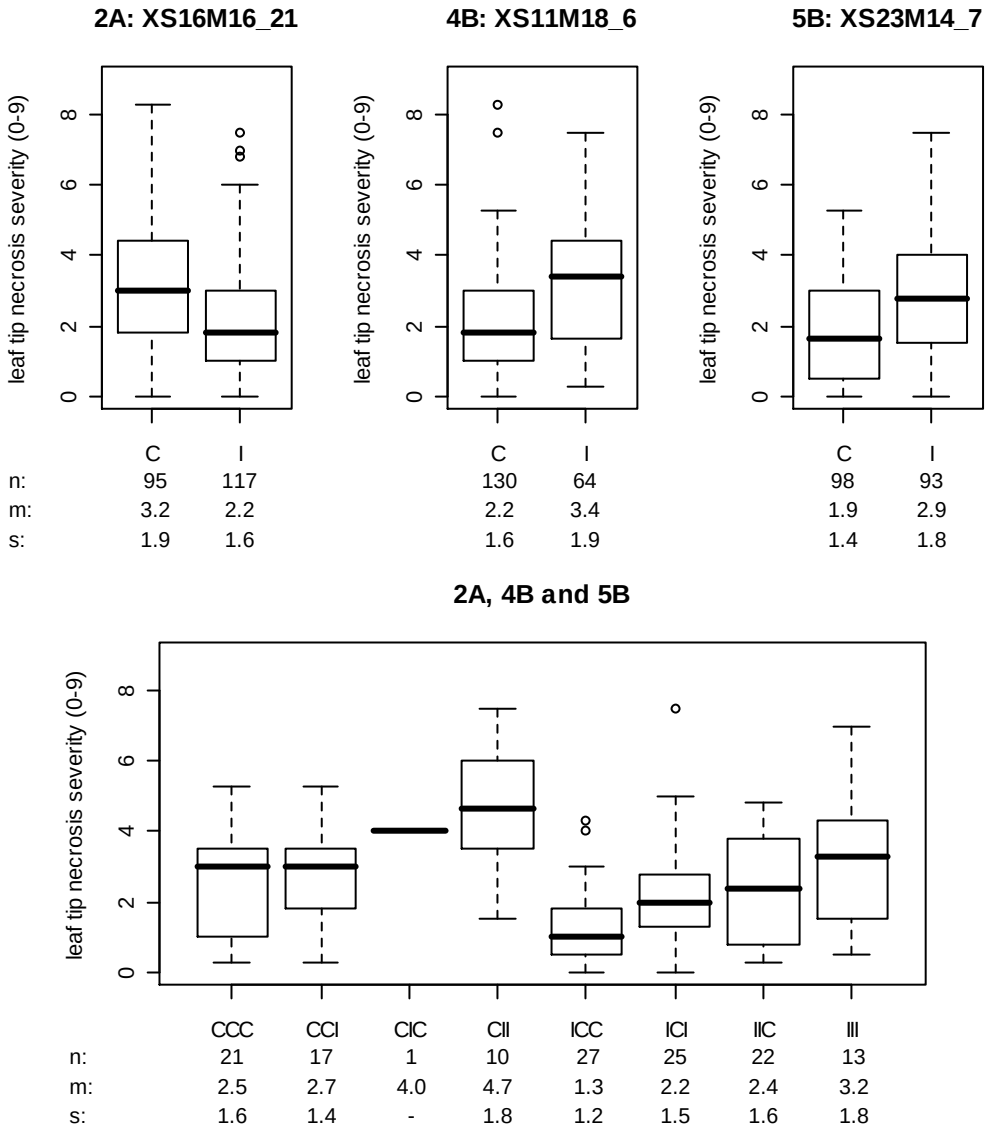

Fig. 73 Leaf tip necrosis severity, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Capo: 0.8, Isengrain: 2.1

5.4.2 Population Arina/Capo

By interval mapping several QTL were detected in the population Arina/Capo. The values of LOD thresholds were only about 2/3 compared to the population Isengrain/Capo. The detected QTL and LOD thresholds are presented in Table 90. For each chromosome the LOD values of the detected QTL are given in the first line, the corresponding R^2 in the second line, the additive effect in the third line and the marker next to the QTL peak in the fourth line. In addition interval mapping was also done for all single experiments. All QTL with a LOD ≥ threshold are listed in Table 88 in black, not significant effects in grey color. Experiments are abbreviated as described in Table 4 on page 40. Means were calculated over the same experiments as in chapter 5.1. For all QTL the parental allele conferring a lower scoring value is given too. In the experiment Piešťany 2008 and for the traits not listed no QTL was identified.

Table 90 Detected QTL, LOD thresholds, chromosomes, LOD values (1st line), R^2 (2nd line), additive effects (3rd line) and nearest marker (4th line) in the population Arina/Capo (interval mapping)

QTL	3A	3B	4B	5A	5B	6B
leaf rust severity		3.69				
1.94		0.084				
		-3.55				
		Xgwm389.1				
relative AUDPC		3.92				
1.97		0.082				
		-2.22				
		Xgwm389.1				
plant height				2.19		2.26
1.90				0.077		0.047
				-1.62		-2.01
				Xgwm1057		*Xgwm518.*1
yellow rust severity		10.90				
1.88		0.200				
		-0.47				
		Xbarc75				
glaucousness	12.61		2.47			
1.98	0.430		0.050			
	-0.58		0.31			
	Xgwm720		*Xgwm149*			
lodging severity				3.09		
1.95				0.093		
				-0.29		
				Xgwm1057		
leaf chlorosis severity	2.61					
1.92	0.051					
	0.42					
	Xgwm1110					
leaf tip necrosis severity		2.43		2.12	4.18	
1.87		0.108		0.091	0.086	
		-0.27		0.28	-0.48	
		Xgwm108		*Xgwm1057*	*Xgwm604*	

A low value of leaf chlorosis severity was inherited from Arina. Low values of leaf rust severity (measured by the relative AUDPC), plant height, awnedness, yellow rust severity and lodging severity originated from Capo. Both parents passed on low values of leaf rust severity (measured by the percentage of infected leaf area), glaucousness and leaf tip necrosis severity to the offspring. The positions of all detected QTL are shown in Fig. 83 to Fig. 94.

As only 21 out of 36 markers scored for the population Arina/Capo were assigned to linkage groups, the remaining 15 markers were tested by means of single point ANOVA. To make results of QTL analysis by interval mapping and single point ANOVA comparable, the risk of the first kind α was set to 0.01 as p-values of markers with LOD values beyond the threshold in QTL analysis were below this level. Five further markers with significant association to the traits leaf rust severity (% infected leaf area and relative AUDPC), *Septoria* leaf blotch severity, glaucousness, lodging severity and leaf tip necrosis severity were detected. For leaf rust severity and glaucousness Capo contributed the alleles associated with a lower value, for *Septoria* leaf blotch severity and lodging severity Arina, and for leaf tip necrosis severity both parents. Table 89 lists the markers, the chromosomes to which these markers were assigned in the population Isengrain/Capo and the additive effect.

In Fig. 74, Fig. 75 and Fig. 77 to Fig. 82 boxplots are shown of all traits for which QTL were detected in the population Arina/Capo. The groups are separated according to the alleles of the marker next to the QTL peak. The alleles are named after the parental line from which they are inherited: A = Arina, C = Capo. Below the boxplots the number of RILs (n), their mean value (m) and their standard deviation (s) are given.

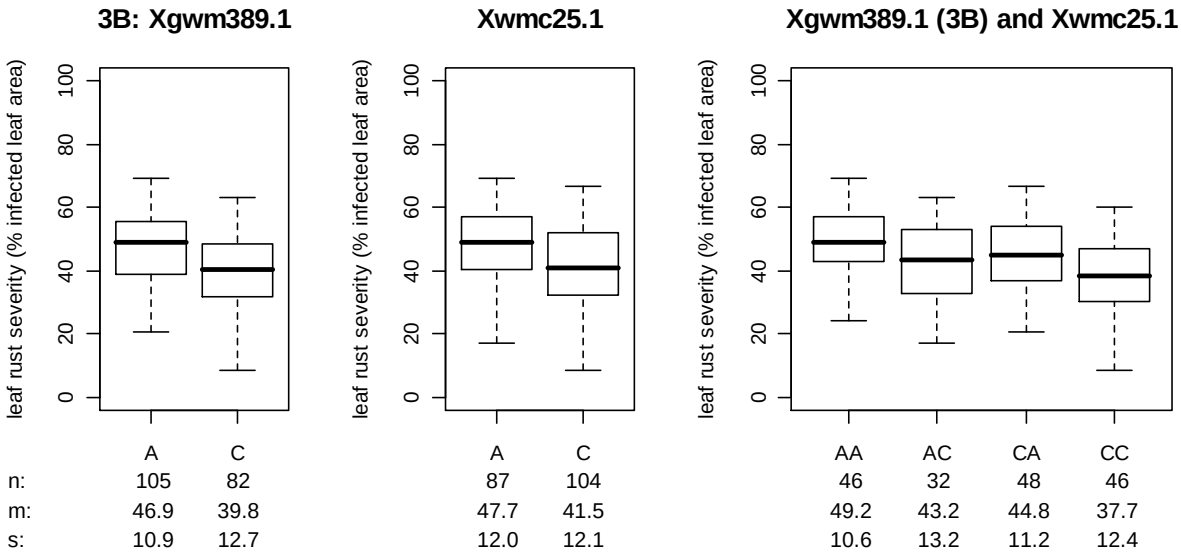

Fig. 74 Leaf rust severity, mean over eleven experiments: Boxplots of the different allele groups of the markers linked to QTL. Arina: 66.3, Capo: 23.4

Fig. 74 illustrates boxplots of the different allele groups and all possible allele combinations of the QTL markers for the trait **leaf rust severity** (measured by the percentage of infected leaf area). Results are based on the experiments in Rust

(2008-2009), Tulln (2007-2009), Fundulea, Martonvásár, Nyon, Piešťany, Probstdorf and Schmida (all 2008) as listed in Table 29. The first marker *Xgwm389.*1 was mapped to a linkage group assigned to the short arm of chromosome 3B (Fig. 45, Fig. 86). As the explained phenotypic variance was between 5-12 % for the single experiments and 8 % for the mean, it was classified as a minor QTL only (Table 90). It was beyond the threshold in seven of these experiments (Table 88). The second marker *Xwmc25.1* remained yet unlinked (Table 89). Also in the population Isengrain/Capo it was associated with a QTL for reduced leaf rust severity, but was not definitely assigned to a chromosome. At both loci the positive allele was inherited from Capo. The mean value and the range for lines carrying at both loci the Arina allele was highest, whereas they were lowest for lines carrying at both loci the Capo allele. Due to the larger difference for the allele combination compared to the single loci it seems that these two loci are independent. Because of the average difference between the parents being much larger than for the different allele groups, and due to the small explained phenotypic variance, it is assumed that there are much more loci involved in leaf rust resistance that have not been detected yet. A further minor effect transmitted by Arina alleles was detected on chromosome 3A (Fig. 44), but only in the experiment Reichersberg 2008; another inherited from Capo on chromosome 5A (Fig. 50) in the experiment Tulln 2009.

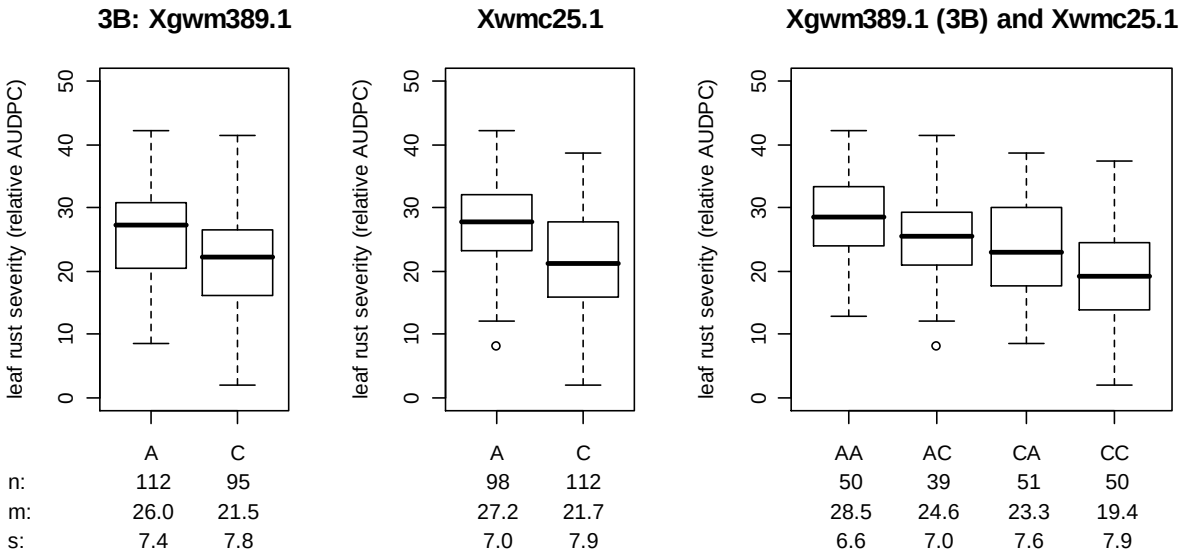

Fig. 75 Leaf rust severity, mean over three experiments: Boxplots of the different allele groups of the markers linked to QTL. Arina: 36.1, Capo: 7.9

Interval mapping for the trait leaf rust severity measured by the **relative AUDPC** revealed that the two detected QTL coincided with those identified for the single assessments (Table 88 to Table 90). The results are based on the experiments in Fundulea, Piešťany and Tulln (all 2008) as described in Table 38. The effect detected on the short arm of chromosome 3B (Fig. 45, Fig. 86) was consistent over the experiments Fundulea and Tulln 2008 and the mean over experiments and accounted for 5-6 % (single experiments)

and 8 % (mean) of the phenotypic variance. At both loci Capo conferred the allele for reduced relative AUDPC. The additive effect of the Capo allele of marker *Xwmc25.1* was slightly bigger than of the 3B allele. Boxplots of the different allele groups of the single markers and all possible allele combinations are illustrated in Fig. 75. As for the single assessments, the average difference between lines carrying the Arina and the Capo allele was slighter for the single loci than for the allele combination. Whereas the mean difference for the 3B marker was larger than for marker *Xwmc25.1*, if leaf rust severity of single assessments was considered, it was the other way round when measured by the relative AUDPC. The bigger average difference for the allele combination compared to the single loci suggests that these two loci are independent.

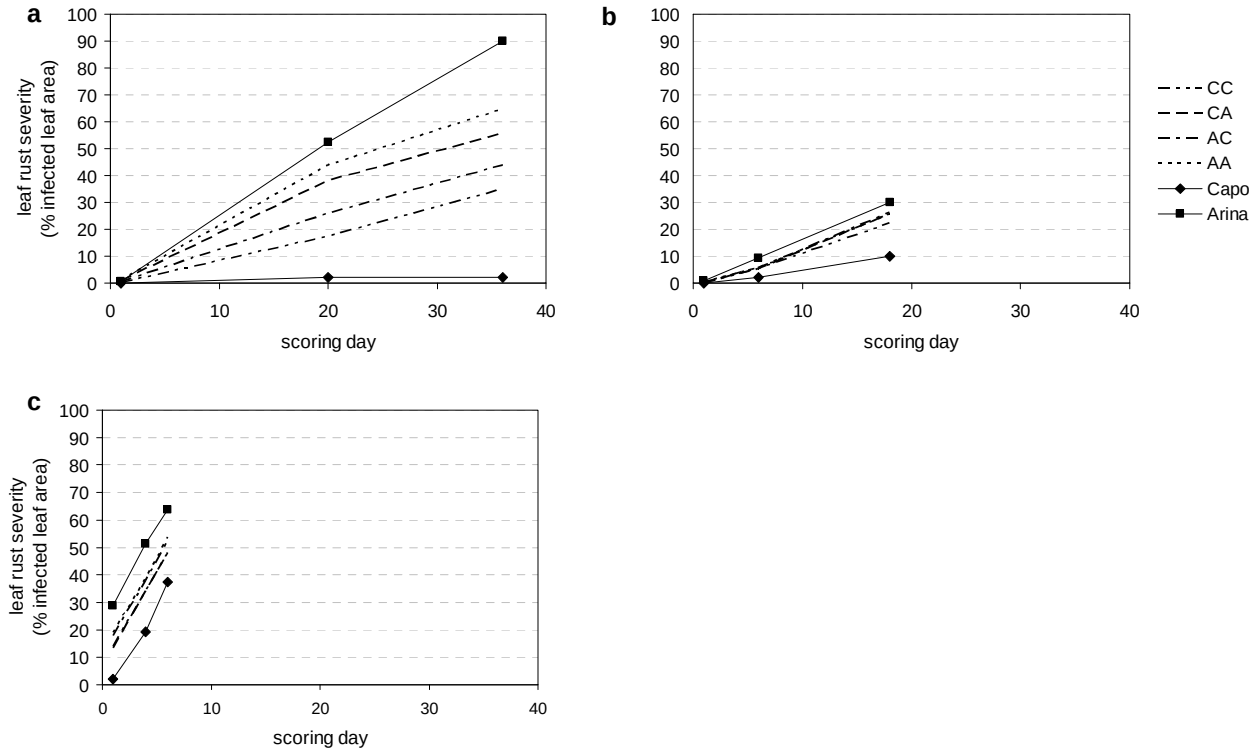

Fig. 76a-c Disease progress curves for leaf rust severity of the population Arina/Capo in the experiments in Fundulea (a), Piešťany (b) and Tulln (c) 2008. Means of the parental genotypes Capo and Arina are shown in comparison to the four RIL group means of the parental allele combinations for the QTL markers *Xgwm389.1* (chromosome 3B) and *Xwmc25.1* (unlinked)

In Fig. 76a-c the disease progress curves for all three experiments with three repeated assessments are plotted. Means of the parental genotypes Arina and Capo are shown in comparison to the RIL population. RILs are divided into four groups according to the alleles of the two markers for leaf rust resistance QTL. The first marker is SSR marker *Xgwm389.1* on chromosome 3B, the second the yet unlinked marker *Xwmc25.1*. For both QTL the allele conferring reduced disease severity originated from Capo. On the first day of assessment in Fundulea 2008 (Fig. 76a) all lines and both parents exhibited almost no symptoms. Until the third assessment more than one month later, infection increased

nearly linearly. As would be expected, the average increase in leaf rust severity of lines carrying at both loci the Arina allele was fastest and of those with the Capo alleles it was lowest. Even on the last scoring day Capo showed almost no symptoms, whereas Arina was most heavily diseased. Similar in the experiment Piešťany 2008 (Fig. 76b) all lines and the parents started from a very low disease level on the first assessment day. The average increase in leaf rust severity of all groups and of Arina was lower than in Fundulea, only of Capo it was higher. It seems that the scoring period was too short for detecting significant differences between the lines with different allele combinations. In the experiment Tulln 2008 (Fig. 76c) the scoring period was much shorter, but it started from a higher infection level. Disease progress curves were almost parallel for the parents and the different groups between the first and the second assessment. The differences between the four allele groups were less than five percent, between the parents almost 30 % with the RIL combination lying about in the middle. From the second to the third scoring disease progress of Arina slightly decreased, whereas it increased for Capo, resulting in an average difference of about 25 %.

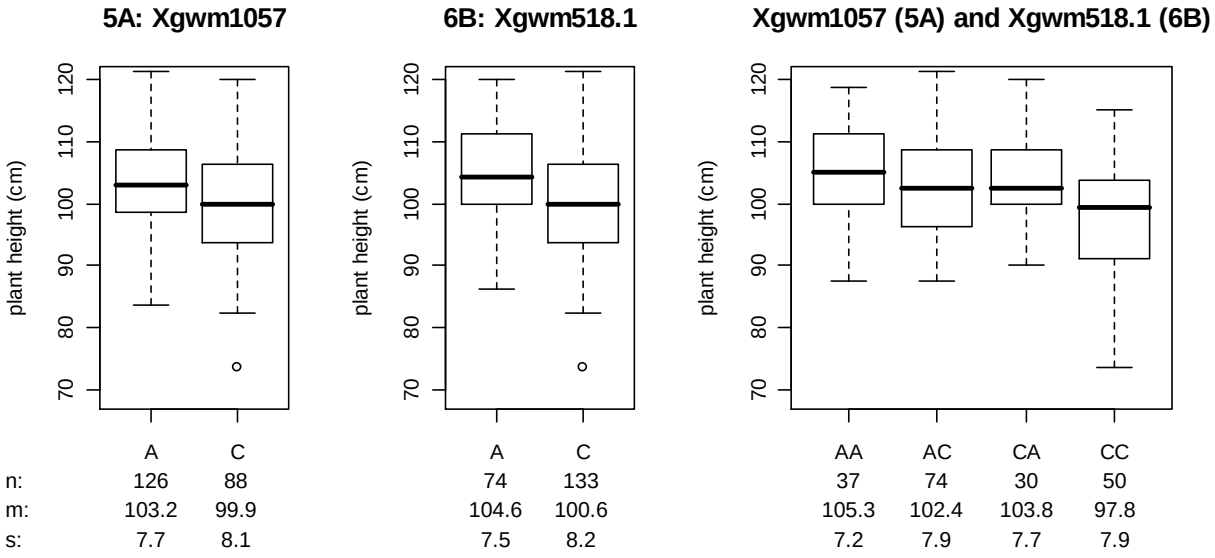

Fig. 77 Plant height, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Arina: 101.2, Capo 102.1

Despite the low difference in **plant height** between the parents Arina and Capo two QTL for plant height were identified in linkage groups assigned to the long arm of chromosome 5A (Fig. 50, Fig. 88) and to the short arm of 6B (Fig. 54). The boxplots of the different allele groups and all possible allele combinations are shown in Fig. 77. Results are based on the experiments in Tulln 2007 and 2008 presented in Table 61. In the first year the mean value of the variety Capo was higher than for Arina, in the second year it was the other way round. The allele for taller plants was in both cases inherited from Arina.
Both QTL were detected for the mean over experiments and explained 8 % (chromosome 5A) and 5 % (6B) of the phenotypic variance. Whereas the QTL on 5A was furthermore identified in the experiment Tulln 2007 (9 %), the effect on 6B was visible in the experiment Tulln 2008 (6 %) (Table 88 and Table 90).

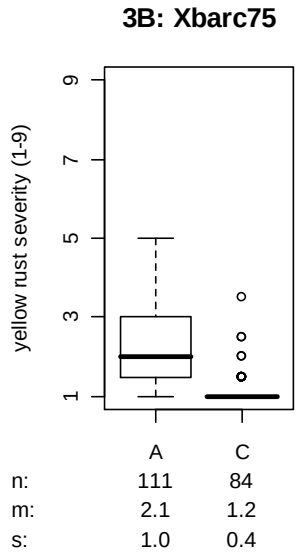

Fig. 78 Yellow rust severity: Boxplots of the different allele groups of marker *Xbarc75* linked to a QTL. Arina: 2.5, Capo: 1.5

For **yellow rust severity** scored on a 1 (= no infection) to 9 (= completely diseased) scale an effect was identified on the short arm of chromosome 3B (Fig. 45, Fig. 86) coinciding with the QTL for leaf rust severity. Results are based only on data of the experiment Nyon 2008 presented in Table 68. The allele associated with increased resistance originated from Capo. Just 19 out of 84 lines carrying the Capo allele were not scored with the best grade one. With a maximum LOD value of 10.90 (Table 88 and Table 90) it was the second most effective QTL detected in the population Arina/Capo, explaining 20 % of the phenotypic variability. Thus it was classified as a major QTL. Fig. 78 illustrates the boxplots of the different allele groups of marker *Xbarc75*.

For the trait **glaucousness** assessed on a 1 (= no wax) to 5 scale in the experiment in Rust 2009 (Table 68) three effects were identified. In the case of the QTL located near the centromere of chromosome 4B (Fig. 48, Fig. 87) the Arina allele was associated with a lower level of glaucousness. For the QTL detected on chromosome 3A (Fig. 44, Fig. 85) and the third near *Xgwm120* (unlinked), reduced glaucousness was inherited from Capo (Table 88 to Table 90). In the population Isengrain/Capo *Xgwm120* was assigned to the long arm of chromosome 2B. The QTL located on chromosome 3A was the most effective in the population Arina/Capo accounting for 43 % of the phenotypic variance, the effect on 4B explained only 5 %. The corresponding boxplots of the different allele groups of the markers *Xgwm720*, *Xgwm149* and *Xgwm120* and all possible allele combinations are shown in Fig. 79. Whereas the mean value of the lines carrying at all three loci the allele for increased glaucousness was the same as of Arina, the mean value of lines with the alleles for low glaucousness was beyond the value of Capo. Due to the large number of different allele combinations sample size for each group was too low for reliable conclusions. For example in the group with the Capo allele at all three loci ten out of the 17 lines were scored with 1. Present results permit the assumption that *Xgwm120* is a

locus not linked to the QTL region on chromosome 3A. Compared to the single loci the average difference between lines with the allele combination for low vs. high glaucousness increased, while the standard deviation remained almost constant.

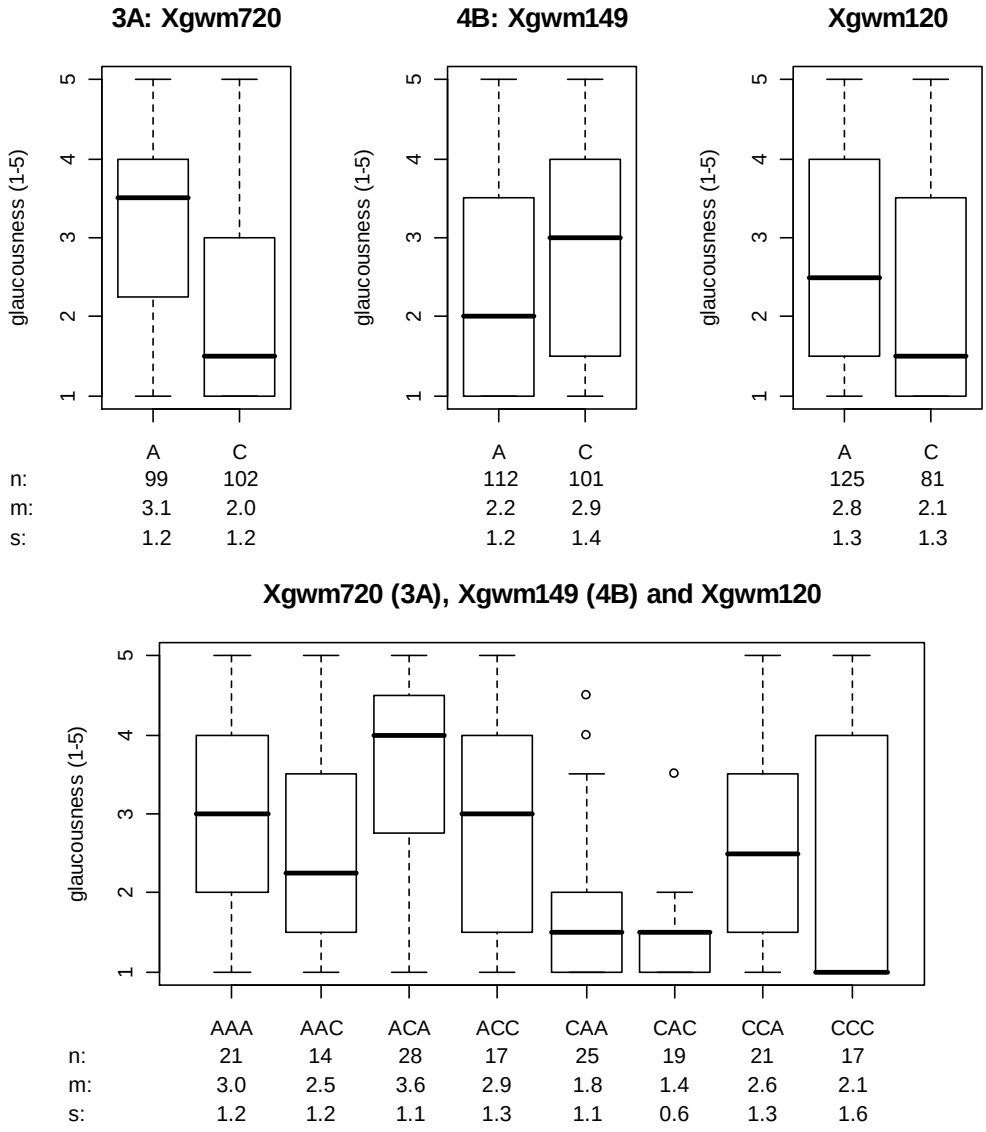

Fig. 79 Glaucousness: Boxplots of the different allele groups of the markers linked to QTL. Arina: 3.6, Capo: 1.0

Marker *Xgwm120* was not only associated with glaucousness, but also with **lodging severity**. This trait was scored in the experiments Probstdorf and Rust 2008, in which severe lodging occurred (Table 68), despite slight difference between the parental lines Arina and Capo. Lines were rated on a 0 (= no lodging) to 5 (= complete lodging) scale. Whereas the positive allele at locus *Xgwm120* was inherited from Arina, in the case of the second QTL located on chromosome 5A (Fig. 50, Fig. 88) the positive allele originated from Capo. These two QTL had almost the same additive effect and were detected in both experiments and the mean (Table 88 to Table 90). The effect on chromosome 5A explained 9 % (6-7 %) of the phenotypic variation. The boxplots of the different allele

groups of the markers *Xgwm1057* and *Xgwm120* and all possible allele combinations are presented in Fig. 80.

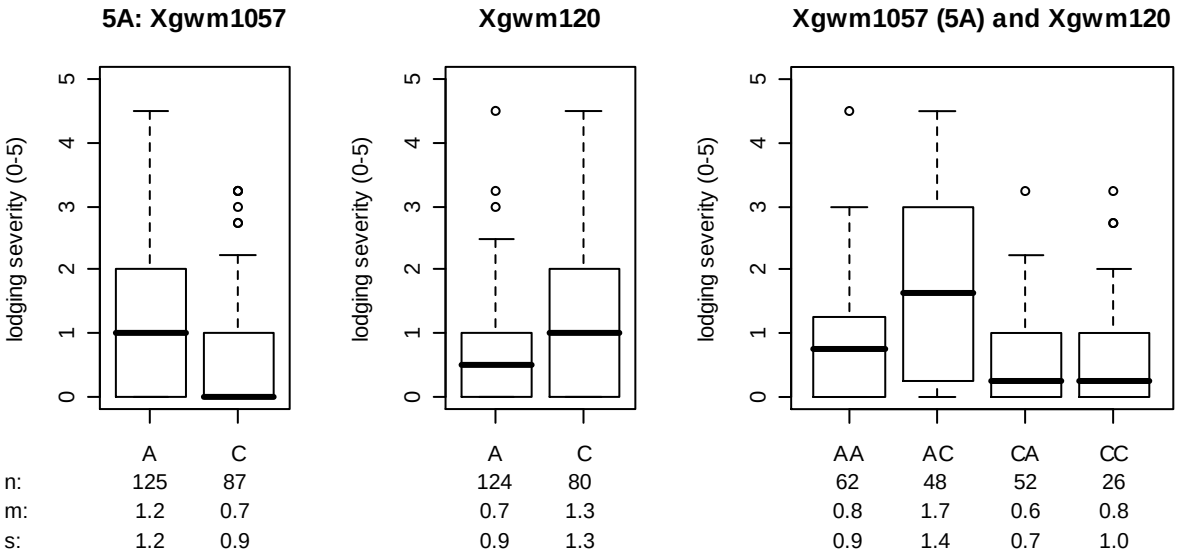

Fig. 80 Lodging severity, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Arina: 0.9, Capo: 0.6

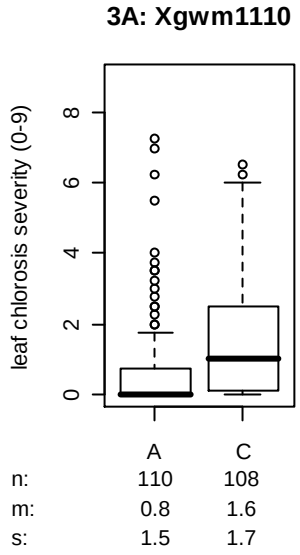

Fig. 81 Leaf chlorosis severity, mean over two experiments: Boxplots of the different allele groups of marker *Xgwm1110* linked to a QTL. Arina: 0.0, Capo: 1.1

For the trait **leaf chlorosis severity** assessed on a 0 (= no visible symptoms) to 9 (= extremely chlorotic) scale in the two experiments Rust and Tulln 2009 (Table 68) an effect was detected on chromosome 3A in a distance of about 5 cM from the QTL for glaucousness (Fig. 44, Fig. 85). This minor effect was consistent over both experiments and the mean over experiments and accounted for 5 % of the phenotypic variation (Table 88 and Table 90). The allele for reduced leaf chlorosis severity was inherited from Arina. The boxplots of the different allele groups of marker *Xgwm1110* are illustrated in Fig. 81.

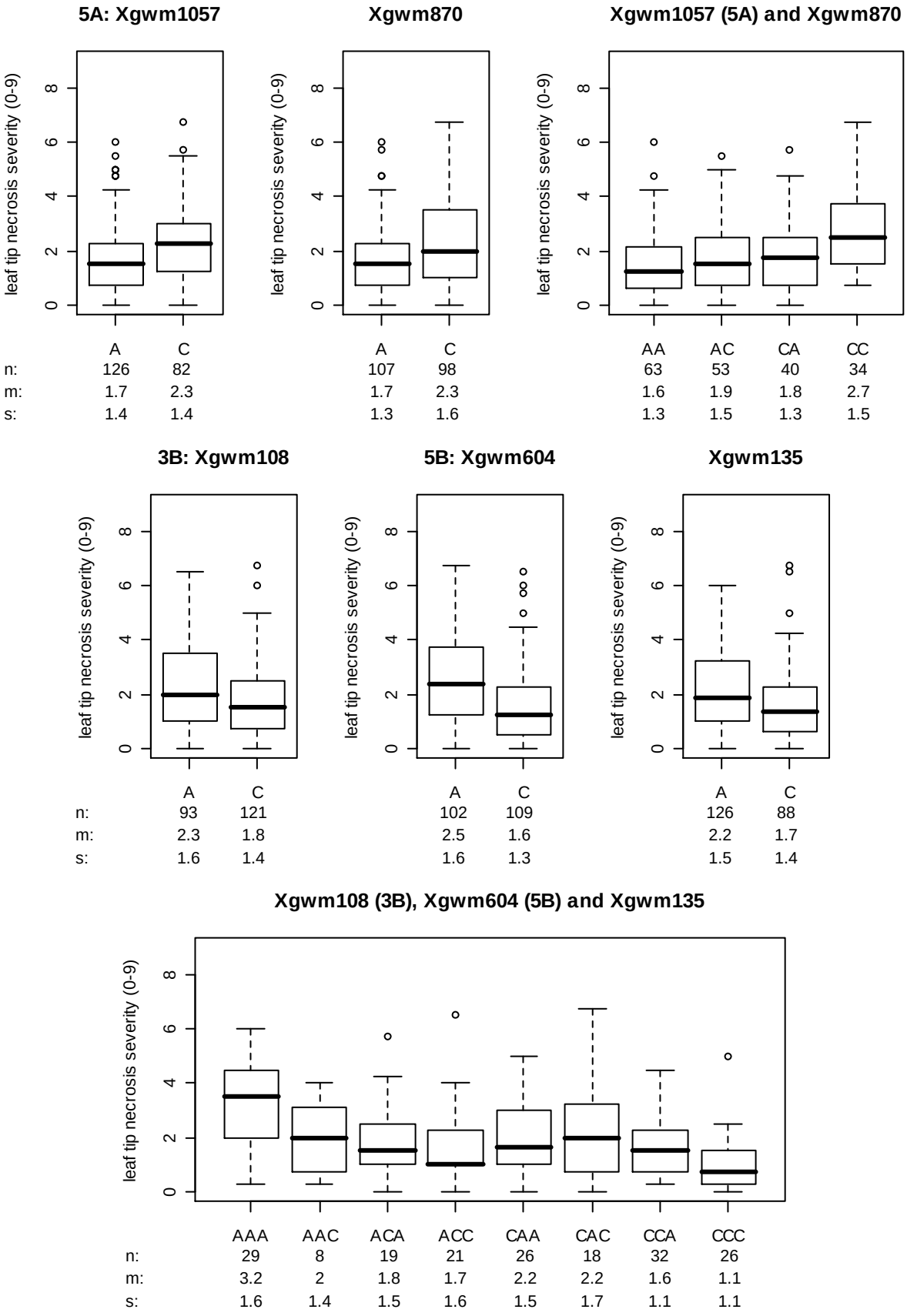

Fig. 82 **Leaf tip necrosis severity, mean over two experiments: Boxplots of the different allele groups of the markers linked to QTL. Upper part: reduced susceptibility conferred by Arina alleles, middle and lower part: transmitted by Capo alleles. Arina: 1.1, Capo: 0.9**

Despite the slight difference between the parental lines Arina and Capo (Table 68) for the trait **leaf tip necrosis severity** rated on a 0 (= no visible symptoms) to 9 (= heavy symptoms) scale in the two experiments Rust and Tulln 2009 (Table 68) five effects were identified. The QTL located on long arm of chromosome 3B (Fig. 45, Fig. 86) explained 11 % of the phenotypic variance, and was thus about as effective as the QTL on 5A, but had a lower additive effect than the QTL on 5B and was not detected in the experiment Rust 2009, whereas the 5A (Fig. 50, Fig. 88) and 5B (Fig. 51, Fig. 89) effects were consistent over both experiments and the mean over them (Table 88 and Table 90). Further effects were associated with marker *Xgwm135* that was assigned to chromosome 1A in the population Isengrain/Capo and *Xgwm870* (chromosome 7A in Isengrain/Capo). In the case of 5A and *Xgwm870* the Arina allele conferred reduced leaf tip necrosis severity, in the case of 3B, 5B and *Xgwm135* the Capo allele. In the upper part of Fig. 82 the boxplots of the allele groups and all possible allele combinations of the markers *Xgwm1057* and *Xgwm870* (Arina effects) are illustrated, in the middle and lower part of *Xgwm108*, *Xgwm604* and *Xgwm135* (Capo effects). In both cases the increased difference for the allele combination compared to the single loci and the at least slightly reduced standard deviation suggests that the not yet definitely assigned locus *Xgwm870* is different from 5A and *Xgwm135* different from 3B and 5B.

5.4.3 Comparison of QTL Detected in Isengrain/Capo and Arina/Capo

Isengrain/Capo

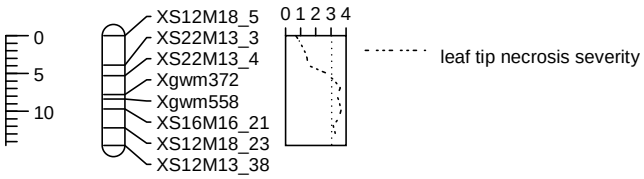

Fig. 83 **Interval analysis of a QTL for leaf tip necrosis severity (mean over two experiments) on a linkage group corresponding to a part of chromosome 2A**

Isengrain/Capo

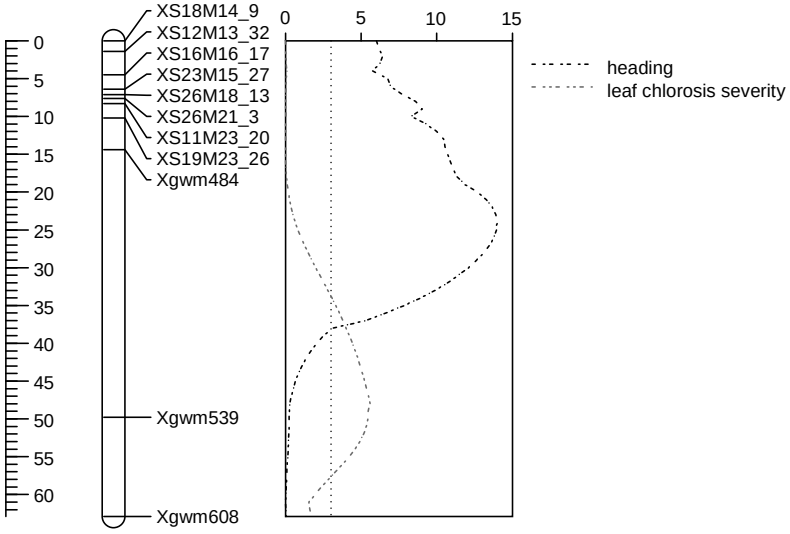

Fig. 84 **Interval analysis of QTL for heading (mean over eleven experiments) and leaf chlorosis severity (mean over two experiments) on a linkage group corresponding to a part of chromosome 2D**

Arina/Capo

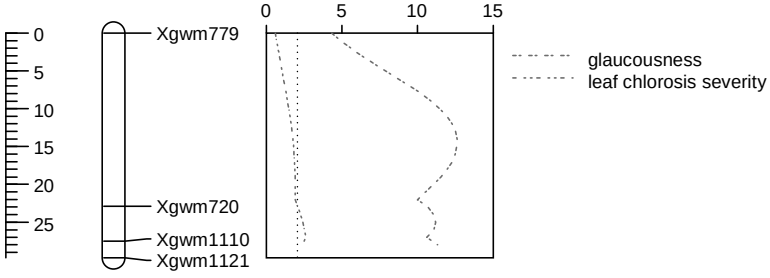

Fig. 85 **Interval analysis of QTL for glaucousness and leaf chlorosis severity (mean over two experiments) on a linkage group corresponding to a part of chromosome 3A**

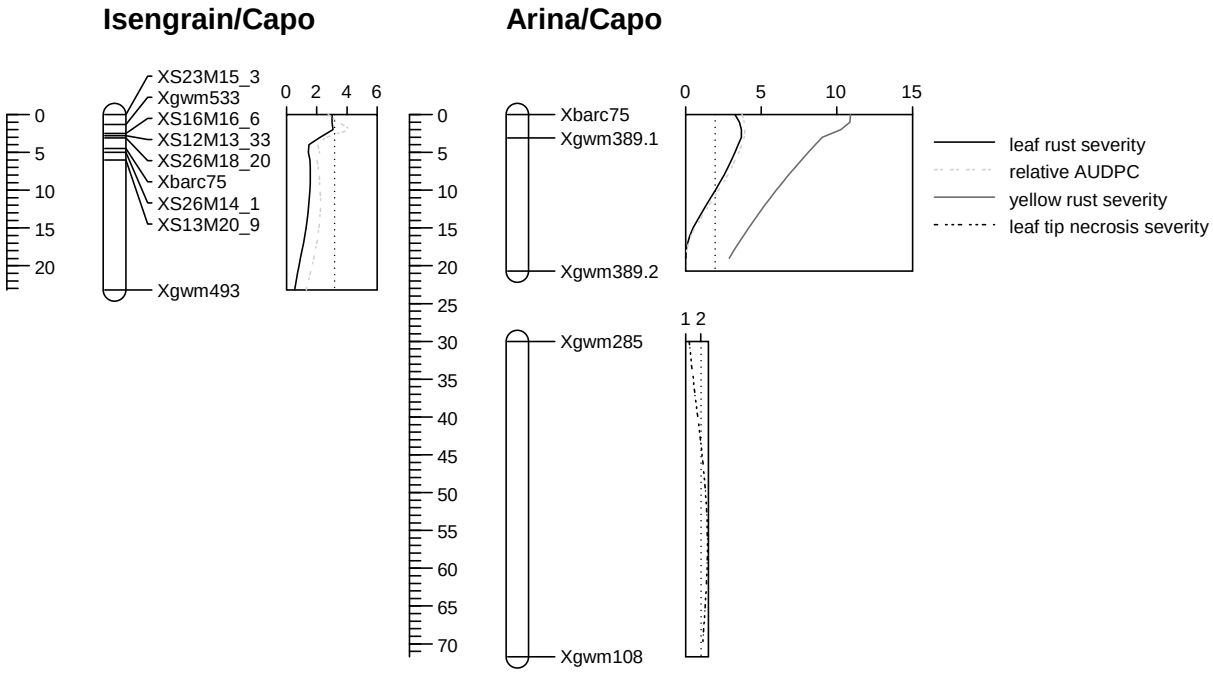

Fig. 86 **Interval analysis of QTL for leaf rust severity (mean over eleven experiments), relative AUDPC (mean over two/ three experiments), yellow rust severity and leaf tip necrosis severity (mean over two experiments) on linkage groups corresponding to parts of chromosome 3B**

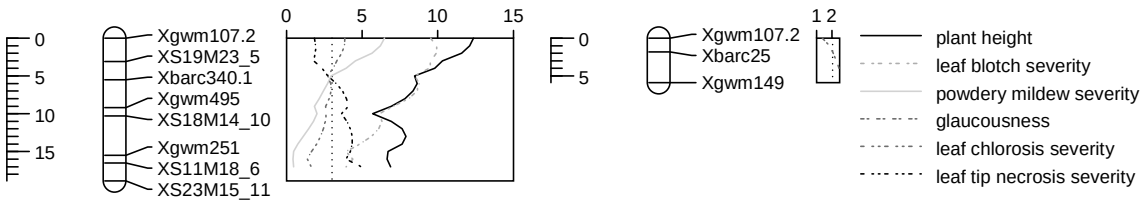

Fig. 87 **Interval analysis of QTL for plant height (mean over five experiments), leaf blotch severity, powdery mildew severity, glaucousness, leaf chlorosis severity (mean over two experiments) and leaf tip necrosis severity (mean over two experiments) on linkage groups corresponding to parts of chromosome 4B**

In Fig. 83 to Fig. 94 graphs of all detected QTL in the populations Isengrain/Capo and Arina/Capo are illustrated. On some chromosomes only a single QTL was identified: On the chromosomes 2A (leaf tip necrosis severity) and three yet not definitely assigned linkage groups (heading and plant height) effects were detected in the population Isengrain/Capo, on 5B (leaf tip necrosis severity) in both populations. QTL for different traits (lodging severity and plant height) were detected in the two populations on chromosome 6B, but for Arina it cannot be shown due to the small linkage group.

On six further chromosomes QTL analysis revealed effects for several traits: On chromosome 2D (heading, leaf chlorosis severity) and 7B (leaf rust severity, leaf chlorosis

severity) only in the population Isengrain/Capo, on 3A (glaucousness, leaf chlorosis severity) and 5A (plant height, lodging severity and leaf tip necrosis severity) solely in Arina/Capo. The QTL detected on chromosome 3B were only partly consistent in both populations, the effects on 4B were different.

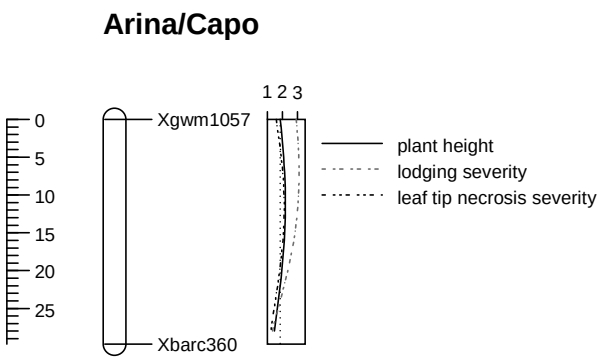

Fig. 88 **Interval analysis of QTL for plant height, lodging severity and leaf tip necrosis severity (each mean over two experiments) on a linkage group corresponding to a part of chromosome 5A**

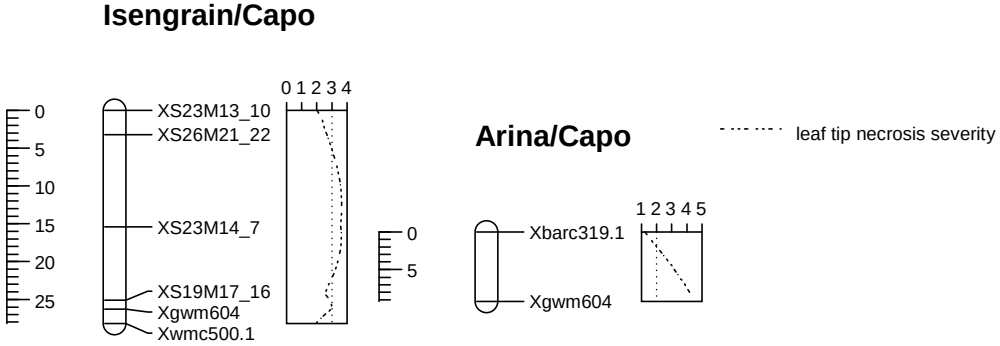

Fig. 89 **Interval analysis of a QTL for leaf tip necrosis severity (mean over two experiments) on linkage groups corresponding to parts of chromosome 5B**

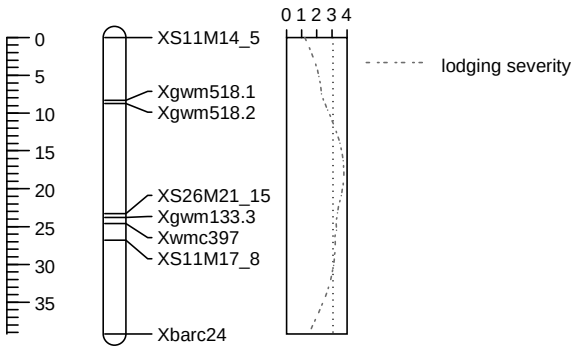

Fig. 90 **Interval analysis of a QTL for lodging severity (mean over four scorings in three experiments) on a linkage group corresponding to a part of chromosome 6B**

Isengrain/Capo

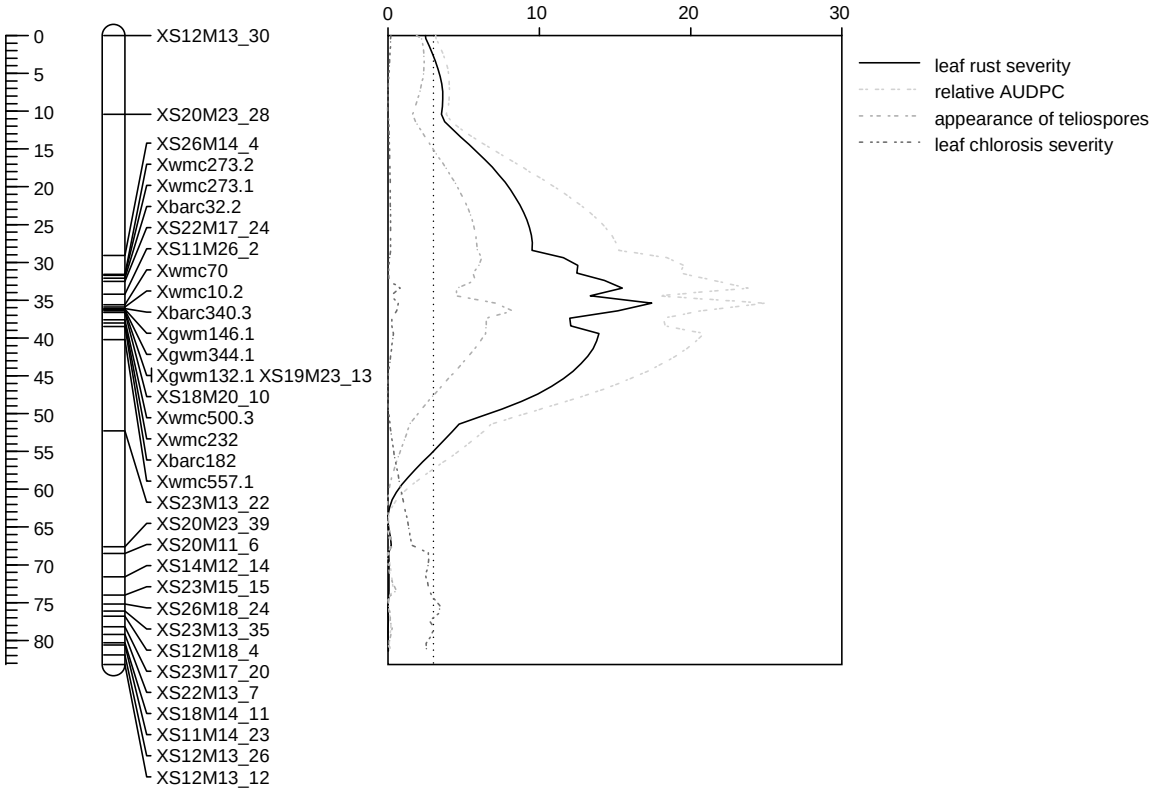

Fig. 91 **Interval analysis of QTL for leaf rust severity (mean over eleven experiments), relative AUDPC (mean over two experiments), the appearance of teliospores and leaf chlorosis severity (mean over two experiments) on a linkage group corresponding to a part of chromosome 7B**

Isengrain/Capo

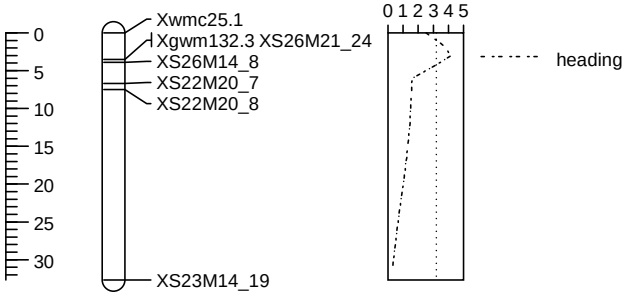

Fig. 92 **Interval analysis of a QTL for heading (mean over eleven experiments) on a linkage group not definitely assigned to chromosomes 2B, 2D, 6A, 6B or 7B**

Isengrain/Capo

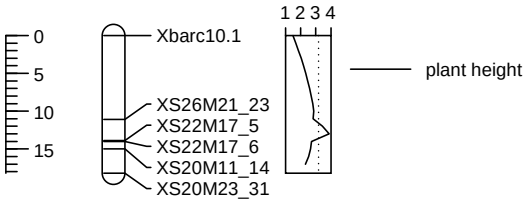

Fig. 93 **Interval analysis of a QTL for plant height (mean over five experiments) on a linkage group not definitely assigned to chromosomes 2B, 4B, 5A or 7B**

Isengrain/Capo

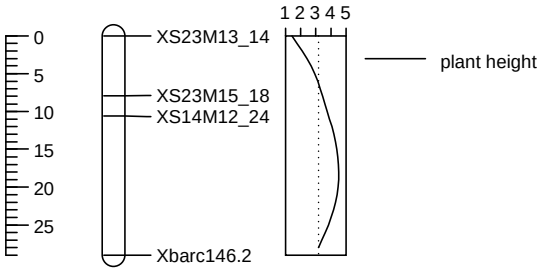

Fig. 94 **Interval analysis of a QTL for plant height (mean over five experiments) on a linkage group not definitely assigned to chromosomes 6A or 6B**

5.5 Epistatic Interactions

As has been shown in the previous results, the QTL of Capo and Isengrain were best seen in different experiments. Therefore epistatic interactions were calculated several times: for the mean of 3, 6 and 11 experiments as well as the 24 single assessments of leaf rust severity measured by the percentage of infected leaf area, leaf rust severity measured by the relative AUDPC (single experiments and the mean over them) and the appearance of teliospores. In total 1,647 significant ($\alpha = 0.0001$) epistatic interactions were detected for these 31 different traits: 311 different markers from all linkage groups were involved in 1,230 pairs. On average one marker showed 21 significant epistatic interactions with 14 other markers, with a maximum of 95 interactions or 52 other markers. Epistatic interactions of a single marker combination were on average significant for just two traits. Because of the extremely high number of tested interactions – the framework map still comprised 318 markers, thus more than 1.6 million tests were performed in total – and despite the very low risk of the first kind ($\alpha = 0.0001$) many of the epistatic interactions that were significant seemed to be detected just by chance. There was no obviously visible pattern: Only in a few cases markers located in two different QTL regions were involved in significant epistatic interactions. Two markers (*XS16M16_6* and *XS26M14_1*) from the 3B QTL region and *Xgwm148* from the 2B QTL region gave significant epistatic interactions, but only for one trait each. Similar epistatic interactions of *XS26M14_1* and a third marker (*XS23M15_3*) with *XS11M26_3* from the 7B QTL were only detected for one trait each. Also in the case of all other QTL markers, epistatic interactions were only significant for one or a few traits, which were not consistent over neighboring markers.

When using the original map for the linkage groups comprising the Isengrain and Capo QTL and all that can possibly be assigned to chromosome 2B, where *Lr13* previously described for Capo is located, only three markers located within the Isengrain QTL were identified that were involved in significant interactions with several other markers. Two of these markers were assigned to different parts of chromosome 2B the other was located in the linkage group 2BD6AB7B.

Calculating ANOVA with and without the interaction term marker1*marker2 the improvement of the model when regarding the interaction term was less than 4 %. Due to these low values and no visible pattern, only additive, but no epistatic interactions between chromosomal regions are assumed in the population Isengrain/Capo.

5.6 SSR Marker Haplotype Comparison

In order to get more information to which resistance genes the QTL for leaf rust resistance on chromosomes 3B and 7B might be related, a haplotype analysis with SSR markers either mapped in these regions or reported for the *Lr* genes of interest (Table 5) was performed. Primer Wmc221 revealed the same fragment size for Capo and Isengrain. Therefore it was possible to use only those markers already mapped in the population Isengrain/Capo.

Capo and Isengrain alleles of the three SSR markers mapped near the QTL identified on the short arm of chromosome 3B were compared to nine different cultivars carrying *Lr27*. In addition fragment sizes of the Furore and Arina alleles were recorded. The results are presented in Table 91. Fragments of the same length as the Capo allele are highlighted in green (codominant markers) or light green if only Isengrain produced a fragment. Isengrain alleles are shown in red color. Fragments differing from both Capo and Isengrain are highlighted in yellow. All fragments that were repeatedly not scorable unambiguously are indicated by a question mark. At the position of the marker *Xgwm493* Capo has the same allele as all lines except Shortim, Timgalen and possibly Gatcher. The size of the Capo fragment associated with *Xbarc75* coincides at least with four cultivars, but at the position of the dominant marker *Xgwm533* that was mapped next to the QTL, in contrast to Capo (and Furore) all lines except Gatcher showed a fragment.

Table 91 Results of the haplotype comparison with lines carrying *Lr27*. For each cultivar the previously described resistance genes (see Table 6 for references) are listed. The order of the markers is the same as in the genetic map (Fig. 45). C: same fragment size as Capo, I: Isengrain, -: no visible fragment, N: size different from C and I, ?: in doubt

Marker	**Capo**	**Isengrain**	Cranbrook	Shortim	Timgalen	Hope	Gatcher	Karl	Kalkee	Rescue	Warigo	Furore	Arina
	Lr	*13+*	*14a*	*27* i.a.	*27* i.a.	*27* i.a.	*14a +27* i.a.	*14a +27* i.a.	*14a +27* i.a.	*14a +27*	*14a +27*	*14a +27*		*13?*
Xgwm533	-	I	N	N	N	N	-	N	N	N	N	-	N
Xbarc75	C	I	C	C		C	N	I	I	C		I	?
Xgwm493	C	I	C	I	?	C	?	C	C	C	C	C	C

For the QTL region on the long arm of chromosome 7B fragment lengths of eleven lines carrying *Lr14a* and ten lines carrying *Lr19* generated by 13 SSR markers were analyzed. In order to know from which parent the Isengrain allele originated, Apollo and Soissons were included in the analysis. In Fig. 95 haplotype comparison is shown on the example of marker *Xgwm132.1*. The primer produces two further fragments polymorphic between the parents, but assigned to other chromosomes. The fragment associated with the Isengrain allele conferring resistance is marked by a red line. In the left part cultivars with *Lr14* are arranged, in the right with *Lr19* and in the middle Isengrain's parents, Isengrain, Capo, Furore and Arina. Fragments of the three lines at the very left are only interesting in the case of 3B markers as these carry *Lr27*. Transfer was confounded with Transfer-12 which carries *Lr19* and included into the analysis although it has only *Lr9*. In addition to the markers previously mapped to the region associated with the QTL on chromosome 7B, *Xgwm44* was used that was assigned to chromosome 7D, but described by Li et al. (2006) as a marker for *Lr19*. In Table 92 colors of the Capo and Isengrain alleles are interchanged compared to Table 91, as in this case the positive allele originated from Isengrain and not from Capo.

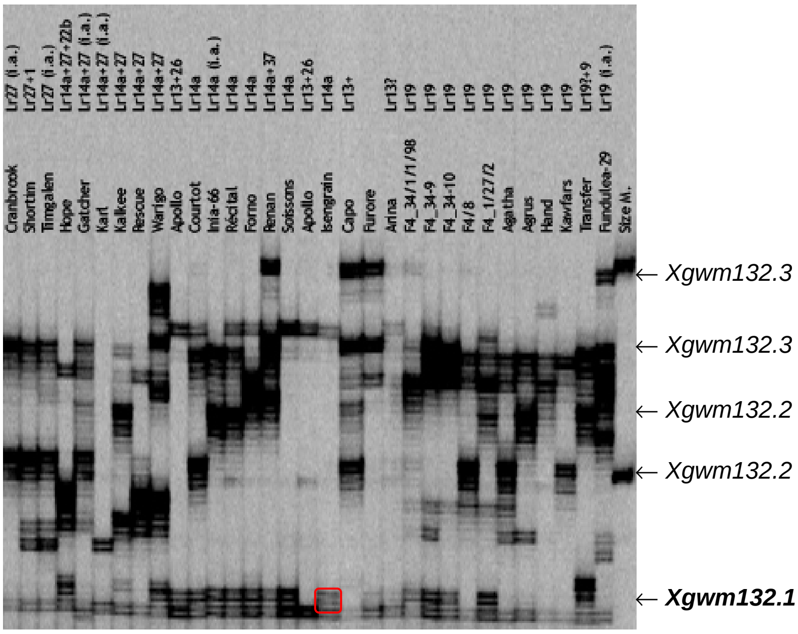

Fig. 95 **Haplotype comparison of SSR marker *Xgwm132.1*. The fragment of the Isengrain allele associated with leaf rust resistance is marked with a red line.**

Table 92 **Results of the haplotype comparison with lines carrying *Lr14a* and *Lr19*. For each cultivar the previously described resistance genes (see Table 6 for references) are listed. The order of the markers is the same as in the genetic map (Fig. 57). C: same fragment size as Capo, I: Isengrain, -: no visible fragment, N: size different from C and I, ?: in doubt**

Marker	**Capo**	**Isengrain**	Apollo	Soissons	Hope	Gatcher	Karl	Kalkee	Rescue	Warigo	Courtot	Inia-66	Récital	Forno	Renan	F_4 34/1/1/98	F_4 34-9	F_4 34-10	F_4/8	F_4 1/27/2	Agatha	Agrus	Hand	Kawfars	Fundulea-29
Lr	*13+*	*14a*	*13+ 26*	*14a*	*14a +27* i.a.	*14a +27* i.a..	*14a +27* i.a..	*14a +27*	*14a +27*	*14a +27*	*14a*	*14a* i.a.	*14a*	*14a*	*14a +37*	*19*	*19*	*19*	*19*	*19*	*19*	*19*	*19*	*19*	*19* i.a.
Xwmc273.2	C	I	?	I	C	N	I	N	I	C	I	I	I	C	N	?				I	?	I	I	N	N
Xwmc273.1	C	-	-	-	C	C	-	C	-	C	?	-	-	C	C	C	-		-	C	-	?	C	-	C
Xbarc32.2	C	I	N	I	N	N	N	N	N	N	I	I	I	N	N	N	N	N	I	N	I	C	C	N	C
Xwmc70	-	I	?	I	I	I	?	-	N	I	I	I	I	I	?	?	N	N	N	-	N	?	?	-	I
Xwmc10.2	-	I	-	I	I	I	-	-	N	I	I	I	I	I	-	I	-	I	N	-	N	I	-	-	I
Xbarc340.3	-	I	-	I	-	-	-	N	N	-	I	I	I	-	-		-	-	I	-	I	-	-	-	?
Xgwm146.1	-	I	-	I	I	?	-	-	N	I	I	I	I	I	-		-	-	N	-	N	-	-	N	
Xgwm344.1	-	I	-	I	I	I	-	N	-	I	I	I	I	I	-		-	-	-	-	-	-	-	-	
Xgwm132.1	-	I	I	I	I	N	?	N	?	?	I	I	I	I	I	I	I	I	-	I	?	?	-	?	?
Xwmc500.3	C	-	?	-	N		?	C	C	-	-	-	C	-	?	N	-	-	C	-	C	C	C		C
Xwmc232	C	-	-	-	-	-	C	-	-	-	-	-	-	-	C	-	-	-	-	-	-	C	-	-	C
Xbarc182	C	-	N	-	N	?		N	?	N	-	-	-	N	N	N	N	N	?	N	?	C	C	N	C
Xwmc557.1	C	-	N	-	-	N	-	N	-	-	-	-	-	-	-	C	-	-	-	-	-	C	N	-	?
Xgwm44 (7D)	C	I	I	C	N	N	N	N	N	N	C	N	N	N	C	C	?	N		N	N	N	N	N	N

Six out of the thirteen markers used for haplotype analysis of the 7B QTL were dominant for the Isengrain allele, four for the Capo allele. Whether the alleleles of two genotypes are the same can only be determined when they produce the same fragment length. Missing fragments can arise from the same or different allelic constitutions. Isengrain shares at least the alleles of all dominant markers with its parent Soissons and the cultivars Courtot, Inia-66 and Récital. At least five alleles of Isengrain, Forno and Hope coincide. With Warigo Isengrain has at least four alleles in common, with Gatcher three and with Karl and Renan one allele. All these lines carry *Lr14a*. The lines carrying *Lr19* share only one or two alleles definitely with Isengrain. Agrus, Hand and Fundulea-29 are more similar to Capo than to Isengrain.

In the case of marker *Xgwm44* that was mapped to chromosome 7D the Isengrain allele was inherited from Apollo. Most of the lines showed a fragment different from Capo and Isengrain, only three lines with *Lr14a* and one or two with *Lr19* had the same fragment size as Capo.

6 Discussion

6.1 Leaf Rust Resistance

No method of statistical analysis can make up for imprecise phenotypic assessment, but gives misleading results. To avoid biased data due to environmental influences as far as possible, altogether 42 field experiments were conducted: 18 for the population Isengrain/ Capo and 12 each for the two validation populations Arina/Capo and Furore/Capo. Artificial inoculation with leaf rust spores was performed to ensure an even disease pressure across the whole experimental site. In the years 2005, 2006, and in 2007 in one experiment infection with leaf rust in Tulln occurred very late in the growing season, however, and it was impossible to evaluate differences between lines before leaf senescence proceeded too far to assess a reasonable amount of lines. One reason was doubtlessly Capo's medium susceptibility to *Septoria* leaf blotch (BAES 2013b). To delay *Septoria nodorum* infection without affecting leaf rust development, especially the antifungal agent Prochloraz® seemed to be suitable. Despite the same procedure for collection and storage of spores and the preparation of water based inoculum, control inoculations in the climate chamber revealed that at least in one case infection with leaf rust did not occur. Obviously these tests should not be omitted as leaf rust development is much faster under the favorable conditions of the climate chamber and the greenhouse. Thus, field inoculation can – if necessary – be repeated considerably earlier. Apart from the aggressiveness of the inoculum itself, inoculation method, plant developmental stage and the environmental conditions seemed to have a big influence on the success of leaf rust infection. Direct spraying of the plants (Micron® Sprayer and inoculation of spreader row plants) appeared to be more effective than planting of infected seedlings. A disadvantage of the Micron® Sprayer is the large amount of spores required. The assistance of many persons is the disadvantage of performing inoculation of individual spreader row plants with subsequent coverage in a reasonable time on a whole experimental site. As it was even impossible to influence humidity because a mist irrigation system was not available, repeated inoculations were necessary. Collection of spores was very laborious and thus availability of inocula limited. Therefore only spreader row plants of every second row were sprayed, but at two occasions within a few days.

Another challenge was the disease assessment itself. Due to the large number of field trials in some years and experiments performed by project partners, scoring had to be done by different persons. Reproducibility of leaf rust assessment by means of the used scoring aid is discussed in a separate chapter.

In 14 experiments of the population Isengrain/Capo leaf rust severity was assessed, as well as in twelve field trials of the population Arina/Capo and seven of Furore/Capo. Not all data were suitable for further statistical analysis, as in a few cases the infection level was too low for detecting clear differences between lines.

6.1.1 Inter-rater and Intra-rater Reproducibility

Studies on reproducibility of disease assessment either focus on whole plots, the whole plant or on single plant parts, mainly leaves. Plants can be observed in greenhouse or field experiments. Different statistical tests can be applied to identify reproducibility of assessments as reviewed by Bock et al. (2010). The effect of field conditions has to be taken into account comparing the results of this work with greenhouse experiments. Field trials to study methods of determining disease severity were conducted e.g. by Large and Doling (1962) and Lipps and Madden (1989), working on powdery mildew of cereals, and Vereijssen et al. (2003) working on *Cercospora* leaf spot in sugar beet.

As Large (1966 cited in Zadoks and Schein 1979) pointed out, plants to be scored for disease severity (% infected leaf area) frequently do not fit the scoring aid: pustules are larger or smaller than shown or they may not be equally distributed over the whole leaf area but more concentrated towards the leaf base. One possible reason for differences between assessments is how a rater assigns disease severity to the classes of the scoring aid in these cases. Sherwood et al. (1983) proved that estimates were different for varying size resp. number of lesions despite that the same leaf area was covered with pustules, and that larger symptoms were estimated more accurately. Additionally Forbes and Jeger (1987) showed that the plant structure influenced estimates. Hau et al. (1989) found influences of leaf shapes on estimates when comparing scores for oval apple and linear barley leaves. However, differences in leaf shapes between different wheat genotypes won't be big enough for such an influence. But it is doubtful, whether disease severity of wheat plants with twisted leaves can be assessed correctly and reproducibly. This possible error can't be tested in experiments with prepared plant parts (or photographs, drawings, sketches,…). In outdoor assessments additional influences may be the position of the sun and whether only the infection of the flag leaf or of all upper leaves is taken into account. In contrast to experiments of repeated disease assessments in the laboratory with constant light, solar radiation on the field is varying much. Differences between green to yellow leaves and reddish-brown pustules can be perceived better in the morning and afternoon or on cloudy days than about noon on sunny days. Wet leaves because of dew or on rainy days reduce the ability to distinguish colors. Vereijssen et al. (2003) detected influences in error susceptibility of the two compared disease assessment methods if plants were wet, varieties darker colored or the sunlight was bright. Hau et al. (1989) did not find any effect neither of leaf nor of lesion color on the estimates, but they were just comparing bright and dark lesions and bright and dark apple leaves under laboratory conditions.

In the course of the study data of repeatedly scored plants by four different raters were gained. In 2004 rater one scored leaf rust severity of more than 3.500 plots. In 2006 and 2007 rater one and two did almost all scorings together, all in all more than 5.000 plots. In the same year or the past few years, rater three and four had scored over 1.000 plots. In every case the same scoring aid was used. Therefore all four raters had pretty much experience in the assessment of leaf rust severity using the same scoring aid before the scorings to be compared.

Based on the available data, there seems to be no clear tendency that either for the classes for lower or higher leaf rust infection reproducibility of the assessment calculated as the percentage of identically scored plots is better. Even if the class with zero infection is not taken into account, it can't be said whether there is a change in reproducibility with increasing severity level or decreasing class width. This is in contrast to the results of e.g. Sherwood et al. (1983), Forbes and Jeger (1987), Hau et al. (1989), and Smith et al. (2005) who studied the accuracy of disease assessment and unveiled a correlation between estimation error and disease severity level.

Intra-rater reproducibility measured by the coefficient of determination for absolute differences (% infected leaf area) was between 0.86 and 0.92. Nutter et al. (1993) who studied dollar spot (*Sclerotinia homoeocarpa*) of bentgrass (*Agrostis palustris*) observed values between 0.83 and 0.93. Regarding the regression parameters, differences were larger: In the present study the values of the intercept varied between -5.64 and 1.13, the values found by Nutter et al. (1993) were lying between -2.66 and 0.74. Values of the slope varied between 0.98 and 1.11, in the study by Nutter et al. (1993) they were lower, lying between 0.80 and 0.93. The value of the correlation coefficient varied between 0.93 and 0.96, thus being higher than observed by Nita et al. (2003), who studied *Phomopsis* leaf blight (*Phomopsis obscurans*) of strawberry (*Fragaria* x *ananassa*). The value using the scale developed by Horsfall and Barratt (1945 cited in Horsfall and Cowling 1978) was 0.81.

Inter-rater reproducibility measured by the coefficient of determination for absolute differences (% infected leaf area) was between 0.72 and 0.94. Nutter et al. (1993) observed slightly lower values (0.70 - 0.89). Godoy et al. (1997) studied different diseases and discovered a dependency on the disease with values of the coefficient of determination lying between 0.43 and 0.95. The values of the intercept were lying between -2.08 and 2.46. Nutter et al. (1993) got values between -0.42 and 6.95. The slope between estimates of different raters in the present study varied between 0.90 and 1.28, in the study by Nutter et al. (1993) between 0.74 and 0.88. The correlation coefficient was between 0.85 and 0.97, thus being higher than the values observed by Nita et al. (2003) that were all below 0.80.

It seems that the intra-rater reproducibility remains constant over the three days of scoring in the year 2008 whereas the inter-rater reproducibility is best on the second day after joint scoring of 52 lines in the morning, and worst on the third day without any joint scoring and after separate rating of several hundred lines. Slightly lower values of the mean and maximum values of the absolute differences between the repeated assessments of rater one and two on the second day in 2008 could indicate that joint scoring of standard lines with different disease severity and different pustule size and distribution helped reducing discrepancies between these two persons despite joint scoring of numerous plots in the previous years. Joint scoring should be done regularly before the start of assessments.

The larger deviations on the third day of assessment may be due to separate scoring of hundreds of plots, different perception of higher disease severity, other light conditions due to changed weather conditions or even other factors not considered yet. Scoring aids depicting different disease severity classes are helpful. Results can be improved using scoring aids not just for different lesion shapes but also for different pustule sizes.
The percentage of identically scored lines is in the range of 30 % to 68 %, meaning that on average (over the whole range of infected leaf area) between one and two third of the plants do not get the same score in a second rating, neither by the same nor by another rater despite using the same scoring aid. It seems that joint scoring before the beginning at least slightly increases this value.

All tests were done despite the problem that these data were not collected with preceding experimental design for these tests from a statistical point of view, but for QTL detection. For that reason all results can only be seen for these particular compared assessments and experiments, and no general statement can be deduced. The most important reasons for this limitation are:

- Not all lines were scored two times on the same day by all four raters as would be required for this kind of experiment. Not even the 136 standard lines repeatedly scored in 2008 were always the same in every comparison for different reasons. This is the cause for all further limitations listed:
- Sample size was unequal for the different comparisons and in most cases larger than necessary for the given precision requirement (α – risk of the first kind, β – risk of the second kind, δ – minimal distance) of the particular statistical test.
- In only one case rating was done by person three and four and once rater three repeated a preceding scoring together with person two. Intra-rater reproducibility can only be determined for rater one (three times) and rater two (two times). On three days rater one and rater two scored the same standard lines. No comparison between rater one and three or four and between rater two and four can be made. Only in two cases a joint rating by two raters was repeated afterwards.
- The range of leaf rust severity was not the same in all assessments. In the first assessment in 2008 the most heavily diseased plants were scored with 40 %, whereas on the third day leaf rust infection progressed up to 80 %.

These results of intra-rater and inter-rater reproducibility suggest that further research is worthy as it has not yet been extensively studied by others considering whole plant plots of cereals in field experiments. The following items have to be regarded:

- Sample size must be determined for the particular statistical test (regression parameters, correlation coefficient or absolute difference of the repeated assessments) based on the difference (δ) that should be detected and the risk of the first kind (α) and of the second kind (β).

- All raters must score the same plants on the same day. They should have about the same experience in using the scoring aid, either having all scored together once before or none of them having experience in joint scoring.
- Before the first scoring there must be a short joint instruction.
- The same plants are scored again a second time by all raters.
- After the second and before the third separate scoring there must be a joint scoring on separately planted lines in order that the raters can adjust their ratings.

If the experiment is designed in this way, it should be possible to answer the following three questions:

- Do scores by one rater fit together?
- Is the scoring aid on its own enough to get comparable scores by different raters?
- Do scores by different raters fit better if there has been an intensive instruction before with joint scoring of lines?

The question, how many lines are necessary for the joint scoring before the beginning can not be answered with this experiment. Therefore a further experiment would be necessary with varying numbers of plants for joint scoring training of different raters.

As the results of QTL analysis show, the number of lines and field experiments was large enough to compensate not just for environmentally caused experimental errors but also for possible deficiencies in reproducibility of disease assessment.

6.1.2 Field Experiments

Data of eleven experiments of the population Isengrain/Capo were used for QTL detection and further statistical analysis. In the other three experiments maximum leaf rust infection was too low for detecting clear differences between lines. Capo, which was used as the resistant parent for developing the RIL population, was on average less infected than Isengrain. In the experiments in Probstdorf the difference was small and in the two experiments Schmida 2007 and Rust 2008, Capo exhibited even more symptoms than Isengrain.

If different populations were tested at the same experimental site, Capo did not always get the same score. Only in a few cases it can be explained by experimental conditions: In the trial Tulln 2004 the population Furore/Capo was assessed about two days later than Isengrain/Capo. In Probstdorf 2006 differences in the average leaf rust severity in different parts of the field were obvious with borders that did not coincide with those between populations, but perhaps with the prevailing wind direction or variable soil conditions. In the experiments Rust and Schmida 2008 the populations Isengrain/Capo and Arina/Capo were rated by different persons. Moreover, in Rust the microclimate at one end of the experimental site was more humid because of a rivulet and tall trees on the other side of the street shading in the morning. Thus there was a decline in humidity from the first replication of the population Isengrain/Capo to the second replication of Arina/Capo.

Although these two populations were rated by the same person in Tulln, Capo's scoring value was higher in the population Arina/Capo, though assessed two days earlier.
In the year 2007 rainfall was infrequent over large parts of the growing season. The experiments in Tulln were planted alongside a street with a shelter belt at one end, a few trees along the street and houses opposite the street. It seemed that differences in leaf senescence – and presumably leaf rust development – were influenced by these factors in periods of water stress of the plants. The population Isengrain/Capo was planted next to the shelter belt and leaf senescence of all cultivars occurred very early, making leaf rust assessment impossible: In the first repetition it was possible to assess leaf rust infection of both parental lines in one plot each, as well as of five standard lines. In the second repetition two plots of Capo, one of Isengrain, 16 standard lines and 13 RILs were scorable. On the same day all plots in the second repetition of the population Furore/Capo and 230 out of 233 RILs of the population Arina/Capo were assessed. Unfortunately two days earlier it was impossible to assess more than the Arina/Capo experiment and the first repetition of Furore/Capo. Between these two days average leaf rust infection increased by about 30 %. The parental lines planted within Arina/Capo – the population located furthermost from the shelter belt – (and 230 out of 233 RILs) were scorable even three days later. This difference in senescence of the parental lines could only be caused by environmental conditions. The other experiment, Tulln (M) 2007, was planted at a greater distance to the shelter belt. In this experiment plants were cut once in the spring in order to prolong vegetation and thus the scoring period for leaf rust severity. Whereas almost all RILs and all parental lines of the populations Isengrain/Capo and Furore/Capo were scorable on the second day of assessment, three days later it was possible to evaluate just ten and two RILs but not a single parental line. In the two rows located next to the street senescence of the experimental plots coincided with those of the neighboring spreader rows, thus being obviously influenced by environmental conditions.
In the experiment Tulln 2004 spreader rows were checked for leaf rust infection between the two scoring dates. Differences across the experimental site appeared to be marginal. In the subsequent years evaluation of the spreader rows was impossible as these plants died earlier than the RILs. Hence, in the year 2008 assessment of spreader row plants was done a few days before the populations. Plots were rated on a 1 (= no visible pustules on any plant) to 5 (= pustules on almost all plants) scale. These data were included as a covariate in an analysis of covariance in order to see whether results changed. The estimated coefficient of heritability calculated by the means of variance components improved by only 0.012 (Isengrain/Capo), 0.003 (Arina/Capo) and was reduced by 0.002 (standard and parental lines). Leaf rust severity of the population Furore/Capo was only assessed in one experiment. In 2008 differences on the experimental sites were not as obvious as in the experiments Probstdorf 2006, Tulln 2007 and Tulln (M) 2007. For a reliable statement whether inclusion of the spreader row infection into the model improves results, data of experiments with remarkable environmental influences would be necessary.

For all three populations correlation coefficients between different experiments were rather low, only in two cases of the population Isengrain/Capo and in one of the Arina/Capo the values of Spearman's rho were significantly beyond 0.71. For Furore/Capo values were generally lower, maybe because of the smaller range of the population due to the close relationship and hence similarity of the two parental lines.

6.1.3 Detection of Leaf Rust Resistance QTL

Five QTL for leaf rust resistance (measured by the percentage of infected leaf area) were identified in the population Isengrain/Capo, but not a single was consistent over all experiments: A minor effect located on chromosome 2B was only detected in the experiment Schmida 2007. The allele conferring reduced susceptibility was inherited from Isengrain. Effects transmitted by Capo alleles were identified on chromosome 1B in the experiment Piešťany 2006 and the unassigned linkage group 2BD6AB7B in the experiments Piešťany 2006 and Rust 2009. These two were also detected in the experiment Piešťany 2006 if leaf rust infection was measured by the relative AUDPC. The major QTL detected on chromosome 3B was consistent over six single assessments of leaf rust severity and the mean, and the relative AUDPC in the experiment Piešťany 2006 and the mean. Whereas the 3B allele associated with reduced leaf rust severity originated from Capo, in the case of the most effective QTL detected in the population Isengrain/Capo on chromosome 7B, the positive allele was inherited from the susceptible parent Isengrain. This QTL was consistent over twelve experiments. The experiments in which the 7B QTL explained much of the phenotypic variance did not coincide with those of the effect on 3B. Ranking the experiments according to the phenotypic variation explained by the QTL, the experiment Piešťany 2006 was first for 3B, but only eleventh (out of twelve) for 7B, Probstdorf 2006 second vs. sixth, Tulln 2004 third vs. fifth.

Based on the present results, it seems impossible to make a reliable prediction about the effectiveness of these two major QTL: Neither can it be said that the 3B QTL was losing effectiveness, although Capo's susceptibility to leaf rust has been slowly increasing since it was brought to market in the year 1989 from 2 to 4 on a 1 (= absent/ very low) to 9 (= very strong) scale (BAES 2013b). In the year 2004 the QTL was less effective than in 2006, although the experiment in Probstdorf was conducted in an area where the pressure on the pathogen is high for adapting to Capo's resistance due to neighboring breeder's fields with a large amount of Capo progeny. Nor is there any obvious common property of the frequency distribution characteristics (mean, range, variance) of the experiments in which the 3B or the 7B QTL was most effective. Regarding the disease progress curves in the experiments Piešťany 2006 and Tulln 2008, it seemed that the 3B QTL was more effective at the beginning of infection causing delayed infection, whereas the 7B QTL reduced speed of spreading or symptom development and was better to recognize the more severe the leaf rust infection was, meaning that genotypes carrying the allele associated with resistance were much less diseased until the end of the vegetation period. These assumptions are only based on the results of two experiments. Further experiments

with repeated assessments starting at that time when the first lines exhibit symptoms of leaf rust infection would be necessary.
Results of repeated leaf rust assessments and the resulting relative AUDPC were highly correlated with the exception of three experiments (Fundulea, Nyon and Piešťany, all 2008): The values of Spearman's rho between two subsequent scorings ranged from 0.69 to 0.94, between first and third assessment correlation was lower (0.49 - 0.90). As not only correlation between data of a single scoring and relative AUDPC was high (0.67 - 0.99), but the same QTL were identified, from this point of view the calculation of AUDPC seemed unnecessary as was also previously stated by Lipps and Madden (1989). In this project it has also been shown, that the effectiveness of QTL varied over the scoring period, and thus repeated assessments seem inevitable for the identification of all effects.
Despite the low number of SSR markers applied to the population Arina/Capo, the QTL located on chromosome 3B and in the linkage group 2BD6AB7B were consistently identified. In both cases the alleles conferring increased resistance were inherited from Capo.
In the population Arina/Capo the QTL on chromosome 3B was detected in seven out of eleven experiments. Using the mean over these seven trials for further analysis, LOD value (5.45) and R^2 (0.14) of the peak marker *Xbarc75* increased compared to the mean over eleven experiments, but heritability dropped to 0.858.

6.1.3.1 Minor Effect on Chromosome 1B

The QTL for leaf rust resistance inherited from Capo detected in the experiment Piešťany 2006 for a single assessment and for the relative AUDPC was located on the short arm of chromosome 1B in the marker interval *Xgwm413–XS26M14_10.* In a similar region QTL inherited from the variety Forno were identified: in a population Forno/Oberkulmer for the amount of infected leaf area (Messmer et al. 2000), in the population Arina/Forno in the marker interval *Xgwm604–OA93* for the AUDPC and for the response to infection (Schnurbusch et al. 2004). In durum wheat a QTL for seedling infection type was associated with SSR marker *Xwmc500.1* (Maccaferri et al. 2010). The resistance gene *Lr26* is located on chromosome 1RS of rye and the short arm of 1B of wheat lines with the 1RS translocation (Mettin et al. 1973, Zeller 1973 both cited in McIntosh et al. 1995, Singh et al. 1990, Hsam 2000, Mago et al. 2002). Mago et al. (2005) located *Lr26* near the centromere of 1RS by high-resolution mapping and mutation analysis. *Lr26* conferring seedling resistance was detected frequently in European wheat cultivars (detailed references in Table 95), but the infection level of Thatcher NILs was generally moderate to very high (detailed references in Table 94). In Hungary they were the most susceptible averaged over the period 2004-2008 with almost 90 % infected leaf area (Vida et al. 2009). In the experiment in Tulln 2007 leaf rust severity of these plants was 50 % infected leaf area. Throughout the world isolates virulent to *Lr26* were found (detailed references in Table 93, Kosman et al. 2004).

Table 93 Studies on the virulence distribution of *Puccinia triticina* in different regions

isolates collected in different years in several regions and identified pathotypes	*Lr2a*	*Lr2b*	*Lr2c*	*Lr13*	*Lr14a*	*Lr14b*	*Lr15*	*Lr19*	*Lr22a*	*Lr23*	*Lr26*	*Lr27+Lr31*	*Lr10+27+31*	*Lr33*	*Lr35*	*Lr41*	*Lr44*	references
850 isolates (53 pathotypes) from Western Europe 1995	x	x	x				x	x		x	x	x						Park and Felsenstein (1998)
isolates from 4 regions of Nebraska (US) 1992-1996	x		x					x			x							Watkins et al. (1998)
575 isolates from 4 regions of Nebraska (US) 1995-1998	x		x							x	x							Watkins et al. (2001)
260 isolates from Israel 1993-1997	x		x				x			x	x							Manisterski et al. (2000)
2608 isolates (105 pathotypes) from 12 European countries 1996-1999	x	x	x				x	x		x	x			x			x	Mesterházy et al. (2000)
259 isolates (12 pathotypes) from Slovakia 1997-2000	x	x	x				x	x		x	x							Huszár et al. (2002)
253 isolates (21 pathotypes) from 4 South Atlantic States 1999	x		x		x						x							Kolmer (2002)
56 isolates (35 pathotypes) from Andalusia (ES, hexaploid + durum wheat) 1998-2000	x	x	x		x		x			x	x							Martínez et al. (2005)
1300 isolates (> 17 pathotypes) from 3 locations of the Ukraine 2002-2003	x	x	x		x	x	x	x	x	x	x	x		x	x			Elyasi-Gomari and Panteleev (2006)
isolates from 4 German regions 2001-2003	x	x	x	x	x	x	x	x		x	x			x	x		x	Lind and Gultyaeva (2007)
isolates from 7 European regions of the Russian Federation 2001-2003	x	x	x	x	x	x	x	x		x	x			x	x		x	Lind and Gultyaeva (2007)
10 isolates from the United States (+ 78 on durum from 7 countries 2000-2003)	x		x		x	x	x	x	x	x	x			x		x	x	Ordoñez and Kolmer (2007)
148 isolates (91 pathotypes) from North America 1980s-2005	x		x		x	x					x							Ordoñez and Kolmer (2009)
178/ 138 isolates from 4 Pakistan regions (10-11 locations) 2002-2004	x	x	x	x	x	x	x	x		x	x		x	x	x			Rattu et al. (2009)
233 isolates (71 pathotypes) from 27 locations of the Czech Rep. 2005-2008	x	x	x				x	x		x	x							Hanzalová (2010)
122 isolates (91 pathotypes) from 11 locations of the Slovak Rep. 2009-2011	x	x	x	x			x	x		x	x							Hanzalová et al. (2012)
124 isolates (44 pathotypes) from 4 regions of Turkey 2009 and 2010	x		x		x	x					x							Kolmer et al. (2013)
74 isolates from Eastern Canada 1987 and 251 from the prairie region 1988	x		x		x						x							Kolmer (1992b)
289 isolates (46 phenotypes) from 4 regions of Canada 1995	x		x		x						x							Kolmer and Liu (1997)
275 isolates (50 phenotypes) from 4 regions of Canada 1996	x		x		x						x							Kolmer (1998)
362 isolates (75 pathotypes) from 4 regions of Canada 1997	x		x		x						x							Kolmer (1999)
268 isolates (34 pathotypes) from 4 regions of Canada 1998	x		x		x						x							Kolmer (2001)
748 isolates from 6 regions of the United States 1990-2006	x		x		x						x					x		Kolmer et al. (2007a)
1120 isolates (54 pathotypes) from 7 regions of the United States 2000	x		x		x						x							Long et al. (2002)
477 isolates (44 pathotypes) from 8 regions of the United States 2001	x		x		x						x							Kolmer et al. (2003)
785 isolates (52 pathotypes) from 8 regions of the United States 2002	x		x		x						x							Kolmer et al. (2004)
580 isolates (52 pathotypes) from 8 regions of the United States 2003	x		x		x						x							Kolmer et al. (2005)
757 isolates (52 pathotypes) from 8 regions of the United States (2000-)2004	x		x		x						x					(x)		Kolmer et al. (2006)
797 isolates (72 pathotypes) from 8 regions of the United States 2005	x		x		x						x					x		Kolmer et al. (2007b)
718 isolates (56 pathotypes) from 8 regions of the United States 2006	x		x		x						x					x		Kolmer et al. (2008)

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near isogenic lines (NILs) tested in different years in several regions or against collected isolates	*Lr2a*	*Lr2b*	*Lr2c*	*Lr13*	*Lr14*	*Lr14a*	*Lr14b*	*Lr14ab*	*Lr15*	*Lr19*	*Lr22*	*Lr22a*	*Lr22b*	*Lr23*	*Lr26*	*Lr27+Lr31*	*Lr10+27+31*	*Lr33*	*Lr44*	*Lr48*	references
4 Indian locations, 1978-1981, NILs/ cultivars								x		x	x				x	x					Sawhney et al. (1982)
8 European locations 1998 and/or 1999, Thatcher (Tc.) NILs	x	x	x	x		x	x		x	x		x		x	x			x	x		Mesterházy et al. (2000)
Eastern Ukrainian forest-steppe isolates 2002-2003, NILs	x	x	x			x	x		x	x		x		x	x	x		x			Elyasi-Gomari and Panteleev (2006)
Omsk (Western Siberia) 2003-2005, Tc, NILs	x	x	x	x			x		x	x		x	x	x	x		x	x			Morgounov et al. (2007)
Adapazari (Turkey) 2005-2006, Tc. NILs + other differentials	x	x	x	x		x	x		x	x		x	x	x	x		x	x			Akin et al. (2008)
5 Pakistan locations 2004-2006, Tc. NILs	x	x	x	x		x	x		x	x		x	x	x	x		x	x			Fayyaz et al. (2008)
Inoculum from 2 Ukrainian locations 2005, NILs	x	x	x			x	x		x	x		x		x	x	x		x			Elyasi-Gomari and Lesovaya (2009)
Central + Eastern Ukrainian forest-steppe 2007, Tc. NILs	x	x	x	x		x	x		x	x		x		x	x	x		x			Elyasi-Gomari and Mikhailovno (2009)
4 Pakistan provinces 2002-2004 (10-11 locations), Tc. NILs	x	x	x	x		x	x		x	x				x	x		x	x			Rattu et al. (2009)
Martonvásár (Hungary) 2004-2008, Tc. NILs	x	x	x	x		x	x		x	x		x		x	x			x	x		Vida et al. (2009)
2002-2005 Aschersleben, 2006-2009 Quedlinburg (DE), Tc. NILs	x	x	x	x	x	x			x	x		x	x	x	x			x	x	x	Serfling et al. (2011)
Ardabil (Iran) 2007, 2008, 2010, 2012, NILs/ cultivars	x	x	x	x		x	x		x	x		x	x	x	x		x	x			Safavi and Afshari (2013)

Another resistance gene, *Lr33*, was mapped on chromosome arm 1BL at a distance of just 3.1 cM from the centromere and 2.6 cM from *Lr26* (Dyck et al. 1987). In durum wheat *Lr33* was associated with SSR marker *Xwmc419* (Maccaferri et al. 2010). High frequencies of virulence to this gene are common (detailed references in Table 93 to Table 95). Average infected leaf area of Thatcher NILs carrying *Lr33* was in Tulln 2007 40 %. In Martonvásár average infection in the period 2004-2008 was 80 % (Vida et al. 2009).

Lr44 is located on chromosome 1BL at a distance of 5.4 cM from *Lr33* (Dyck and Sykes 1994). In most of the regions almost all isolates are virulent to *Lr44* and effectiveness is generally low (detailed references in Table 93 to Table 95). Average infected leaf area of Thatcher NILs carrying *Lr44* was in Tulln 2007 35 %.

In the terminal region of 1BL *Lr46* was identified (Singh et al. 1998, William et al. 2003, Rosewarne et al. 2006). *Lr51* is a resistance gene from *Triticum speltoides* that was transferred by Dvořák and Knott (1980) to the long arm of chromosome 1B (Helguera et al. 2005), *Lr55* is derived from *Elymus trachycaulis* (McIntosh et al. 2005).

Singh D et al. (2013) mapped *Lr71*, which they proved to be different from *Lr33*, *Lr44* and *Lr46*, on chromosome 1B close to the centromere between the markers *Xgwm18* and *Xbarc187*. Compared to the wheat consensus map (Somers et al. 2004), marker order in this region was inverted.

Regarding the marker region of the detected QTL on 1B, the effect might correspond to *Lr26*, *Lr33*, *Lr71*, the QTL identified in the population Forno/Oberkulmer (Messmer et al. 2000), in durum wheat (Maccaferri et al. 2010), on 1RS in a population Beaver/Soissons

by Singh et al. (2009) or the temporarily designated *LrZH84* identified by Zhao et al. (2008). As Capo does not possess the 1RS translocation, the effect can not be due to *Lr26* or the 1RS QTL.

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number of wheat cultivars tested against several pathotypes	*Lr2a*	*Lr2b*	*Lr2c*	*Lr13*	*Lr14a*	*Lr14b*	*Lr15*	*Lr19*	*Lr22a*	*Lr22b*	*Lr23*	*Lr26*	*Lr27*	*Lr27+Lr31*	*Lr10+27+31*	*Lr33*	*Lr41*	*Lr44*	references
71 Indian and Pakistani cultivars, 14 Mexican pathotypes	x	x	x	x			x				x	x		x					Singh and Gupta (1991)
50 Mexican cultivars, 18 Mexican pathotypes	x	x	x	x	x		x				x	x		x					Singh and Rajaram (1991)
48 Indian + 63 exotic cultivars, 6 Indian pathotypes	x	x	x	x	x		x		x	x	x	x		x		x			Kaur et al. (2000)
72 European cultivars, 10 Australian pathotypes	x		x	x	x		x	x			x	x		x					Winzeler et al. (2000)
91 Western European cultivars, 20 Australian pathotypes	x		x	x	x		x	x			x	x		x					Park et al. (2001)
70 cultivars from the UK, 10 Australian pathotypes				x							x	x							Singh D et al. (2001)
37 Japanese cultivars, 11 Mexican pathotypes	x	x	x	x	x	x	x	x	x		x	x		x		x			Singh RP et al. (2001b)
35 cult.+ 17 breeding lines from the US, 16 isolates from the US	x		x		x							x							Kolmer (2003)
225 cult. from KZ, MN, RU, 10 Europ. and Mediterranean isolates	x	x	x				x	x			x	x	x				x	x	Singrün et al. (2004)
84 cult. from DK, FI, NO, SE, 12 Mexican pathotypes	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x			Hysing et al. (2006)
105 European cultivars, 5 Australian pathotypes	x	x	x	x	x		x	x			x	x		x					Pathan and Park (2006)
28 Spanish + 41 durum wheat cult., 12 (durum: 7) pathotypes	x	x		x							x	x							Martínez et al. (2007)
76 Pakistani cultivars, 14 Mexican pathotypes	x	x	x	x	x	x	x	x	x		x	x		x	x	x			Rattu et al. (2010)
36 Iranian cultivars, 13 Iranian isolates	x	x	x	x	x	x	x	x			x	x				x			Boroujeni et al. (2011)
66 Argentine cultivars, 17 Argentine pathotypes	x		x		x			x			x	x		x			x		Vanzetti et al. (2011)
43 Turkish cultivars, 13 pathotypes from CA, US, TK	x		x		x	x						x							Kolmer et al. (2013)

6.1.3.2 Minor Effect on Chromosome 2B

A minor effect on leaf rust severity inherited from Isengrain was detected in the experiment Schmida 2007 and located on chromosome 2B around the position of marker *Xgwm148*. Several resistance genes have been mapped to 2B: *Lr13* (McIntosh et al. 1995, Seyfarth et al. 2000, Bansal et al. 2008b), *Lr16* (Dyck and Kerber 1971, McCartney et al. 2005b) and *Lr23* (McIntosh and Dyck 1975 cited in McIntosh et al. 1995, Nelson et al. 1997) on the short arm, as well as *Lr50* (Brown-Guedira et al. 2003) and *Lr58* (Kuraparthy et al. 2007) on the long arm. Also *Lr35* derived from *Aegilops speltoides* (Kerber and Dyck 1990) and *Lr48* (Bansal et al. 2008b, Singh A et al. 2011) are located on chromosome 2B. *Lr48* first detected by Saini et al. (2002) was mapped by Bansal et al. (2008b) to the short arm between the markers *Xgwm429b* and *Xbarc7*, thus in the region of *Xgwm148* if compared to the wheat consensus map (Somers et al. 2004). Proximal to *Lr48* they

mapped *Lr13*. Singh A et al. (2011) located *Lr48* on the long arm between the markers *Xwmc175* and *Xwmc332*, and thus did not confirm the mapping to the short arm. Seyfarth et al. (2000) located *Lr13* distal to marker *Xgwm630*. *Lr23* transferred from *Triticum turgidum* var. *durum* to hexaploid wheat was found to be closely linked to *Lr13* (McIntosh et al. 1995). Nelson et al. (1997) detected a gene transferred from *Triticum tauschii* on the homoeologous chromosome arm 2DS suppressing *Lr23*. In durum wheat *Lr48* was mapped distal to SSR marker *Xgwm148*, *Lr13* distal and *Lr23* proximal to *Xbarc55.1* (Maccaferri et al. 2010).

Lr13 is abundant in wheat cultivars, but effectiveness is low as virulence to *Lr13* is common. Also isolates virulent to *Lr23* have frequently been detected and effectiveness is similar, although it occurs in fewer varieties (detailed references in Table 93 to Table 95, Park and McIntosh 1994). Leaf rust infection of Thatcher NILs carrying *Lr13* was 60 % infected leaf area in the experiment Tulln 2007 and about 50 % in Martonvásár averaged over the period 2004-2008 (Vida et al. 2009). For *Lr23* leaf rust severity was much lower: about 2 % in Tulln and likewise not differing significantly from zero in Martonvásár (Vida et al. 2009). *Lr48* was just included in a single study by Serfling et al. (2011) and belonged to the group of lines with medium infection level.

Further effects on leaf rust development have been identified on chromosome 2B: Messmer et al. (2000) detected a QTL for infected leaf area on 2BS 15 cM proximal to the centromere and maybe allelic to *Lr13*, but maps are not comparable. Also Rosewarne et al. (2008) identified a QTL for slow rusting resistance on 2BS in the region of the *Lr13* locus. Xu et al. (2005c) found significant effects on slow rusting resistance measured by the AUDPC, infection rate measured by the daily disease progress rate (AUDPC/day) and final severity (%) in the region proximal to SSR marker *Xbarc167*. It coincided with a QTL for prolonged latent period measured by the average number of days from inoculation to epidermis rupture of the first uredinium (Xu et al. 2005b). In a similar region Nelson et al. (1995) detected an effect on mean latent period. Leonova et al. (2007) detected a QTL for adult plant resistance on chromosome 2B in the marker interval *Xgwm257-Xgwm120* coinciding with a QTL for seedling resistance. Compared to the wheat consensus map (Somers et al. 2004) and the Isengrain/Capo map the markers *Xgwm148* and *Xgwm257* mapped in reversed order and closer to each other. A QTL for leaf rust severity was identified by Rosewarne et al. (2008) in the region between marker *Xgwm257* and *Xgwm120*. Unfortunately the SSR markers located closer to the detected effect on leaf rust infection were not mapped by Somers et al. (2004). In durum wheat Maccaferri et al. (2010) found association of *Xwmc770* and the tightly linked loci *Xgwm410.1*/*Xgwm148*/*Xbarc183.1* with differences in seedling infection type. The QTL for relative disease severity of durum identified by Marone et al. (2009) in a single field experiment was located on the long arm in the marker region *Xbarc101-Xwmc441*, in the growth chamber in the *Xcfd73-Xwmc441* interval.

The other resistance genes were mapped clearly outside the region in which the minor effect was identified in the experiment Schmida 2007: *Lr16* was the terminal locus of 2BS in three populations, distal to *Xwmc764* (McCartney et al. 2005b). *Lr50* was transferred

from *Triticum timopheevii* ssp. *armeniacum* and located on the distal end of 2BL near marker *Xgwm382* (Brown-Guedira et al. 2003). Also *Lr58* occurred on the distal end of the long arm of chromosome 2B, but in a wheat-*Aegilops triuncialis* translocation (Kuraparthy et al. 2007). *Lr35* was mapped by Seyfarth et al. (1999). Even by means of the *Triticum aestivum* maps Synthetic x Opatha (Nelson et al. 1995) and Synthetic x Opatha, BARC available from the GrainGenes database (USDA 2014) their map is hardly comparable with the wheat consensus map (Somers et al. 2004). It seems that *Lr35* is located in the region around SSR marker *Xgwm120*.
As the detected QTL originated from Isengrain and not from Capo, who carries *Lr13* (Winzeler et al. 2000, Park et al. 2001, Mesterházy et al. 2002), and due to the marker position, it seems more likely to correspond to *Lr48* or one of the other identified effects than to *Lr13*, *Lr23* or any of the resistance genes mapped to the distal ends of chromosome 2B, although *Triticum timopheevii* occurs in the pedigree of Isengrain's parent Apollo (Martynov et al. 2006).

6.1.3.3 Minor Effect on Chromosome 3A

On chromosome 3A the only minor leaf rust resistance QTL (% infected leaf area) inherited from Arina was detected in the experiment Reichersberg 2008. It was associated with SSR marker *Xgwm720*. In the population Arina/Forno no effect was identified on this chromosome (Schnurbusch et al. 2004), maybe because a QTL transmitted by Forno alleles was detected in the population Forno/Oberkulmer on the long arm near the centromere (Messmer et al. 2000). As far as the Forno/Oberkulmer map (Messmer et al. 1999) is comparable to the Arina/Capo map, it seems possible that the effects are located in a similar region. In durum wheat Maccaferri et al. (2008) detected a QTL for leaf rust severity measured by the percentage of infected leaf area effective at the early and medium stage and also for the AUDPC in the marker interval *Xbarc45-Xwmc664* and distal (Mantovani et al. 2008). In a subsequent study the SSR markers *Xwmc664* and *Xwmc388.2* were associated with effects on seedling infection type and adult plant resistance (Maccaferri et al. 2010).
Lr63 was introgressed from *Triticum monococcum* to common wheat and located on the short arm of chromosome 3AS, very closely linked to *Xbarc321* and *Xbarc75* (Kolmer et al. 2010). From *Aegilops speltoides* ssp. *ligustica Lr66* was transferred to chromosome 3A of wheat (Marais et al. 2010b). As these introgressions happened just recently, it is unlikely that Arina, which was released in 1981 (Martynov et al. 2006), carries one of these genes.

6.1.3.4 Major Effect on Chromosome 3B

The only major leaf rust resistance QTL transmitted by Capo alleles was mapped to the short arm of chromosome 3B. It was consistent over single assessments of infected leaf area (six single experiments, mean over three and six experiments) and the relative AUDPC (experiment Piešťany 2006, mean over two experiments). The results of the

population Isengrain/Capo were also verified in the population Arina/Capo, for which it was consistent over single assessments of seven experiments and the mean over eleven experiments as well as for the relative AUDPC of both experiments and the mean over them. As the marker order of the 3BS region of the Isengrain/Capo map differed from the wheat consensus map (Somers et al. 2004), the sequence of the few and partly not linked markers in the population Arina/Capo might differ from that shown in Fig. 45. Depending on the experiment, two different AFLP markers were closest in the population Isengrain/Capo with the SSR marker *Xgwm533* lying in-between them. In single point ANOVA for means over eleven experiments and the mean relative AUDPC (two experiments) of the population Arina/Capo *Xgwm533* was not significant. *Xgwm389.1* which was throughout next to the QTL peak in this population revealed no polymorphism between the cultivars Isengrain and Capo and thus was not usable for genotyping.

On the short arm of chromosome 3BS the resistance gene *Lr27* is located (Singh and McIntosh 1984). It is only effective if the complementary gene *Lr31* on 4BS (Singh and McIntosh 1984, McIntosh et al. 1995) or 4BL (Nelson et al. 1997) is present, too (Singh and McIntosh 1984). Just recently, Ingala et al. (2012) mapped a gene from the Argentinean durable leaf rust resistant cultivar Sinvalocho MA to this 3BS region. It was completely linked to marker *Xgwm533* and designated *LrSV2*. As it confers adult plant resistance in contrast to the seedling resistance gene *Lr27*, further studies are required to find out, whether they are allelic.

Faris et al. (1999) detected an effect on seedling leaf rust resistance in the *Lr27* region in an Opata85/W-7984 (synthetic wheat) cross. In a population Frontana/Remus Steiner (2003) identified a major QTL for leaf rust resistance inherited from Frontana distal to *Xgwm389*. The most distal marker in the map was *Xgwm493*, thus marker order was inverted compared to the wheat consensus map (Somers et al. 2004) and the Isengrain/ Capo map. Distal to *Xgwm493* Marza et al. (2006) identified a QTL for leaf rust reaction (% severity) consistent across environments in a population Ning7840/Clark (Chinese facultative wheat/US soft red winter wheat). In only one experiment Messmer et al. (2000) detected an effect in the population Forno/Oberkulmer transmitted by the Oberkulmer allele, which they assigned to the long arm of chromosome 3B near the centromere. In a similar region Maccaferri et al. (2010) found effects on adult plant resistance of durum measured by the AUDPC associated with the SSR markers *Xgwm685* and *Xbarc203* located in the centromeric and the proximal region of the long arm. Also the QTL for leaf rust resistance identified by Singh et al. (2009) on the short arm in a population Beaver/Soissons was effective in just one field experiment, another on the long arm in two experiments. Unfortunately no map with the linked AFLP and DArT markers is available. A further QTL for field infection type and infected area was detected on the long arm of chromosome 3B in a region distal to SSR marker *Xbarc164* (Chu et al. 2009).

WMS493 is a probe for *Lr27* and *Xgwm493-3B* (USDA 2014). Marker *Xgwm493* was mapped at a distance of about 20 cM from the peak marker, but in all experiments the LOD value has dropped below the threshold at this map position. Unfortunately markers mapped to 3BS in the population Isengrain/Capo clustered at the telomere and the

nearest markers were located about 17 cM distal and 21 cM proximal to *Xgwm493*. Vast homology between Isengrain and Capo in this chromosomal region might be the reason for this low marker density: Out of eight SSR markers described for the terminal region of 3BS and tested for polymorphism between the parental lines Isengrain and Capo only five were applicable to the population, but two of them were mapped to other linkage groups. Nelson et al. (1997) mapped *Lr27* in the marker interval *Xbcd907-Xtam61*, thus distal to *Xgwm493* – as far as the maps are comparable in that detail. Therefore from this point of view it seems impossible to make any reliable statement whether Capo might carry *Lr27* or not. As a consequence the haplotype comparison was performed (see 6.1.4 SSR Marker Haplotype Comparison).
Australian pathotypes virulent to *Lr27* and *Lr31* occurred soon after the release of the decreasingly resistant cultivar Gatcher (McIntosh et al. 1995). The frequency of virulent isolates and the effectiveness differed considerably between studies (detailed references in Table 93 to Table 95).

6.1.3.5 Minor Effect on Chromosome 5A

In the population Arina/Capo a minor effect on leaf rust severity (% infected leaf area) located on chromosome 5A near SSR marker *Xgwm1057* and transmitted by Arina alleles was detected in the experiment Tulln 2009.
Tsomin et al. (1990) identified two complementary dominant genes for leaf rust resistance on the chromosomes 5A and 1D of the line Yantar. To the short arm of chromosome 5A (and to four further chromosomes) *Lr38* from *Agropyron intermedium* was translocated (Friebe et al. 1993, 1996). In the population Forno/Oberkulmer a QTL for leaf rust resistance (leaf area covered with pustules) associated with the short arm of chromosome 5A and transmitted by Forno alleles was identified in a single experiment (Messmer et al. 2000), but it was not detected in the population Arina/Forno (Schnurbusch et al. 2004). On the same chromosome arm, proximal to SSR marker *Xbarc10*, Singh et al. (2009) detected a QTL for adult plant leaf rust resistance. Furthermore an APR gene and *LrM* (Tankimanova et al. 1993), *LrM7* (Shulembaeva et al. 1997) and a resistance gene inherited from *Triticum monococcum* ssp. *aegilopoides* (Hussien et al. 1998) have been located on chromosome 5A of wheat.
In durum wheat a QTL for seedling infection type on 5AS associated with SSR marker *Xwmc489.1* was identified by Maccaferri et al. (2010). At the distal end of 5AL in the marker interval *Xgwm595-Xgwm291* a QTL for relative latency period of adult plants was detected in the growth chamber (Marone et al. 2009).
In the experiment Tulln 2007 Thatcher NILs with *Lr38* were highly resistant with only 3 % infected leaf area, whereas in Martonvásár averaged infection over the period 2004-2008 was more than 80 %. Apart from that just in South America the effectiveness has been studied and *Lr38* was reported to confer seedling resistance in Uruguay (Germán et al. 2007).

6.1.3.6 Major Effect on Chromosome 7B

The most effective leaf rust resistance QTL detected in the population Isengrain/Capo was transmitted by Isengrain alleles and assigned to the long arm of chromosome 7B. It was consistent over single assessments of the percentage of infected leaf area of twelve experiments, the relative AUDPC and the appearance of teliospores. The QTL peak was located in the marker interval *XS20M23_28-XS23M13_22* with *Xgwm132.1* being closest in most of the experiments. As for the wheat consensus map (Somers et al. 2004) and the Arina/Forno map (Paillard et al. 2003), in the Isengrain/Capo map it is also a region of very high marker density: 28 AFLP and SSR markers were mapped within just 11 cM, thus about 2.5 markers/cM.

In this chromosomal region the resistance gene *Lr14a* derived from Yaroslav emmer (Law and Johnson 1967, Kolmer 2007) and *Lr14b* from common wheat were identified (McIntosh et al. 1995). They were tightly linked with a recombination value of just 0.16 % and therefore considered as alleles, although it was possible to combine both in one single line (Dyck and Samborski 1970). Dyck and Samborski (1970) furthermore studied the inheritance of pathogenicity to the two alleles of *Lr14* and found out that virulence to both was inherited by a single gene, which is recessive in the case of *Lr14a*, but dominant in the case of *Lr14b*. *Lr14a* showed high sensitivity to the environment (Browder 1980), and was more effective at temperatures below 20°C (Dyck and Johnson 1983 cited in McIntosh et al. 1995). Herrera-Foessel et al. (2008) mapped *Lr14a* 1 cM proximal to SSR marker *Xgwm344* in durum wheat.

Lr14a is a gene for seedling resistance present in many European wheat cultivars (Winzeler et al. 2000, Park et al. 2001, Pathan and Park 2006). In Northern Europe it is among the most common genes (Hysing et al. 2006). In the Canadian prairie region the release and large scale cultivation of the cultivar Renown and derivatives carrying *Lr14a* was followed by a rapid increase of virulence frequency to this gene (Kolmer 1991). This seems to be true for all wheat growing areas. All surveys revealed that virulence to *Lr14a* – and similar to *Lr14b* – is very common throughout the world (Table 93). Thus the level of protection in the field is very low (Table 94). Cultivars with *Lr14ab* were moderately resistant in India in the late 1970s/beginning 1980s (Sawhney et al. 1982), but susceptibility increased during the following decade (Sawhney et al. 1992). In durum wheat virulence to *Lr14b* is even more common than to *Lr14a* (Ordoñez and Kolmer 2007).

In seedling tests Isengrain and its parental line Soissons showed a susceptible infection pattern to different isolates of *Puccinia triticina* similar to Thatcher NILs carrying *Lr14a* and were thus postulated to have *Lr14a* as a single resistance gene (Błaszczyk et al. 2004). Furthermore Yaroslav emmer, transmitting *Lr14a*, occurs in Apollo's pedigree (Martynov et al. 2006). In the experiment in Tulln in 2007 about 60 % of the leaf area of Thatcher NILs with either of these two alleles exhibited leaf rust symptoms, in Martonvásár averaged infection over the period 2004-2009 of NILs carrying *Lr14a* was almost 90 % (Vida et al. 2009).

Marone et al. (2009) identified a major QTL for reduced disease severity of adult durum wheat plants (measured by the percentage of leaf area covered with rust pustules) and for increased latency period (the time between inoculation and appearance of 50 % of the uredinia) on the long arm of chromosome 7B in a region very similar to *Lr14a*. As the used isolates of *Puccinia triticina* were virulent on *Lr14a* they concluded that the allele inherited from the cultivar Creso must be different and named it *Lr14c*. In the Creso derivative Colosseo Maccaferri et al. (2008) detected a major QTL effective across the entire cycle of disease development (*QLr.ubo-7B.2*) in the same chromosomal region. A much weaker but similar effect (*QLr.ubo-7B.1*) was identified in the proximal region of the short arm in the marker region *Xwmc405.1-Xgwm573*. But in this case Creso inherited the susceptible allele. Association mapping of elite durum lines revealed similar results: The main effect on seedling leaf rust infection type and adult plant response consistent across different field trials and isolates was associated with the marker interval *Xgwm577-Xwmc10* in which *Lr14* and *QLr.ubo-7B.2* were located (Maccaferri et al. 2010). On the distal end of the short arm a further effect was associated with SSR marker *Xwmc323* and another influence on seedling infection type with *Xgwm1184* at the proximal end.

In the population Arina/Forno Schnurbusch et al. (2004) identified a QTL for leaf rust resistance measured by the AUDPC using the Cobb scale (% infection) derived from Arina on the short arm of chromosome 7B in the marker interval *sfr.BE427461-Xgwm573b*. On the long arm in the region *XksuD2-XgbxG218b* they detected a QTL for leaf rust resistance measured by the AUDPC of response to infection inherited from Forno. As Park et al. (2001) found out that Forno carries *Lr14a*, Schnurbusch et al. (2004) assumed that the QTL detected on 7BL might correspond to *Lr14a*. In the population Forno/Oberkulmer several QTL for the leaf area covered with pustules were identified on chromosome 7B (Messmer et al. 2000): On the short arm in the marker interval *Xpsr952-Xgwm46* the effect was transmitted by Oberkulmer alleles. It most likely corresponds to the QTL inherited from Arina in the population Arina/Forno, thus Forno seems to carry the adverse allele for this QTL. The other detected QTL were identified on the long arm. A major QTL which was effective in three out of four experiments was located in the *Xpsr593c-Xpsr129c* region. In the fourth experiment a major effect was detected in the proximal neighboring marker interval *Xpsr350-Pwir232b*. A further minor effect was identified in the distal *Xglk750-Xmwg710a* interval. In all three cases Forno transmitted the alleles associated with increased resistance. Unfortunately the Forno/Oberkulmer and the Arina/Forno map are hardly comparable in this chromosomal region. In a similar region as Schnurbusch et al. (2004) detected a QTL on 7BL in the population Arina/Forno an effect on field leaf rust severity in a population Synthetic/Opata was positioned (Nelson et al. 1997) and a QTL for adult plant reaction to leaf rust under conditions of natural infestation in another population Synthetic/Opata (Faris et al. 1999). In a single-seed-descent RIL population CI 13227/ Suwon 92 Xu et al. (2005b+c) revealed effects on latent period (average number of days from inoculation to epidermis rupture of the first uredinium), final leaf rust severity (%), AUDPC and infection rate (daily disease progress rate, AUDPC/day) associated with the marker interval *Xbarc50-Xbarc182*. Rosewarne et al. (2008) identified a QTL for leaf rust

severity (%) in a RIL population Avocet-S/Attila in the marker interval *Xgwm146-Xwmc273*, Nyori (2010) a QTL for slow rusting resistance in a RIL population Avocet-S/Amadina proximal to *Xwmc276*.

Davoyan and Tenovskaya (1996) introgressed two genes from *Triticum miguschovea* to common wheat, of which one seems to be located on chromosome 7B. William et al. (1997) detected a QTL for leaf rust resistance in the population Parula/Siete Cerros on 7BL which they speculated to be homoeoallelic to *Lr34* on 7DS.

As elaborated above, in several studies with different wheat and durum populations QTL were identified in or very tightly linked to the *Lr14* region (Nelson et al. 1997, Faris et al. 1999, Messmer et al. 2000, Schnurbusch et al. 2004, Xu et al. 2005b+c, Maccaferri et al. 2008, Marone et al. 2009) and the region harboring the Isengrain QTL. As *Lr14* is no longer very effective throughout the world (detailed references in Table 93 to Table 95), it seems that there might be alleles different from *Lr14a* and *Lr14b* as suggested by Marone et al. (2009), very closely located QTL or yet unknown factors influencing the effectiveness of this resistance gene.

Sharma and Knott (1966) introgressed the resistance gene *Lr19* from *Agroypron elongatum* (= *Thinopyrum ponticum*, *Lophopyrum ponticum*) to chromosome 7DL of wheat. By double crossover it was relocated to chromosome 7BL in the recombinant line Lr19-149 (Prins et al. 1997). In another case *Lr19* was translocated to 7AL (Eizenga 1987). Also in durum *Lr19* was located on 7AL (Zhang et al. 2005).

Virulence to *Lr19* occurs very rarely and thus the level of protection in the field is high. Leaf rust infection of lines carrying *Lr19* has just been reported from India (Sawhney et al. 1982, Bhardwaj et al. 2005), Mexico (Huerta-Espino 1994), Ukraine (Elyasi-Gomari and Panteleev 2006, Elyasi-Gomari and Lesovaya 2009, Elyasi-Gomari and Mikhailovno 2009), Germany, the Russian Federation (Lind and Gultyaeva 2007), Western Siberia (Morgounov et al. 2007), Turkey (Akin et al. 2008), Pakistan (Rattu et al. 2009, 2010), Czech Republic (Hanzalová 2010), Argentina (Vanzetti et al. 2011) and Iran (Safavi and Afshari 2013), but the frequency of virulent isolates and the level of infection were very low. Neither in Tulln nor in Martonvásár Thatcher NILs with *Lr19* were infected (Vida et al. 2009) and similar in all the other studies lines carrying *Lr19* did not show any symptoms of leaf rust infection aside necrotic or chlorotic flecks (detailed references in Table 93 to Table 95).

Several SSR markers located within the Isengrain QTL were described as nearby loci for *Lr19* on 7BL in the GrainGenes database (USDA 2014). To gain more information of the Isengrain allele on 7BL conferring leaf rust resistance, haplotype comparison with lines carrying either *Lr14a* or *Lr19* was performed (5.6 SSR Marker Haplotype Comparison).

Just recently Herrera-Foessel et al. (2012) showed that the slow rusting adult plant resistance gene in the *Lr14* region they designated *Lr68*, is close to *Xgwm146*, but different from the *Lr14a* and *Lr14b* loci. *Lr68* or another tightly linked minor gene on 7B seems to be also the source of the French durum wheat Sachem's resistance in addition to *Lr14a* and just as *Lr14a* located closely to *Xgwm146* (Singh A et al. 2013).

Zhang et al. (2011) identified a resistance gene closely linked to *Xgwm344*, but with a seedling reaction pattern different from Thatcher lines carrying *Lr14a* and *Lr14b* and temporarily designated it *LrBi16*.

6.1.3.7 Minor Effect Associated with *Xwmc25.1* (Unassigned Linkage Group)

A second minor QTL originating from Capo was detected in the population Isengrain/Capo in the yet not definitely assigned linkage group 2BD6AB7B in the marker interval *Xwmc25.1-XS22M20_8* in the experiments Piešťany 2006 and Rust 2009. By single point ANOVA it was also verified in the population Arina/Capo, in which it was consistent over single assessments (% infected leaf area) of five experiments and the relative AUDPC of the experiment Fundulea 2008. It was associated with SSR marker *Xwmc25.1*. As *Xwmc25.2* was unambiguously mapped to chromosome 2BS, but not linked to *Xwmc25.1*, it is unlikely that this effect is related to chromosome 2B and thus to *Lr13* that has already been described for Capo (Winzeler et al. 2000, Mesterházy et al. 2002, Park et al. 2001). A marker produced by the primer pair Wmc25 was also mapped to the short arm of chromosome 2D (Somers et al. 2004). *Xgwm132.3* was a neighboring SSR marker in the linkage group 2BD6AB7B. Singh et al. (2010) mapped markers produced by the primer pair Gwm132 to chromosomes 2B, 6A, 6B and 7B. To the linkage group assigned to chromosome 6B marker *Xgwm132.2* was assigned, to 7B *Xgwm132.1*. No linkage groups were assigned to the particular regions of chromosomes 2D and 6A. Therefore it is more likely that the effect is located there.

Several effects on leaf rust resistance have already been identified on the short arm of chromosome 2D. For *Lr2* (Luig and McIntosh 1968) three alleles (*Lr2a*, *Lr2b* and *Lr2c*) were detected (Soliman et al. 1964 cited in McIntosh et al. 1995, Dyck and Samborski 1968). In several surveys *Lr2a* appeared to be the most and *Lr2c* the least effective allele, but virulence to all three alleles has been detected frequently throughout the world (detailed references in Table 93 to Table 95).

McIntosh and Baker (1968 cited in McIntosh et al. 1995) assumed that also *Lr15* (Luig and McIntosh 1968) is allelic to *Lr2*. Whereas Manisterski et al. (2000) found low virulence frequency to *Lr15* in Israel, others found widespread virulence in Europe and throughout the world. Effectiveness likewise varied between experiments (detailed references in Table 93 to Table 95).

Furthermore *Lr22* introgressed from *Aegilops squarrosa* is located on 2DS (Rowland and Kerber 1974). Another gene first described by Bartoš et al. (1969) was identified by Dyck (1979) to be an allele and was thus designated as *Lr22b*. Hiebert et al. (2007) mapped *Lr22a* 2.9 cM proximal to SSR marker *Xgwm296*. Virulence to *Lr22b* has been very common since at least the early 1960s and *Lr22b* was furthermore detected in the cultivar Thatcher (Dyck 1979). Moreover *Lr22* confers adult plant resistance, thus virulence studies are rare. Two studies testing *Lr22a* revealed widespread virulence. The effectiveness of *Lr22b* seems to be generally lower compared to *Lr22a*. Whereas lines carrying *Lr22a* were highly susceptible to Ukrainian and Western Siberian inoculum, plants

showed good protection against pathotypes from other European regions (detailed references in Table 93 to Table 95).

Raupp et al. (2001) located *Lr39* translocated from *Aegilops tauschii* distal and *Lr2* as well as *Lr22* proximal to SSR marker *Xgwm210*. Compared to the wheat consensus map (Somers et al. 2004) they mapped the markers *Xgwm210* and *Xgwm296* in reverse order and the distances both between them and to the next marker *Xgwm455*, were larger. By molecular mapping Singh S et al. (2004) also assigned *Lr41* to chromosome 2DS, distal to SSR marker *Xgwm210* and found strong evidence that it is allelic to *Lr39*, which was confirmed by Gill et al. (2006). Both, Singh S et al. (2004) and Sun et al. (2009), mapped *Lr41* distal to *Xgwm210* and *Xbarc124*, but with a much smaller distance (1.9 and 0.5 cM) compared to the wheat consensus map (Somers et al. 2004). Unfortunately no virulence data for *Lr39* were available with the only exception of one German study testing isolates collected from certain Thatcher NILs (Serfling et al. 2011). Pathotypes virulent to *Lr41* have been detected in South Africa (Pretorius 1997) and the United States, but the frequency was generally low and effectiveness high (detailed references in Table 93 and Table 95).

Just recently Ingala et al. (2012) mapped *LrSV1*, a resistance gene of Sinvalocho MA, close to *Xgwm296*. Compared to the wheat consensus map (Somers et al. 2004) marker order was inverted. Whereas *Lr22a* can be excluded due to Sinvalocho MA's available pedigree, it still has to be elucidated, whether *LrSV1* is an allele different from *Lr22b* or a different locus.

The percentage of infected leaf area of Thatcher NILs tested in Tulln 2007 and the averaged infection over the period 2004-2008 in Martonvásár (Vida et al. 2009) were (Tulln/ Martonvásár): 40/~55 % (*Lr2a*), 50/~63 % (*Lr2b*), 40/almost 80 % (*Lr2c*), 50/~65 % (*Lr15*) and 30/~15 % (*Lr22*/*Lr22a*).

Additionally several studies detected effects on leaf rust infection that were assigned to the short arm of chromosome 2D. Schnurbusch et al. (2004) identified a QTL for leaf rust resistance transmitted by Forno alleles in a population Arina/Forno in the 2DS region around the SSR markers *Xgwm296* and *Xgwm261*. They assumed that this effect might correspond to *Lr2* or *Lr22*. Distal to *Xgwm261* Xu et al. (2005c) identified a QTL for leaf rust infection duration (length of the sporulation period). Also the QTL associated with seedling and adult plant resistance transferred from *Triticum tauschii* and detected by Leonova et al. (2007) was located around *Xgwm261*. Proximal to *Xgwm455* – the peak marker *Xbarc124* is not contained in the wheat consensus map (Somers et al. 2004) – Xu et al. (2005b) detected an effect on latent period (mean number of days from inoculation to when a uredinium ruptured the epidermis).

Lr54 transferred to wheat from *Aegilops kotschyi* was mapped by Marais et al. (2005) to the long arm of chromosome 2D.

Regarding both the mapped region and the quite high infection level of these resistance genes, it is rather unlikely that the effect identified in the population Isengrain/Capo associated with *Xwmc25.1* might correspond to one of them, most presumably to *Lr39*, *Lr41* or one of the QTL identified on 2DS.

Hussien et al. (1998) located a resistance gene inherited from *Triticum monococcum* ssp. *monococcum* on chromosome 6A. From *Aegilops sharonensis Lr56* was introgressed into hexaploid wheat. This translocation involved the whole short and the proximal long arm of chromosome 6A (Marais et al. 2006). It is located near the telomere of the long arm (Marais et al. 2010a). A similar part of this chromosome was replaced by a spontaneous translocation from *Aegilops neglecta* carrying the resistance gene *Lr62* (Marais et al. 2009). *Lr64* was identified on the long arm of chromosome 6A in a wheat-*Triticum turgidum* ssp. *dicoccoides* cross (McIntosh et al. 2009).
Due to lack of information on the pedigree of Purdue line PD-5517-1-8-2-1 (Martynov et al. 2006) and little knowledge on Martin, it is unclear, whether Capo can possess resistance genes from *Triticum aestivum* relatives.

6.1.4 SSR Marker Haplotype Comparison

Only two major QTL for leaf rust resistance that were consistent over several experiments and the mean were identified in the population Isengrain/Capo. The effect located on chromosome 3B in the marker interval XS23M15_3-*XS13M20_9* was inherited from Capo and was verified in the population Arina/Capo. The second QTL originated from Isengrain and was assigned to chromosome 7BL. These two were further investigated by haplotype analysis of SSR markers in those regions.

6.1.4.1 Chromosome 3B

Near the region of the 3B QTL *Lr27* was described (Singh and McIntosh 1984). In the GrainGenes database (USDA 2014) WMS493 is described as a probe for *Lr27*, WMS493 and WMC493 are quoted as probes for *Xgwm493-3B*, but marker *Xgwm493* was in all experiments clearly outside the QTL region. For the haplotype comparison nine lines carrying *Lr27* were analyzed with the two SSR markers located within the QTL as well as *Xgwm493*. Only for the latter most of the lines carrying *Lr27* showed the same fragment as Capo. For *Xgwm533*, the marker closest to the QTL peak and dominant for the Isengrain allele, just a single line produced no fragment similar to Capo, the eight other lines showed a fragment different to the Isengrain allele. In the case of *Xbarc75*, the second SSR marker mapped within the QTL, the alleles of four lines corresponded to Capo, two to Isengrain, and the others produced either no fragment or fragments with a different length.
Based on these results it seems very unlikely that Capo carries *Lr27*, but further investigation will be necessary.

6.1.4.2 Chromosome 7B

In the region, where the QTL on the long arm of chromosome 7B was detected, *Lr14a* was identified (Law and Johnson 1967, McIntosh et al. 1995), which was also described for Isengrain (Błaszczyk et al. 2004).

Lr14a was no longer effective in Tulln or in other studies (Table 93 and Table 94), and Isengrain was in most of the experiments clearly more susceptible than Capo. Five of the SSR markers located within the QTL (*Xgwm344*, *Xbarc340*, *Xbarc182*, *Xgwm146*, *Xwmc232*) and five further markers (*Xgwm611*, *Xgwm577*, *Xbarc50*, *Xwmc276*, *Xwmc166*) were described as nearby loci for *Lr19* on 7BL in the GrainGenes database (USDA 2014). The latter were not usable for mapping because – if at all – they produced fragments that were too difficult to score or monomorphic.

Numerous studies have been done on the genetics of *Lr19*, but in most of them endopeptidase, RFLP, STS, SCAR, RAPD or AFLP markers were used (e.g. Winzeler et al. 1995, Prins et al. 1996, 1997 and 2001, Prins and Marais 1998, Chełkowski 2003, Cherukuri et al. 2003, Groenewald et al. 2003 and 2005, Šliková et al. 2003 and 2004, Błaszczyk et al. 2004 and 2008, Prabhu et al. 2004, Singh R et al. 2004, Zhang et al. 2005, Gupta et al. 2006, Li et al. 2007, Uhrin et al. 2008). Only a few molecular genetic analyses were performed with SSR markers (e.g. Li et al. 2006, Dreisigacker 2009). WMC221 (Dreisigacker 2009) produced just a monomorphic fragment, *Xgwm44* (Li et al. 2006) was already mapped to chromosome 7D in the population Isengrain/Capo.

In total eleven lines carrying *Lr14a* and ten lines carrying *Lr19* as well as Isengrain's parents Apollo and Soissons, the latter transmitting *Lr14a* (Błaszczyk et al. 2004), were characterized with the 13 SSR markers mapped to the 7BL QTL and *Xgwm44*. The cultivar Hope occurs in Apollo's pedigree, Courtot in Soissons' (Martynov et al. 2006). These two were therefore included in the analysis.

The results of the haplotype analysis indicate that Isengrain does not carry *Lr19*. There was good agreement of Isengrain with six lines carrying *Lr14a* and Isengrain's parental line Soissons, but hardly any congruence between Isengrain and lines carrying *Lr19*. The effect is obviously inherited from Courtot via Soissons and not from Hope via Apollo. Regarding the level of infection of Thatcher NILs, and the parental lines Isengrain and Capo in field experiments, and the results of the haplotype analysis, it seems that the 7BL region in common wheat is as complex and yet not fully understood as in durum wheat.

Maccaferri et al. (2010) performed haplotype analysis of different durum wheat elite accessions with main emphasis on the distal region of chromosome 7BL, where *Lr14a* (Law and Johnson 1967), *Lr14c* of Creso (Marone et al. 2009) and *QLr.ubo-7B.2* inherited from the Creso derivative Colosseo (Maccaferri et al. 2008) were located. Though cultivated for over thirty years, Creso is still resistant in most of the durum growing areas (Maccaferri et al. 2010). They discovered that Creso, Creso derivatives and cultivars with *Lr14a* shared at least the alleles of the six most distal markers of 7BL and that just the most distal three of them, *Xcfa2257.2*, *Xgwm344.2* and *Xwmc10*, were associated with the Creso phenotype of leaf rust infection type. Nevertheless they found analogously to Marone et al. (2009) that certain isolates of leaf rust were virulent on Thatcher NILs with

Lr14a, but avirulent on Creso. Therefore Maccaferri et al. (2010) suggested that their relationship should be resolved by an allelism test plus cloning and sequencing of the genes and their upstream sequences as this 7B QTL region is currently one of the most important resistance sources in durum.
Because of the strong effect also further research on the 7BL QTL inherited from Isengrain appears very attractive. It is worthwhile clarifying, whether Isengrain carries an *Lr14* allele different from those already known or if there are any other not yet identified factors in the population Isengrain/Capo positively influencing the resistance of lines carrying the *Lr14a* allele.

6.1.5 Epistatic Interactions

No epistatic interactions, but only additive effects between QTL identified in the population Isengrain/Capo were detected. The maximum difference between best and worst allele combination is 13.9 %. This is smaller compared to e.g. the difference for the two alleles at the 7BL QTL (17.4 %). Therefore it can be said that the two major QTL act additively and are not biased by epistatic interactions. Not even between a QTL and another chromosomal region epistatic effects were detected that were consistent over tightly linked markers, although for several resistance genes that were described for regions of or close to QTL of the population Isengrain/Capo interactions with other genes have previously been reported.
For the leaf rust resistance gene *Lr14a* Law and Johnson (1967) detected modifying genes in the cultivar Hope. They were located on the short arm of chromosome 7B and two possibly homoeologous genes on 7A and 7D. It was observed that cultivars carrying *Lr13* identified in Capo (Winzeler et al. 2000) and *Lr14a* inherited by Isengrain (Błaszczyk et al. 2004) were highly resistant to Australian pathotypes that were virulent on *Lr13* alone (McIntosh et al. 1995). Kolmer (1992a) reported several combinations of *Lr13* with other resistance genes governing enhanced resistance compared to the parental lines with the individual *Lr* gene, with some further *Lr* genes no interactions were detected (Pretorius and Roelfs 1996, Bender et al. 2000). Some studies revealed that it depends on the virulence pattern of the rust pathotype, whether resistance is improved when *Lr13* is combined with other genes or not (Kloppers and Pretorius 1997, Kolmer 1997, Park et al. 2002). Studying the resistance inherited from the cultivars Frontana and Manitou, both carrying *Lr13*, Dyck et al. (1966) found out that modifiers increased resistance and changed the gene from recessive to partially dominant, but that *Lr13* as well as the modifiers showed high sensitivity to the environmental conditions.
If *Lr27* is responsible for the QTL on 3BS, then *Lr31* located on chromosome 4B needs to be present for expression of resistance (Singh and McIntosh 1984). Singh et al. (1999) observed that cultivars with the gene combination *Lr27* and *Lr12* had a resistant phenotype similar to those with the complementary genes *Lr27* and *Lr31*. They concluded that *Lr12* and *Lr31* are either the same genes or closely linked as has been previously suggested by Park and McIntosh (1994). *Lr12* (Dyck and Kerber 1971, McIntosh et al.

1995) as well as *Lr31* (McIntosh et al. 1995) were located on chromosome 4B. Nelson et al. (1997) mapped *Lr31* on the long arm next to RFLP marker *XksuG10*. *Lr12* was identified in the marker interval *Xgwm251-Xgwm149* about 1 cM proximal to the marker identified for *Lr31* (Singh and Bowden 2011). Compared to the wheat consensus map (Somers et al. 2004) the two SSR markers were mapped in reverse order. Under high disease pressure the complementary genes *Lr27* and *Lr31* seemed to interact with *Lr34* governing increased resistance (Sawhney 1992).

Cappelle Desprez, which occurs three times in the pedigree of Isengrain's parent Apollo (Martynov et al. 2006), was postulated to carry *Lr34* (McIntosh 1992 cited in McIntosh et al. 1995). The leaf rust infection of Thatcher NILs with *Lr34* in Tulln was between the level of Capo and Isengrain in 2007, and in Martonvásár average infection was more than 40 % (Vida et al. 2009). It appears very unlikely that both lines – Capo and Isengrain – carry this resistance gene and that *Lr34* was therefore not detected in this population. It is tightly linked with leaf tip necrosis (Singh 1992), but both cultivars exhibited just few symptoms, and also the average level of the population was just about 2 on a 0-9 scale. Furthermore preliminary tests revealed that Capo does not carry *Lr34* (Matiasch et al. 2007, 2010).

Marker density of the Isengrain/Capo map is very variable, with an average marker distance of 3.5 cM. Thus it might be possible that a few epistatic interactions were not detected because of too large gaps. Nevertheless the absence of epistatic interactions might also be a further evidence that Capo does not carry *Lr27* and Isengrain some resistance allele different from *Lr14a*.

6.2 Heading, Plant Height and Other Traits

6.2.1 Field Experiments

In all three populations Isengrain/Capo, Arina/Capo and Furore/Capo in addition to leaf rust severity heading was assessed in most of the experiments and plant height assessment performed in all trials in Tulln. If remarkable differences of the traits leaf blotch severity, *Septoria* leaf blotch severity, powdery mildew severity, yellow rust severity, frost heaving severity, lodging severity, leaf chlorosis severity and leaf tip necrosis severity between the genotypes occurred, they were evaluated in the particular population(s). In the population Arina/Capo furthermore the traits awnedness and glaucousness were assessed as Arina is an awned cultivar and clearly more waxy than Capo. For all these traits except awnedness all three populations showed continuous variation, suggesting that either genes are acting quantitatively, several genes are involved, or the traits are influenced by environmental factors. Generally there were not just significant ($\alpha = 0.05$) effects of the genotypes, but differences between the experiments and interactions between genotype and experiment. In a few cases (Isengrain/Capo: leaf chlorosis severity and leaf tip necrosis severity; Arina/Capo: plant height and leaf tip necrosis severity) no significant interactions were detected. For both populations no difference between the experiments was detected for leaf chlorosis and leaf tip necrosis severity. This can be due

to the fact that these two traits were assessed in only two experiments in the same year with a distance of about ten kilometers only between the fields. More remarkable was that in the population Furore/Capo no influence of the genotype on powdery mildew severity was detected, but significant differences between the two experiments and interactions between genotype and experiment. Differences between the parental lines were very low and thus obviously differences in powdery mildew severity between plots were primarily due to environmental influences.

6.2.2 QTL Detection

In the population Isengrain/Capo 17 QTL for seven different traits were identified in eleven linkage groups. In the population Arina/Capo eleven QTL were detected in linkage groups and five were associated with single markers, thus twelve different mapped or supposed linkage groups were associated with QTL for eight different traits. Table 96 gives an overview on the detected QTL and the relevant linkage groups in the two populations. “?” refers to markers with significant effects detected in single point ANOVA in the population Arina/Capo that were not identified in the population Isengrain/Capo. In these cases the linkage group to which the particular marker was mapped in the population Isengrain/Capo is given.

With the exception of the leaf rust resistance QTL discussed in 6.1.3 no QTL identified in the population Isengrain/Capo was detected in the population Arina/Capo in the same chromosomal region. In the case of the leaf tip necrosis severity QTL associated with chromosome 5B different marker intervals seemed to be involved.

The differences concerning chromosomes 2A_2 (leaf tip necrosis severity), 2B_2 (plant height), 2D (heading, leaf chlorosis severity), 4A (leaf tip necrosis severity), 4B (leaf tip necrosis severity), 6B (lodging severity), 7B_2 (leaf chlorosis severity) and the three yet not definitely assigned linkage groups 2BD6AB7B (heading), 2B4B5A7B (plant height), 6AB (plant height, lodging severity) can be explained by the lack of a corresponding group or chromosomal region mapped for the population Arina/Capo. The traits *Septoria* leaf blotch severity, glaucousness and yellow rust severity were only assessed in the population Arina/Capo, leaf blotch severity in the population Isengrain/Capo. A further reason for differences in QTL detection might be the significantly lower threshold in the population Arina/Capo. Thus several QTL with a maximum LOD value below three were detected that were not identified in the population Isengrain/Capo: on chromosomes 3A (leaf chlorosis severity), 3B_2 (leaf tip necrosis severity), 5A (plant height, lodging severity, and leaf tip necrosis severity), and 6B (plant height).

Table 96 Comparative view on the QTL detected in the populations Isengrain/Capo and Arina/Capo

trait	Isengrain/Capo	Arina/Capo
leaf rust severity	1B_1^{C}, 2B_1^{I}, 2BD6AB7B^{C}, 3B^{C}, 7B_2^{I}	2BD6AB7B?^{C},3A^{A}, 3B_1^{C}, 5A^{C}
relative AUDPC	1B_1^{C}, 2BD6AB7B^{C}, 3B^{C}, 7B_2^{I}	2BD6AB7B?^{C}, 3B_1^{C}
teliospores	7B_2^{I}	not tested
heading	2BD6AB7B^{I}, 2D^{I}	
plant height	2B_2^{I}, 2B4B5A7B^{I}, 4B^{I}, 6AB^{I}	5A^{C}, 6B^{C}
awnedness	not tested	4D^{C}
leaf blotch severity	4B^{C}	not tested
Septoria leaf blotch severity	not tested	7A_2?^{A}
powdery mildew severity	4B^{I}	
yellow rust severity	not tested	3B_1^{C}
glaucousness	not tested	2B_1?^{C}, 3A^{C}, 4B^{A}
lodging severity	6AB^{I}, 6B^{I}	2B_1?^{A}, 5A^{C}
leaf chlorosis severity	2D^{C}, 4B^{C}, 7B_2^{I}	3A^{A}
leaf tip necrosis severity	2A_2^{I}, 4A^{I}, 4B^{C}, 5B_2^{C}	1A_2?^{C}, 3B_2^{C}, 5A^{A}, 5B^{C}, 7A_2?^{A}

? The effect was detected in single point ANOVA. Given is the linkage group to which the marker was mapped in the population Isengrain/Capo

^{A} lower score associated with the Arina allele

^{C} lower score associated with the Capo allele

^{I} lower score associated with the Isengrain allele

6.2.2.1 Heading

In the population Isengrain/Capo two QTL for heading were identified. Both were consistent over those eleven experiments in which the date of heading (or flowering) was recorded and the mean over them as well as the experiment Piešťany 2006, in which heading was assessed on just three occasions, and Probstdorf 2006, in which it was rated on a 1-9 scale. The QTL on chromosome 2D in the marker interval *XS18M14_9-Xgwm593* was classified as a major QTL as it explained 52-94 % of the phenotypic variability. The effect associated with the yet not definitely assigned linkage group 2BD6AB7B in the *Xwmc25.1-XS22M20_8* marker region contributed to 8-14 % of the phenotypic variance. In both cases earliness was associated with the Isengrain allele. The Arina/Capo map lacks corresponding linkage groups.

On the short arm of the homoeologous chromosomes 2B and 2D *Ppd-B1* (formerly *Ppd2*) and *Ppd-D1* (formerly *Ppd1*), genes for response to photoperiod, are located (Welsh et al. 1973 cited in Law et al. 1978). Suenaga et al. (2005) mapped *Ppd-B1* between *Xwmc257* and *Xgwm429*. Hanocq et al. (2004) identified a QTL for photoperiod sensitivity and vernalization requirement in the *Ppd-B1* region on 2BS near *Xgwm148*. It is unlikely that the QTL identified in the linkage group 2BD6AB7B is allelic to *Ppd-B1* or one of the QTL for photoperiod sensitivity, heading date, flowering time, narrow-sense earliness, and vernalization requirement detected on 2BS (Sourdille et al. 2000a, 2003, Börner et al. 2002, Gervais et al. 2003, Shindo et al. 2003, Hanocq et al. 2004, Kuchel et al. 2006, Wang et al. 2009, Zhang et al. 2009), as another group of the population Isengrain/Capo was definitely assigned to this or a tightly linked chromosomal region.

Ppd-D1 was mapped to the marker interval *Xgwm455-261* (Sourdille et al. 2000b), *Xwmc112-Xbarc168* (Suenaga et al. 2005), but in both maps marker order was inverted compared to the wheat consensus map (Somers et al. 2004). Hanocq et al. (2004) located *Ppd-D1* in the marker interval *Xgwm261-Xgwm484*, where they identified a QTL for photoperiod sensitivity and vernalization requirement. Huang et al. (2003) mapped *Xgwm484* closest to the peak of a QTL for ear emergence time. This is the same marker as in the population Isengrain/Capo for the QTL on 2D. Huang et al. (2003) assumed a QTL for increased yield located more distal on 2DS to be a pleiotropic effect of *Ppd-D1*. In several other studies QTL related to heading time were detected in the *Ppd-D1* region. Distal to *Xgwm261* Xu et al. (2005a) identified a QTL for early heading. Proximal to *Xgwm261* Liu et al. (2007) found a QTL for days to anthesis. A QTL for days to heading was detected by Kulwal et al. (2003) proximal to *Xgwm261*, by Narasimhamoorthy et al. (2006) around *Xgwm261*. Börner et al. (2002) found a QTL for ear emergence time and flowering time. Depending on the exact mapping of these genes and QTL, they might either correspond to the detected effect on 2D or the linkage group 2BD6AB7B.

On the long arm of chromosome 2D distal to marker *Xgwm539* Huang et al. (2004) mapped a QTL for ear emergence time. In the population Isengrain/Capo the linkage group assigned to 2D also spans this region, thus this QTL can not correspond to the effect detected in 2BD6AB7B. More likely the 2BD6AB7B QTL corresponds to the QTL for ear emergence time identified by Kuchel et al. (2007) on 2BS in the marker interval *Xgwm614-Xbarc200*.

Marker distances of published 6A maps (Somers et al. 2004, Singh et al. 2010) vary considerably and it is impossible to say, whether the QTL located by Huang et al. (2003) near *Xgwm494* might correspond to the effect on heading date detected in the linkage group 2BD6AB7B of the population Isengrain/Capo. On chromosome 6B Hoogendorn (1985) identified a factor with a major effect on earliness per se (expressed in days to ear emergence). Islam-Faridi et al. (1996) detected genes for sensitivity to day-length on the long arm of chromosome 6BL and furthermore genes for inhibition of ear emergence on the long arms of all group 6 chromosomes. If group 2BD6AB7B can be mapped to a group 6 chromosome at all, then most likely to the short arm of 6A. Thus it seems impossible that the detected QTL corresponds to one of these genes. On chromosome 6B Marza et al. (2006) detected a QTL for heading date, but unfortunately markers are not comparable. None of the QTL for flowering time identified by Lin et al. (2008) on chromosome 7B can be allelic to the effect associated with this unassigned linkage group as other linkage groups were mapped to these regions. More likely 2BD6AB7B corresponds to the 7BS chromosomal region, in which Sourdille et al. (2000a, 2003) detected a QTL for heading time or maybe earliness per se, and *Vrn3* (formerly *Vrn-B3*, *Vrn-B4*, *Vrn-5* or *Vrn5*), a gene for vernalization response, is located (McIntosh 1988 cited in Sourdille et al. 2000a, Chao et al. 1989, McIntosh et al. 2008 and 2010). Law (1966) identified an effect on ear emergence on 7BS.

6.2.2.2 Plant Height

QTL for plant height were detected in the populations Isengrain/Capo and the Arina/Capo. The major QTL located on chromosomes 4B and 6AB were consistent over all five experiments and the mean over them. Two further effects were identified on chromosome 2B in the experiment Tulln 2007 and in the not yet definitely assigned linkage group 2B4B5A7B consistent over two experiments and the mean over five experiments. In the case of these four QTL the Isengrain allele was associated with reduced plant height and they were only detected in the population Isengrain/Capo. Whereas Arina had about the same height as Capo, Isengrain was almost 25 cm shorter. The effects on chromosome 5A and 6B were identified in the population Arina/Capo only. Maximum LOD values were clearly below the threshold of the population Isengrain/Capo and also the additive effect was less strong. This might be the reason, why these QTL were not be detected in the population Isengrain/Capo.

The major QTL located in the marker interval *Xgwm107.2-XS11M18_6* on the short arm of chromosome 4B explained 14-27 % of the phenotypic variance. The gibberellin insensitive dwarfing gene *Rht1* originally by mistake located on 4A (Gale and Marshall 1976, Blanco and Simeone 1982) was mapped to the distal part of 4BS, a region including *Xgwm107* (Börner et al. 1996a, Somers et al. 2004, Williams et al. 2006). Börner et al. (1996b) gave a good overview on the conventional and the newly proposed nomenclature of all previously described alleles of this gene, partly formerly regarded as distinct genes (Gale and Youssefian 1985 cited in Börner et al. 1996b, Worland 1986, Worland and Petrovic 1988, Börner et al. 1995 cited in Börner et al. 1996b). The semi-dwarfing allele *Rht-B1b* (formerly *Rht1*) has been described for Isengrain's parent Soissons (Worland et al. 1998). Several further studies revealed QTL in the same or a nearby chromosomal region that might also be allelic (e.g. Cadalen et al. 1998, Rebetzke et al. 2001, Huang et al. 2003 and 2004, Sourdille et al. 2003, McCartney et al. 2005a, Liu et al. 2006, Marza et al. 2006, Rebetzke et al. 2008, Zhang et al. 2008, Wu et al. 2010). As allele specific PCR primers are already available (Ellis et al. 2002), it would be possible to verify whether the *Rht-B1b* allele was transmitted from Soissons to Isengrain or Isengrain carries another allele.

The second major plant height QTL explaining 11-15 % of the genotypic variance of the population Isengrain/Capo is either located on chromosome 6A or 6B. It was associated with SSR marker *Xbarc146.2*. In the population Arina/Capo a minor QTL was detected on chromosome 6B in the experiment Tulln 2008 and the mean over two experiments. It was associated with SSR marker *Xgwm518.1*. Several QTL for plant height have been identified on chromosome 6A and 6B: On the short arm of 6A around *Xpsr563a* (Keller et al. 1999), and in the marker intervals *Xwmc182-Xpsp3029* (Griffiths S et al. 2012) and *Xgwm334-Xwmc297* (Wu et al. 2010); on the long arm of 6A around *Xgwm786* (Huang et al. 2004), proximal to *Xbarc3* (Spielmeyer et al. 2007), and in the marker intervals *Xpsp3071-Xgwm570*, *P4232.4-Xcwm306* and *Xgwm617-Xcwm487* (Wu et al. 2010); on the short arm of 6B in the marker intervals *Xwmc486-Xwmc417* and *Xgwm132-Xwmc104* (Wu et al. 2010); on the long arm of 6B in the intervals *Xwmc269.3-P4232.1* and *Xgwm644.1-Xwmc417.2* (Wu et al. 2010). Unfortunately maps and markers of the

detected QTL on chromosome 6A by Marza et al. (2006), and the short arm of 6A by Börner et al. (2002) and Liu et al. (2005) are not comparable. As another linkage group was unambiguously mapped to the 6B region harboring *Xbarc146*, the detected plant height QTL is more likely to be located on 6AS and thus correspond to the QTL identified by Börner et al. (2002), Griffiths S et al. (2012) or Wu et al. (2010) in the distal part of this chromosome arm. The plant height QTL detected in the population Arina/Capo might also correspond to one of them.

A minor plant height QTL was detected in the population Isengrain/Capo on the long arm of chromosome 2B. It was associated with AFLP marker *XS14M18_20*. In the Wheat, Synthetic x Opata, BARC map available from the GrainGenes database (USDA 2014), *Xbarc114*, the only SSR marker of this linkage group, was mapped near the centromere, in the Wheat, Physical, SSR map (USDA 2014) on the short arm of 2B. As another linkage group was unambiguously assigned to the chromosomal regions in question, it is unclear, where *Xbarc114* is located exactly in the population Isengrain/Capo. The gibberellin sensitive dwarfing gene *Rht4* (Hu et al. 1972 cited in McIntosh et al. 2003) was located on 2BL near marker *Xwmc317* (Ellis et al. 2005). In this region several QTL have been detected: Around *Xgwm47* by Schmolke et al. (2005), and in the interval *Xcwm529-Xwmc317* by Wu et al. (2010). More proximal on 2BL Mathews et al. (2008) found a QTL associated with *Xgwm388*. On the short arm Gervais et al. (2003) identified a plant height QTL in the marker interval *Xgwm429-Xgwm148*. Regarding both linkage groups assigned to 2B in the population Isengrain/Capo, the identified QTL most likely corresponds to *Rht4* or one of the two QTL located nearby.

A further minor plant height QTL was detected in the population Arina/Capo in the experiment Tulln 2007 and the mean over experiments. It was associated with SSR marker *Xgwm1057* on chromosome 5AS and thus possibly corresponds to an epistatic QTL identified by Wu et al. (2010) in the marker interval *P2470-Xgwm154*.

Another plant height QTL found in the population Isengrain/Capo was not assigned to a certain chromosome. It was associated with AFLP marker *XS22M17_5* and might be located on chromosome 2B, 4B, 5A or 7B. *Xbarc10.1* was the only SSR marker included in this linkage group. As *Xbarc10.3* was mapped to the distal region of 2BS and *Xbarc10.2* of 5AS, and another linkage group was assigned to the region in question of 7BL, the linkage group most likely corresponds to the distal part of chromosome 4BL. In this region Verma et al. (2005) identified QTL for height of the first internode and plant height in the marker interval *Xgwm538-Xgwm6*. Marker order differs slightly from the wheat consensus map (Somers et al. 2004). Liu et al. (2005) detected a major QTL for plant height with a peak next to *Xbarc125*, the most distal marker in this 4BL map. With the exception of *Xbarc10*, mapped to the short arm of chromosome 4B, markers are not comparable with the wheat consensus map (Somers et al. 2004) or the Isengrain/Capo map.

6.2.2.3 Other traits

Awnedness

In the population Arina/Capo a QTL for awnedness was detected on chromosome 4D. It was only identified in the experiment Tulln 2008, but not in the three other experiments in which awnedness was rated. The effect was associated with SSR marker *Xgwm624* and explained 4 % of the phenotypic variability. As awnedness is not subject to environmental influences, differences between experiments can just be explained by heterozygosity. Literature on awnedness attributable to chromosome 4D was not available.

Leaf Blotch Severity and *Septoria* Leaf Blotch Severity

In the population Isengrain/Capo leaf blotch severity was assessed in the experiment Aumühle 2004. A major QTL explaining 19 % of the phenotypic variability was identified on chromosome 4B. The LOD values of all markers of this linkage group were beyond the threshold, with a peak near *Xgwm107.2*. Reduced severity was transmitted by the Capo allele. More than 17 genes for resistance to *Septoria tritici* blotch (*Stb*) have been identified, but none on chromosome 4B (Goodwin and Thompson 2011, USDA 2014). Risser et al. (2011) detected a QTL for *Septoria tritici* blotch rating in a population Tuareg/Biscay on chromosome 4B in the marker interval *Xwmc0471-Xwmc0238*, thus in a similar region as in the population Isengrain/Capo. The QTL was inherited from Tuareg and consistent across experiments.

In the population Forno/Oberkulmer Aguilar et al. (2005) detected a QTL for adult plant resistance to *Stagnospora nodorum* leaf blotch. It was located on chromosome 4B in the marker interval *Xglk348-Xpsr921*. Friesen et al. (2009) identified a QTL for flag leaf reaction type to *Stagnospora nodorum* on the short arm of chromosome 4BS. The effect was consistent across two experiments and located in the marker interval *Xwmc47-Xfcp301*. As far as these maps are comparable with the Isengrain/Capo map by the means of the wheat consensus map (Somers et al. 2004) and the Arina/Forno map (Paillard et al. 2003), these effects seem to be associated with chromosomal regions different from that harboring the leaf blotch severity QTL identified in the population Isengrain/Capo.

In single point ANOVA for the population Arina/Capo an effect on *Septoria tritici* blotch resistance was identified in the experiment Nyon 2008. It was associated with the Arina allele of SSR marker *Xgwm870* that was mapped to linkage group 7A_2 in the population Isengrain/Capo. Goodwin et al. (2007) located *Stb3* on chromosome 7AS linked to *Xwmc83*. Previously it was erroneously assigned to chromosome 6DS as it was linked to microsatellite marker *Xgdm132* (Adhikari et al. 2004). An Arina/Capo map in which *Xgwm870* is assigned to a certain chromosome is necessary for locating the identified QTL exactly.

Two *Stb* genes and a QTL have previously been identified in Arina: *Stb6* on chromosome 3A (Chartrain et al. 2005) mapping close to *Xgwm369* on the short arm (Brading et al. 2002), *Stb15* on 6AS with *Xpsr904* being closest, and a QTL on 7DS in the marker interval *Xcdo475b-Xswm5* (Arraiano et al. 2007). None of them were detected in the population

Arina/Capo. A linkage group was mapped to chromosome 3A, but there is no congruence with the wheat consensus map (Somers et al. 2004). To the two other chromosomes no linkage group was assigned. The very low number of markers that was not sufficient for a detailed genotyping of the population Arina/Capo is most probably the reason for not detecting these genes.

Powdery Mildew Severity

The major QTL for powdery mildew severity identified in the population Isengrain/Capo was another effect associated with SSR marker *Xgwm107.2* on the short arm of chromosome 4B. It explained 12 % of the phenotypic variance in the experiment Probstdorf 2006.

The powdery mildew gene *Pm7* derived from rye (*Secale cereale*) was translocated to the distal end of the long arm of wheat chromosome 4B (Heun and Friebe 1990). Investigating the adult plant powdery mildew resistance of the cultivar Knox 62, Johnson et al. (2003) found out that amongst others chromosome 4B was responsible. In a population Fukuho-komugi/Oligoculm Liang et al. (2006) identified a QTL for maximum powdery mildew severity on the long arm of chromosome 4B. It was located in the marker interval *Xgwm375-Xgwm251*, thus distal to *Xgwm495*, but significant in one experiment only. In a similar region Lillemo et al. (2008) detected a major QTL in a population Saar/Avocet transmitted by the Avocet allele. It was consistent over all experiments and explained 21 % to 40 % of the phenotypic variance. The SSR markers *Xgwm251* and *Xgwm375* were closest to the QTL peak.

In the population Isengrain/Capo SSR marker *Xgwm495* was clearly distal to the QTL region. Furthermore several surveys showed that virulence to *Pm7* is common (Niewoehner and Leath 1998, Imani et al. 2002, Parks et al. 2008) and lines carrying *Pm7* are susceptible (Wang et al. 2005). Thus the effect identified in the population Isengrain/ Capo is most probably different from the previously detected QTL and *Pm7*.

Yellow Rust Severity

In the experiment Nyon 2009 a major QTL for yellow rust resistance was identified in the population Arina/Capo . It was located on the short arm of chromosome 3B close to SSR marker *Xbarc75* and beyond the threshold across the whole linkage group spanning the 20 cM marker interval *Xbarc75-Xgwm389.1-Xgwm389.2*.

Suenaga et al. (2003) detected a QTL for yellow rust severity and infection type in the distal region of 3BS close to marker *Xgwm389* and effective across all five experiments. It was considered to be the same QTL as identified by Singh et al. (2000) and corresponding to *Yr30* (Singh RP et al. 2001a cited in Suenaga et al. 2003). Several studies revealed QTL in a similar region: In a population Frontana/Remus Steiner (2003) detected a major QTL for yellow rust severity transmitted by Remus alleles near marker *Xgwm493*, the most distal marker of the map. By single marker analysis Badakhshan et al. (2008) identified a QTL for yellow rust AUDPC associated with *Xgwm389*. Dedryver et al. (2009) detected a QTL linked to *Xgwm533* just for the last of three scoring times (grain filling stage of one

parental line), but not for AUDPC. William et al. (2006) found a QTL for yellow rust severity and infection type with the maximum next to AFLP marker *XPstAATMseCAC2*, thus about 5 cM distal to *Xgwm533*. Bariana et al. (2010) identified the QTL in just one out of six experiments. It explained 5 % of the phenotypic variance, but was beyond the threshold in a region spanning more than 80 cM between the markers *Xgwm533* and *XwPt-5906* distal to *Xgwm376* on 3BL. Lowe et al. (2011) detected a QTL for reaction type and disease severity close to *Xgwm533.1* and most probably different from *Yr30*.

Furthermore several yellow rust resistance genes were identified on chromosome 3B: *Yr4* (Worland 1988 cited in McIntosh et al. 1995) was located distal to *Xbarc75* (Bansal et al. 2010). In the cultivar Stephens the dominant race-specific resistance gene named *YrS* (Chen et al. 1994) or *YrSte2* (Chen et al. 1996) was identified on chromosome 3B. In a breeding line with stable resistance since the 1970s Börner et al. (2000) detected *Yrns-B1* about 20 cM proximal to *Xgwm493*. By mapping with additional SSR markers Khlestkina et al. (2007) located *Yrns-B1* in a distance shorter than 2 cM to *Xgwm493*. Also *Xgwm533* and three further markers were closely linked to the gene.

SSR marker *Xgwm533* was not linked to the group harboring the yellow rust resistance QTL in the population Arina/Capo. As the fragment size was different from the population Isengrain/Capo, it possibly maps to another chromosome. It seems likely that the yellow rust resistance QTL detected in the population Arina/Capo corresponds to one of the described QTL or resistance genes. For a clarification a marker enhanced map and possibly a haplotype and/or virulence analysis will be necessary.

Glaucousness

In the population Arina/Capo three QTL for glaucousness were detected. Capo inherited the alleles for reduced glaucousness of the effect associated with SSR marker *Xgwm120* that was assigned to chromosome 2B in the population Isengrain/Capo and the major QTL located on chromosome 3A, whereas Arina transmitted the allele for reduced glaucousness identified on 4B.

Allan and Vogel (1960) located a dominant factor for waxy culms of durum selection 396 on chromosome XIII. Tsunewaki (1964 cited in Tsunewaki and Ebona 1999) located *W1*, a gene for wax production in wheat, on chromosome 2B. Driscoll (1966) showed that the genes for glaucousness and a dominant inhibitor of wax on the nonstandard arm of chromosome XIII (i.e. the short arm of chromosome 2B) are not allelic. These results were confirmed by Tsunewaki and Ebona (1999). Rong et al. (2000) used RFLP-markers to map the glaucousness inhibitor $W1^1$ in the distal region of 2BS of the Israeli accession TTD140 of *Triticum turgidum* var. *dicoccoides* (wild emmer). The inhibitor of glaucousness on chromosome 2B inherited from the *Aegilops speltoides* accession k-389 showed to be allelic to $W1^1$ (Pshenichnikova et al. 2007). In a similar region Simmonds et al. (2008) mapped a gene for viridescense *(Vir)* derived from wild emmer in the wheat cultivar Shamrock. It was located on the distal end of 2BS 2 cM proximal to microsatellite marker *Xgwm614*. According to the wheat genes consensus map (Triticum-Genes-2B) available from the GrainGenes database (USDA 2014), *W1* is positioned 4.3 cM distal to *Lr16* and

Sr23, which were mapped by Somers et al. (2004) 12 cM distal to *Xgwm614*. Thus it is not clear, whether *W1* and the *Vir* gene are either closely linked or the same gene (Simmonds et al. 2008).
As marker *Xgwm120* was mapped on the long arm of chromosome 2B (Somers et al. 2004), it seems that all three effects on glaucousness identified in the population Arina/Capo are different from those previously described. For verifying the results, glaucousness needs to be rated in further experiments and mapping repeated with a marker enriched Arina/Capo map.

Lodging Severity

In both populations two QTL for lodging severity were detected. In the population Isengrain/Capo the effect on chromosome 6B was identified in the experiment Probstdorf 2008 and the mean over the three experiments, the effect not definitely assigned to chromosome 6A or 6B in the experiment Probstdorf 2008 only. One QTL detected in the population Arina/Capo was detected on chromosome 5A, another by means of single point ANOVA. Both were consistent over the experiments Probstdorf and Rust 2008 and the mean over these two experiments. The maximum LOD values of the population Arina/Capo were clearly beyond the threshold for the population Isengrain/Capo, but the additive effects were slightly larger. Regarding the scores of the parental lines (mean over experiments), Capo was the less susceptible in the Arina/Capo experiments (Capo: 0.6, Arina: 0.9), but clearly more prone to lodging in the Isengrain/Capo trials (Capo: 1.8, Isengrain: 0.4). Therefore the differences in the identified QTL might be explained by field data from different experiments used for QTL detection.
In the first of two years of investigation, Verma et al. (2005) found a QTL for lodging severity in the marker interval *Xgwm374-Xgwm344* on chromosome 2B. Whereas they located this region on the long arm, Somers et al. (2004) placed them into the centromeric region towards the short arm. *Xgwm120*, which was associated with lodging severity in the population Arina/Capo, was mapped by Somers et al. (2004) clearly more distal on 2BL. In the population Isengrain/Capo and by Verma et al. (2005) it was much closer to *Xgwm374* and *Xgwm344*, thus the effect identified in the population Arina/Capo might correspond to the QTL detected by Verma et al. (2005).
In the distal part of chromosome 5AL Keller et al. (1999) located a QTL for lodging severity in the population Forno/Oberkulmer. SSR marker *Xgwm1057*, which was associated with the effect in the population Arina/Capo, was mapped to the short arm in the population Isengrain/Capo. Regarding the chromosomal region, the QTL identified in the population Arina/Capo most likely corresponds to the QTL for lodging severity detected near *Xbarc180* by Marza et al. (2006).
On the long arm of chromosome 6B Keller et al. (1999) identified another QTL for lodging severity. As far as the maps are comparable, it might correspond to the QTL on chromosome 6B identified in the population Isengrain/Capo.

No literature on lodging severity attributable to chromosome 6A was available. As another group was unambiguously assigned to chromosome 6B, the linkage group 6AB more likely corresponds to the 6A chromosome.

Leaf Chlorosis Severity

QTL for leaf chlorosis severity were detected in the population Isengrain/Capo on chromosomes 2D, 4B and 7B, in the Arina/Capo on 3A. Leaf chlorosis can be caused by various influences such as nutrients and pathogens. Due to a lack of knowledge about the causal agent(s), these QTL are not discussed in detail.

Leaf Tip Necrosis Severity

For the trait leaf tip necrosis severity four QTL were identified in the population Isengrain/Capo. The effect on chromosome 2A was detected in the experiment Rust 2009 and the mean over both experiments, the effect on 4A only in the experiment Rust 2009, and the major QTL on 4B was detected in the experiment Tulln 2009 and the mean. Just the effect on 5B was consistent over both experiments and the mean. In the population Arina/Capo five QTL for leaf tip necrosis severity were detected: On chromosome 3B, 5A, 5B and by means of single point ANOVA two further associated with markers mapped to 1A and 7A in the population Isengrain/Capo.

The only study investigating leaf tip necrosis severity on chromosomes different from 7D was done by Messmer et al. (2000) on the population Forno/Oberkulmer. Amongst others they identified QTL on chromosome 1A, 2A, 3B, 4B, 5A and 5B. Even by the means of the wheat consensus map (Somers et al. 2004) and the Arina/Forno map (Paillard et al. 2003), the map by Messmer et al. (2000) is hardly comparable with the Isengrain/Capo and Arina/Capo map. It seems that the QTL on chromosome 1A was mapped closer to the centromere than *Xgwm135*. On 2A the effect was located on the distal part of the short arm, whereas AFLP marker *XS16M16_21* is close to the centromere on the long arm. Both QTL on 3B were identified on the short arm, but *Xgwm108* assigned to the long arm. Similar for chromosome 4B two effects were detected on the short arm, but the QTL with a peak close to *XS11M18_6* in the population Isengrain/Capo was located on the long arm. Messmer et al. (2000) identified one QTL in the distal region of the short and another in the proximal region of the long arm of 5A. The effect found in the population Arina/Capo might correspond to the proximal 5AL QTL. The QTL on the long arm of chromosome 5B seemed to be associated with different markers in the populations Isengrain/Capo and the Arina/Capo. It is possible that one of them corresponds to the QTL detected by Messmer et al. (2000). All in all it seems that at least six of the QTL for leaf tip necrosis severity identified in the two Capo derived populations are different from those previously published.

6.3 Trait Associations

In all three populations correlations between different traits were generally weak. With a value of about r = 0.40 to 0.50, Spearman's rho as a measure of the monotone relationship was highest between leaf chlorosis severity and leaf tip necrosis severity in the populations Isengrain/Capo and Arina/Capo. In some experiments the correlation between plant height and heading in the population Isengrain/Capo reached similar levels, whereas in the two other populations it was almost zero. Leaf rust severity and leaf tip necrosis severity showed a negative correlation. Depending on the date of assessment, the absolute value of Spearman's rho was slightly larger than 0.50 for both populations. Also leaf rust severity and leaf chlorosis severity were negatively correlated, but the absolute value of the population Arina/Capo was slightly, of Isengrain/Capo clearly, lower.

With only a few exceptions the values of Spearman's rho for all other trait associations in all experiments were below 0.40. Unfortunately plant height and lodging severity were never assessed in the same experiment. Between these two traits associations seem possible, even though Verma et al. (2005) estimated Pearson's correlation coefficients of less than 0.30 in two experimental years. Keller et al. (1999) found correlation and coincident QTL not just for lodging severity and plant height, but also for days to ear emergence, culm stiffness, culm thickness, leaf width and leaf-growth habit. In the population Isengrain/Capo a major QTL for plant height and a minor QTL for lodging severity were identified on chromosome 6AB with the same marker being closest to the peak. The allele for reduced plant height was transmitted from Isengrain, the allele for reduced lodging severity from Capo. Also in the population Arina/Capo minor QTL for plant height and lodging severity were detected in the same region. Furthermore the QTL for leaf rust severity and leaf tip necrosis severity shared the same peak marker on chromosome 5A. Reduced plant height, lodging severity and leaf rust severity were associated with the Capo allele. Only reduced leaf tip necrosis severity was inherited from Arina. In both cases marker density in the particular QTL region was very low and results need to be verified with a more saturated map. Heading and lodging severity were assessed five times in the same experiments, at least once for each population. Correlation was low (-0.08 to 0.35), QTL were not detected in similar regions. In the population Arina/Capo another effect on lodging severity was coincident with an effect on glaucousness.

Simón et al. (2004) observed that multiple regression models including plant height and heading date accounted for 44.3 to 99.1 % of the variation in necrosis percentage caused by *Septoria tritici*. In their study the positive correlation between heading date and necrosis severity seemed to be caused by environmental conditions. As leaf blotch or Septoria leaf blotch severity and plant height were not assessed in the same experiment for any of the three Capo derived populations, it was impossible to test this relationship. The correlation with heading was for the populations Arina/Capo and Furore/Capo almost zero. In the case of the population Isengrain/Capo, there was a negative, but low correlation (Aumühle 2004: -0.35).

Van der Wal et al. (1970) and Zadoks and Schein (1979) described an interaction between leaf rust and *Septoria nodorum* on wheat: If *Septoria* inoculation occurred first, the urediniospores production of leaf rust was reduced. If leaf rust infection started earlier, the plants became even more susceptible to *Septoria nodorum*. Neither a relevant correlation between leaf blotch or *Septoria* leaf blotch and leaf rust severity nor a coincident QTL was detected in the populations Isengrain/Capo or the Arina/Capo. For both populations these traits were just once assessed in the same experiment. Furthermore, in the years 2005 to 2009 fungicides were applied against *Septoria nodorum* at the Austrian trial sites. Moreover, before the third year of field experiments, the number of tested lines of the population Isengrain/Capo was reduced: Lines difficult to score for leaf rust resistance in the first experimental years due to e.g. *Septoria* infection or other leaf diseases were excluded. All these facts may explain that no interaction between leaf rust and *Septoria nodorum* were detected.

The only relevant trait correlations with $r > 0.59$ ($\alpha = 0.05$) were between single assessments of leaf rust severity measured by the percentage of infected leaf area and the relative AUDPC. In some experiments correlation coefficients between all single assessments were significant; in a few not even those between subsequent scorings. The high correlation between a single assessment of the percentage of infected leaf area and the AUDPC at a certain growth stage is in congruence to Lipps and Madden (1989), who found a high correlation studying powdery mildew of wheat (r of 0.87-0.98). But still, for the detection of QTL repeated scoring seems inevitable, as data from the ideal date, when differences between genotypes are largest and the number of missing values due to leaf senescence as lowest as possible, are necessary.

In the population Arina/Capo QTL for leaf rust severity and yellow rust severity were identified in a similar region on the short arm of chromosome 3B. In the case of both diseases, reduced susceptibility was inherited by the Capo allele. Whether the same locus is responsible for the resistance to both rust diseases is unclear yet. Further experiments to assess yellow rust resistance in all populations are in progress. *Sr2*, a stem rust resistance gene, has been found to be tightly linked to *Lr27*, to which the leaf rust QTL of Capo may correspond (Singh et al. 2000, Sharp et al. 2001). The marker *Xgwm533* was described by Spielmeyer et al. (2003) to be diagnostic of *Sr2*. An effect on yellow rust resistance was identified by William et al. (2006) on 3BS distal to marker *Xgwm533*. This and other effects on yellow rust resistance are discussed in detail in chapter 6.2.2.3. Suenaga et al. (2003) suggested the same loci on 3BS close to *Xgwm389* to effect leaf and yellow rust severity. Singh et al. (2000) identified not only effects on leaf, yellow and stem rust severity, but also on powdery mildew severity in the same chromosomal region and concluded that it is maybe not only race- but pathogen-non-specific. This is in contrast to the results of Steiner (2003), who identified effects on leaf and yellow rust severity in a similar region on 3BS, but not inherited from the same parental line. A high resolution map of all three populations together with the additional data of the yellow rust experiments can help to clarify the association of the effects on leaf and yellow rust severity on 3BS inherited from Capo.

No leaf rust QTL identified in the populations Isengrain/Capo or the Arina/Capo was associated with leaf tip necrosis. This provides further evidence that Capo does not carry *Lr34*. Steiner (2003) found an association of leaf rust and leaf tip necrosis not just for the QTL on 7D, but also on 3B.
A QTL for leaf tip necrosis severity was associated in the population Arina/Capo with a QTL for *Septoria* leaf blotch severity. In the population Isengrain/Capo the QTL for leaf blotch severity on 4B was coincident with the QTL for plant height, powdery mildew severity and leaf chlorosis severity. Shorter plants and reduced powdery mildew severity were associated with the Isengrain allele, whereas reduced leaf chlorosis and leaf tip necrosis severity were inherited from Capo. Thus, it can not be concluded that generally increased disease severity was associated with reduced plant height. Maybe the excess of the Capo alleles for all markers of this linkage group can be an explanation for the colocalization of these four effects. At any rate, further research would be necessary as powdery mildew severity has been assessed only in a single experiment, leaf chlorosis and leaf tip necrosis severity in two experiments.

6.4 Estimation Methods for the Coefficient of Heritability

Two different calculation methods for estimating the coefficient of broad sense heritability (h^2) were compared: The simplified formula after Nyquist (1991) that is only valid if all experiments have the same number of replications and no data are missing, and the exact calculation method by means of variance components. For all traits repeatedly assessed in one population the values after both calculation methods are summarized in Table 97. The number of experiments and the degrees of freedom of the error term are listed. If not all experiments had two replications or if there were no missing data, it is specified in the comment. The difference was in most of the cases less than 0.005. The only exception was ANOVA and analysis of covariance of the standard lines across four experiments including the data of spreader row infection. In these two cases the coefficient of heritability increased by 0.012 and 0.014. Independet of the number of experiments and the number of replications, if data of only a small part of lines, varying between the different experiments, are missing, the estimated coefficient of heritability after the simplified formula is at most solely incorrect at the second position after the decimal point.

Table 97 Estimated coefficient of heritability calculated after the simplified formula by Nyquist (1991) (N) and by the means of variance components (vc) for different traits assessed in the three populations. df_ε = degrees of freedom (error)

trait	h^2 (N)	h^2 (vc)	population	no. of exp.	df_ε	comment
leaf rust severity (%)	0.90	0.91	Isengrain/Capo	11	1979	1 exp. only 1 rep.
leaf rust severity (%)	0.89	0.90	Arina/Capo	11	2014	2 exp. only 1 rep.
leaf rust severity (%)	0.79	0.80	Furore/Capo	6	914	1 exp. only 1 rep.
LR severity (relative AUDPC)	0.55	0.55	Isengrain/Capo	2	477	
LR severity (relative AUDPC)	0.61	0.62	Arina/Capo	3	688	
LR severity with spreader (%)	0.85	0.85	Isengrain/Capo	4	766	
LR sev. without spreader (%)	0.84	0.84	Isengrain/Capo	4	798	
LR severity with spreader (%)	0.84	0.84	Arina/Capo	4	846	
LR sev. without spreader (%)	0.83	0.83	Arina/Capo	4	864	
LR severity with spreader (%)	0.94	0.96	standard lines	4	337	
LR sev. without spreader (%)	0.95	0.96	standard lines	4	345	
heading (day of the year)	0.98	0.98	Isengrain/Capo	11	2080	2 exp. only 1 rep.
heading (day of the year)	0.96	0.96	Arina/Capo	9	1382	3 exp. only 1 rep.
heading (day of the year)	0.86	0.87	Furore/Capo	9	1395	2 exp. only 1 rep.
plant height (cm)	0.95	0.95	Isengrain/Capo	5	947	1 exp. only 1 rep.
plant height (cm)	0.83	0.83	Arina/Capo	2	232	1 exp. only 1 rep.
plant height (cm)	0.84	0.84	Furore/Capo	5	800	1 exp. only 1 rep.
powdery mildew severity (1-9)	0.02	0.02	Furore/Capo	2	400	no missing data
leaf chlorosis severity (0-9)	0.93	0.93	Isengrain/Capo	2	477	
leaf chlorosis severity (0-9)	0.92	0.92	Arina/Capo	2	464	no missing data
leaf tip necrosis severity (0-9)	0.92	0.92	Isengrain/Capo	2	476	
leaf tip necrosis severity (0-9)	0.91	0.91	Arina/Capo	2	462	

6.5 Genetic Map

The genetic map of the population Isengrain/Capo is in good congruence with the wheat consensus map (Somers et al. 2004). In a few regions two loci were mapped in reversed order, namely in linkage groups assigned to the chromosomes 2A (*Xgwm558* ↔ *Xgwm372*), 2B (*Xwmc500.2* ↔ *Xgwm 120*), 2D (*Xgwm608* ↔ *Xgwm539*) or 3B (*Xgwm247* ↔ *Xgwm 340*). In the case of 2A and 3B these loci map very close to each other. Also in the region of the QTL for leaf rust resistance on chromosome 7B the marker density is very high and the order in the Isengrain/Capo map differs slightly from the consensus map. The largest deviations appear on chromosome 5A, where *Xbarc197* maps to a rather distant region, as well as *Xgwm295* on 7D. Primer Gwm295 and Gwm130 produced two fragments with only one being polymorphic. Using nulli-tetrasomic lines of hexaploid wheat, it was proven that the mapped loci are located on chromosome 7D (Matiasch 2005).

In the Arina/Capo map the markers *Xgwm1110* and *Xgwm1121* mapped in reverse order compared to the Isengrain/Capo map. The polymorphic fragments produced by Gwm533 have a different length for the Isengrain and Arina allele. As Capo produced no fragment, the locus *Xgwm533* in the population Arina/Capo at present can not be mapped definitely to chromosome 3B.

The ratio of Isengrain : Capo allele in the RIL population is 1 : 1.06 if calculated across all mapped loci. Calculated for all codominant loci the ratio is 1 : 1.07, for those where only Capo produced a visible fragment 1 : 1.17 and for those with only the Isengrain allele yielding a visible fragment 1 : 0.93. The difference between the latter two may be due to difficulties to distinguish, whether a fragment was erroneously not produced or because of the allelic constitution, as in case of doubt the particular line was not rated and thus more of these lines were “missing”.
In parts of several linkage groups segregation did not follow the Mendelian ratio. In most of the cases too many lines carried the Capo allele or were heterozygous. Also in the region of some QTL the assumption of a Mendelian segregation was violated: Whereas in the region of the QTL for leaf rust resistance on chromosome 3B clearly less than half of the lines carried the Capo allele in both populations, at the lower end of the 7B QTL less than fifty percent had the Isengrain allele. At the loci associated with QTL for plant height on the chromosomes 2B and 4B and in the linkage group 6AB more lines carried the Capo fragment than would be expected. Maybe taller plants had more chance to be selected at an early stage of RIL population development. Another reason for the general excess of the Capo allele might be Isengrain’s susceptibility to frost heaving, thus lines not enduring a cold winter. For all loci mapped to chromosome 4B, the ratio Isengrain : Capo allele was > 1 : 2. In the case of the QTL for leaf blotch severity, leaf chlorosis severity and leaf tip necrosis severity identified in this linkage group, the positive allele originated from Capo. Only for powdery mildew severity increased resistance was associated with the Isengrain allele. Maybe this fact explains the excess of the Capo allele as lines that were difficult to assess in the field experiments in the years 2004 and 2005 because of various leaf diseases were excluded from marker analysis and the subsequent field trials, although for leaf chlorosis severity and leaf tip necrosis severity also other QTL were detected, for which the Isengrain allele conferred resistance.

6.6 Conclusion

The aim of the present study was to identify the leaf rust resistance source of the Austrian winter wheat cultivar Capo. The results suggest that Capo’s leaf rust resistance is based on the QTL on chromosome 3B and several further genes with minor effects. It is very unlikely that a major Capo derived QTL has been overseen as the Isengrain/Capo map is rather dense and should cover the whole genome. If it was not detected due to the strong effect of the QTL inherited from Isengrain, it should be possible to identify it in another Capo derived population. Molecular mapping of the populations Arina/Capo and the Furore/Capo is already in progress.
The 3B QTL appears to be a promising resistance source as it is likely to be effective against leaf and yellow rust and working in a quantitative manner. Further field studies on yellow rust resistance of Capo derived populations are already underway to find out, whether the same QTL on 3B is effective against both rust fungi or the two effects are only tightly linked. If further, preferably codominant, markers for the 3B QTL region can be

identified in these populations, they may help clarifying in another haplotype analysis, whether Capo carries *Lr27* and *Yr30*. At any rate, markers for the Capo derived leaf rust QTL are valuable for rust resistance breeding as this cultivar and its relatives still play a decisive role in cultivar development for the Pannonian Region.

As Isengrain was rather susceptible to leaf rust as well as Thatcher NILs carrying *Lr14a*, the QTL on chromosome 7B ought to be studied in more detail. Either must there be another locus influencing the effectiveness of this region not detected yet and/or there are more not yet known alleles at the *Lr14* locus. Its power makes this effect interesting for resistance breeding.

Due to difficulties in assessment of true leaf rust severity and troubles because of late infection or varying environmental influences, it is even more important than for other plant diseases to compare results of several experiments. Molecular markers tightly linked to major QTL facilitate breeding for leaf rust resistance as testing for this trait in field experiments becomes less important. The good leaf rust severity rating of Capo in recent field experiments suggests that its resistance is durable. After validation, breeding companies can use the identified markers for a more precise selection of durable leaf rust resistant offspring.

7 Literature

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Appendix A: Lab Instructions and Protocols

This part is mainly aimed at students or other future workers at the Institute of Biotechnology in Plant Production – IFA Tulln. Hopefully it is helpful when doing similar work.
Some of these protocols are based on protocols already used at the institute, some lab instructions are –as far as I know– written down in detail for the first time. A few are based on various published protocols. Please have a look in the chapter “Materials and Methods” for the exact citations if you are interested in the published master protocols.

Abbreviations used in this appendix:

APS	ammoniumpersulfate
ATP	adenosin-5’-triphosphate
BBCH	coding system for plant growth stages (Maier 2001)
BME	β-mercaptoethanol
bp	base pairs
BSA	bovine serum albumin
CTAB	cetyltrimethylammoniumbromide
ddH2O	double-distilled water
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide-5’-triphosphate
EDTA	ethylendiaminetetraacetate
EtOH	ethanol
mA	milliampere
MgCl2	magnesium chloride
mL	milliliter
mM	millimol
Mse	restriction enzyme from Micrococcus species
NaCl	sodiumchloride
NaOH	sodiumhydroxide
NH4OAc	ammoniumacetate
OD	optical density (absorption)
PCR	polymerase chain reaction
rpm	rounds per minute
RXN	reaction
Sse	restriction enzyme from Streptomyces species
SM	size marker
SSR	simple sequence repeat (or microsatellite)
Taq	DNA-polymerase from the bacterium Thermus aquaticus
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TEMED	N,N,N’,N’-tetramethylethylendiamine
Tris	Tris(hydroxymethyl)amino-methane
UV	ultra violet radiation
V	Volt
W	Watt

Genomic DNA Isolation

Harvesting Plant Material

- Plant 8-10 grains of each line in 6x8 multi plates in the greenhouse.
- After about 2 weeks (BBCH 11 to 13) harvest leaves of all plants into parchment bags.
- Let them lyophilize for about 19 hours.
- If the leaves were afterwards stored at -80°C, let them dry for one more hour at maximum 40°C in the airing cupboard.
- Weigh about 300-400 mg of the lyophilized tissue into a 15 mL polypropylene centrifuge tube.
- Add 5-7 metal beads (∅ 3-4 mm) and put it into the shaking mill for about 15 minutes.

DNA Extraction

- Add 9.0 mL of warm (64°C) freshly made CTAB[1] extraction buffer to the ground, lyophilized tissue. Distribute tissue along the sites of the tube before adding buffer to avoid clumping of dry tissue on the bottom. Mix well with a vortex mixer.
- Incubate for 90 min with continuous gentle rocking in a 65°C water bath. During this time mix again one time.
- Remove tubes from water bath, wait some minutes to cool down before adding 4 mL of chloroform/isoamylalcohol (3-methyl-1-butanol), 23+1. Rock gently to mix for 10 min.
- Spin in a laboratory centrifuge for 10 min at 4000 rpm and room temperature.
- Pour off aqueous layer into new 15 mL tubes. Add 3 mL chloroform[2]/isoamylalcohol[3] (3-methyl-1-butanol), and rock gently for 10 min.
- Spin in a laboratory centrifuge for 10 min at 4000 rpm and room temperature.
- Pipette off top aqueous layer into new 15 mL tubes.
- Add 4.8 mL isopropanol (2-propanol[4]) (0.6 times the volume of the aqueous layer). Mix by gentle inversion.

---

[1] **CTAB buffer**

- 100 mM Tris-pH 7.5 (tris(hydroxymethyl)amino-methane) Pufferan® Buffer Grade, Roth #AE15.3
- 700 mM NaCl (sodiumchloride) reinst
- 50 mM EDTA-pH 8.0 (ethylendiamintetraacetate) p.a., Roth #8043.2
- 1% CTAB (hexadecyltrimethylammonium bromide), Sigma-Aldrich CHEMIE Gmbh, Steinheim, DE #H-8882
- 140 mM BME (2-mercaptoethanol) p.a., Roth #4227.3

⇒ heat buffer to 60-65°C before adding the CTAB and BME.

[2] reinst, Merck, KGaA, Darmstadt, DE #6340.2

[3] zur Synthese, Merck #8.18969.1000

[4] zur Synthese, Carl Roth GmbH + Co. KG, Karlsruhe, DE #9866.5

- Remove precipitated DNA with glass hook. Place hook with DNA in 15 mL plastic tube containing 1 mL of TE; gently twirl hook until DNA slides off the hook. Cap tube and rock gently overnight at room temperature to dissolve DNA.
- Precipitate DNA by adding 50 µl of 5 M NaCl and then 2.5 mL absolute ethanol[5] (2.5 times the original TE volume). Mix by gentle inversion.
- Remove precipitated DNA with glass hook. Place hook with DNA in 15 mL glass tube containing 3.5 mL Wash 1[6]. Leave DNA on hook in tube for about 20 min.
- Rinse DNA on hook briefly in 1.5 mL of Wash 2[7] and carefully dry it to get rid of the alcohol. Transfer DNA to 2 mL plastic tube containing 1 mL TE[8]; gently twirl hook until DNA slides off the hook. Cap tube and rock gently overnight at room temperature to dissolve DNA. Store samples at –20°C.

Measuring and Adjusting DNA Concentration

- Small sample numbers can be measured on the lab photometer (e.g. Pharmacia GeneQuant™), for larger amounts the use of a microplate reader (e.g. TECAN Multichannel Photometer, TECAN® Trading AG, CH) is advisable.
- Values of OD_{260} (absorption at a wavelength of 260 nm) should be in the range of 0.1 to 3.0. Therefore samples have to be diluted with TE-buffer and mixed well.
- At least one blind value (TE-buffer only) has to be included in the measurement.
- Using Pharmacia Gene Quant, DNA concentration can be calculated according to following formula:

$$DNA\ concentration\ [\mu g / \mu l] = \frac{OD_{260} * dilution\ factor * 50\ [\mu g / ml]}{1000}$$

- Using TECAN photometer, measurement depth had to be calculated first:

$$measurement\ depth\ [cm] = \frac{measured\ volume\ per\ well\ [\mu l] * 1.09\ (max.\ depth\ [cm])}{392\ (max.\ volume\ per\ well\ [\mu l])}$$

Then DNA concentration can be calculated according to the formula

$$DNA\ concentration\ [\mu g / \mu l] = \frac{(OD_{260}^{sample} - OD_{260}^{blind\ value}) * dilution\ factor * 50}{measurement\ depth\ [cm]}.$$

---

[5] p.a., Merck #1.00988.2500

[6] **Wash 1**
- 76% EtOH (ethanol) p.a.
- 0.2 M NaOAc (sodiumacetate trihydrate) Sigma Ultra, Sigma #S-7670

[7] **Wash 2**
- 76% EtOH (ethanol) p.a.
- 10 mM NH_4OAc (ammoniumacetate) p.a., Roth #7869.2

[8] **TE-buffer (pH 8.0)**
- 10 mM Tris: Pufferan® buffer grade
- 1 mM EDTA p.a.

- The ratio OD_{260}^{sample} / $OD_{260}^{blind\ value}$ is a measure for DNA quality. The value should be between 1.8 and 2.0. If the ratio is above 2.0, samples may still be contaminated with chloroform. In this case, the steps ethanol precipitation and rinsing with Wash 1 and Wash 2 should be repeated. A lower ratio may result from proteins or other substances absorbing UV radiation. Then samples should be precipitated in ethanol (2.5 times the volume) again.
- Samples are adjusted with TE buffer to a uniform concentration of 200 ng/µl.

Checking DNA Quality by Neutral Agarose Gel Electrophoresis

Depending on the DNA size of the samples to be separated, different concentrations of agarose are used. To separate genomic DNA, 0.7% is common, for smaller fragments e.g. after digestion with restriction enzymes, agarose concentration can be increased to 2%.
As ethidiumbromide is extremely mutagenic, always wear gloves and handle buffer, gel and equipment with care! Never throw used gels into the "normal" waste or pour used buffer into the sink! Liquids have to be preprocessed first!

- Prepare the gel casting unit.
- Weigh 0.7 g (resp. 2.0 g) of agarose[9] on a weighing paper.
- Dissolve in 100 ml 1xTAE-buffer[10] (the use of a graduated cylinder is exact enough).
- Melt agarose in a microwave oven. You have to heat it several times until boiling and shake inbetween to get a clear solution.
- For a small gel (2x12 samples) pour 50 ml into a 200 ml Erlenmeyer flask, for a big gel (2x6x8 samples), 100 ml.
- Cool down to <50°C. Rinsing the outside of the Erlenmeyer flask with tap water will fasten cooling.
- For a small gel, add 2.5 µl ethidium bromide[11], for a big one 5 µl. Mix carefully.
- Pour gel and take care not to produce too many bubbles. If there are some bubbles near the future slots for the samples, try to remove them with some plastic pipette tip.
- Let the gel polymerize for at least 20 minutes.
- Cover the gel by at least 0.5 cm with 1xTAE-buffer.
- Load your samples:

  The amount of DNA that can be loaded differs with the expected size range. The maximum volume is 20 µl. As larger amounts are easier to handle, dilute with dest. H_2O. When loading a big gel you can use an 8-channel pipette.

---

[9] Biozym LE Agarose for gel electrophoresis, Biozym Scientific GmbH, Hess. Oldendorf, DE #840004

[10] **10xTAE-buffer (pH 8.0)**
- 400 mM Tris: Pufferan® buffer grade
- 50 mM NaOAc, trihydrate Sigma Ultra
- 7.7 mM EDTA ethylendiamintetraacetate p.a.

[11] Sigma #E8751-1G

Digested DNA: 5°µl DNA + 1 µl 6x MassRuler™ Loading Dye (supplied with size marker)
(e.g. after restriction or preselective amplification for AFLPs)
Size marker: FastRuler™ DNA Ladder, Low Range, ready-to-use (Fermentas Life Sciences #SM1103)
1 µl SM + 6 µl H_2O + 2 µl loading dye

Genomic DNA: 2 µl DNA (50 ng/µl) + 5 µl H_2O + 2 µl 6x loading dye (e.g. Fermentas)
Size marker: λ DNA/*Hind* III Fragments (Invitrogen #15612-013)
1 µl SM + 6 µl H_2O + 2 µl loading dye

- Let the gel run for half an hour:

Digested DNA:		Genomic DNA:	
	90 V		85 V
	85 mA		60 mA
	100 W		100 W

- Running buffer can be used several times. But if you want to discard it, don't forget to pour it into the bottle for preprocessing.
- Put the gel without any plastic tray directly onto the cleaned glass plate of the Gel Doc™ XR 170-8170 (Biorad, Milan, IT).
- Start Programm "Quantity One" → Select Scanner → Gel Doc xr.
- Take care that the iris is not too much closed. Exposure time to UV radiation would be too long and the ethidium-DNA-complex destroyed before you could get a first glimpse on it.
- Select Auto "Exposure" to get a picture, then "Freeze" and "Save".
- If your samples have not already been exposed for too long, you can try to get a nicer picture by changing iris, zoom or focus either direct with the lens control buttons on the Geldoc or in the scanning program and/or exposure time with "Manual Acquire".
- Clean the glass plate of the scanning unit.

PCR Protocols

Microsatellites – SSRs

Reaction Mix for M13-tailed Primers

Table A 1 Concentrations and Volumes for 1 reaction (RXN) for M13-tailed SSR primers

	stock	final	1 RXN
ddH_2O			5.464 µl
PCR-buffer	10 x	1 x	1 µl
dNTPs (ech)	2 mM	0.2 mM	1 µl
$MgCl_2$	500 mM	1.8 mM	0.036 µl
R-primer	10 µM	0.2 µM	0.2 µl
F-primer	10 µM	0.02 µM	0.02 µl
M13-30 (labeled)	10 µM	0.18 µM	0.18 µl
Taq	5 U/µl	0.05 U/µl	0.1 µl
DNA	50 ng/µl	10 ng/µl	2 µl
Total			10 µl

Sequence M13-30 oligo: 5' IRD700/800 - CCC AGT CAC GAC GTT G 3'

Reaction Mix for Direct Labelled Primers

Table A 2 Concentrations and Volumes for 1 reaction (RXN) for direct labelled SSR primers

	stock	final	1 RXN
ddH_2O			5.564 µl
PCR-buffer	10 x	1 x	1 µl
dNTPs (each)	2 mM	0.2 mM	1 µl
$MgCl_2$	500 mM	1.8 mM	0.036 µl
F-primer (labeled)	10 µM	0.12 µM	0.12 µl
R-primer	10 µM	0.2 µM	0.2 µl
Taq	5 U/µl	0.04 U/µl	0.08 µl
DNA	50 ng/µl	10 ng/µl	2 µl
Total			10 µl

PCR-Program for M13-tailed Primers

• Denaturation	94°C	2 min
30 Cycles:		
• Denaturation	94°C	1 min
• Cooling	auf 51°C	0,5°C/sec
• Annealing	51°C	30 sec
• Heating	auf 72°C	0,5°C/sec
• Extension	72°C	1 min
Final:	72°C	5 min
	10°C	∞ min

PCR-Program for Direct Labelled Primers

• Denaturation	94°C	2 min
35 Cycles:		
• Denaturation	94°C	1 min
• Annealing	55°C	1 min
• Extension	72°C	2 min
Final:	72°C	10 min
	10°C	∞ min

Amplified Fragment Length Polymorphisms – AFLPs

Quality control: 100 ng of each diluted sample (2 µl DNA, 50 ng/µl + 5 µl H2O + 2 µl 6x loading dye) are loaded on a 0.7% agarose gel.

Preparation of Adapters

Table A 3 Mix for *Mse*-adapters

	stock	final	per 40 RXN
Adapter *Mse*I-1	500 µM	50 µM	2 µl
Adapter *Mse*I-2	500 µM	50 µM	2 µl
PCR-H_2O			16 µl
Total			20 µl

Table A 4 Mix for *Sse*-adapters

	stock	final	per 40 RXN
Adapter *Sse*8387I-1	50 µM	5 µM	2 µl
Adapter *Sse*8387I-2	50 µM	5 µM	2 µl
PCR-H_2O			16 µl
Total			20 µl

Sequences:
adapter *Mse*I-1: 5' GAC GAT GAG TCC TGA G 3'
adapter *Mse*I-2: 5' TAC TCA GGA CTC AT 3'
adapter *Sse*8387I-1: 5' CTC GTA GAC TGC GTA CAT GCA 3'
adapter *Sse*8387I-2: 5' TGT ACG CAG TCT AC 3'

The mix is heated in the cycler to 95°C and then cooled down to room temperature within 10 min.

Restriction/Ligation in 96-plates

Table A 5 Restriction/Ligation mix for 1 reaction (RXN)

	stock	final	per 1 RXN
genomic DNA (**200 ng/µl**)	50 ng/µl	250 ng	2.5 µl
Sse 8387 I (Takara)	10 U/µl	5 U	0.5 µl
*Mse*I (New England Biolab)	10 U/µl	2.5 U	0.25 µl
T4-ligase	5 U/µl	1 U	0.2 µl
BSA (10x Takara)	10x=0.1%	0.01%	2 µl
10xrestricion buffer for *Sse*8387I (NEB2)	10x	1x	2 µl
Mse-adapter	50 µM	2 µM	0.5 µl
Sse-adapter	5 µM	0.2µM	0.5 µl
ATP	100 mM	1 mM	0.2 µl
PCR-H_2O			11.35 µl
Total		20 µl	20 µl

- 17.5 µl of the ligation mix is added to each restriction mix and incubated at 37°C for 3 hours and then over night at room temp.
- After that 5 µl are loaded on a 2% agarose gel. A smear should appear at the range between 100 to 800 bp.
- Dilute with water four fold (e.g. add 60 µl H_2O to 20 µl R/L mix).

Pre-selective PCR Amplification in 96 or 384-plates

Table A 6 Pre-amplification mix for 1 reaction (RXN)

	stock	final	per 1 RXN
Pre*Mse*I-primer +0	10 µM	0.3 µM	0.6 µl
Pre*Sse*8387I-primer +0	10 µM	0.3 µM	0.6 µl
dNTP	2 mM (each)	0.2 mM	1.5 µl
PCR buffer (incl. $MgCl_2$)	10x	1x	2 µl
$MgCl_2$	50 mM	1.5 mM	0 µl
Taq-polymerase	5 U/µl	0.05U	0.1 µl
ligated DNA			4.5 µl
PCR-H_2O			10.7 µl
Total		20 µl	20 µl

Sequences: preselective primer Pre*Mse*I: 5' GAT GAG TCC TGA GTA A 3'
preselective primer Pre*Sse*8387I: 5' GTA GAC TGC GTA CAT GCA G 3'

- 15.5 µl is added to each sample.
- Amplify using the following program:

	2 min	72°C
20 cycles:	30 sec	94°C
	60 sec	60°C
	2 min	72°C
hold at:		4°C

Note: All temperature ramps must be 1°C per second.

- After that 5 µl are loaded on a 2% agarose gel. A smear should appear at the range between 100 to 800 bp.
- Dilute with water twenty fold (add 95 µl H_2O to 5 µl Pre-sel mix).

Selective PCR Amplification

Table A 7 Selective amplification mix for 1 reaction (RXN)

	stock	final	per 1 RXN
PCR-H_2O			4.24 µl
PCR buffer	10x	1x	1 µl
dNTP	2 mM (each)	0.2 mM	1 µl
$MgCl_2$	50 mM	1.5 mM	0 µl
*Mse*I-primer +2	10 µM	0.3 µM	0.3 µl
*Sse*83871I-primer +2*	10 µM	0.3/0.5 µM	0.3/0.5 µl
Taq-polymerase	5 U/µl	0.05U/µl	0.16 µl
preamplified DNA (5+95µl H_2O)			3 µl
Total		10 µl	10 µl

* For the dyes Fam, IRD700 and IRD800 the concentration is 0.3 µM, for Cy3 and Cy5 0.5µM

Sequences: selective primer *Mse*I: 5' GAT GAG TCC TGA GTA AA**N N** 3'
selective primer Pre*Sse*8387I: 5' **label** - GAC TGC GTA CAT GCA GG**N N** 3'

- 7 µl is added to each sample.
- Amplify using the following program:

	2 min	94°C
10 cycles:	30 sec	94°C
	30 sec	63°C
	2 min	72°C
23 cycles:	30 sec	94°C
	30 sec	54°C
	2 min	72°C
hold at:		4°C

Note: All Temperature ramps must be 1°C per second.

Separation of DNA-Fragments Using the LI-COR®[12] – 4200 DNA Analyzer

Operating Instruction and Gimmicks

by L. Matiasch

modified: 2009-08-04

Gel preparation

Cleaning and assembling of the glass plates

- Carefully clean the glass plates. To get rid of dried gel-remainders, wash the plates with hot water. If they are rather clean, it is sufficient to spray the inner sides[13] of the plates that will be in direct contact with the gel with aqua dest. and wipe off the water with "Roth"[14] tissue. To improve later the filling with the liquid gel, clean the inner sides with ethanol ("70% Ethanol vergällt") and wipe off with the tissue.
- Clean spacer with aqua dest. and put them on the very left and right sides of the glass.
- Put the other glass plate on top and fix the two plates. Leave the top screw open for pouring the gel. Always close one of the left and one of the right side at the same time and do not fix it too tightly to prevent both clamps and class plates from breaking.

Pouring the gel

- Move to the fume hood for pouring the gel[15].
- Put the assembled glass plates in an angle of about 15° on a styrofoam plate (see Fig. A 1, left).
- In general we use a 7% acrylamide gel[16]. For gels with a length of 25 cm and a thickness of 0.25 mm you have to mix:

12 LI-COR Biosciences GmbH, Bad Hamburg, DE

13 Always take care to use the same side of the plates as inner respectively outer side and never touch the plates without gloves. Fat on the inner side of the plates will result in bubbles and prevent you from getting a nice gel.

14 Carl Roth GmbH + Co. KG, Karlsruhe, DE

15 Acrylamide is carcinogenic – **never work with acrylamide outside the fume hood** until it has polymerized to prevent inhalation and wear gloves!

16 If for some primer combinations bands do not separate well, you can increase the amount of acrylamide up to 10%.

Urea+TBE[17]	19.5 ml
Acrylamide[18]	3.5 ml
DMSO[19]	250 µl
APS[20]	175 µl
TEMED[21]	25 µl

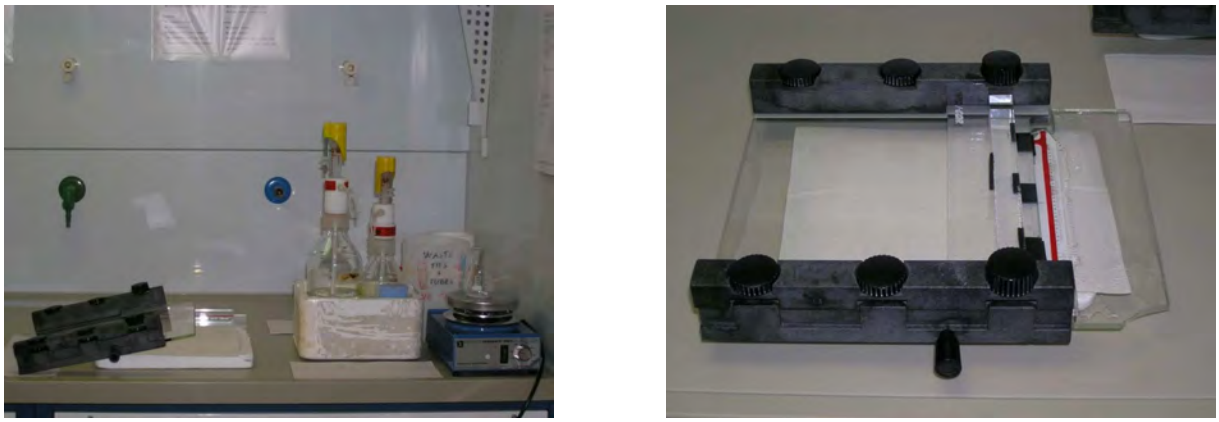

Fig. A 1 Preparing a gel for LI-COR® – 4200 DNA Analyzer

- Mix urea+TBE and acrylamide in first thoroughly with a magnetic stirrer. Add DMSO, APS and TEMED and be ready for pouring the gel. It polymerizes soon!
- Pour the gel beginning at one side of the cavity. Whilst pouring, gently knock on the glass plates to prevent development of bubbles.
- If there are –despite following all the advises above– some bubbles in the gel, you can try to get them out with the very thin wire. But be quick. As soon as the gel starts polymerizing, you will cause even more harm!
- Where later the sharktooth comb for loading the samples will be inserted, place the opposite side of an old one comb to get a straight edge.
- Insert the plastic plate and fix the very top screws (see Fig. A 1, right)
- Let the gel polymerize for at least half an hour.
- Clean up fume hood.

---

[17] **8 M urea**
- 210 g urea: Rotiphorese® NF-Harnstoff f automatische Sequencer, Roth #A120.1;
- 50 ml 10x TBE ⇒ Dissolve in 180 ml and fill then to 420 ml with osmotic H_2O.

10x TBE
- 162.0 g Tris base (tris(hydroxymethyl)amino-methane): Pufferan® buffer grade, Roth #AE15.3
- 27.5 g boric acid p.a., Roth #6943.1
- 9.3 g EDTA (ethylendiamintetraacetate) p.a., Roth #8043.2

⇒ Fill to 1000 ml with osmotic H_2O.

[18] Long Ranger ® Gel Solution, Cambrex Bio Science, Rockland Inc., Rockland, US, #50615

[19] dimethylsulfoxide, p.a., Fluka Chemika, Chemia AG, Buchs, CH, #41640

[20] 10% ammoniumpersulfate: Dissolve 1 g (Roth #9592.2) in approx. 180 ml dest. H_2O and freeze in quantities of 190 µl.

[21] N,N,N',N' tetramethylethylendiamin, Sigma-Aldrich #87687

- Remove the old sharktooth comb.
- Replace the plastic plate with a buffer tank.
- Remove gel particles on the outer parts by washing the glass plates. Clean with ethanol ("70% Ethanol vergällt") and wipe off with the tissue.

Starting the LI-COR®

- Switch on PC.
- Insert the glass plates into the LI-COR® unit. Some plates have been used with both sides up and there are scratches from the gel loading syringe. Take care that this "band" is not at the same level where the laser is scanning.
- Fill both buffer reservoirs with running (1x TBE)-buffer up to the marks.
- Carefully clean the upper edge from all gel particles. These would later prevent loading of the samples. Washing with a 5 ml-syringe with running buffer, or using the teeth of an old comb or an old wire for getting out bubbles may be helpful.
- Insert the sharktooth comb. If it efforts much strength to insert it, wait until the temperature of the gel has increased.
- Cover the buffer tanks and connect the wires.
- Switch on LI-COR®.
  - Select "V4.10 Data Collection Model 4200 Dual Dye DEV1"
  - Select File → New. In the "Project" window write a "Project name" and take care that for "Pixel size (bits)" 8 is selected. In the windows "700"' resp. "800" write an "Image name".
  - To adjust scanning intensity, select in the "Image" window Options → Auto gain →Auto After auto gain has finished, select Done. **You have to do this for both images (wavelengths)!**
- Before loading the first time let the gel run for about half an hour. At least the temperature should rise to the default 48°C. It seems the longer you wait until loading the first samples, the nicer the first picture. But if a gel is running for a too long time, it will become damaged.
- **Settings:**

Voltage (V)	1500
Current (ma)	50
Power (W)	40
Heater (degC)	48
Signal Filter	3
Scan Speed	3

Sample preparation

In general you get nice pictures when adding 1.2 µl of PCR samples to 5 µl loading buffer[22]. If the bands are too weak, you can raise the amount of samples of up to 5 µl. Larger amounts of samples do not seem to improve the intensity of the bands.[23]

- Centrifuge mixture for some seconds at 3000 rpm.
- Denature at 95°C for 5 minutes.
- Immediately place on ice for fast cooling. After cooling down centrifuge again[24].

Setting the Focus

- At first better do auto gain once more for both images (wavelengths): Options → Autogain → Auto.
- In the "Scanner Control" window select Options → Focus[25]. The "ideal focus" is shown in Fig. A 2. But despite a less "symmetric" bend or a smaller peak, gel pictures can be very nice.

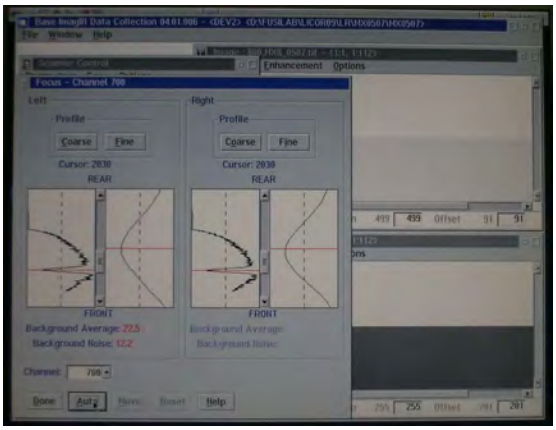

Fig. A 2 Setting the Focus on LI-COR® – 4200 DNA Analyzer

Loading the samples

- It is possible to load samples labeled with 700 and 800 nm at the same time.
- 1.2 – 1.5 µl can be loaded.

---

[22] **Loading buffer:**

- 35.625 ml formamide Ultra for molecular biology, Fluka #47671
- 1.875 ml EDTA (0.5 mol/l, pH=8.0): Dissolve 186.12 g EDTA in approx. 750 ml of dest. H_2O. Add conc. NaOH solution (dissolve NaOH pellets in a small volume of dest. H_2O) slowly to bring pH to 7.95 **Note: EDTA will dissolve only while adding NaOH**. After EDTA is in solution, bring to 950 ml, **check pH again and bring it to 8.0, fill up to 1000 ml.** Autoclave
- 225 µl fuchsin, Merck für die Mikrobiologie und Mikroskopie #1358, dissolved in methanol

[23] Bands will also be more intense, if you do not load a "fresh" PCR right from the cycler, but store it for one or more days at 4°C

[24] But not for too long before loading!

[25] The better results you get when selecting Channel 700.

- Always clean the gel-loading syringe ("Hamilton[26]") carefully by pipetting running buffer several times.
- Always check, whether the needles are at the right places.
- After loading the first PCR, run the gel for about 3 minutes until the samples are in the gel. Then no dye of the loading buffer should be seen above the gel.
- Before loading the second PCR, wash the above edge of the gel again with running buffer.
- It is possible to load a gel several times. If the bands of the two samples are of very different length and you decide to load at different times, do not forget that the primer front is that intense that it is also detected by the laser of the other wavelength and can cover the fragments!
- You can adjust the number of frames either to let the gel run for a longer time or to stop it automatically without having to wait.

Cleaning up

- To get both glass plates apart, the black plastic device may be helpful.
- Generally the whole gel is sticking to one of the plates. The best way to remove it is to "curl" it off the plate by pressing first a tissue on the whole area.
- Clean glass plates, spacers and sharktooth comb by rinsing with hot water first.
- Afterwards rinse with osmotic water as the normal tap water will cause white spots when drying.
- Buffer in the lower reservoir can be used several times.
- Clean up the desk all around the LI-COR®.
- Save your pictures.
  - Select "PC Datenverbindung"
  - In the upper part of the window you see the directories of the computer connected to your LI-COR® machine.
  - In the lower part, the directory of the corresponding folder on the lab-PC should be shown, e.g. /ron. Select your own folder with the right mouse button.
  - With the left mouse button select the *.tif-files you want to copy. Then drag them with the right mouse button to your folder. **Take care to use the correct "Übertragungsmodus" (ASCII or Binär)!**
  - You will find your files on the lab-PC in C:\ftproot\Ron (resp. Maxime, Hagrid, Hermine).
- Close the Data Collection program by selecting ⊠
- Select "Systemabschluss" ⏏
- Switch off LI-COR® and PC.

[26] Hamilton Company, Reno, US

Separation of DNA-Fragments using the C.B.S.®[27] System and Typhoon Trio™[28] scanner

Operating Instruction and Gimmicks

by L. Matiasch, G. Stift, A.I. Alimari and K. Herzog

modified: 2008-11-21

Gel preparation

Cleaning and assembling of the glass plates

- Carefully clean the glass plates. To get rid of dried gel-remainders, wash the plates with hot water. If they are rather clean, it is sufficient to spray the inner sides[29] of the plates that will be in direct contact with the gel with aqua dest. and wipe off the water with "Roth"[30] tissue. To improve later the filling with the liquid gel, clean the inner sides with some drops of ethanol abs.[31] Use a plastic Pasteur pipette and wipe off with the tissue.
- Clean spacer with aqua dest. and put them on the very left and right sides of the glass plate with the cavity for the sharktooth comb (as this is the broader of the two plates).

Put the other glass plate on top and fix the two plates with 5 black clamps on each side. Leave a space for a sixth clamp on the down end of the plates. It is best to fix the clamps on the very outer part of the spacers to prevent the liquid gel from spilling (see Fig. A 3, left).

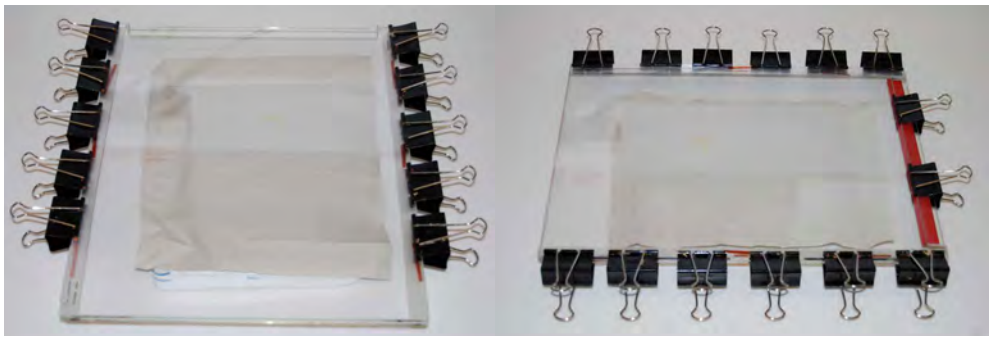

Fig. A 3 Preparing a gel for C.B.S.® chamber

[27] Vertical Electrophoresis System (Sequencer), C.B.S. Scientific Company, Del Mar, US

[28] Typhoon TRIO Variable Mode Imager, Amersham Biosciences, Buckinghamshire, GB

[29] Always take care to use the same side of the plates as inner respectively outer side and never touch the plates without gloves. Fat on the inner side of the plates will result in bubbles and prevent you from getting a nice gel.

[30] Carl Roth GmbH + Co. KG, Karlsruhe, DE

[31] Never use denatured ethanol ("70% ethanol vergällt"). It contains fluorescing substances that influence the scanning.

Pouring the gel

- Move to the fume hood for pouring the gel[32].
- Put the assembled glass plates in an angle of about 15° on a styrofoam plate.
- In general we use a 7% acrylamide gel[33]. For big gels (e.g. for AFLPs) you have to mix:

Urea+TBE[34]	60 ml
Acrylamide[35]	13 ml
APS[36]	400 µl
TEMED[37]	64 µl

- Mix Urea+TBE and acrylamide in first thoroughly with a magnetic stirrer. Add APS and TEMED and be ready for pouring the gel. It polymerizes soon!
- Pour the gel beginning at one side of the cavity. Whilst pouring, gently knock on the glass plates to prevent development of bubbles.
- If there are –despite following all the advises above– some bubbles in the gel, you can try to get them out with the very thin wire. But be quick. As soon as the gel starts polymerizing, you will cause even more harm!
- Insert a red spacer at the cavity to get a straight edge, where later the sharktooth comb for loading the samples will be inserted. Fix the red spacer with two more black clamps[38] and also add a sixth clamp on the left and right side of the very down part of the gel (see Fig. A 3, right).
- Let the gel polymerize for at least half an hour.
- **Clean up fume hood.**
- Remove gel particles on the outer parts by washing the glass plates. Dry the plates carefully.

---

[32] Acrylamide is carcinogenic – **never work with acrylamide outside the fume hood** until it has polymerized to prevent inhalation and wear gloves!

[33] If for some primer combinations bands do not separate well, you can increase the amount of acrylamide up to 10%.

[34] **8 M urea**

- 240.24 g urea: Rotiphorese® NF-Harnstoff f automatische Sequencer, Roth #A120.1
- 59 ml 10x TBE ⇒ Fill to 500 ml with osmotic H_2O (for the exact concentration 514.7 ml).

10x TBE

- 162.0 g tris base (tris(hydroxymethyl)amino-methan): Pufferan® Buffer Grade, Roth #AE15.3;
- 27.5 g boric acid p.a., Roth #6943.1
- 9.3 g EDTA (ethylendiamintetraacetate) p.a., Roth #8043.2

⇒ Fill to 1000 ml with osmotic H_2O.

[35] Rotiphorese® Gel 40 (19:1), Roth #3030.1

[36] 10% ammoniumpersulfate: Dissolve 1 g (Roth #9592.2) in approx. 180 ml dest. H_2O and freeze in quantities of 190 µl.

[37] N,N,N',N' tetramethylethylendiamin, Sigma-Aldrich #87687

[38] The clamps must not reach further from the boarder of the glass plates than the spacer!

Running-in the gel

- Insert the glass plates into the C.B.S.® unit. The plate with the cavity has to be at the side of the buffer reservoir.
  - Fix it at both sides with two white spring clamps.
  - Tighten the gasket of the upper reservoir.
  - Fill both buffer reservoirs with running (1x TBE)-buffer up to the marks.

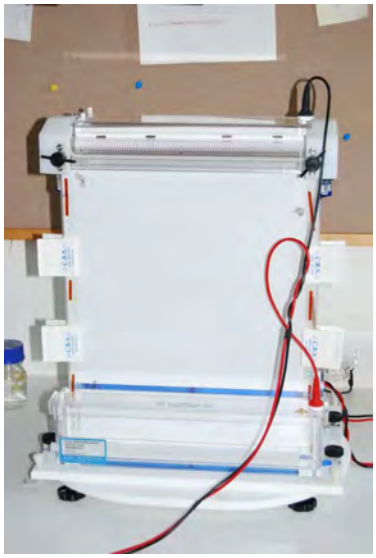

Fig. A 4 C.B.S.® chamber ready for running the gel

  - Carefully clean the upper edge from all gel particles. These would later prevent loading of the samples. Washing with a 5ml-syringe with running buffer, or using the teeth of an old comb or an old wire for getting out bubbles may be helpful.
  - Insert the sharktooth comb. If it efforts much strength to insert it, wait until the temperature of the gel has increased.
  - Cover the buffer tanks and connect the other end of the cable to the power supply. Take care to use the correct adapters for the chosen power supply. If you are not sure, ask somebody to help you!!!
- Let the gel run for at least 40 minutes. The temperature of the gel should rise to about 45-50°C. It seems the longer you wait until loading the samples, the nicer the picture. But if a gel is running for a too long time, it will become damaged starting from the down side.
- **Settings:**

  2500 V (never go beyond this!)
  150 mA (or maximum of the power supply)
  100 W[39]

[39] If the temperature is rising too much, reduce to 65 W. High temperature can make the glass break and/or the bands on the gel have the shape of a smile.

Sample preparation

In general you get nice pictures when adding 5 µl loading buffer[40] to the original PCR[41].

- Centrifuge mixture for some seconds at 3000 rpm.
- Denature at 95°C for 5 minutes.
- Immediately place on ice for fast cooling. After cooling down centrifuge again[42].

Loading the samples

- It is possible to load up to 3 PCR labeled with different dyes.

  Possible combinations:

  - Fluorescein and HEX or
  - Cy5, Fluorescein and TAMRA or
  - Cy5 and FAM or
  - Cy3 and FAM (But very intense FAM-band will be visible as very weak bands when scanning Cy3!) or
  - Cy5, Cy3 and FAM (But **only** if you want to use your data **for mapping**! When scanning Cy5, intense Cy3 bands will be visible too.)

  If you are loading more than one PCR on the same gel, always take care, that the **same sample** will be at the **same position**!

- Depending on the used loading-comb you can load the following amounts:
  - 126-comb: 1.4 – 1.5 µl[43]
  - 94-comb[44]:2.0 – 2.5 µl
  - 62-comb: 2.0 – 3.0 µl

  Even despite loading from behind, you can always check, whether the needles are at the right places.

[40] **Loading buffer:**
- 35.625 ml formamide ultra for molecular biology, Fluka Chemica, Chemia AG, Buchs, CE, #47671
- 1.875 ml EDTA (0.5 mol/l, pH=8.0): Dissolve 186.12 g EDTA in approx. 750 ml of dest. H_2O. Add conc. NaOH solution (dissolve NaOH pellets in a small volume of dest. H_2O) slowly to bring pH to 7.95 **Note: EDTA will dissolve only while adding NaOH**. After EDTA is in solution, bring to 950 ml, **check pH again and bring it to 8.0, fill up to 1000 ml.** Autoclave
- 225 µl fuchsin, Merck für die Mikrobiologie und Mikroskopie #1358 or Bromphenol blue, United States Biochemical Corp, Cleveland, US, #12370

[41] **Bands will be more intense, if you do not load a "fresh" PCR right from the cycler, but store it for one or more days at 4°C.**

[42] But not for too long before loading!

[43] Bands are not too nice, so use for testing parents only!

- Always clean the gel-loading syringe ("Hamilton[45]") carefully by pipetting running buffer several times.
- After loading the first PCR, run[46] the gel for several minutes until the samples are in the gel. Then no dye of the loading buffer should be seen above the gel[47].
- Before loading the second PCR, wash the above edge of the gel again with running buffer.

Running the gel

- **Settings:**

  2500 V (never go beyond this!)
  150 mA (or maximum of the power supply)
  60 W (when running 1 gel)
  80 W (when running 2 gels) Temperature should remain at about 45-50°C. If it is becoming too hot, you have to reduce to 65 W or less to prevent the gel from a smile or even more damage and the glass from breaking.

  In general an AFLP-gel is scanned after 2 and 4 hours. After 2 hours, small fragments can be scored. After 4 hours, also the big fragments are well separated, but small fragments have already reached the end of the gel and escaped. Depending on the pattern of the special case, other times may be better.

Scanning

- Switch on computer and Typhoon Trio™ scanner. It takes about 20 minutes until the scanner is ready (lamps stable).
- Take out some buffer of the upper reservoir with a big syringe or a 50ml-tube until the level is deep enough not to float out when taking off the gasket and the glass plates.
- Carefully clean the glass plates with aqua dest. Before scanning, the gel should cool down for 10 minutes or you should accelerate cooling by washing with cold water first.
- Control the glass plate of the scanner. If necessary, clean with aqua dest. You may use alcohol too, but **never use "70% Ethanol vergällt" (denatured ethanol)**!
- The thinner glass plate (the front one without the cavity) has to be the down one when scanning. Label your plates with a number and "left" or "right" that also on the scanned picture you can be sure that you did not change the gels or the side.
  - To start the program, select the icon "Typhoon Scanner Control v 5.0" on the computer's desktop. With "Template" → "Load" you can select the suitable template.
  - If you want to change scanning intensity, choose "Setup" and "PMT".
  - Start with "Scan".

---

[44] Be aware that a PCR-Plate has 96 wells!

[45] Hamilton Company, Reno, US

[46] For settings see next section.

[47] If you use the pink loading buffer, it won't enter into the gel. In this case you should load at least one lane with blue loading buffer to recognize the entering of the bands into the gel.

Table A 8 Settings for Scanning AFLPs on Typhoon Trio™ Scanner

Fluorochrome	Emission Filter	PMT	Laser	Sensitivity	Beam Splitters
Fluorescein	526 SP	1000	Green (532)	Normal	
HEX				Normal	
Cy5	670 BP 30	800	Red (633)	Normal	580
Fluorescein	526 SP	1000	Green (532)	Normal	
TAMRA	580 BP 30	800	Green (532)	Normal	
Cy5	670 BP 30	800	Red (633)	Normal	630
Cy3	580 BP 30	800	Green (532)	Normal	
FAM	520 BP 40	700	Blue (488)	Normal	

- The intensity of the bands is not the same for all possible AFLP-primer combinations. As a complete scan for 3 dyes takes about 20 minutes, it can save time, if you check the settings on a small part (about 4 rows) of the plate before scanning the entire one.
- If your bands are too weak or too dark, adjust scanning intensity by choosing “Setup” and “PMT”[48].

Cleaning up

- To get both glass plates apart, the red C.B.S.® plastic device may be helpful.
- Generally the whole gel is sticking to one of the plates. The best way to remove it is to “curl” it off the plate.
- Clean glass plates, spacers and sharktooth comb by rinsing with hot water first.
- Afterwards rinse with osmotic water as the normal tap water will cause white spots when drying.
- Buffer in the lower reservoir can be used several times. So it is sufficient to empty the upper reservoir.
- Clean up the desk all around the C.B.S.® units.
- Switch off power supply.

Have a look at the calendar: When does the next one want to use the scanner? To prevent dusting of the magnetic bands, Typhoon Trio™ should not be switched on for more than about 2 hours if not used.

[48] If your picture is not too nice, check again if you diluted the primers correctly. Some of them should only be diluted 1:5!

Appendix B: R© Functions and SAS® Codes

R© function for the generation of histograms for the traits heading, plant height and leaf rust resistance (mean over experiments)

```
function (file, cols=c(5:9))
{
        ## file ... character, filename
        ## cols ... vector, columnindexes, default: column 5 to 9

        data <- read.Dummy(file)    ## reading data from a .csv file, function: read.Dummmy

        M <- as.matrix(data[, cols])
        col.names <- dimnames(M)[[2]]

        win.metafile(filename = paste("histogram_", col.names[1], ".wmf", sep=""),
              width = 4, height = 4, pointsize = 7,
              restoreConsole = TRUE)
        hist(M[,1], breaks=c(130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150,
                                152, 154, 156, 158, 160, 162, 164),
              labels=TRUE,
              xlab="heading [day of the year]",
              main=paste("histogram of", col.names[1]), ylim=c(0, 120))
        dev.off()

        win.metafile(filename = paste("histogram_", col.names[2], ".wmf", sep=""),
              width = 3, height = 3, pointsize = 6,
              restoreConsole = TRUE)
        hist(M[,2], breaks=c(60, 70, 80, 90, 100, 110, 120, 130, 140),
              labels=TRUE,
              xlab="plant height [cm]",
              main=paste("histogram of", col.names[2]), ylim=c(0, 120))
        dev.off()

    for (i in 3:5) {
        win.metafile(filename = paste("histogramm_", col.names[i], ".wmf", sep=""),
              width = 3, height = 3, pointsize = 6,
              restoreConsole = TRUE)
        hist(M[,i], breaks=c(0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100),
              labels=TRUE,
              xlab="diseased area [%]",
              main=paste("histogram of", col.names[i]), ylim=c(0, 160))
        dev.off()
    }
}
```

Depending on the range of the data it might be necessary to adjust the height of the y-axis (ylim) or the graduation of the x-axis (breaks). If the columns different from 5 to 9 contain the traits of interest, the range has to be adjusted in the first line of this function. Histograms are stored as .wmf files and named "histogram_columnname".

R© function "read.Dummy"

```
function (file)
{
        df1 <- read.csv(file)
        df1 <- df1[-1,]
        return(df1)
}
```

If this function is used for reading .csv files into R©, the first line must contain the variable names and the second line is a dummy that is deleted after reading. The decimal sign is the point.

SAS® code for single point ANOVA for the trait heading (mean over experiments)

```
data Data2;
set Work.Data;
data d;
set Work.Data;
array c(36) Xbarc25 -- Xwmc25_2;        *name of the first marker -- name of the last marker
keep genotype marker response mheading;
do marker= 1 to 36;             * number of markers
        response=c(marker);
        if response=0 then response=missing;
output;
end;
run;
proc sort;
by marker;
run;

proc glm data=d outstat=Work.Anova_out;
by marker;
class response;
model mheading = response;
means response;
run;
quit;
proc means noprint data=d;
        class response ;
        by marker;
        var mheading ;
        types response ;
        output out=Work.Markermeans
             n=          nmheading
             mean=       mmheading
             StdDev=     smheading ;
run;
quit;
```

```
data F_Values;
 set Work.Anova_out;
 if _TYPE_ ='SS1' then delete;
 keep marker df ss f prob;
run;
data t;
set Work.F_Values;
proc sort;
by prob;
run;
```

Appendix C: Trait Correlations

Abbreviations used in the following tables:

P	Pearson's product moment correlation coefficient
S	Spearman's rho
Head	heading (day of the year)
PH	plant height (cm)
LR	leaf rust severity (LR1, LR2,... if assessed several times) (% infected leaf area)
AUDPC	area under the disease progress curve for leaf rust resistance
Seedling	seedling resistance against leaf rust (0 = immune – 4 = susceptible)
CD	crop density (1 = no missing plants – 5 more than half of the plants missing)
Leaf	leaf blotch severity (1 = no visible symptoms – 9 = completely diseased)
PM	powdery mildew severity (1 = no visible symptoms – 9 = completely diseased)
Sep	*Septoria* leaf blotch severity (1 = no visible symptoms – 9 = completely diseased)
YR	yellow rust severity (1 = no visible symptoms – 9 = completely diseased)
Glauc	glaucousness (1 = no wax – 5)
FH	frost heaving severity (1 = no damage – 9 all plants frozen)
Lodg	lodging severity (Lodg1,... if assessed several times) (different scales)
Chl	leaf chlorosis severity (0 = no visible symptoms – 9 = extremely chlorotic)
LTN	leaf tip necrosis severity (0 = no visible symptoms – 9 = heavy symptoms)

Table A 9 Trait correlations population Isengrain/Capo: mean over experiments

		P PH	S PH	P LR	S LR	P AUDPC	S AUDPC	S LTN
Head	n	227	227	238	238			
	r	0.40	0.44	-0.17	-0.17			
	H_0	acc.	acc.	acc.	acc.			
PH	n			226	226			
	r			-0.01	-0.01			
	H_0			acc.	acc.			
LR	n					240	240	
	r					0.94	0.96	
	H_0					rej.	rej.	
Chl	n							240
	r							0.48
	H_0							acc.

The means over the experiments were calculated as follows:

Head – PH:	Tulln 2004, 2006, 2007 (M), 2007, 2008
Head – LR:	Tulln 2004, Probstdorf 2007, Tulln (M) 2007, Rust 2008, Schmida 2008, Tulln 2008
PH – LR:	Tulln 2004, Tulln 2007 (M), Tulln 2008,
LR – AUDPC:	Piestany 2006, Tulln 2008
Chl – LTN:	Rust 2009, Tulln 2009

Table A 10 Trait correlations population Isengrain/Capo: Aumühle 2004

		P LR	S LR	P Leaf
Head	n	204	204	239
	r	-0.02	-0.18	-0.35
	H_0	acc.	acc.	acc.
LR	n			204
	r			-0.10
	H_0			acc.

Table A 11 Trait correlations population Isengrain/Capo: Tulln 2004

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	S CD
Head	n	240	240	234	234	143	143	240
	r	0.28	0.30	-0.11	-0.14	-0.05	-0.07	-0.01
	H_0	acc.	acc.	acc.	acc.	not t.	not t.	acc.
PH	n			234	234	143	143	240
	r			0.11	0.10	0.03	0.07	-0.19
	H_0			acc.	acc.	not t.	not t.	acc.
LR1	n					143	143	234
	r					0.80	0.87	-0.05
	H_0					not t.	not t.	acc.
LR2	n							143
	r							-0.09
	H_0							not t.

Table A 12 Trait correlations population Isengrain/Capo: Piestany 2006

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	240	240	240	240	240	240	240	240
	r	-0.14	-0.18	-0.55	-0.28	-0.15	-0.14	-0.23	-0.25
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			240	240	240	240	240	240
	r			0.72	0.73	0.60	0.60	0.77	0.77
	H_0			rej.	rej.	acc.	acc.	rej.	rej.
LR2	n					240	240	240	240
	r					0.75	0.74	0.98	0.97
	H_0					rej.	rej.	rej.	rej.
LR3	n							240	240
	r							0.87	0.87
	H_0							rej.	rej.

Table A 13 Trait correlations population Isengrain/Capo: Probstdorf 2006

		S LR1	P LR2	S LR2	S PM	S FH
Head[1)]	n	239		130	240	240
	r	-0.15		0.05	0.02	0.22
	H_0	acc.		not t.	acc.	acc.
LR1	n		130	130	239	239
	r		0.75	0.77	0.22	-0.13
	H_0		not t.	not t.	acc.	acc.
LR2	n				130	130
	r				0.30	0.02
	H_0				not t.	not t.
PM	n					240
	r					0.08
	H_0					acc.

[1)] scored on a 1 (= early) to 9 (= late) scale

Table A 14 Trait correlations population Isengrain/Capo: Tulln 2006

		P PH	S PH
Head	n	240	240
	r	0.42	0.41
	H_0	acc.	acc.

Table A 15 Trait correlations population Isengrain/Capo: Piestany 2007

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	234	234	234	234	234	234	234	234
	r	0.15	0.11	0.26	0.27	0.38	0.43	0.33	0.33
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			234	234	234	234	234	234
	r			0.66	0.71	0.44	0.49	0.64	0.67
	H_0			rej.	rej.	acc.	acc.	acc.	rej.
LR2	n					234	234	234	234
	r					0.72	0.77	0.92	0.92
	H_0					rej.	rej.	rej.	rej.
LR3	n							234	234
	r							0.89	0.89
	H_0							rej.	rej.

Table A 16 Trait correlations population Isengrain/Capo: Probstdorf 2007

		P LR1	S LR1	P LR2	S LR2	S Lodg[1)]
Head	n	239	239	232	232	239
	r	-0.04	-0.05	-0.08	-0.09	0.21
	H_0	acc.	acc.	acc.	acc.	acc.
LR1	n			232	232	239
	r			0.64	0.69	0.06
	H_0			acc.	rej.	acc.
LR2	n					232
	r					0.06
	H_0					acc.

[1)] scored on a 1 (= no lodging) – 5 (= complete lodging scale)

Table A 17 Trait correlations population Isengrain/Capo: Schmida 2007

		P LR2	S LR2	P LR3	S LR3
LR1	n	234	234	49	49
	r	0.74	0.81	0.61	0.69
	H_0	rej.	rej.	not t.	not t.
LR2	n			49	49
	r			0.85	0.87
	H_0			not t.	not t.

Table A 18 Trait correlations population Isengrain/Capo: Tulln (M) 2007

		P PH	S PH	P LR1	S LR1
Head	n	227	227	213	213
	r	0.48	0.50	-0.26	-0.27
	H_0	acc.	acc.	acc.	acc.
PH	n			213	213
	r			-0.08	-0.06
	H_0			acc.	acc.

Table A 19 Trait correlations population Isengrain/Capo: Tulln 2007

		P PH	S PH
Head	n	240	240
	r	0.29	0.32
	H_0	acc.	acc.

Table A 20 Trait correlations population Isengrain/Capo: Probstdorf 2008

		S Lodg[1)]
LR	n	213
	r	-0.14
	H_0	acc.

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)

Table A 21 Trait correlations population Isengrain/Capo: Rust 2008

		P LR	S LR	S Lodg[1)]
Head	n	234	234	240
	r	-0.02	0.00	0.35
	H_0	acc.	acc.	acc.
LR	n			234
	r			0.13
	H_0			acc.

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)

Table A 22 Trait correlations population Isengrain/Capo: Schmida 2008

		P LR	S LR
Head	n	216	216
	r	-0.06	-0.04
	H_0	acc.	acc.

Table A 23 Trait correlations population Isengrain/Capo: Tulln 2008

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	240	240	240	240	240	240	240	240	240	240
	r	0.15	0.11	-0.19	-0.17	-0.23	-0.24	-0.24	-0.23	-0.23	-0.24
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
PH	n			240	240	240	240	240	240	240	240
	r			0.11	0.08	0.09	0.11	0.12	0.13	0.11	0.12
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n					240	240	240	240	240	240
	r					0.87	0.92	0.76	0.90	0.90	0.94
	H_0					rej.	rej.	rej.	rej.	rej.	rej.
LR2	n							240	240	240	240
	r							0.89	0.94	0.99	0.99
	H_0							rej.	rej.	rej.	rej.
LR3	n									240	240
	r									0.93	0.97
	H_0									rej.	rej.

Table A 24 Trait correlations population Isengrain/Capo: Rust 2009

		P LR2	S LR2	S Chl	S LTN
LR1	n	192	192	240	240
	r	0.64	0.66	-0.33	-0.38
	H_0	not t.	not t.	acc.	acc.
LR2	n			192	192
	r			-0.32	-0.51
	H_0			not t.	not t.
Chl	n				240
	r				0.42
	H_0				acc.

Table A 25 Trait correlations population Isengrain/Capo: Tulln 2009

		S Chl	S LTN
Head	n	240	240
	r	-0.02	-0.02
	H_0	acc.	acc.
Chl	n		240
	r		0.47
	H_0		acc.

Table A 26 Trait correlations population Arina/Capo: mean over experiments

		P PH	S PH	P LR	S LR	S Chl	S LTN	P AUDPC	S AUDPC	S Lodg[1)]
Head	n	232	232	209	209	232	232	230	230	
	r	0.03	0.04	-0.30	-0.29	0.21	0.19	-0.33	-0.30	
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	
PH	n			230	230					
	r			0.12	0.11					
	H_0			acc.	acc.					
LR	n					225	225	228	228	227
	r					-0.52	-0.58	0.95	0.95	-0.11
	H_0					acc.	acc.	rej.	rej.	acc.
Chl	n						233			
	r						0.56			
	H_0						acc.			

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)

The means were calculated over the experiments as follows:

Head – PH: Tulln 2007, 2008
Head – LR: Tulln 2007, Fundulea 2008, Nyon 2008, Rust 2008, Schmida 2008, Tulln 2008, Rust 2009, Tulln 2009
Head – Chl: Rust 2009, Tulln 2009
Head – LTN: Rust 2009, Tulln 2009
Head – AUDPC: Fundulea 2008, Tulln 2008
PH – LR: Tulln 2007, Tulln 2008
LR – Chl: Rust 2009, Tulln 2009
LR – LTN: Rust 2009, Tulln 2009
LR – AUDPC: Piestany 2008, Fundulea 2008, Tulln 2008
LR – Lodg: Probstdorf 2008, Rust 2008
Chl – LTN: Rust 2009, Tulln 2009

Table A 27 Trait correlations population Arina/Capo: Tulln 2007

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	P LR3	S LR3
Head	n	232	232	232	232	229	229	183	183
	r	-0.05	-0.03	-0.14	-0.14	-0.15	-0.14	-0.14	-0.12
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	not t.	not t.
PH	n			233	233	230	230	184	184
	r			-0.01	-0.02	0.06	0.07	0.10	0.08
	H_0			acc.	acc.	acc.	acc.	not t.	not t.
LR1	n					230	230	184	184
	r					0.77	0.79	0.76	0.77
	H_0					rej.	rej.	not t.	not t.
LR2	n							184	184
	r							0.92	0.96
	H_0							not t.	not t.

Table A 28 Trait correlations population Arina/Capo: Fundulea 2008

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC	S Seedling
Head	n	230	230	230	230	230	230	230	230	220
	r	-0.13	-0.13	-0.15	-0.17	-0.11	-0.11	-0.15	-0.17	-0.13
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			230	230	230	230	230	230	220
	r			0.26	0.26	0.20	0.22	0.28	0.29	0.23
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR2	n					230	230	230	230	220
	r					0.75	0.76	0.97	0.97	0.07
	H_0					rej.	rej.	rej.	rej.	acc.
LR3	n							2320	230	220
	r							0.88	0.89	0.07
	H_0							rej.	rej.	acc.
AUDPC	n									220
	r									0.09
	H_0									acc.

Table A 29 Trait correlations population Arina/Capo: Nyon 2008

		P LR1	S LR1	P LR2	S LR2	S PM	S Sep	S YR
Head	n	228	228	225	225	228	228	228
	r	-0.03	-0.05	-0.15	-0.14	-0.06	0.02	-0.05
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			226	226	229	229	229
	r			0.40	0.19	0.10	0.02	0.06
	H_0			acc.	acc.	acc.	acc.	acc.
LR2	n					226	226	226
	r					0.22	-0.14	0.16
	H_0					acc.	acc.	acc.
PM	n						229	229
	r						-0.03	0.23
	H_0						acc.	acc.
Sep	n							229
	r							0.23
	H_0							acc.

Table A 30 Trait correlations population Arina/Capo: Piestany 2008

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	229	229	229	229	229	229	229	229
	r	-0.18	-0.19	-0.13	-0.19	-0.28	-0.27	-0.25	-0.25
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			229	229	229	229	229	229
	r			0.59	0.59	0.58	0.54	0.66	0.64
	H_0			acc.	acc.	acc.	acc.	rej.	acc.
LR2	n					229	229	229	229
	r					0.63	0.67	0.85	0.87
	H_0					acc.	rej.	rej.	rej.
LR3	n					229	229	229	229
	r					0.63	0.67	0.95	0.94
	H_0					acc.	rej.	rej.	rej.

Table A 31 Trait correlations population Arina/Capo: Probstdorf 2008

		S Lodg[1)]
LR	n	231
	r	-0.09
	H_0	acc.

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)

Table A 32 Trait correlations population Arina/Capo: Reichersberg 2008

		P LR	S LR
Head	n	213	213
	r	-0.16	-0.14
	H_0	acc.	acc.

Table A 33 Trait correlations population Arina/Capo: Rust 2008

		P LR	S LR	S Lodg[1)]
Head	n	225	225	230
	r	-0.09	-0.08	0.11
	H_0	acc.	acc.	acc.
LR	n			226
	r			0.00
	H_0			acc.

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)

Table A 34 Trait correlations population Arina/Capo: Schmida 2008

		P LR	S LR
Head	n	232	232
	r	-0.22	-0.21
	H_0	acc.	acc.

Table A 35 Trait correlations population Arina/Capo: Tulln 2008

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	233	233	233	233	233	233	233	233	233	233
	r	0.08	0.08	-0.37	-0.35	-0.40	-0.34	-0.35	-0.31	-0.40	-0.35
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
PH	n			233	233	233	233	233	233	233	233
	r			0.10	0.12	0.17	0.19	0.14	0.16	0.15	0.17
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n					233	233	233	233	233	233
	r					0.80	0.86	0.76	0.82	0.90	0.94
	H_0					rej.	rej.	rej.	rej.	rej.	rej.
LR2	n							233	233	233	233
	r							0.88	0.87	0.98	0.97
	H_0							rej.	rej.	rej.	rej.
LR3	n									233	233
	r									0.92	0.91
	H_0									rej.	rej.

Table A 36 Trait correlations population Arina/Capo: Rust 2009

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P LR4	S LR4	P LR5	S LR5	S Chl	S LTN	S Glauc
Head	n	232	232	232	232	232	232	226	226	223	223	232	232	232
	r	-0.37	-0.41	-0.43	-0.44	-0.45	-0.48	-0.34	-0.35	-0.26	-0.28	0.18	0.17	-0.19
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			233	233	233	233	227	227	224	224	233	233	233
	r			0.82	0.85	0.76	0.80	0.57	0.66	0.54	0.63	-0.39	-0.36	0.26
	H_0			rej.	rej.	rej.	rej.	acc.	rej.	acc.	acc.	acc.	acc.	acc.
LR2	n					233	233	227	227	224	224	233	233	233
	r					0.90	0.90	0.76	0.81	0.73	0.78	-0.45	-0.43	0.26
	H_0					rej.	rej.	rej.	rej.	rej.	rej.	acc.	acc.	acc.
LR3	n							227	227	224	224	233	233	233
	r							0.73	0.76	0.70	0.74	-0.40	-0.41	0.23
	H_0							rej.	rej.	rej.	rej.	acc.	acc.	acc.
LR4	n									223	223	227	227	227
	r									0.92	0.92	-0.52	-0.49	0.2188
	H_0									rej.	rej.	acc.	acc.	acc.
LR5	n											224	224	224
	r											-0.53	-0.52	0.20
	H_0											acc.	acc.	acc.
Chl	n												233	233
	r												0.51	-0.40
	H_0												acc.	acc.
LTN	n													233
	r													-0.26
	H_0													acc.

Table A 37 Trait correlations population Arina/Capo: Tulln 2009

		P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P LR4	S LR4	S Chl	S LTN
Head	n	233	233	228	228	222	222	231	231	233	233
	r	-0.19	-0.21	-0.34	-0.37	-0.37	-0.37	-0.23	-0.22	0.21	0.17
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n			228	228	222	222	231	231	233	233
	r			0.67	0.71	0.65	0.73	0.53	0.70	-0.26	-0.34
	H_0			rej.	rej.	acc.	rej.	acc.	rej.	acc.	acc.
LR2	n					222	222	227	227	228	228
	r					0.91	0.91	0.75	0.78	-0.37	-0.50
	H_0					rej.	rej.	rej.	rej.	acc.	acc.
LR3	n							221	221	222	222
	r							0.84	0.85	-0.40	-0.54
	H_0							rej.	rej.	acc.	acc.
LR4	n									231	231
	r									-0.44	-0.55
	H_0									acc.	acc.
Chl	n										233
	r										0.52
	H_0										acc.

Table A 38 Trait correlations population Furore/Capo: mean over experiments

		P PH	S PH	P LR	S LR
Head	n	201	201	181	181
	r	0.05	0.07	-0.15	-0.17
	H_0	acc.	acc.	not t.	not t.
PH	n			193	193
	r			0.04	-0.01
	H_0			not t.	not t.

The means were calculated over the experiments as follows:

Head – PH: Tulln 2004, 2007, 2007 (M), 2008

Head – LR: Tulln 2004, Probstdorf 2007, Tulln 2007, Tulln 2007 (M), Tulln 2008

PH – LR: Tulln 2004, 2007, 2007 (M), 2008

Table A 39 Trait correlations population Furore/Capo: Aumühle 2004

		P LR	S LR
Head	n	201	201
	r	0.00	-0.03
	H_0	acc.	acc.

Table A 40 Trait correlations population Furore/Capo: Tulln 2004

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	S CD
Head	n	201	201	200	200	114	114	201
	r	0.09	0.07	-0.03	-0.07	-0.09	-0.10	0.03
	H_0	acc.	acc.	acc.	acc.	not t.	not t.	acc.
PH	n			200	200	114	114	201
	r			0.07	0.05	0.03	-0.01	0.02
	H_0			acc.	acc.	not t.	not t.	acc.
LR1	n					114	114	200
	r					0.68	0.70	0.28
	H_0					not t.	not t.	acc.
LR2	n							114
	r							0.33
	H_0							not t.

Table A 41 Trait correlations population Furore/Capo: Probstdorf 2006

		S LR1	P LR2	S LR2	S PM	S FH
Head[1)]	n	201		38	201	201
	r	-0.14		0.03	0.00	0.07
	H_0	acc.		not t.	acc.	acc.
LR1	n		38	38	201	201
	r		0.82	0.81	0.42	0.13
	H_0		not t.	not t.	acc.	acc.
LR2	n				38	38
	r				0.38	0.15
	H_0				not t.	not t.
PM	n					201
	r					-0.05
	H_0					acc.

[1)] scored on a 1 (= early) to 9 (= late) scale

Table A 42 Trait correlations population Furore/Capo: Tulln 2006

		P PH	S PH
Head	n	201	201
	r	0.07	0.02
	H_0	acc.	acc.

Table A 43 Trait correlations population Furore/Capo: Probstdorf 2007

		P LR1	S LR1	P LR2	S LR2	S Lodg[1)]
Head	n	201	201	189	189	201
	r	-0.05	-0.04	0.03	-0.08	-0.08
	H_0	acc.	acc.	not t.	not t.	acc.
LR1	n			189	189	201
	r			0.60	0.74	-0.15
	H_0			not t.	not t.	acc.
LR2	n					189
	r					0.01
	H_0					not t.

[1)] scored on a 1 (= no lodging) – 5 (= complete lodging scale)

Table A 44 Trait correlations population Furore/Capo: Tulln 2007

		P PH	S PH	P LR	S LR
Head	n	201	201	201	201
	r	-0.15	-0.15	-0.17	-0.18
	H_0	acc.	acc.	acc.	acc.
PH	n			201	201
	r			0.01	0.02
	H_0			acc.	acc.

Table A 45 Trait correlations population Furore/Capo: Tulln (M) 2007

		P PH	S PH	P LR	S LR
Head	n	201	201	194	194
	r	0.10	0.12	-0.11	-0.11
	H_0	acc.	acc.	not t.	not t.
PH	n			194	194
	r			-0.01	-0.03
	H_0			not t.	not t.

Table A 46 Trait correlations population Furore/Capo: Probstdorf 2008

		S PM	S Sep	S Lodg1[1)]	S Lodg2[2)]
Head	n	201	201	201	201
	r	0.23	-0.04	0.11	0.14
	H_0	acc.	acc.	acc.	acc.
PM	n		201	201	201
	r		-0.07	0.15	0.21
	H_0		acc.	acc.	acc.
Sep	n			201	201
	r			0.00	-0.20
	H_0			acc.	acc.
Lodg1	n				201
	r				0.04
	H_0				acc.

[1)] scored on a 0 (= no lodging) – 5 (= complete lodging scale)
[2)] scored on a 1 (= no lodging) – 9 (= complete lodging scale)

Table A 47 Trait correlations population Furore/Capo: Tulln 2008

		P PH	S PH	P LR1	S LR1	P LR2	S LR2	P LR3	S LR3	P AUDPC	S AUDPC
Head	n	201	201	201	201	201	201	201	201	201	201
	r	-0.11	-0.09	-0.04	-0.06	-0.08	-0.08	-0.12	-0.14	-0.09	-0.09
	H_0	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
PH	n			201	201	201	201	201	201	201	201
	r			0.17	0.18	0.17	0.15	0.17	0.18	0.18	0.17
	H_0			acc.	acc.	acc.	acc.	acc.	acc.	acc.	acc.
LR1	n					201	201	201	201	201	201
	r					0.74	0.75	0.56	0.68	0.79	0.81
	H_0					rej.	rej.	acc.	rej.	rej.	rej.
LR2	n							201	201	201	201
	r							0.82	0.87	0.98	0.98
	H_0							rej.	rej.	rej.	rej.
LR3	n									201	201
	r									0.90	0.93
	H_0									rej.	rej.

Appendix D: Thatcher Near Isogenic Lines (NILs)

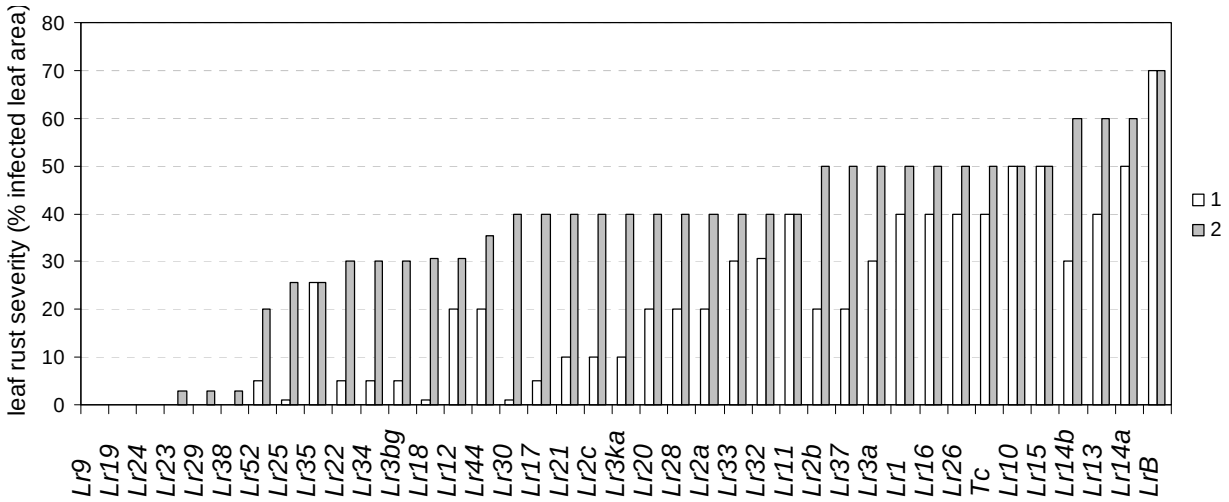

Table A 48 Leaf rust infection (% infected leaf area) of Thatcher (Tc) and NILs with different *Lr* genes in the experiment Tulln 2007 on two assessment days. 1: June 15th, 2: June 18th

Molecular Genetic Analysis of Durable Adult Plant Leaf Rust Resistance in the Austrian Winter Wheat Cultivar 'Capo'

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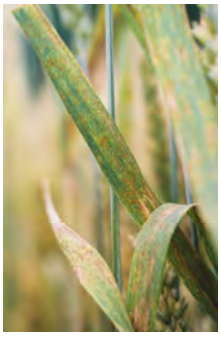
Introduction

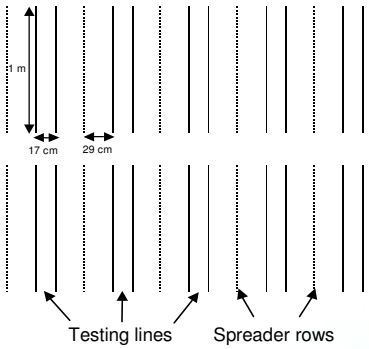
Leaf rust (see Fig. 1) caused by *Puccinia recondita f. sp. tritici* is a worldwide occurring disease of wheat. In some areas the fungus can cause yield losses of 40 to 90% (Hoffmann, 1999), in Austria losses of up to 20% are possible (Zwatz 1998). An ecologically and economically sound option for rust control is the use of resistant cultivars. Leaf rust resistance can be governed by major genes (so called *Lr*-genes) and/or quantitative (minor) resistance genes. There are already more than 50 known *Lr*-genes (McIntosh 2003).
The Austrian winter wheat cultivar Capo, developed by Probstdorfer Saatzucht, seems to possess durable adult plant leaf rust resistance (APR). As APR is often difficult to evaluate, there is little information about the genetic background. Besides *Lr13,* which is not effective in Europe (Winzeler 2000), it seems that Capo does not carry any known effective *Lr*-gene. This project aims at characterizing durable adult plant leaf rust resistance in Capo and at developing tools for use in marker assisted selection.

Materials and Methods

Recombinant inbred line populations from crosses between Capo (Diplomat/Purdue5517//Extrem/HP3517) and two susceptible cultivars – Isengrain (Apollo/Soissons) and Furore (Carolus//Pokal/Martin) – have been developed. These are tested at several locations during three seasons in replicated field experiments. To provoke an even disease pressure over the whole experimental area, spreader rows (a mix of rust susceptible lines) are sown between the tested lines (see Fig. 2).
Additionally susceptible plants that are artificially inoculated in the greenhouse will be planted into the spreader rows.
Evaluation of leaf rust severity will be done using the scoring scheme (see Fig. 3) by Walther (2000).



↑ **Fig. 1**: Symptoms of leaf rust



↑ **Fig. 2**: Layout of the field plots for evaluation of leaf rust resistance

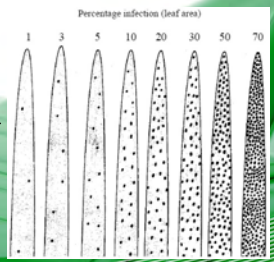
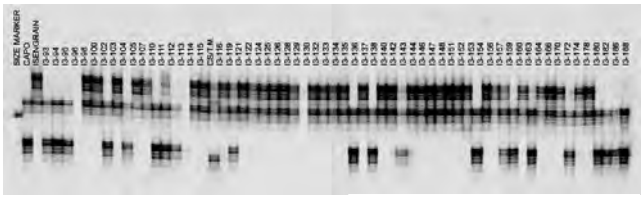


Fig. 3: Scoring scheme for evaluation of leaf rust severity (Walther 2000) →

In parallel 240 lines of the population Capo/Isengrain are characterized with molecular markers, mainly SSR- (microsatellite-) and AFLP- (amplified fragment length polymorphism) markers to construct a linkage map of this cross. A biometrical analysis of the resistance and the marker data will allow the detection of quantitative trait loci (QTLs) for leaf rust resistance and the quantification of their effects and interactions. The second population (Capo/Furore) will be used for QTL validation.

Preliminary Results and Future Work

In the first year of this project resistance data for leaf rust severity could be collected on two locations. Although disease pressure was very high, there were clear differences between the tested lines.
About 160 SSR-primers have been tested on the parental lines. Out of them more than 50% proved to be polymorphic between Capo and Isengrain; half of these markers have already been analyzed on the population. In the long run the majority of the genome should be covered with SSRs (at least one to three per chromosome arm) and AFLPs.
A potential candidate gene for Capo's resistance could be *Lr34*. Therefore, the SSR markers GWM130 and GWM295 mapping near *Lr34* (Suenaga 2003) have been analyzed already. Analysis of variance revealed no significant association of leaf rust severity with either GWM130 (see Fig. 4 and 5) or GWM295 alleles. Hence *Lr34* does not seem to be involved in APR of Capo.



↑ **Fig. 4**: PCR amplification pattern of SSR-marker GWM130 on part of the population Capo/Isengrain

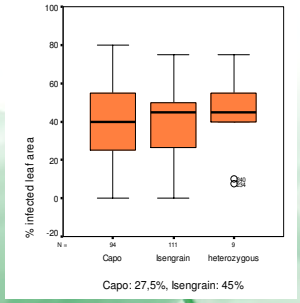


Fig. 5: Boxplots for leaf rust severity for the different groups of the alleles for SSR-marker GWM130 →

Capo: 27,5%, Isengrain: 45%

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Molecular genetic analysis of durable adult plant leaf rust resistance in the Austrian winter wheat cultivar 'Capo'

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Introduction

Leaf rust (*Puccinia recondita f.* sp. *tritici*) is a worldwide occurring disease of wheat. The ecological and economical best option for rust control is the use of resistant cultivars. Leaf rust resistance can be governed by major genes (*Lr-genes*) and/or quantitative (minor) resistance genes. The Austrian winter wheat cultivar Capo seems to possess durable adult plant leaf rust resistance (APR). As APR is often difficult to evaluate, there is little information about the genetic background. This project aims to genetically characterize durable adult plant leaf rust resistance in Capo and at developing tools for use in marker assisted selection.

Materials and Methods

Recombinant inbred line populations from crosses between Capo and two susceptible cultivars - Isengrain and Furore - have been developed. These are tested at several locations during three seasons in replicated field experiments. To provoke disease pressure over the whole experimental area, spreader rows (a mix of rust susceptible lines) are sown between double rows of the tested lines. Additionally susceptible plants that are artificially inoculated in the greenhouse will be planted into the spreader rows. In parallel 240 lines of the population Capo/Isengrain are characterized with molecular markers, mainly SSR- and AFLP-markers to construct a linkage map of this cross. A biometrical analysis of the resistance and the marker data will allow the detection of quantitative trait loci (QTLs) involved in leaf rust resistance. The second population (Capo/Furore) will be used for QTL validation.

Preliminary Results and Future Work

In the first year of this project resistance data for leaf rust severity could be collected at two locations. Although disease pressure was very high, there were clear differences between the tested lines. Half of the tested SSR-primers proved to be polymorphic between Capo and Isengrain. Analysis of the population has started.

A potential candidate gene for Capo's resistance could be *Lr34*. Therefore, the SSR markers *Xgwm130* and *Xgwm295* mapping near *Lr34* (SUENAGA, 2003) have been analyzed already. Analysis of variance revealed no significant association of leaf rust severity with either *Xgwm130* or *Xgwm295* alleles. Hence, *Lr34* does not seem to be involved in APR of Capo.

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Kartierung der Braunrostresistenz in einer Kreuzungspopulation aus Capo x Isengrain
Mapping of leaf rust resistance in a Capo x Isengrain population

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Abstract

The fungal disease leaf rust, caused by *Puccinia recondita* f. sp. *tritici*, is occurring wherever wheat is grown. The best option to reduce yield losses due to earlier senescence of leafs is the cultivation of resistant varieties. Leaf rust resistance can be based upon one or more major genes (*Lr*-genes) and/or minor resistance genes acting quantitatively (quantitative trait loci, QTL). Capo is an Austrian winter wheat cultivar grown for 20 years with still low susceptibility to leaf rust. It seems to possess durable adult plant leaf rust resistance (APR). As this type of resistance can not be tested on seedling plants, molecular markers could facilitate early selection of new resistant varieties. 240 $F_{6:7}$ recombinant inbred lines (RILs) of the cross Capo x Isengrain have been tested over a period of 6 years on different locations. A QTL for leaf rust severity inherited from Isengrain could be detected at all experiments. Whether this is *Lr14a*, previously detected in seedling tests, needs further investigation, as Thatcher NILs with *Lr14a* were rather susceptible. One minor QTL inherited from Capo could only be detected in a few experiments. We could prove that *Lr34*, a gene conferring APR, is not present in Capo. Diversity array technology (DArT) marker will be added to the present microsatellite (SSR) and amplified fragment length polymorphism (AFLP) map. Two further Capo cross derived populations are also under investigation.

Keywords

Puccinia recondita, QTL mapping, *Triticum aestivum*

Einleitung

Braunrost, hervorgerufen durch den Pilz *Puccinia recondita* f. sp. *tritici*, ist eine weltweit verbreitete Krankheit von Weizen. Ein starker Befall führt aufgrund der verfrühten Blattseneszenz zu Ertragseinbußen. In Österreich ist dieser Pilz besonders im Osten, im pannonischen Trockengebiet, verbreitet. Hier sind Reduktionen des Kornertrags von bis zu 20 % möglich (ZWATZ 1998). Vielfach ist der Einsatz von Fungiziden jedoch nicht wirtschaftlich bzw. im ökologischen Landbau überhaupt nicht zulässig. Daher ist der Anbau resistenter Sorten von großer Bedeutung.

Braunrostresistenz kann zwei Ursachen haben: Die Resistenz beruht entweder auf einem oder wenigen Hauptgenen (*Lr*-Gene) oder auf sogenannten QTLs (quantitative trait loci). Bisher sind über 50 *Lr*-Gene beschrieben (MACINTOSH 1995, MCINTOSH et al. 1995, SCHNURBUSCH et al. 2004). Nur wenige davon gelten als dauerhaft, so zum Beispiel *Lr46* (SINGH et al. 1998) und *Lr34* insbesondere in Kombination mit *Lr12* oder *Lr13* (ROELFS 1988). Gegenüber den meisten anderen bekannten *Lr*-Genen sind früher oder später virulente Pilzrassen aufgetreten, sodass diese heute nicht mehr bzw. nur mehr regional wirksam sind. Als dauerhafter haben sich quantitative Resistenzen der erwachsenen Pflanze erwiesen. Diese führen nie zu einer vollständigen Resistenz, sondern sie bewirken z.B. eine längere Latenzperiode, eine langsamere Ausbreitung auf der Pflanze oder eine geringere Sporulationsfähigkeit. Dadurch sind die Pflanzen nicht völlig befallsfrei, weshalb auch der Selektionsdruck auf den Pilz geringer ist.

Braunrostresistenz kann nur in aufwändigen Inokulationsversuchen getestet werden. Sämlingstests sind nicht ausreichend, wenn die Resistenz erst in der erwachsenen Pflanze wirksam ist (APR, adult plant resistance). Deshalb könnten molekulare Marker für *Lr*-Gene bzw. QTLs die praktische Pflanzenzüchtung vereinfachen. Schon in einem sehr frühen Züchtungsstadium können große Pflanzenzahlen in kurzer Zeit vorselektiert werden, da dazu nur DNS (Desoxyribonukleinsäure) von wenigen Blättern notwendig ist.

Material und Methoden

Untersucht wurden 240 Inzuchtlinien (RILs, recombinant inbred lines), ($F_{6:7}$) der Kreuzung Capo x Isengrain. Die von Hermann Hänsel gezüchtete und 1989 zugelassene Qualitätswinterweizensorte Capo (Pokal/Martin) der Probstdorfer Saatzucht scheint dauerhaft braunrostresistent zu sein. Im Jahr der Zulassung wurde ihre Anfälligkeit gegenüber Braunrost auf einer Skala von 1 (fehlend/sehr gering) - 9 (sehr stark) mit 2 (sehr gering bis gering) beurteilt, bis vor wenigen Jahren mit 3 (gering) und erst seit kurzem wird sie

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mit 4 (gering bis mittel) eingestuft (AGES 2009). Außerdem zeigt sie eine sehr geringe Anfälligkeit gegenüber Gelbrost (*Puccinia striiformis*) sowie mittlere Anfälligkeit gegenüber Mehltau (*Erysiphe graminis*) und Septoria (*Septoria* spp.). Aufgrund der Summe seiner agronomischen Eigenschaften ist Capo auch 20 Jahre nach seiner Zulassung immer noch die dominante Qualitätswinterweizensorte in Österreich. Sie macht fast ein Viertel der Vermehrungsfläche für Z-Saatgut aus. Im pannonischen Trockengebiet bzw. für den ökologischen Landbau macht Capo sogar ca. ein Drittel der Fläche aus, das ist mehr als die Flächen der nächsten drei Sorten zusammen (BAES 2009). In Sämlingstests wurde in Capo *Lr13* nachgewiesen (WINZELER et al. 2000). Dieses allein ist allerdings in weiten Teilen Europas nicht mehr wirksam und erklärt damit nicht die geringe Anfälligkeit dieser Sorte (MESTERHÁZY et al. 2000). In einem ersten Schritt sollte abgeklärt werden, ob Capo zusätzlich *Lr34* hat, das in Sämlingstests nicht gut untersucht werden kann (MESTERHÁZY et al. 2002), da es erst in der erwachsenen Pflanze wirksam ist. Capo zeigt allerdings nicht die typische Blattspitzendürre, die mit *Lr34* eng gekoppelt ist (SINGH 1992). Isengrain (Apollo/Soissons) ist eine französische Weizensorte, gezüchtet von Florimond Desprez. Aufgrund ihrer Anfälligkeit gegenüber Braunrost wurde sie als Kreuzungspartner gewählt.

In den Jahren 2004 bis 2009 wurden die 240 RILs sowie die Eltern- und Standardlinien in insgesamt 18 Versuchen auf 6 Standorten (Aumühle, Tulln und Rust im Tullnerfeld; Schmida bei Hausleiten im Bezirk Korneuburg; Probstdorf im Marchfeld; Piešťany in der Slowakei) getestet. Das Versuchsdesign war eine vollständig randomisierte Blockanlage mit 2 Wiederholungen. Um einen gleichmäßigen Krankheitsdruck auf der gesamten Versuchsfläche zu gewährleisten und auch um diesen überprüfen zu können, wurde jeweils links und rechts der Doppelreihe jedes Prüfglieds ein sogenannter Infektionsstreifen angebaut (*Abbildung 1*). Hierfür wurde ein Gemisch braunrostanfälliger Sorten verwendet.

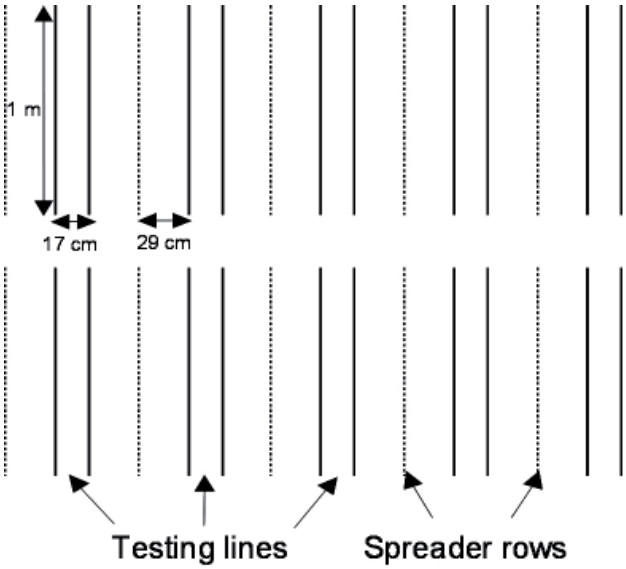

Abbildung 1: **Versuchsdesign. Links und rechts jeder Doppelreihe einer Prüflinie befindet sich ein Infektionsstreifen aus einem Gemisch braunrostanfälliger Sorten.**

Figure 1: **Experimental design. Left and right of each double row of testing lines is a spreader row of leaf rust susceptible cultivars.**

Verschiedene künstliche Inokulationsmethoden wurden ausprobiert: Auspflanzen von infizierten Sämlingspflanzen, die zuvor im Glashaus durch Ansprühen mit einer wässrigen Sporensuspension inokuliert wurden, in die Infektionsstreifen; Injektion der Sporensuspension direkt in die Blattscheide von Pflanzen in den Infektionsstreifen; flächendeckendes Sprühen einer ölbasierten Sporensuspension mit ULVA+ (Micron Sprayer Ltd., Bromyard, Herefordshire, UK). Am effektivsten war das direkte Ansprühen einer Pflanze je Infektionsstreifen während des Bestockens (BBCH 20-29) am späten Nachmittag mit ca. 2 ml einer wasserbasierten Sporensuspension (ca. 10 Mio. Sporen/ml) und anschließender Abdeckung über Nacht.

Für die Bonitur wurde das Schema von *Abbildung 2* verwendet, wobei die Skala, sofern notwendig, in 10er Schritten bis 100 % Befall erweitert wurde.

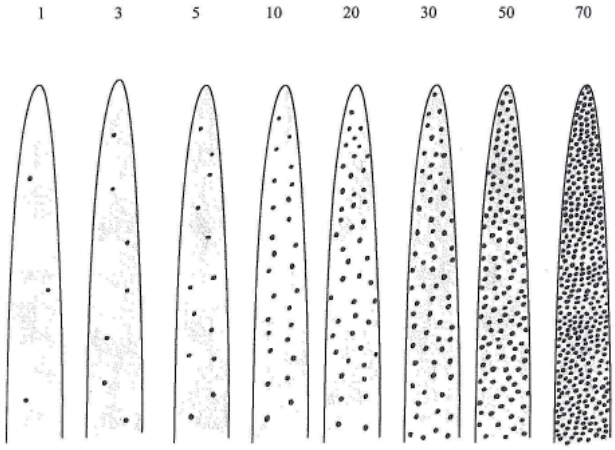

Abbildung 2: **Boniturschema zur Schätzung des Prozentanteils mit Braunrost befallener Blattfläche (MOLL et al. 1996; zitiert in BARTELS und BACKHAUS 2000)**

Figure 2: **Scoring aid for estimating percentage of leaf rust infected leaf area**

Der Befall mit Braunrost war leider nicht in allen Jahren ausreichend stark bzw. früh genug, um bonitiert werden zu können bzw. ausreichend zu differenzieren. Deshalb konnten für die weiteren Berechnungen nur die Daten von 11 Versuchen verwendet werden.

Parallel dazu wurde die Population mit molekularen Markern (SSR, simple sequence repeats; AFLP, amplified fragment length polymorphism) charakterisiert. Die Berechnung der aktuellen genetischen Karte basiert auf 130 SSR- und 483 AFLP-Marken. Verwendet wurde JoinMap® Vers. 4 mit Evaluation License (Haldane's mapping function, ansonsten Grundeinstellungen). Die resultierenden Kopplungsgruppen decken alle Chromosomen ab. Die anschließende QTL-Analyse erfolgte mit dem Programm Cartographer© Vers. 2.5. Für die Auswertung der Feldversuche mittels Varianzanalyse (ANOVA, analysis of variances) bzw. die Einzelmarker-ANOVA wurde das Programm SAS® Vers. 9.2 TS Level 1M0 verwendet.

Ergebnisse und Diskussion

Aufgrund der vorliegenden Ergebnisse lässt sich ausschließen, dass Capo *Lr34* enthält. Die beiden bei SUENAGA et al. (2003) für dieses Resistenzgen beschriebenen Marker *Xgwm130* und *Xgwm295* wurden auch in dieser Population auf Chromosom 7D in geringem Abstand kartiert. Die Allele unterscheiden sich nicht signifikant hinsichtlich des Braunrostbefalls (MATIASCH et al. 2007). Boxplots der Population für die Allele der beiden Eltern Capo (C) und Isengrain (I) sowie Heterozygote (H) von *Xgwm*130 sind in *Abbildung 3* gezeigt. Die Verteilung des Braunrostbefalls (Mittelwert über alle 11 Experimente) ist für alle 3 Allelgruppen nahezu ident.

Der bisher stärkste gefundene QTL konnte auf Chromosom 7BL lokalisiert werden. Diese Resistenz wird von Isengrain vererbt. In Sämlingstests konnte bereits von BŁASZCZYK et al. (2004) *Lr14a* in Isengrain nachgewiesen werden. Sowohl Isengrain selber als auch Thatcher NILs mit *Lr14a* und *Lr14b* waren im Versuch in Tulln 2007 mit bis zu 60 % befallener Blattfläche wesentlich stärker infiziert als die RILs mit dem entsprechenden Isengrain-Allel. Zu ähnlichen Ergebnissen kamen VIDA et al. (2009). Der Koeffizient durchschnittlichen Befalls lag für *Lr14a* bei fast 90 %, für *Lr14b* bei 60 %. Hingegen zeigten Thatcher NILs mit *Lr19* weder in Tulln noch in Martonvásár (VIDA et al. 2009) Befall mit Braunrost. Bei Durum wurde *Lr19*, das ursprünglich aus *Agropyron elongatum* (syn. *Thynopyrum ponticum*) (CHERUKURI et al. 2003) stammt, auf Chromosom 7B eingekreuzt. Ein Vergleich der Isengrain-Allele der in der Population signifikanten Marker mit denen von verschiedenen Sorten, für die *Lr14a* bzw. *Lr19* nachgewiesen wurde, ist auf jeden Fall noch notwendig, um diesen QTL besser einordnen zu können.

Für Capo konnte bisher nur ein QTL gefunden werden, der über dem Grenzwert liegt. Dieser ist jedoch nicht in allen Versuchen signifikant. Dass kein stärkerer QTL gefunden wurde, könnte daran liegen, dass die Karte in einzelnen Bereichen noch nicht ausreichend dicht ist. Deshalb werden aktuell noch zusätzlich DArT (diversity array technology) Marker gemacht, um die Kopplungskarte zu verfeinern.

Eine andere Ursache könnte sein, dass für die Resistenz von Capo sehr viele Gene verantwortlich sind, von denen jedes einzelne nur einen derart geringen Beitrag leistet, dass sie in der Kreuzungspopulation mit Isengrain nicht als QTL erkennbar sind. Zwei weitere Kreuzungspopulationen - Arina x Capo und Furore x Capo - sollen noch mit genetischen Markern charakterisiert werden. Es ist zu erwarten, dass Capo QTLs aufgrund der stärkeren Braunrostanfälligkeit der Kreuzungspartner verglichen mit Isengrain besser zu sehen sind.

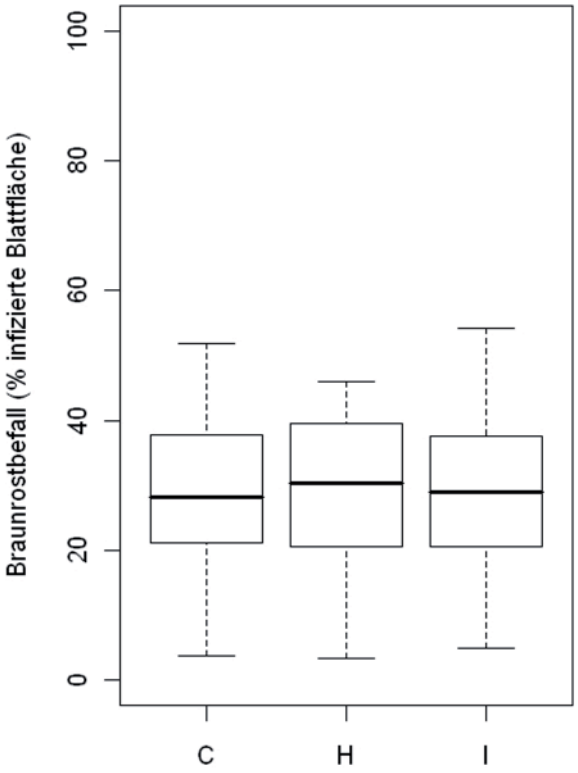

Abbildung 3: **Boxplotdarstellung des Braunrostbefalls (Mittelwert über alle 11 Experimente) der 240 RILs der Kreuzung Capo x Isengrain. Die Gruppen sind getrennt nach den Allelen C (Capo), H (Heterozygot) und I (Isengrain) für Mikrosatellitenmarker *Xgwm130*. Mittelwert für Capo 26,7 %, Isengrain 35,5 %**

Figure 3: **Boxplots for leaf rust infection (mean over all 11 experiments) of the 240 Capo x Isengrain RILs. Groups are separated for the alleles C (Capo), H (heterozygous) and I (Isengrain) of microsatellite marker *Xgwm130*. Mean of Capo 26.7 %, Isengrain 35.5 %**

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Mapping of adult plant leaf rust and stripe rust resistance in the Austrian winter wheat cultivar 'Capo'

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INTRODUCTION

The Austrian cultivar Capo possesses quantitative and durable adult plant leaf rust resistance, but does not possess any effective major *Lr* gene to our knowledge. Aim of this work was to clarify the genetics of the durable rust resistance of the cultivar Capo.

MATERIALS AND METHODS

Plant material

F_6 derived RIL populations from the crosses: Capo/Isengrain (240 lines), Capo/Furore (201 lines), Capo/Arina (233 lines)

Resistance evaluation

Leaf rust resistance testing was done at field trials in Austria, Slovakia, Switzerland, Romania and Hungary. *Lr* was provoked by spreading field collected rust spores from the previous seasons. Rust severity was scored once or twice in each experiment using a percent scale. In addition the Capo/Furore population was tested for adult plant stripe rust resistance in one field test and for seedling resistance in a greenhouse test.

Genotyping and QTL mapping

The populations were genotyped with SSR, AFLP and DArT markers. QTL analysis was done in *QTL Cartographer* and/or *Qgene*.

RESULTS AND DISCUSSION

For the QTL mapping of leaf rust resistance QTL data from 6 field experiments were informative for the Capo/Isengrain population, 11 experiments for the Capo/Arina population and 6 experiments for the Capo/Furore population. Quantitative variation for leaf rust severity was evident in all three populations. The broad sense heritability estimate for leaf rust severity across experiments was 0.8 (Capo/Furore) to 0.9 (Capo/Isengrain and Capo/Arina), indicating that this trait segregated in these populations.

In the Capo/Isengrain population the largest effect QTL was derived from the susceptible parent Isengrain, mapping to chromosome 7BL, this QTL corresponds most likely to the gene *Lr14a.*

Capo-derived reproducible QTL for *Lr* resistance mapped to chromosomes 2A, 2B and 3B. QTL for stripe rust resistance were detected at 2B and 3BS. The 3BS rust resistance QTL from Capo is close to the *Lr27* and *Yr30* resistance loci.

Figure 1: symptoms of leaf rust (left) and stripe rust (right) on wheat leaves.

Table 1: QTL estimates for mean *Lr* severity (means over 6 informative experiments) in the population **Capo/Isengrain**

Chrom.	Closest marker	Resistance source	no of sign. exp.	SIM	
				LOD	R^2
3BS	*XS12M13_33*	*Capo*	4	4.4	8
7BL	*Xgwm132.1*	*Isengrain*	6	33.9	48

Table 2: QTL estimates for mean *Lr* severity (means over 11 informative experiments) in the population **Capo/Arina**

Chrom.	Closest marker	Resistance source	no of sign. exp.	SIM	
				LOD	R^2
2A	*wPt-665330*	*Capo*	10	7.2	19
2B	*wPt-8548*	*Capo*	5	4.4	13
3BS	*wPt-10192*	*Capo*	4	3.3	9.3

Table 3: QTL estimates for mean *Lr* severity (means over 6 informative experiments) and *Yr* severity (1 greenhouse and 1 field experiment) in the population **Capo/Furore**

Trait	Chrom.	Closest marker	Resistance source	no of sign. exp.	SIM	
					LOD	R^2
Lr	3BS	*wPt-7984*	*Capo*	6	13.5	32
Yr	2B	*wPt-743307*	*Capo*	1	12.4	27
(seedling)	3BS	*wPt-798970*	*Capo*	1	9	21
Yr	2B	*wPt-743307*	*Capo*	1	25.8	49
(adult plant)	3BS	*wPt-798970*	*Capo*	1	3.5	8.6

Summary

The obtained results indicate that CAPO possesses adult plant leaf rust resistance, with QTL mapping to chromosomes 2A, 2B and 3B and QTL for stripe rust resistance mapping to chromosomes 2B and 3B. Notably, the 3B QTL confers resistance to leaf rust and stripe rust and seems the most stable source of leaf rust resistance in Capo, significant over three populations. Whether some of the detected QTL are associated with already known *Lr* or *Yr*-genes needs further investigations. This QTL region therefore appears especially attractive for further genetic analysis. Markers for resistance breeding are available now.

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QTL mapping of adult plant leaf rust and stripe rust resistance derived from the Austrian winter wheat cultivar Capo

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Abstract

Leaf rust caused by *Puccinia triticina* is among the most prevalent leaf diseases of wheat worldwide. The Austrian cultivar Capo possesses quantitative and durable adult plant leaf rust resistance, but does not possess any effective major *Lr* gene to our knowledge. We developed and tested three recombinant inbred line populations: Capo×Isengrain, Capo×Furore and Capo×Arina for adult plant leaf rust resistance in well replicated field experiments over locations and years under high disease pressure. In addition, the Capo×Furore population was tested for stripe rust (*P. striiformis*) resistance. In parallel the three populations were genetically fingerprinted with molecular markers (SSR, AFLP and DArT). We genotyped and genetically mapped at least 620 polymorphic markers per population. We calculated linkage maps of all three populations and detected quantitative trait loci (QTL) for leaf rust resistance. Highly significant Capo derived QTL for leaf resistance were located on chromosomes 2A, 2B and 3B, supporting the hypothesis of quantitative rust resistance in this cultivar. Interestingly in the Capo×Isengrain population the strongest QTL derived from the susceptible parent (Isengrain) and mapped to chromosome 7B, corresponding most likely to the gene *Lr14a*. In addition we found two major QTL for stripe rust resistance in the Capo×Furore population on chromosomes 2B and 3B. The obtained results will be useful for selection and breeding of new cultivars with durable adult plant resistance to leaf rust and stripe rust.

Keywords

Puccinia striiformis, *Puccinia triticina*, QTL mapping, resistance breeding, *Triticum aestivum*

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