MASTER THESIS

Improving common bunt resistance in bread wheat by marker-assisted selection

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Abstract

Common bunt is a seedborne fungal disease caused by *T. caries* and *T. laevis* occurring in wheat production worldwide, causing severe yield losses and a significant reduction in seed quality, especially under organic growing conditions due to the absence of chemical seed treatment. The deployment of host resistance is therefore a major component for a sustainable disease management in organic plant production systems. Hence, this study focuses on the analysis of a diverse panel of adapted and improved resistant wheat lines derived by crossing the exotic resistant donor lines Blizzard, Bonneville and PI119333 with five susceptible but adapted bread wheat cultivars. The aim was to validate the known resistance QTL on chromosomes 1A and 1B by phenotypic and genotypic characterization. For this purpose, 359 recombinant inbred lines were developed and grown in the field after artificial seed inoculation with common bunt teliospores and scored for their disease resistance as well as multiple agro-morphological traits. All lines were genotyped with the SSR markers *Xgwm374* and *Xgwm264* indicative for the known resistance QTL 1BS, and with the markers *Xbarc83* and *Xcfa2129* flanking the resistance QTL on chromosome 1A. Phenotyping results revealed that a great amount of the lines did not develop any disease. 67% of the lines developed from the parental line Bonneville can be classified as resistant and 75% of those from Blizzard. The highest amount of resistant lines was achieved in the population derived from PI119333 with 83%. Genotyping resulted in an association of all four tested SSRs with the bunt resistance trait in the mapping populations derived from Bonneville. Marker *Xgwm374* and *Xgwm264* explained 18.6% and 16.6% of the phenotypic variation while *Xbarc83* and *Xcfa2129* explained 9.8% and 14.2%. In the population Blizzard the markers *Xbarc83* and *Xcfa2129* were also significantly associated with common bunt resistance with R^2 values of 15.9% and 17.6%. *Xgwm374* explained 16.5% and *Xgwm264* 11% but were not significant for this population. Notwithstanding, for populations derived from PI119333 all four markers were not significant which confirms the assumption that the resistance QTL of this line are located in other chromosomal regions. The established lines represent highly breeding relevant germplasm that can be used for further resistance breeding and for developing new adapted organic wheat cultivars.

Keywords: common bunt, *T. caries, T. laevis,* resistance breeding, molecular marker

Zusammenfassung

Weizensteinbrand ist eine durch *T. caries* und *T. laevis* verursachte Pilzkrankheit, welche weltweit in der Weizenproduktion auftritt. Besonders in der ökologischen Landwirtschaft führt sie zu hohen Ertrags- und Qualitätsverlusten, weshalb die Verwendung resistenter Sorten von großer Bedeutung für eine nachhaltige Krankheitsbekämpfung ist. Um verbesserte resistente Linien zu entwickeln wurden die exotischen resistenten Linien Blizzard, Bonneville und PI119333 mit fünf anfälligen, jedoch angepassten Weizensorten gekreuzt. Das Ziel war die bereits bekannten QTL auf den Chromosomen 1A und 1B mit Hilfe einer Phänotypisierung und Genotypisierung zu validieren. Dafür wurden 359 rekombinante Inzuchtlinien entwickelt, welche nach künstlicher Inokulation mit Teliosporen auf dem Feld angebaut wurden und auf Steinbrandbefall, sowie andere agronomische Merkmale bewertet wurden. Des Weiteren wurden alle Linien mit den molekularen Markern X*gwm374* und *Xgwm264* auf Chromosom 1B und *Xbarc83* und *Xcfa2129* auf 1A genotypisiert. Die Phänotypisierung zeigte, dass bei einem Großteil der entwickelten Linien kein Krankheitsbefall auftat. 67% der Linien abstammend von Bonneville können als resistent eingestuft werden und 75% von Blizzard. Den größten Anteil resistenter Linien erreichte die Population PI119333 mit 83%. Durch die Genotypisierung wurde festgestellt, dass alle vier Marker mit der Steinbrandresistenz in den Populationen Bonneville assoziiert werden können. Marker *Xgwm374* und *Xgwm264* erklären 18.6% bzw. 16.6% der phänotypischen Varianz und *Xbarc83* und *Xcfa2129* erklären 9.8% bzw. 14.2%. Auch in der Population Blizzard sind die Marker *Xbarc83* und *Xcfa2129* signifikant, mit R^2 Werten von 15.9% bzw. 17.6%. Marker *Xgwm374* und *Xgwm264* erklären 16.5% bzw. 11% sind jedoch nicht signifikant für diese Population. Für die Population abstammend von PI119333 waren alle vier Marker nicht signifikant, was die Annahme bestätigt, dass sich die Resistenz QTL dieser Linie in anderen Regionen im Genom befinden. Die erzeugten Linien können für die weitere Resistenzzüchtung verwendet werden, um angepasste und steinbrandresistente Sorten für die ökologische Landwirtschaft zu entwickeln.

Schlüsselwörter: Weizensteinbrand, *T. caries, T. laevis,* Resistenzzüchtung, molekulare Marker

List of Abbreviations

ANOVA	Analysis of variance
APS	Ammoniumperoxodisulfat $(NH_4)_2S_2O_8$
AW	Awns
BBCH	Scale to describe the phenological growth stage of plants (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie)
BOKU	University of Natural Resources and Life Sciences
CB	Common bunt incidences
CN	Ear type (compacted/ normal)
CTAB	Cetyltrimethylammonium bromide
Cy5	Cyanine dye
Df	Degree of freedom
DH	Date of heading
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
F_x	x^{th} generation after crossing
FAM	Fluorescein amidite
FAO	Food and Agriculture Organization of the United Nations
FHB	Fusarium Head Blight
h^2	Heritability
HCB	Hexachlorbenzene
LH	Leaf health
LOD	Lodging
LSD	Least significant difference
MAS	Marker-assisted selection
$MgCl_2$	Magnesium chloride
NA	Not available
NaOAc	Sodium acetate
NH_4OAc	Ammonium acetate
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
PH	Plant height

RIL	Recombinant inbred lines
SSD	Single seed descent
SSR	Single sequence repeat
TE	Buffer of Tris and EDTA
Tris	Tris(hydroxymethyl)aminomethane
TEMED	Tetramethylethylenediamine $C_6H_{16}N_2$
USDA-ARS	United States Department of Agriculture – Agricultural Research Service

Units

°C	Centigrade
cM	Centimorgan
dt, t, Mt	Decitonne, tonne, million tons
ha	Hectare
l, ml, µl	Liter, milliliter, microliter
M, mM	Molar concentration (mol/L), millimolar
m^2	Square meter
min	Minute
ng, mg, g, kg	Nanogram, milligram, gram, kilogram
nm, µm, mm, cm, m, km	Nanometer, micrometer, millimeter, centimeter, meter, kilometer
V	Volt

Contents

1 Introduction

1.1 Wheat

Wheat is one of the most important crops produced worldwide and belongs together with rice and maize to the “big three” cereal crops playing a major role for human nutrition and livestock feed (Shewry 2009). In the year 2017 the global yield production amounted accordingly to over 770 million tons (FAO 2018). The cultivation of wheat began already about 10 000 years ago during the Neolithic Revolution with the earliest forms originating in the south-eastern part of Turkey, which had either a diploid (AA) or tetraploid (AABB) genome (Shewry 2009). About 9 000 years ago the hexaploid (AABBDD) wheat (*Triticum aestivum* L.) which carries a set of 2n=6x=42 chromosomes arose from a cross between the cultivated emmer with the wild grass *Aegilops tauschii (Triticum tauschii)* (DD) (Miedaner 2014). The additional D genome of the wild grass *T. tauschii* had an important impact on the cultivation of wheat as it partly confers the excellent backing quality and the adaption to different climatic zones. This hybridization event made the spreading of the wheat growing area from its origin in the Fertile Crescent to cooler regions, into today’s cultivation areas of Asia and East- and Central Europe feasible (Miedaner 2014). During the domestication of bread wheat directional selection for major agronomic traits took place, which differentiated the cultivated forms from their wild type. The two most important traits are the loss of shattering of spikes at maturity, which leads to lower seed losses at harvesting and the change to free-threshing naked forms, which is important for post-harvest processing (Shewry 2009; Miedaner 2014). A major contribution to higher yield and plant production management was made during the Green Revolution in 1960s. Important steps were the development of fertilizer-responsive and short-strawed varieties with higher lodging resistance (Shiferaw et al. 2013; Miedaner 2014). Wheat has an optimum growing temperature of about 25°C and is adaptable to a broad range of moisture conditions. However, an adequate water availability during the growing season is necessary to achieve high yields (Curtis et al. 2002).

Today 95% of the wheat grown worldwide is hexaploid bread wheat (Miedaner 2014). Its major characteristics is the baking quality as wheat flour can be formed into doughs which allow a processing into many baking products like bread, pastries or cookies. The specific structure of the unique gluten protein fractions amongst others is responsible for these properties and gives wheat an advantage over other crops (Shewry 2009). The other 5% of grown wheat is mainly tetraploid durum wheat, which is more adapted to a Mediterranean climate and is primarily used for pasta production (Shewry 2009).

Wheat is a very important crop for human nutrition as it contains minerals, vitamins and fats (lipids) which are beneficial for the diet (Curtis et al. 2002). It contributes to 20% of the daily calories and provides about 21% of the daily protein intake which makes it the world's most important protein source as e.g. it accounts for 75% of cereal intake in developed countries (Shiferaw et al. 2013). Wheat cultivars with lower quality properties furthermore represent a relevant source of animal feed, aside from their importance for human consumption (Curtis et al. 2002). According to estimates of the FAO the world would require about 840 million tons of wheat by 2050, i.e. almost 100 million tons more than the current production level (Sharma et al. 2015). Since 1961 wheat yield increased on average about 0.9% per year, whereas the annual yield gain was high at the first decades of the Green Revolution with about 4%, it stagnated during the last decade. However, to feed the increasing population an increase of 2.4% of yield would be necessary (Ray et al. 2013; Shiferaw et al. 2013). Despite that, the global wheat production area only slightly increased during the 1960s and 1970s and declined significantly in the recent years (Figure 1-1) (Shiferaw et al. 2013).

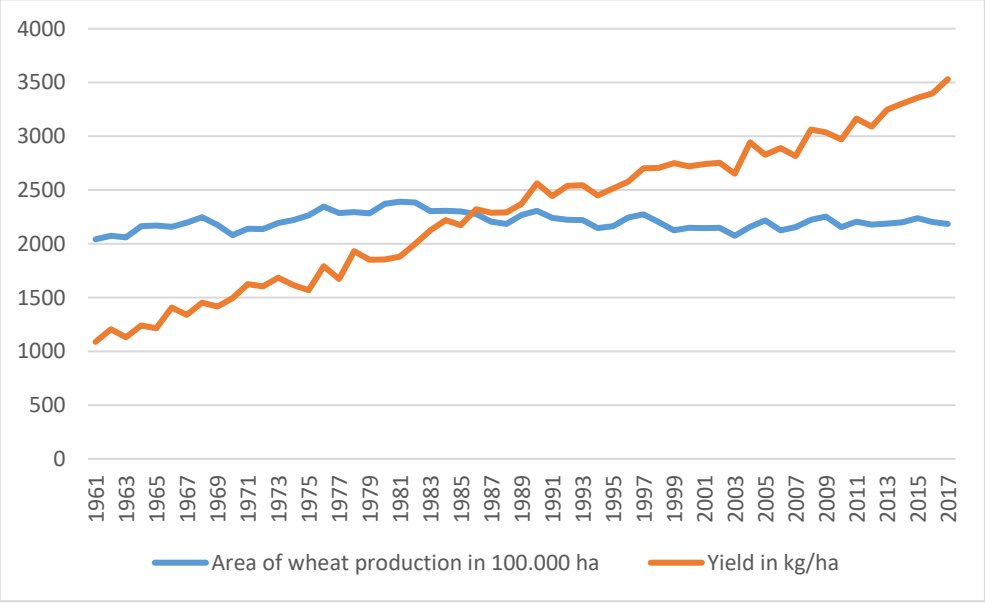

Figure 1-1 Development of global wheat production from 1961 to 2017 (FAO 2018)

In 2017, the top 5 wheat producing countries were China (134 Mt), India (99 Mt), Russia (86 Mt), the United States of America (47 Mt) and France (37 Mt). Asia is responsible for 44% of the world's wheat production, followed by Europe with 35% (FAO 2018). The average yield in 2017 was 35.31 dt/ha worldwide but shows great differences between developed and developing countries (FAO 2018; Shiferaw et al. 2013). The highest yields were reached in Ireland with 101.75 dt/ha followed by New Zealand with 98.64 dt/ha, Austria reached an average yield of 48.71 dt/ha (FAO 2018).

Climate change is considered to be a potential threat for the global wheat production since less grain is produced at temperatures above 30 degrees Celsius (Sharma et al. 2015). It is estimated that for each °C of further temperature increase the production will decrease by 6% (Asseng et al. 2015). Rising temperatures and changes in rainfall will cause yield losses especially in South Asia endangering food security of billions of people. Additionally, challenges like new threats of diseases and pests, new weed flora, herbicide resistance, soil health and stagnated productivity levels will most likely arise (Sharma et al. 2015). Sustainable management practices will thus be important to avoid loss of productive land due to water scarcity and soil degradation (Shiferaw et al. 2013).

Hence, organic agricultural systems will potentially play a major role to sustain ecological balance and maintain genetic and agricultural diversity in the near future. The use of natural ecological processes, beneficial organisms, natural pest controls, diversified crop rotation and ground covers, together with recycling of farmyard manure and promoting biological activity in the soil are key elements of organic agriculture (David et al. 2012). Globally, 1.2% of farmland is currently organically managed with the highest shares of total agricultural land being in Oceania (6.5%) and Europe (2.7%) (European Union 6.7%), and in 2016 the organic farmland increased by 7.5 million hectares which is a rise of 15% (Willer and Lernoud 2018). Cereals are the largest crop group with wheat covering almost the half of the area in Europe. Looking at the percentage, Austria has the highest organic shares of agricultural land in the European Union, as every fifth acreage is managed organically (Figure 1-2 (a)) and almost half of the organically produced cereal is wheat (Figure 1-2 (b)) (Willer and Lernoud 2018; EUROSTAT 2019); AgrarMarkt Austria 2019). Reflecting the issue that in the past 20 years the production area of organic bread wheat in Austria has risen from 5000 ha up to 35000 ha (Figure 1-3) (AgrarMarkt Austria 2019).

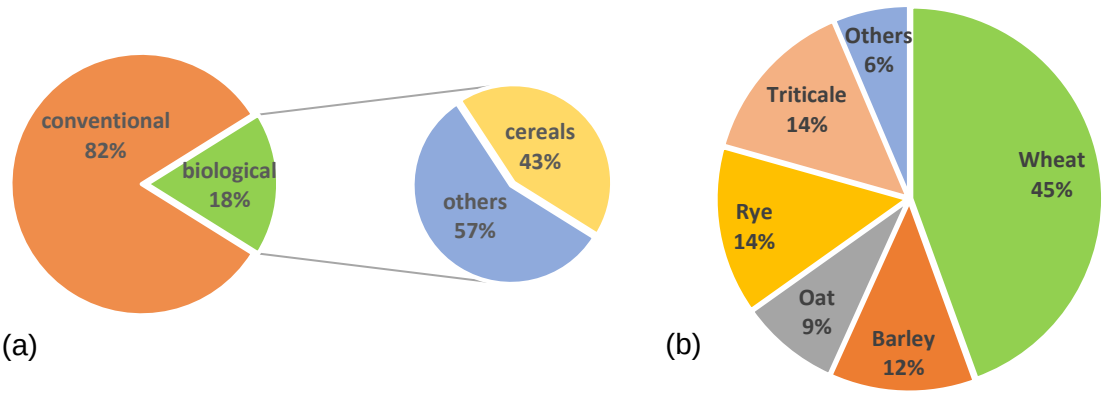

Figure 1-2 (a) Distribution of conventional and biological managed acreage in Austria in 2018 (b) Distribution of cereal types produced organically in Austria in 2018 (AgrarMarkt Austria 2019)

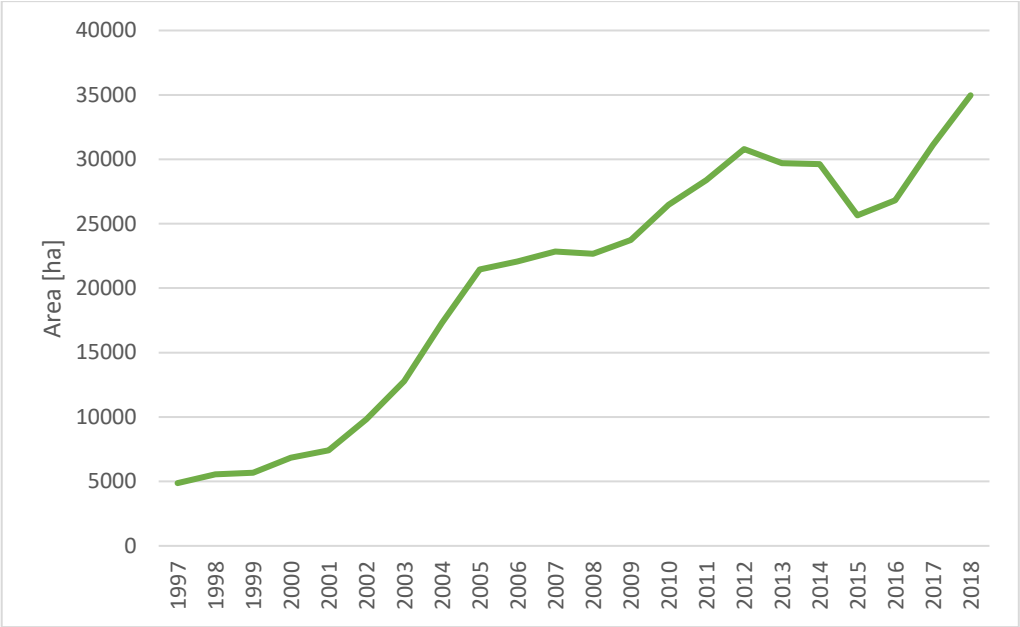

Figure 1-3 Development of the organic bread wheat production area in Austria from 1997 to 2018 (AgrarMarkt Austria 2019)

Since the organic production is increasing, breeding for genotypes which are better adapted to these growing conditions is pivotal to achieve high gains in yield potential in combination with an acceptable quality of the end products. Important success factors for growing under organic conditions are nutrient efficiency, the ability for weed suppression and resistance to seedborne diseases (Löschenberger et al. 2008).

1.2 Common bunt

Since agriculture in Europe is moving towards organic and sustainable, low-input farming systems, common bunt has re-emerged in organic wheat production systems during the last two decades (Lammerts van Bueren et al. 2008). Due to regulatory constrains, many seedborne diseases, including common bunt, can no longer be controlled with the use of chemical seed treatments in such systems. The number of bunt incidences is accordingly increasing and organic seed lots are especially prone to contamination with common bunt spores as under dry conditions they are germinable up to 20 years (Spieß et al. 2015; McNeil et al. 2004). Common bunt contaminated wheat causes considerable loss of yield and seed quality and plant stands established with untreated seeds can suffer a common bunt incidence of up to 80% with severe yield losses of 40% (Cota et al. 2009). Since wheat kernels are replaced with bunt balls, yield losses almost equal disease incidence and even cleaning the seeds cannot totally prevent their occurrence (Waldow and Jahn 2007). Additionally, the legal requirements for organic seed production has enhanced the bunt

problem in Europe as with January 2004 it is no longer possible to use conventionally produced seeds in organic agriculture following the Commission Regulation (EC) No. 1452/2003 stipulating that all used plant material must be produced under organic farming conditions. This regulation together with the prohibition of chemical protection aggravates the disease management and makes it necessary that planting materials are pathogen-free and of high quality (Lammerts van Bueren et al. 2003).

Due to the use of chemical seed treatments in conventional agriculture, common bunt is almost completely under control, however in organic production systems alternative treatments are necessary. Only a few organic seed treatments are effective, with some variation in their efficacy, which increase though the production costs and, in many cases, can only be applied on a small scale. That is the reason why the use of host resistance is a major component for a sustainable disease management in organic systems (Matanguihan et al. 2011).

1.2.1 Historical aspects of common bunt

Before effective control measures were developed, common bunt was ranked as one of the most destructive wheat diseases (Sholberg et al. 2006). During the 18th century, bunted wheat was so prevalent that it was used to make bread for the poor and was fed to animals. The produced flour of infested grain is discolored with an unpleasant smell and taste but was edible and not proven to be harmful (Gaudet and Menzies 2012). In 1750 Mathieu Tillet was one of the first who conducted fundamental experiments about the “smutting of wheat”. For his experiment he planted seeds which were dusted with black spores and others that were not. From those seeds who were coated he observed 50% more smutted heads whereby he proved that smut spores were infective. Furthermore, he tried to prevent the disease by washing seed grain in water, cattle urine, lye solutions, lime and salt and copper sulfate. Each helped to suppress it but non eliminated smut entirely. For his pioneering work the genus of the bunt fungi was named *Tilletia* (Fischer and Holton 1957; Goates 1996). At the beginning of the 20th century, common bunt was the most destructive wheat disease in the Pacific Northwest region of the United States. It caused higher yield losses and reduction in quality than any other wheat disease (Fischer and Holton 1957). Its management, the pathogen genetics, seed treatments and resistance were therefore intensively studied. W. J. Farrer was the first one who applied systematic breeding methods and released the first bunt resistant wheat cultivar ‘Florence’. Gaines and Flor studied the genetics of bunt resistance and established the existence of physiologic races of the pathogen (Matanguihan et al. 2011). From the observations that resistant varieties were attacked by new virulent races of bunt and resistant genes influenced the racial population dynamics of bunt pathogens, it transpired that a gene-for-gene interaction between wheat

and the common bunt pathogens is present. Thus, bunt resistance genes (*Bt*) in wheat possess corresponding avirulence genes (*avr* genes) in the fungus (Matanguihan et al. 2011). Races of *T. caries* are designated with the letter “T” and those of *T. laevis* with the letter “L” and 30 races of *T. caries* and 10 different races of *T. laevis* have been identified so far (Goates 2012). A set of differential wheat lines has already been established by Hoffmann and Metzger in 1979 containing 10 differential lines carrying the resistance genes *Bt1* to *Bt10* in order to evaluate the virulence characteristics of common bunt with respect to newly bred varieties. Over the years this set was expanded with five additional lines carrying the resistance genes *Bt11* to *Bt15* (Goates 2012; Matanguihan et al. 2011). Germplasm resistance screening showed that most of the European bunt populations were not able to break the *Bt* resistance genes 5, 8, 9, 10 and 11, while they showed virulence against *Bt* genes 1, 2, 3 and 7 (Matanguihan et al. 2011). This also matches with experiments conducted in Austria where teliospores of *T. caries* were virulent to *Bt* genes 2 and 7 but differential lines carrying *Bt4, Bt5, Bt6, Bt8, Bt9, Bt10, Bt11* and *Bt12* showed low *T. caries* disease incidence (Huber and Bürstmayr 2006).

1.2.2 Worldwide distribution of common bunt

Common bunt is widespread and effectively perpetuated with seeds, either on seed surfaces or as infections. Dispersal of common bunt by wind was probably not important for early wheat farmers due to hand harvesting and threshing at a central site (Saari and Mamluk 1996). However, wind may be an important factor of common bunt distribution in modern agriculture, as combine harvesting liberates teliospores, depositing them onto the surface of the field, as well as releasing them into the atmosphere, where they can be carried long distances (Saari and Mamluk 1996). From the center of origin in the Near East infected seeds were spread to other parts of the world by human activities such as trade and migration causing a worldwide spread of the disease (Figure 1-4). In the Near East region, common bunt may be the most widespread and important wheat disease aside from rusts and close to the center of origin the highest frequency of common bunt resistance sources can be found as from Serbia and Montenegro through Macedonia and Turkey to Iran a concentration of landraces resistant to bunt is evident (Bonman et al. 2006).

Already little common bunt infection of grain is sufficient to reduce quality and cause marketing problems, even when yield losses are minor (Saari and Mamluk 1996). In Turkey bunt diseases are of major importance since it was ascertained that about 10% of the wheat fields were infected with common bunt. Some fields even show disease incidence of 90% (Parlak 1981). Furthermore, common bunt causes severe losses in some areas of South Asia, North Africa, Asia, North America and South America, especially in Argentina, and it has been a major disease throughout Europe. Also, in Australia significant losses were

caused due to this disease, before the extensive use of chemical seed treatments. Although bunt incidence has been reduced, epidemics can quickly develop since soils are still infested with low levels of inoculum (Saari and Mamluk 1996). Even though wheat is the primary host of common bunt other crops like rye, red fescue, barley and several grasses can also serve as hosts (Gaudet and Menzies 2012).

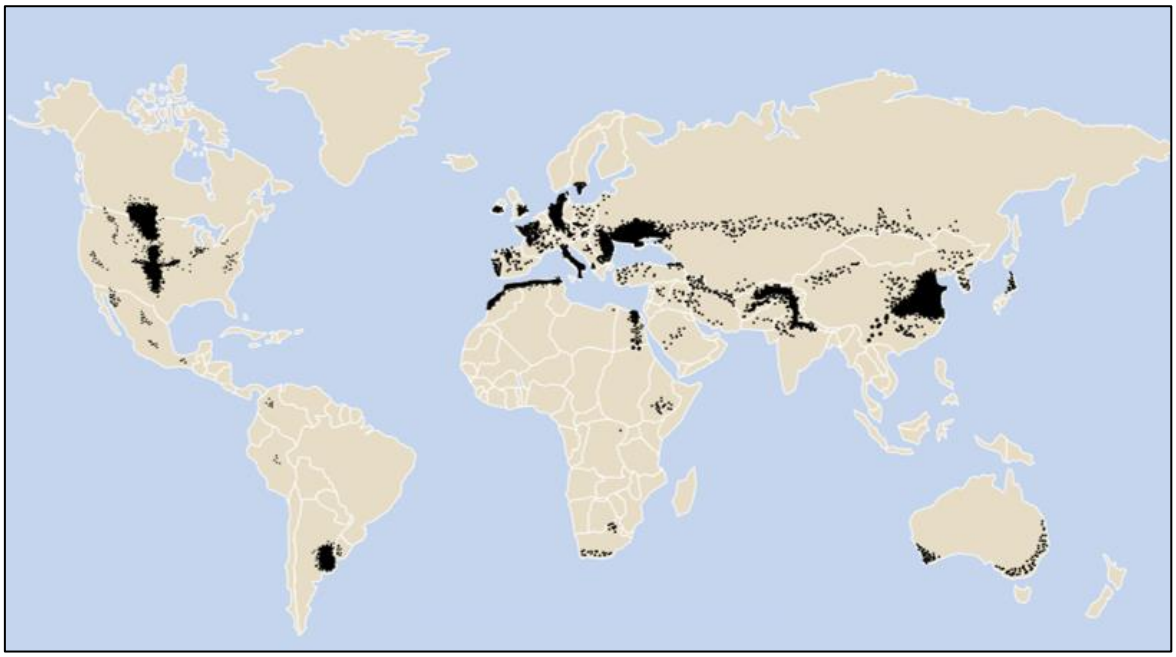

Figure 1-4 Worldwide distribution of common bunt (Saari and Mamluk 1996)

1.2.3 Disease cycle and symptoms

Common bunt is caused by the two closely related fungi *Tilletia caries* (D.C.) Tul. &C. Tul. (syn. *Tilletia tritici* (Bjerk.) G. Winter) and *Tilletia laevis* J.G. Kühn (syn. *T. foetida* (Wallr.) Liro) (Matanguihan et al. 2011). They are Heterobasidiomycetes in the order Ustilaginales and belong to the family Tilletiaceae (Goates 1996). The two species are very similar in germination requirements, life cycles and disease symptoms, but differ in their shape of teliospores. Those of *T. caries* have reticulated walls, as opposed to this those of *T. laevis* which have smooth walls (Figure 1-5) (Matanguihan et al. 2011). The teliospores of *T. caries* are light pale yellow to grey or reddish brown and generally globose. The diameter is 14-23.5 µm, occasionally up to 25 µm. The polygonal reticulations are usually 0.5-1.5 µm deep. On the other hand, the teliospores of *T. laevis* are light pale to dark olivaceous brown and globose or ovoid, with a diameter of 14-22 µm. The species usually can be distinguished with the use of a light microscope (Goates 1996). *T. caries* is more widespread in the Northwestern Europe, whereas *T. laevis* is more common in Eastern Europe (Matanguihan et al. 2011). Contamination with *T. caries* also occurs all over Austria, but there is no evidence for wheat infection with *T. laevis* (Huber and Bürstmayr 2006; Zwetko et al. 2004).

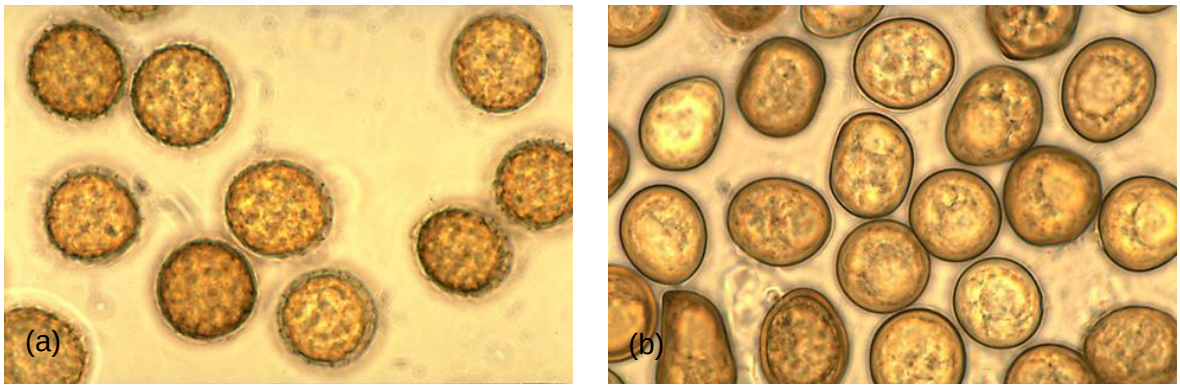

Figure 1-5 (a) Teliospores of Tilletia caries (b) Teliospores of Tilletia leavis (Mathre 2000)

Common bunt infection mainly occurs from seedborne spores but also from spores present in the soil. The optimum soil temperature for infection ranges from 5 to 10°C. The infection level is reduced at 22°C (Matanguihan et al. 2011; Goates 1996). In temperate and northern regions early seeding of spring wheat and late seeding of winter wheat at cooler soil temperatures increases common bunt infection level (Gaudet and Menzies 2012). The cycle of infection starts when teliospores on the seed or in the soil germinate and produce infection hyphae. Shortly after seed germination, the produced hyphae penetrate the wheat coleoptiles which generally occurs 7-10 days after seeding (Goates 1996). After the germination of a spore, a basidium is formed together with haploid sporidia that fuse to produce dikaryotic secondary hyphae and secondary sporidia. The secondary hyphae then form an appressorium that penetrates the coleoptile (Figure 1-6) (Gaudet and Menzies 2012). The hyphae is initially established both in resistant and susceptible cultivars, but in resistant ones it does not progress to the apical meristem before internode elongation, which is necessary for a disease development (Goates 1996). The seedlings are vulnerable for infection up to a size of 2 cm in which stage the hyphae systematically grows in susceptible plants (Spieß et al. 2015). When ovaries begin to form, the fungus proliferates in the spikes and sporulates in the endosperm tissue until the entire kernel is converted into a sorus, called bunt ball. Theses bunt balls contain 4 to 5 million spores and can easily break. Especially during harvest or grain handling spores are released and thus causing contamination of seeds and soil infestation due to deposition on fallow land. Throughout the dry summer the spores remain on the field to germinate and infect wheat sown in the fall and therefor initiates the next cycle of infection (Matanguihan et al. 2011; Spieß et al. 2015; Hoffmann 1982). Common bunt teliospores are viable for about two years under natural field conditions in the soil. Their survival is favored at low soil moisture and wheat monoculture. Hence, fields are considered pathogen free when wheat did not enter into the crop rotation for at least two years, and if they are located distant from possible sources of windborne inoculum (Goates 1996; Gaudet and Menzies 2012).

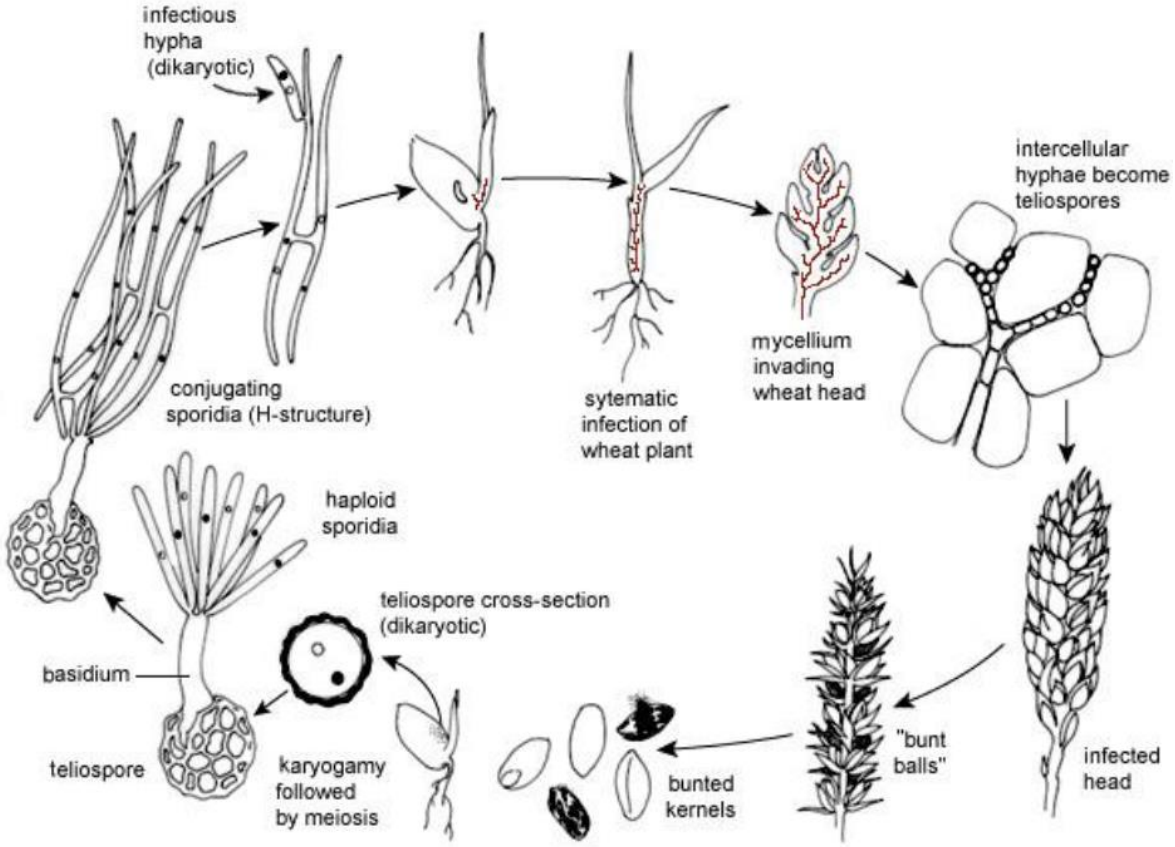

Figure 1-6 Disease cycle of common bunt caused by Tilletia caries and Tilletia leavis (Mathre 2000)

Symptoms of common bunt infection first appear after heading, when sporulation begins in the very young ovary. Compared to healthy spikes, infected ones are colored with a darker green tint and remain green for a longer time period, whereas at maturity they are usually slightly lighter with a bluish-gray color. The sori almost look like wheat kernels but have a more rounded shape (Figure 1-7) (Goates 1996). Mourad et al. (2018) found that common bunt infection increases the seedling vigour, delays heading, increases head length, increases root length and decreases the biological yield. The produced teliospores have a strong fishy smell hence common bunt is also called stinking smut. The cause for this severe smell is the production of trimethylamine which is even noticeable at a very low contamination level as 0.1% by volume causing high loss of quality even at low infection levels (Matanguihan et al. 2011). Common bunt infested grain often is generally downgraded to animal feed, also leading to a reduction in pricing. Producers most likely refuse to accept bunted grain because grain handling systems will get contaminated (Gaudet and Menzies 2012). At high infection levels feeding to animals should be done with caution since toxic adverse effects can occur and such grain is recommended to be burned or fermented as washing of infected grain is very costly and only economically with high quality wheat (Brandstetter and Weinhappel 2011).

Figure 1-7 (a) Common bunt infected spikelet (b) open bunt balls with spores and bunt balls with intact pericarp compared to healthy wheat kernels

Dwarf bunt is a disease closely related to common bunt, caused by *Tilletia controversa.* The two species are difficult to distinguish from each other, and sometimes can occur in the same field or even on the same plant. But dwarf bunt only occurs in higher altitude areas and regions with permanent snow cover and it is only present in winter wheat. The teliospores of *T. controversa* require cooler temperatures and are viable in the soil for many years. Infection mainly occurs through the soil, infection from seedborne inoculum is rare (Brandstetter and Weinhappel 2011; Goates 1996).

1.3 Control of common bunt

To prevent common bunt incidence seeding material of high quality should be used. For organic fields a cultivation break of two years for wheat is recommended and in case of common bunt appearance all harvesting material needs to be cleaned carefully to prevent further contamination (Brandstetter and Weinhappel 2011). Sowing seeds at higher soil temperature can decrease infection levels as seeds germinate faster, and bunt spores have not enough time to germinate and infect the seedlings. Early sowing of winter wheat and late sowing of spring wheat is preferable while seeding shallow can prevent common bunt incidence (Clark and Cockerill 2011; Gaudet and Menzies 2012; Goates 1996). After an occurrence of common bunt infection, infested fields should be ploughed deeply to bury the spores (Spieß et al. 2015).

In Austria the threshold for common bunt treatment is at 10 spores per seed and at an infestation level of up to 300 spores per seed they must be treated with registered seed treatments. If an even higher contamination level is present, seeds will not be certified (Clark and Cockerill 2011; Brandstetter and Weinhappel 2011).

1.3.1 Chemical seed treatment

Since genetic specificity made it difficult to only control the disease by host resistance, various seed treatment methods have been used. During the last century, it progressed from formaldehyde, copper carbonate, organic mercuries, and polychlorobenzenes to systematic fungicides like carboxin. It turned out that the most effective agent against common bunt was hexachlorbenzene (HCB). The usage of this plant protection agent was readily adapted in many plant production systems to control diseases such as common bunt. With the appearance of this chemical seed treatment, not only seedborne but also soilborne inoculum could be controlled (Hoffmann 1982). However, there are restrictions regarding chemical plant protection, thus its application is forbidden nowadays (Matanguihan et al. 2011; Goates 1996).

In Austria most of the registered chemical seed treatments contain the active compounds fludioxonil and difenoconazole (Bundesamt für Ernährungssicherheit 2019).

1.3.2 Physical methods

Common bunt incidences can generally be reduced if bunt contamination in seed lots is decreased, since the spores of common bunt are placed loosely on the surface of the grains i.e. the grain as such is healthy and not infected until germination. Common bunt incidence can thus be reduced by decreasing the spore load with the use of physical treatments such as air-screen and brush cleaning of the seeds (Borgen 2004). Combining both can remove up to 99.8% of the spores (Borgen 2005). In the nineteenth century hot water treatments were used to control seedborne diseases, but this method is costly and not applicable on large quantities, hence other types of thermal treatment of seeds were developed e.g. in Germany, experiments on a combination of vapor and microwave treatment and experiments on irradiation of seeds with electro-rays were carried out (Matanguihan et al. 2011; Borgen 2004). Furthermore, the technology “SonoSteam” which was initially used to eliminate pathogens on food surfaces was tested, in which the effect of surface heat sterilization was used to control common bunt by exposing contaminated seeds to a combination of steam and ultrasound. At the Swedish University of Agricultural Sciences a high precision treatment with hot, humid air, called ThermoSeed, was developed to kill seedborne fungi including *T. caries* (Matanguihan et al. 2011).

1.3.3 Organic seed treatment

In experiments conducted by Koch et al. (2006) the most efficient organic seed treatment with the highest level of bunt control (94%) was Tillecur, which is a yellow mustard powder product that is applied as slurry to seeds before sowing (Matanguihan et al. 2011). Waldow and Jahn (2007) reported similar results for the plant growth promoting agent Tillecur that provided efficient bunt control compared to hot water treatment which was less effective and only showed effects at high inoculum level. They also recommend that susceptible cultivars should be treated from a threshold of 1-5 spores/seed and moderately susceptible cultivars should be treated at a contamination level of 20 spores to avoid disease accumulation, especially in seed production (Waldow and Jahn 2007).

In West Asia and North Africa seed treatments with organic nutrients such as powdered skimmed milk, hucket (local skimmed milk) and wheat flour were used to prevent bunt incidence caused by *T. caries* and *T. laevis*. Even though these substances did not kill the teliospores there was a significant reduction in bunt incidence possibly referable to an increase of unknown antagonistic microorganisms or production of toxic metabolites that inhibited teliospore germination (El-Naimi et al. 2000).

Another alternative could be the treatment of seeds with acetic acid solutions or the use of acetic acid vapors as fumigants, as both methods were reported to reduce common bunt incidence (Borgen and Nielsen 2001; Sholberg et al. 2006). Acetic acid is a naturally occurring substance with high biodegradability and due to its low toxicity it could substitute conventional fungicides to reduce the general environmental impact of seed treatments. In the conducted experiments common bunt infection was reduced up to 96% without effecting germination vigor of the seeds (Borgen and Nielsen 2001).

1.3.4 Biological control

With the introduction of biological control mechanisms it might be possible to successfully control common bunt incidence without negative effects on seed germination and vigor (Borgen and Davanlou 2001). It was already observed in 1976 that *Bacillus* species can reduce diseases incidence of common bunt as they significantly reduced the teliospore germination (Kollmorgen 1976). It was also reported that inoculation with *Pseudomonas fluorescens* inhibits teliospore germination of *T. laevis* and reduces common bunt incidence in the field by 65% (McManus 1993). Johnsson et al. (1998) found that one isolate of *Pseudomonas chlororaphis*, strain MA 342, suppresses common bunt incidence in the field, which has been further developed into commercial biopesticides. One of them is Cerall which is already used against seedborne diseases in wheat, rye and triticale, including *T. caries* (Matanguihan et al. 2011). Dromph and Borgen (2001) tested the effect of collembolans on the viability of soilborne inoculum of *T. caries* in an experiment in which

teliospores were fed to different species of collembolans. The results showed that ingestion by collembolans almost completely inhibits germination of bunt teliospores and thereby reduces infection of wheat.

1.4 Host resistance and plant breeding

In the past years breeding for bunt resistance had low priority in wheat growing countries as the disease could be readily controlled by chemical seed treatment. Nevertheless, for organic agriculture the development of host resistance is a major component to reduce the occurrence of common bunt and achieve high yields and a suitable end-use quality (Matanguihan et al. 2011). Although fungicides are effective to control the disease, they are expensive and may present problems associated with toxicity, environmental hazards and availability or distribution. Furthermore, resistant cultivars could control the disease more effectively than chemicals (Goates 1996). Organic farmers largely depend on crop varieties produced for conventional farming systems with high inputs of artificial, mineral fertilizers and chemicals for crop protection. Nevertheless, it has been suggested that for organic wheat production corresponding breeding programs are necessary to develop robust varieties that are better adapted to low-input conditions. such programs might especially aim to improve traits like improved rooting systems, stronger interspecific competition ability for weed suppression and yield stability (Lammerts van Bueren et al. 2008). One of the challenges is the introgression of bunt resistance genes from exotic wheat cultivars and wheat relatives into adapted cultivars as most sources of bunt resistance possess poor agronomic traits (Matanguihan et al. 2011). Current research continued with monitoring bunt incidence and pathogen races, screening cultivars for bunt resistance, conducting studies on the mode of inheritance of bunt resistance and searching for new sources of resistance. With the use of molecular techniques, resistance genes have been identified and mapped. 15 bunt resistance genes are currently identified, although it is not always known which of those genes a cultivar possesses (Matanguihan et al. 2011). High disease levels only develop if complementary virulence genes of the pathogen exist for all the resistance genes of a particular host. If a plant has any resistance genes other than the avirulence genes of the pathogen it will be resistant against this bunt race. With the selective increase of virulence races and by the development of new combinations of virulence genes in the bunt population, resistance may be overcome. Thus the continuous search for new sources of resistance and incorporation into cultivars is necessary (Goates 1996).

1.5 Marker assisted selection

DNA-based technologies allow the development of more rapid and efficient methods for screening for bunt resistance, making molecular markers a very useful tool in breeding programs. Marker-assisted selection (MAS) uses DNA sequences which are closely linked with the bunt resistance genes to identify the resistance phenotype of the plant, which can speed up the development of resistant cultivars enormously as disease symptoms of common bunt only get visible at plant maturity making screening for resistance in the field very time consuming (Gaudet and Menzies 2012). Additionally, environmental effects can influence the infection level, or if disease pressure is low difficulties in classifying lines as resistant or susceptible might arise. Marker assisted selection can facilitate and hasten this screening process as it can already be applied at the seed or seedling stage (Gaudet and Menzies 2012; Matanguihan et al. 2011). Notwithstanding, only a few *Bt* genes have markers associated with them and not all resistance genes have been mapped to their chromosomal location at the moment (Matanguihan et al. 2011).

Many different types of markers are available including PCR-based DNA markers such as microsatellites and DNA-based techniques can be applied using F_2 and backcross populations, near-isogenic lines (NILs), double haploid lines (DH) as well as recombinant inbred lines (RILs). The first PCR-based marker for bunt was developed for resistance gene *Bt10* in wheat, located on chromosome 6D (Gaudet and Menzies 2012). The *Bt10* gene is widely used in breeding programs since it is effective against all known races of common bunt in western Canada (Wang et al. 2009). Furthermore, markers have been developed for a bunt resistance gene present in the winter wheat cultivar Blizzard (Gaudet and Menzies 2012). This resistance is effective against all North American races of dwarf bunt and European and current United States races of common bunt. Wang et al. (2009) found that the microsatellite markers *Xgwm374, Xbarc128* and *Xgwm264*, located on wheat chromosome 1BS, are significantly linked to the resistance locus. The calculated genetic distance between the bunt resistance locus and the overlapping markers was 3.9 cM. These three markers can be useful for selecting common bunt resistance from Blizzard and identifying new sources of resistance (Wang et al. 2009).

Up to now it is known that the *Bt1* gene is located on chromosome 2B; *Bt4, Bt5* and *Bt6* on 1B; *Bt7* on 2D; *Bt9* and *Bt10* on 6D and *Bt11* on 3B. Further quantitative trait loci (QTL) have been identified on chromosomes 1B, 3A, 4B, 4D, 5B, 7A, 7B, and 7D (Bhatta et al. 2018). Chen et al. (2016) also reported resistance loci on chromosome 1A and showed that among others markers *Xcfa2129* and *Xbarc83* are linked with putative resistance QTL.

1.6 Aims of this master thesis

Common bunt is a seedborne fungal disease which causes severe yield losses of wheat, where resistance breeding makes a major contribution to a sustainable disease management. For this study the three exotic common bunt resistant lines Bonneville, PI119333 and Blizzard were crossed with five susceptible wheat cultivars adapted to the agroclimatic conditions in Austria. Regarding to their resistant parent the resulting RIL populations can be classified into three mapping populations. To validate known resistance QTL by phenotypic and genotypic characterization, all lines were screened for their common bunt resistance in the field and analyzed with SSR markers linked to known resistance genes.

The objectives of this master thesis can be summarized as followed:

- Phenotypic evaluation of the three mapping populations regarding common bunt infection level, number of resistance genes and traits correlated with common bunt incidence

- Genotypic evaluation of the three mapping populations with SSR markers *Xgwm374, Xgwm264, Xbarc83* and *Xcfa2129* and evaluation if these markers are linked with the common bunt resistance genes present in the RIL populations

- Identification of possible breeding relevant lines for further resistance breeding against common bunt

2 Materials and methods

2.1 Plant material

A total of thirteen recombinant inbred line (RIL) populations of winter wheat (*Triticum aestivum L. subsp. aestivum*) were developed from crosses between three exotic resistant lines (Bonneville, PI119333 and Blizzard) with five susceptible, but adapted lines (Rainer, Midas, Tommy, Pannonikus and 20568.1.2). These populations comprised 359 recombinant inbred lines (RILs) that were generated at the University of Natural Resources and Life Sciences, Department of Agrobiotechnology, Institute of Biotechnology in Plant Production, in Tulln, Austria, since 2007. Depending on their resistant parental line they can be grouped into three mapping populations (Table 2-1). Population 1 consists of crosses with the line Bonneville and comprised 87 RILs, Population 2 was generated from the line PI119333 with 240 RILs and Population 3 containing 32 RIL progenies derived from crosses with Blizzard. The recombinant inbred lines were generated by Single Seed Descent (SSD) up to the F_5 generation (S-genotypes) and some lines further with bulk propagation to a F_8 generation (P-genotypes), and can thus be expected to have a degree of homozygosity of approximately 93.75% (F_5) and 99.22% (F_8).

Table 2-1 Recombinant inbreed lines tested for common bunt resistance; P-genotypes = Propagation (Bulk), S-genotypes = Single Seed Descend

Mapping population	Cross	Pedigree	Number of RILs per population	Year of crossing
Population 1 Bonneville	P101	Bonneville/Rainer	10	2007
	S1	Rainer/Bonneville//20568.1.2	28	2010
	S2	Midas/Bonneville//Rainer	47	2010
	S7	Bonneville/Rainer	2	2010
Population 2 PI119333	P106	PI119333/Rainer	86	2007
	P107	PI119333/Tommi	19	2007
	P109	PI119333/Tommi	27	2007
	S12	PI119333/Pannonikus	40	2010
	S13	PI119333/Midas	30	2010
	S14	PI119333/Tommi	38	2010

Population 3 Blizzard	S3	Rainer/Blizzard//Midas	20	2010
	S4	Blizzard/Rainer	5	2010
	S5	Blizzard/Rainer	7	2010

2.1.1 Resistant parental lines

The resistant lines Bonneville, PI119333 and Blizzard were screened by Huber and Bürstmayr (2006) to evaluate their resistance against *Tilletia caries* and *Tilletia controversa.* Bonneville, Blizzard and PI119333 showed a high resistance to both species *T. caries* and *T. controversa* and thus are potential genotypes for resistance breeding.

Bonneville (*Triticum aestivum L. subsp. aestivum*) is an awned hard red winter wheat variety developed in Idaho, United States at the Idaho Agricultural Experiment Station in cooperation with the USDA-ARS, in 1994 (Table 2-2). It was especially bred for dryland conditions and a high resistance to bunt but possesses long stems with a tendency for lodging. Furthermore, it shows a tolerance to snow mold, is resistant to stripe rust, displays a late heading date and a good milling and baking quality characteristics (Figure 2-1(a)) (USDA 2018).

PI119333 (*Triticum aestivum L. subsp. aestivum*) is a winter wheat landrace collected in Elazig, Turkey in 1936. PI119333 is a line recommended as source of resistance, containing the bunt resistance gene *Bt12 (Goates and Bockelman 2012)*. The landrace is awned and tall with a tendency for lodging (Figure 2-1(b)).

Blizzard (*Triticum aestivum L. subsp. aestivum*) is a hard red winter wheat variety with tall plants and awned heads (Figure 2-1(c)). Blizzard shows a high resistance against common bunt and dwarf bunt. It is tolerant to snow mold but shows a susceptibility to stripe rust (USDA 2018). Blizzard was developed in Idaho, United States at the Idaho Agricultural Experiment Station in cooperation with the USDA-ARS, in 1989.

Table 2-2 Description of exotic resistant lines Bonneville, PI119333 and Blizzard (USDA (2018), Grain Genes (n.d.))

Name	Origin	Pedigree	Release
Bonneville (PI557015)	Idaho Agricultural Experimental Station and USDA-ARS	Utah216c-12-10/Cheyenne/5/PI476212/4/Burt/3/Rex/Rio//Nebred/6/Kiowa/Utah222a-437-2//Dm/3/PI476212/MT6619/4/McCall/El Gaucho/3/Kiowa/Utah233-3-10/Burt	1994
PI119333	Elazig Turkey	Collected	Landrace 1936
Blizzard (PI512302)	Idaho Agricultural Experimental Station and USDA-ARS	((Orfed / Elgin /3/ (UT112a-520-6-1, Ridit /2/ Kanred / Sevier), UT216c-12-10) /4/ Cheyenne /5/ PI476212 /4/ Burt /3/ Rio / Rex /2/ Nebred, A68203W-E-1-3-3) /6/ (A68203W-1-6-1, (Orfed / Elgin /3/ (UT112a-520-6-1, Ridit /2/ Kanred / Sevier), UT216c-12-10) /4/ Cheyenne /5/ PI476212 /4/ Burt /3/ Rio / Rex /2/ Nebred)	1989

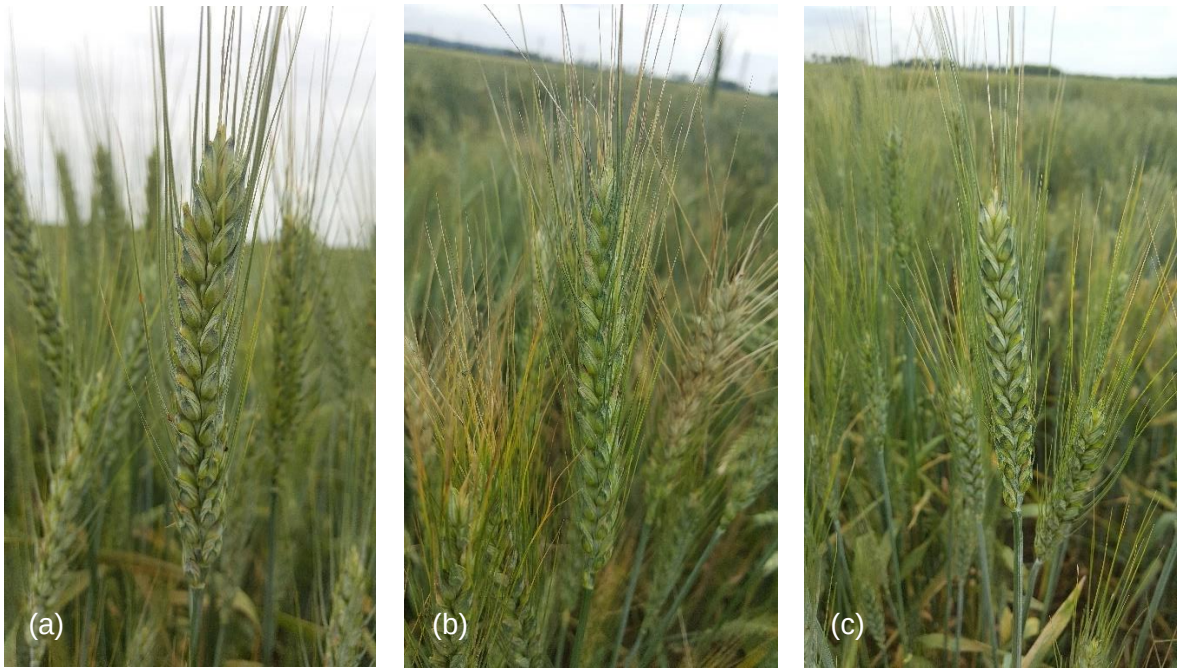

Figure 2-1 Exotic resistant parental lines (a) Bonneville (b) PI119333 (c) Blizzard

2.1.2 Susceptible parental lines

All selected susceptible parental lines are winter wheat (*Triticum aestivum L. subsp. aestivum*) cultivars and are already adapted to Austrian growing conditions. Rainer, Midas and Pannonikus were developed by Saatzucht Donau and possess a high baking quality (Table 2-3). Rainer is an awnless milling wheat and was released in 2006. Midas and Pannonikus are awned and belonging to the group of quality wheat. Both varieties were released in 2008 (Saatzucht Donau n.d.).

The cultivar Tommi was developed by Nordsaat and released in 2002. It is awnless and belongs to the category of quality wheat and was described by Wächter et al. (2004) as being resistant against *Tilletia caries* (Bundessortenamt 2019). Huber and Bürstmayr (2006) came to the same result and classified this cultivar as resistant to *T. caries,* but at the same time have shown that the cultivar Tommi is highly susceptible to *T. controversa.*

20568.1.2 was developed at the University of Natural Resources and Life Sciences, Department of agrobiotechnology IFA-Tulln, Institute of Biotechnology in Plant Production, from a cross between the cultivars Capo and Sumai-3. Sumai-3 is known for its high resistance against Fusarium Head Blight (FHB) and as well shows high resistance against common bunt. Capo is moderately resistant to FHB but is highly susceptible to common bunt.

Table 2-3 Description of susceptible parental lines Rainer, Midas, Tommi, Pannonikus and 20568.1.2

Cultivar	Origin	Characteristics	Release
Rainer	Saatzucht Donau GesmbH & CoKG, Probstdorf, Austria	Milling wheat	2006
Midas	Saatzucht Donau GesmbH & CoKG, Probstdorf, Austria	Quality wheat	2008
Tommi	Nordsaat Saatzucht GmbH	Quality wheat	2002
Pannonikus	Saatzucht Donau GesmbH & CoKG, Probstdorf, Austria	Quality wheat	2008
20568.1.2	IFA-Tulln	Pre-breeding line	

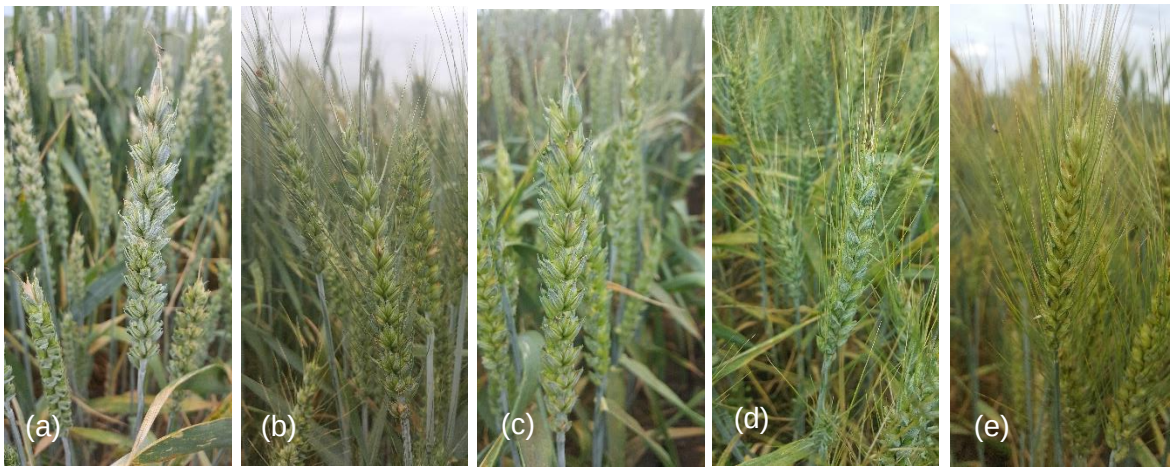

Figure 2-2 Susceptible parental lines (a) Rainer (b) Midas (c) Tommi (d) Pannonikus (e) 20568.1.2

2.2 Field experiment

The field experiment was located in Tulln (48°30' N, 16°04'), Austria, on the fields of the University of Natural Resources and Life Sciences (BOKU), Department of Agrobiotechnology, Institute of Biotechnology in Plant Production. Tulln is a town at the Danube, at 175m above sea level, in the Pannonian climate region. The long-term average temperature in Tulln – Langenlebarn is 10.3°C and the mean precipitation is 597mm (ZAMG 2002). In the growing season 2017/18 the mean monthly temperature was higher and with less precipitation compared to the long-term average (Figure 2-3).

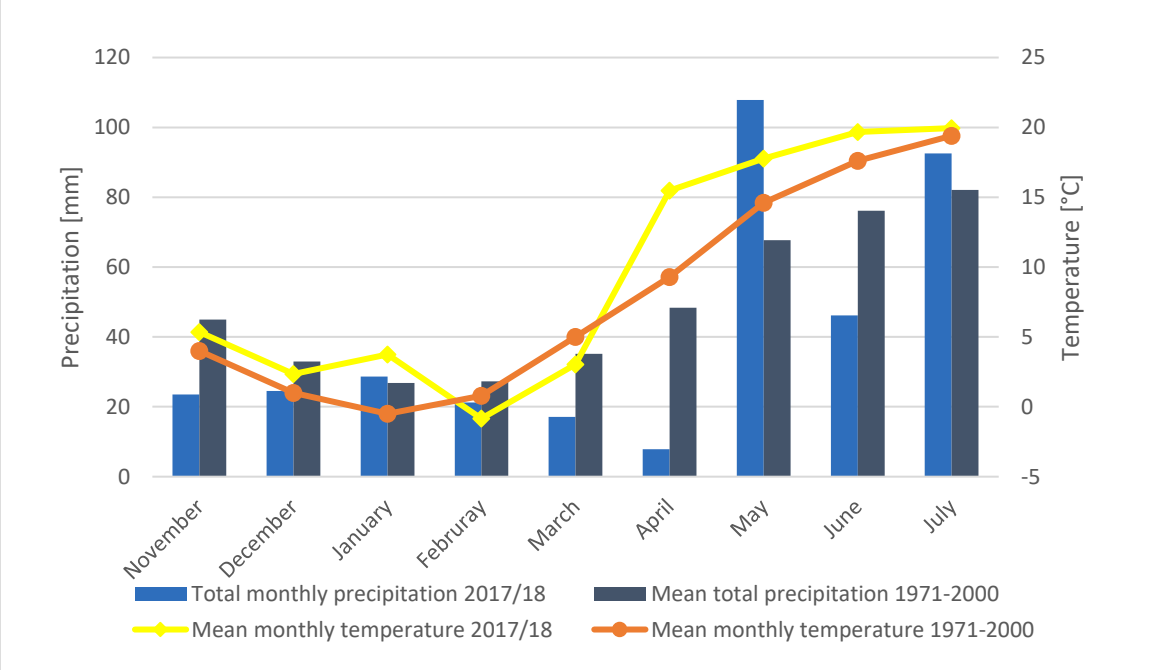

Figure 2-3 Mean monthly temperature and monthly precipitation in Tulln, Austria during the vegetation period 2017/18 (November 1st 2017 to July 31st 2018) compared to long-term mean monthly temperature and precipitation in Langenlebarn, Austria 1971 – 2000 (Data from BOKU Department of Agrobiotechnology 2018, (ZAMG 2002))

2.2.1 Inoculation of seeds

All seeds of the different genotypes were inoculated with a mix of common bunt teliospores (*Tilletia caries*) before planting. The teliospores were obtained from infected plant material from prior common bunt trials in Tulln, Austria in 2016 and 2017. The preparation of the inoculum and the inoculation of seeds was conducted after an adapted protocol from Goates (1996). Spores were isolated from bunt balls of the infected spikelet and cleaned through sieving and to ensure they are only spores from *Tilletia caries,* a sample was inspected under a light microscope. After the preparation of a 0.05% Methylcellulose solution, 90g of spores were added to 300ml of the solution. Finally, 0.6ml of the inoculum was added to each sample consisting of 20g of seeds resulting in a concentration of 0.3g spores/ml and 0.9g of spores per 100g of seeds.

2.2.2 Experimental design

All mapping populations and the parental lines were planted in a randomized complete block design in two replications. The first replication was planted on November 3rd, 2017 and the second replication on November 10th, 2017. All genotypes were grown in plots consisting of two rows with 160cm length and a sowing density of 10g per plot. The distance within these two rows was 17cm, the distance between the plots 33cm and 50cm to the next row (Figure 2-4).

Figure 2-4 Common bunt trial 2017/18 in Tulln, Austria

Fertilizer containing nitrogen, phosphorus, potassium and sulfur (330kg/ha, NPK 17:6:18 + 7S) was applied on March 3rd, 2018. A second fertilizer was applied on May 5th, 2018 containing calcium ammonium nitrate (150kg/ha, CAN with 27% nitrogen). The crop

management included moreover the applications of two herbicides. Andiamo Flexx (33.3 g/l Diflufenican, 500 g/l Mecoprop-P, 50 g/l Florasulam) was applied with a rate of 1.35 l/ha on March 20th, 2018 and Puma Extra (69 g/l Fenoxaprop-P-ethyl, 75 g/l Mefenpyr-Diethyl (Safener)) with a rate of 0.85 l/ha on May 23rd, 2018. Furthermore, the insecticide Decis forte (100 g/l Deltamethrin) was applied on May 23rd,2018 with a rate of 75 ml/ha. Lastly, the growth regulator Cerone (660 g/l Ethephon) was applied on May 4th, 2018 with a rate of 0.7 l/ha. The preceding crop was maize.

2.2.3 Common bunt assessment

Common bunt incidences were evaluated from July 6th, 2018 to July 20th, 2018 (BBCH stage 85) by visual estimation. From each of the two rows per plot 75 heads were counted, cut open and investigated for the presence of bunt balls and black spores. In common bunt infected heads, the bunt balls and black spores were clearly visible (Figure 2-5). If there was no infection at all in the first 75 heads, the second row was only looked at sporadically. The common bunt incidences were then expressed as percentage of infected heads out of the 150 counted ones (or 75 if no infection was present).

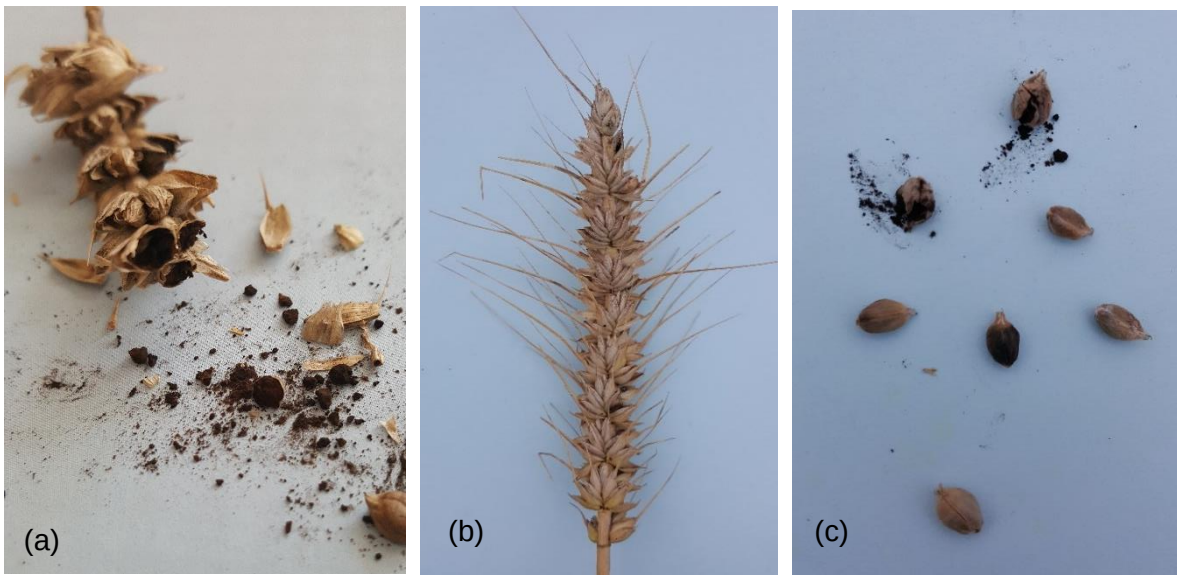

Figure 2-5 (a) infected spikelet cut open (b) common bunt infected spikelet of variety Midas (c) bunt balls

2.2.4 Assessment of other traits

Besides the evaluation of the common bunt incidences, several additional traits were evaluated in order to investigate their correlation with common bunt resistance.

Date of heading (DH): Starting on May 21st, 2018 until July 13th, 2018 every second day the plants were evaluated for their earliness, and the date of heading was noted as soon as 50 percent of the heads reached BBCH stage 55. For standardization days were counted

beginning with May 1st (May 21st equals 21 days after May 1st; July 13th equals 44 days after May 1st).

Plant height (PH): The plant height was measured with a measuring stick, in the middle of each plot, from the ground to the end of the heads excluding awns. It was measured in steps of 5cm. The assessment took place on July 4th, 2018.

Lodging (LOD): Each plot was assessed for lodging on June 18th, 2018. All plots were evaluated on a scale from 1 to 9. 1 meaning all plants are in an upright position with no lodging at all, 9 meaning all plants in the plot are completely on the ground.

Awns (AW): In addition, on July 24th, 2018 the plots were evaluated for the absence or presence of awns. If awns were present, they were classified with 1, awnless plots with 0. If the plots were mixed and showed both, plants with and without awns, they were classified as 0.5.

Fusarium Head Blight (FHB): All plots were checked for infection with Fusarium spp. fungi. Symptoms of this infection are premature bleaching of spikelets, the shriveling of kernels and the whitening of the ears. Each plot was evaluated on a scale from 1 to 9. 1 meaning no infection visible at all, 9 meaning all plants within the plot showed an infection. The plants were assessed on June 22nd, 2018.

General leaf health assessment (LH): On June 18th, 2018 all plots were assessed for their general leaf health. The assessment was performed on a scale from 1 to 9. Plots where all leaves were healthy were assessed with 1, while plants where the whole leaf area was infected, or dead were assessed with 9.

Homogeneity: All plots were assessed for their homogeneity of the plants, using a scale from 1 to 5. Plots with complete homogeneity were assessed with 1, plots with almost complete heterogeneity within the plants were assessed with 5. The scoring was performed on June 20th, 2018.

Plant stand: On June 18th, 2018 the plant stand was evaluated, using a scale from 1 to 9. Plots with the maximum plant density were scored with 1. Plots where no plants were present were scored with 9.

2.3 Genotyping of parental lines and RIL populations

2.3.1 Sample preparation

Ten seeds of every genotype were planted separately in 6x8-well pots and grown in the greenhouse. After two weeks, when the plants reached a height of about 10cm they were cut into labeled 96-well plates for the DNA-extraction (Figure 2-6). All leaf samples were subsequently dried for two days in a drying chamber at 37°C.

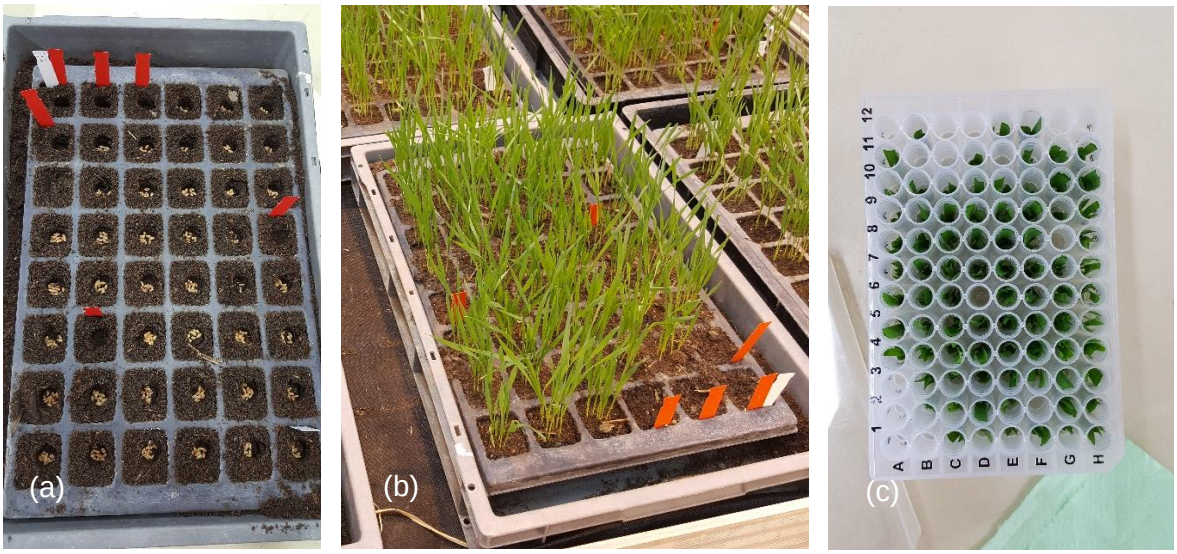

Figure 2-6 Sample preparation (a) seeds in 6x8-well pots (b) plants after two weeks (c) leaves cut into 96-well plate

2.3.2 DNA-Extraction

Each of the 96 wells was filled with 4 glass beads so that the leaves could then be grind in a mill (Mixer Mill MM301, Retsch GmbH) for ten minutes. The orientation of the plates was changed after five minutes. Afterwards the DNA-Extraction was performed according to a modified protocol outlined by Saghai-Maroof et al. (1984). Firstly, the CTAB-Buffer was prepared (Table 2-4) and 600µl were added to each sample. The tubes were then put into a water bath at 65°C, with gentle rocking for 90 minutes.

Table 2-4 Preparation of the CTAB-Buffer

Stock	Final concentration	Amount for 250ml (four 96-well plates)
dH_2O		162.5 ml
1M Tris-7.5	100 mM	25 ml
5M NaCl	700 mM	35 ml
0.5 M EDTA-8.0	50 mM	25 ml
CTAB	1%	2.5 ml
14M BME	140 mM	2.5 ml

Afterwards the tubes were cooled down to room temperature and 300µl of a chloroform:isoamylalcohol mixture (24:1) were added to each tube and shook by gentle inversion for five minutes. Afterwards several centrifugation steps were conducted, all at room temperature using the Eppendorf Centrifuge 5804R. To induce the phase separation (Figure 2-7(a)) the first centrifugation lasted for 10 minutes at 4000rcf. 300µl of the top aqueous layer were pipetted off into a new tube and 300µl of isopropyl alcohol were added and mixed well by gentle inversion. After another centrifugation for 8 minutes at 1200rcf, the liquid was poured off, leaving the DNA-pellet at the bottom of the tube (Figure 2-7(b)). For the first washing step 100µl of Wash 1 (76% EtOH, 200mM NaOAc) were added and after gently mixing for 15 minutes it was centrifuged for 8 minutes at 1200rcf and then the liquid was poured off. A second washing step with 100µl of Wash 2 (76% EtOH, 10mM NH_4OAc) followed, and after 5 minutes of gently shaking and centrifuging for 8 minutes at 1200rcf the liquid was poured off. For the last step the remaining DNA-pellet which has dried over night was dissolved in 100µl 0,1 TE buffer (1 mM Tris, 0.1mM EDTA) and mixed for some hours at room temperature. The DNA was finally stored at 4°C.

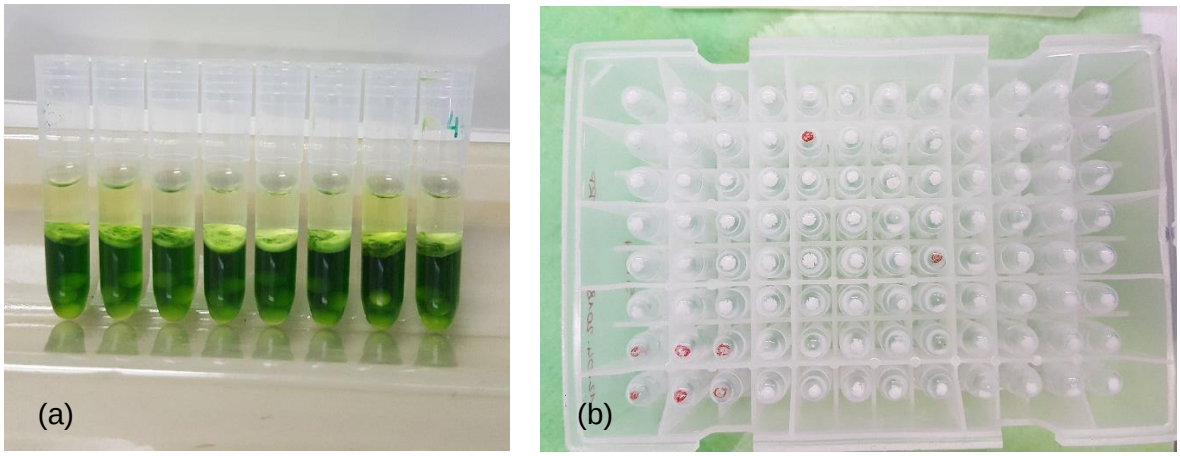

Figure 2-7 (a) phase separation after first centrifugation (b) tubes with DNA-pellets

2.3.3 Adjusting the DNA-Concentration

To ensure the quantity and quality of the obtained DNA, their optical density was measured on a photometer (BioSpec-nano UV-VIS Spectrophotometer) and the purity of the sample was determined via the ratio of the optical density at 260nm to 280nm. The samples were then diluted to a concentration of 150ng/µl for storage at -20°C by adding the appropriate amount of 0.1M TE Buffer. For the DNA amplification samples were adjusted to a concentration of 50ng/µl by adding water (dH_2O).

2.3.4 SSR markers

Several SSR markers located on chromosomes 1A, 1B, 7A and 7D were tested on all parental lines for their association with common bunt resistance (Table 2-6). Out of the 25 tested markers only *Xgwm374, Xgwm264, Xcfa2129* and *Xbarc83* (Table 2-5) showed clear visible polymorphisms between the parental lines and thus were further applied on all RILs.

The markers *Xgwm374* and *Xgwm264* were selected based on the study of Wang et al. (2009), who reported them to be located at chromosome 1BS and closely linked to the resistant locus of the cultivar Blizzard. Marker *Xcfa2129* and *Xbarc83* are located on chromosome 1A and also linked with the resistance according to Chen et al. (2016).

The labeling of the PCR-fragments during PCR was done with the fluorescence dye 6-Carboxyfluorescein (FAM) and cyanine dye Cy5. Three different primers were used: a M13 tailed forward primer, a reverse primer and a universal fluorescence FAM or Cy5 labeled M13 primer.

Table 2-5 Used SSR markers and their primer sequences

Marker	Forward and reverse primer sequence
Xgwm374	5’ ATAGTGTGTTGCATGCTGTGTG 3’
	5’ TCTAATTAGCGTTGGCTGCC 3’
Xgwm264	5’ GAGAAACATGCCGAACAACA 3’
	5’ GCATGCATGAGAATAGGAACTG 3’
Xcfa2129	5’ GTTGCACGACCTACAAAGCA 3’
	5’ ATCGCTCACTCACTATCGGG 3’
Xbarc83	5' AAGCAAGGAACGAGCAAGAGCAGTAG 3'
	5' TGGATTTACGACGACGATGAAGATGA 3'

Table 2-6 List of tested SSR markers

Marker	Chromosomal location
Xcfa2129	1A
Xwmc312	1A
Xwmc550	1A
Xbarc1022	1A
Xbarc83	1A
Xwmc135	1A
Xwmc159	1A
Xgwm374	1B
Xgwm264	1B
Xgwm273	1B
Xwmc404	1B
Xwms33	1B
Xbarc194	1B
Xwmc172	1B
Xbarc312	1B
Xbarc8	1B
Xbarc119	1B
Xwms344	7A
Xbarc1028	7A
Xwmc308	7A
Xcfa2040	7A
Xbarc184	7D
Xbarc244	7D
Xbarc153	7D
Xgdm88	7D

2.3.5 Polymerase chain reaction

The amplification of the DNA through polymerase chain reaction (PCR) was conducted in 384 well PCR-plates with a volume of 10µl for each well. The wells were filled with 2µl of DNA with a concentration of 50ng/µl and mixed with 8µl of master mix (Table 2-7). DNA amplification was then performed according to the PCR-protocol shown in Table 2-8.

Table 2-7 PCR mastermix components

	Stock concentration	Final concentration	Amount for a 10µl reaction
PCR-Buffer incl. 15mM MgCl2	10 X	1 X	1.0 µl
dNTP Mix (10X)	2 mM	0,2 mM	1.0 µl
R-Primer (10µM)	10 µM	0.2 µM	0.2 µl
F-Primer (10µM)	10 µM	0.02 µM	0.02 µl
M13-30 Primer (10µM)	10 µM	0.18 µM	0.18 µl
Taq-Enzyme (5U/µl)	5 U/µl	0.05 U/µl	0.1 µl
ddH_2O			5.5 µl
Template DNA	50 ng/µl	10 ng/µl	2 µl
Total			10 µl

Table 2-8 PCR amplification protocol

Step	Temperature	Duration [min]	Number of cycles
1. Initial denaturation	95°C	04:00	1
2. Denaturation	95°C	00:50	7
3. Primer annealing	65°C	01:00	7
4. Elongation	72°C	01:00	7
5. Denaturation	95°C	00:30	25
6. Primer annealing	51°C	00:30	25
7. Elongation	72°C	00:30	25
8. Final Elongation	72°C	05:00	1
9. Cool down	14°C	∞	

2.3.6 Polyacrylamide gel electrophoresis

The PCR-product was separated by polyacrylamide gel electrophoresis, for which a CBS system consisting of a running-gel and a stacking-gel was prepared. The 12% polyacrylamide (PAA) running-gel was prepared with 3.5ml 10x TBE buffer, 21ml dH_2O, 10.50ml acrylamide (29:1, 40%), 368µl APS (10%) and 18.2µl Temed, while the stacking-gel was prepared with 250µl 10x TBE buffer, 1.94ml dH_2O, 310µl acrylamide (29:1, 40%), 52.5µl APS (10%) and 2.7µl Temed. To prepare the samples for loading on the gel, 2.5µl

of loading buffer was added to each sample. The electrophoresis was conducted for two hours at 400V and 80mA.

For the visualization of the PCR fragments the Typhoon Trio Variable Mode 41 Imager (Amersham Biosciences) was used. The fragments were labeled with fluorescent dye and detected at 488nm for FAM and at 633nm for Cy5.

For the scoring of the results, lines showing the same band as the resistant parent were scored as 1 and lines showing the band from the susceptible parent were scored as 2. Heterozygous genotypes were scored as 3.

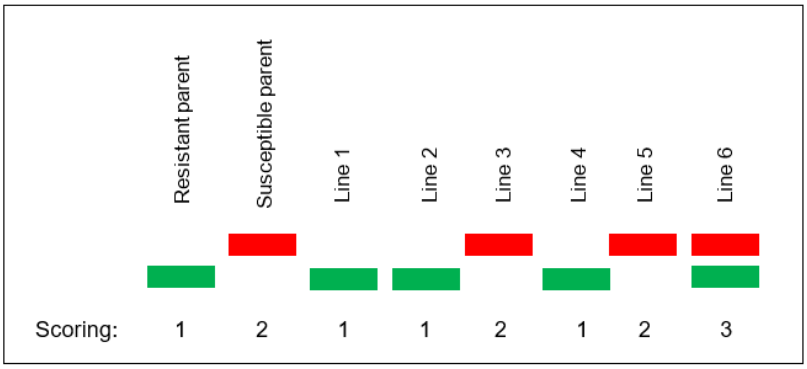

Figure 2-8 Illustration of the marker scoring

2.4 Statistical analysis

2.4.1 Evaluation of phenotypic data

An analysis of variance (ANOVA) was performed separately for the mapping populations Bonneville, PI11933 and Blizzard, for the traits common bunt incidence, date of heading, plant height, lodging, awns, fusarium head blight and leaf health. For each of the traits a linear model was fitted:

$$x_{ij} = \mu + G_i + R_j + \varepsilon_{ij}$$

x_{ij} …phenotypic value of the i^{th} genotype in the j^{th} replicate
μ …population mean
G_i …effect of i^{th} genotype
R_j …effect of j^{th} replicate
ε_{ij} …residuals

To determine the critical value for common bunt incidence between resistant and susceptible lines the least significant difference (LSD) at a significance level of α=0.05 was calculated using the following formula:

$$LSD = t * \sqrt{\frac{2 * ve}{n}}$$

t …t-value
ve …residual variance
n …number of replications

Recombinant inbred lines showing common bunt incidence below this value were classified as resistant, RILs with higher common bunt incidence were classified as susceptible.

The coefficient of determination R^2 was calculated with the formula:

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}}$$

SS_{res} …sum of squares of residuals
SS_{tot} …total sum of squares

A Chi^2-test was used to check the segregation pattern with ratios 1:1, 3:1 and 7:1 of resistant versus susceptible lines i.e. if common bunt resistance was conferred by one, two or three

independently segregating genes. Therefore, the number of observed resistant RILs and susceptible RILs was tested against the expected number of resistant and susceptible lines with the formula:

$$X^2 = \sum_{i=1}^{n} \frac{(O - E)^2}{E}$$

O …observed frequency
E …expected frequency

The heritability (h^2) was estimated with the use of the variance components which were calculated with a linear mixed model. For this trial the heritability corresponds to the repeatability and was calculated with the formula:

$$h^2 = \frac{vg}{vg + \frac{ve}{n}}$$

vg …genotypic variance
ve …residual variance
n …number of replications

2.4.2 Evaluation of genotypic data

To test for associations between molecular markers and common bunt incidence, the recombinant inbred lines were divided into two groups either showing the allele from the resistant or the susceptible parent respectively. Lines carrying both alleles (heterozygous) were excluded from the analysis. To test for significant differences between genotypes, a one-way analysis of variance (ANOVA) was performed using the following model:

$$x_i = \mu + M_i + \varepsilon_i$$

x_i …mean phenotypic value
μ …population mean
M_i …effect i^{th} marker genotype
ε_i …residuals

All statistical analyses were conducted with the statistical package R (R Core Team 2019).

The R script of the analysis of the genotypic and phenotypic data is shown in the appendix.

3 Results

3.1 Genetic variation and heritability

In Figure 3-1 the correlation between replication 1 and replication 2 for common bunt incidence is shown for the mapping populations Bonneville, PI119333 and Blizzard. The correlation coefficient was high for all three populations, ranging from 0.809 for population 2 (PI119333) and 0.834 for population 1 (Bonneville), to 0.906 for population 3 (Blizzard). For most of the other traits the correlation was not as high as for common bunt incidence (Appendix 1).

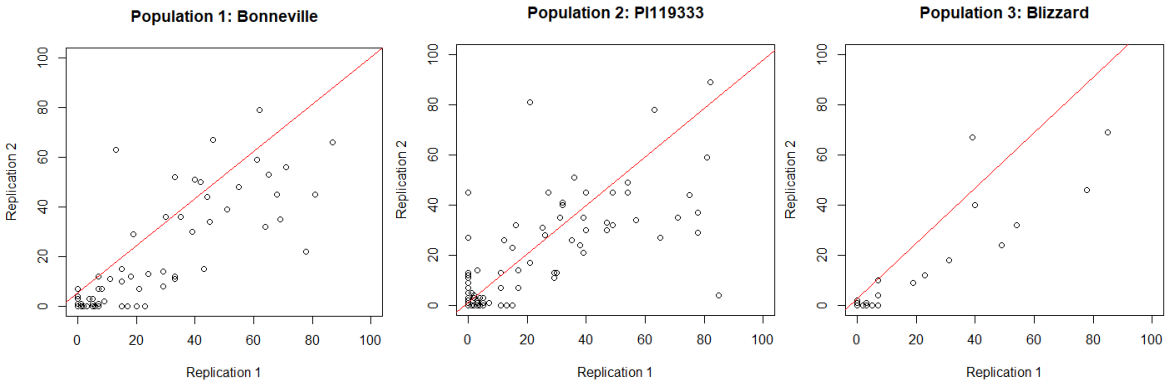

Figure 3-1 Pearson's correlation for common bunt incidence (%) for replication 1 and replication 2 for the mapping populations Bonneville, PI119333 and Blizzard

3.2 Common bunt incidences

3.2.1 First and second degree statistical parameters

The exotic resistant lines Bonneville, PI119333 and Blizzard showed an average common bunt infection of less than 1% which clearly confirms the classification of these lines as resistant. The susceptible parental lines showed a much higher rate of common bunt incidences, with 64.92% for Rainer, 73.50% for Midas and 64.50% for Pannonikus. Only the lines Tommi and 20568.1.2 were similar to the resistant lines with an infection level of 1.25% and 0% (Appendix 2).

As presented in Figure 3-2 all mapping populations showed differences of the mean common bunt incidences. The highest infection rate was found in Population 1 (Bonneville) with 17.51%, although within this population the lines of the cross Bonneville/Rainer showed a very low infection rate of 0.87%, while the highest resistance was achieved for Population 2 (PI119333) with an average of 7.36% common bunt incidence. The mean common bunt incidence for Population 3 (Blizzard) is 12.31% but there was also substantial variation

between the crosses within this population. The lines resulting from the cross Rainer/Blizzard//Midas had a higher level of infection (19.32%) compared with the lines from the cross Blizzard/Rainer which had a very low infection rate of 0.63% (Table 3-1).

Table 3-1 General statistic distribution parameters for common bunt incidence (%) for all recombinant inbred lines and for each mapping population (Population 1 Bonneville; Population 2 PI119333; Population 3 Blizzard)

Population	N	Mean	Minimum	Maximum
All populations	359	10.56	0.00	85.50
Population 1	87	17.51	0.00	76.50
Bonneville/Rainer	12	0.87	0.00	10.00
Rainer/Bonneville//20568.1.2	28	13.27	0.00	76.50
Midas/Bonneville//Rainer	47	24.74	0.00	70.50
Population 2	240	7.36	0.00	85.50
PI119333/Rainer	86	6.94	0.00	49.50
PI119333/Tommi	84	5.94	0.00	85.50
PI119333/Pannonikus	40	6.26	0.00	51.50
PI119333/Midas	30	14.22	0.00	70.50
Population 3	32	12.31	0.00	77.00
Rainer/Blizzard//Midas	20	19.32	0.00	77.00
Blizzard/Rainer	12	0.63	0.00	5.50

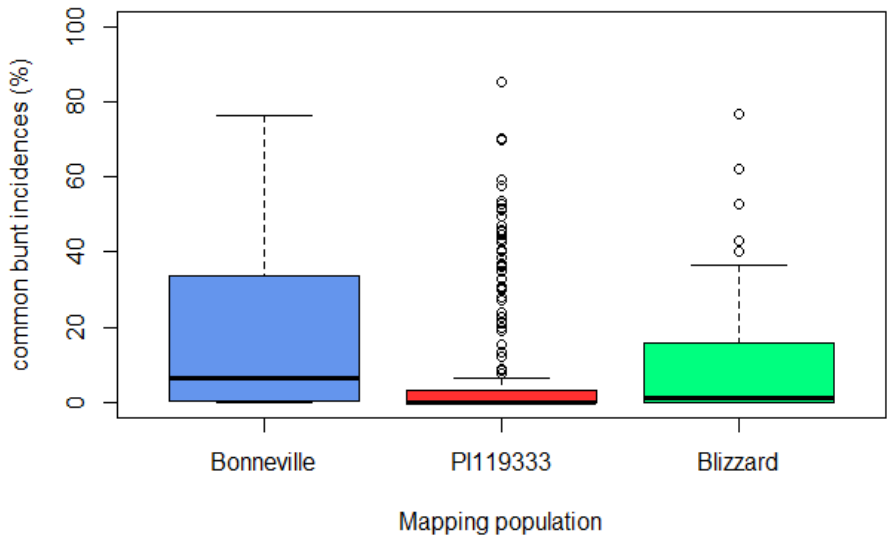

Figure 3-2 Boxplot of common bunt incidence for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

The heritability (h^2) for this case corresponds to the repeatability and was estimated with the variance component analysis. In all mapping populations the heritability for common bunt incidence was high with a value of 0.91 for population 1 (Bonneville), 0.89 for population 2 (PI119333) and 0.94 for population 3 (Blizzard) (Table 3-2).

Table 3-2 Heritability (h^2) for common bunt incidence of mapping population Bonneville, PI119333 and Blizzard

Trait	Population	h^2
CB	All populations	0.91
	Population 1, Bonneville	0.91
	Population 2, PI119333	0.89
	Population 3, Blizzard	0.94

The results from the analysis of variance (ANOVA) performed from the phenotypic data for common bunt incidence are shown in Table 3-3, Table 3-4 and Table 3-5. In all three mapping populations the genotypes were significantly different ($p<0.001$) from each other for this trait.

Table 3-3 Results analysis of variance and coefficient of determination (R^2) for common bunt incidence for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	83	78669	947.82	10.5493	<2.2e-16
Replication	1	887	886.88	9.8711	0.002319
Residuals	84	7547	89.85		
R^2	0.9134				

Table 3-4 Results analysis of variance and coefficient of determination (R^2) for common bunt incidence for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	120188	507.12	9.3489	<2e-16
Replication	1	102	102.48	1.8892	0.1705
Residuals	255	13832	54.24		
R^2	0.8969				

Table 3-5 Results analysis of variance and coefficient of determination (R^2) for common bunt incidence for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	27488.8	886.73	16.8772	2.714e-12
Replication	1	210.2	210.25	4.0014	0.05428
Residuals	31	1628.7	52.54		
R^2	0.9445				

The frequency distribution looks similar for all three mapping populations (Figure 3-3, Figure 3-4 and Figure 3-5). The least significant difference at α=5% is marked with a red line and the parental lines are visible due to the black dotted lines. Only a few lines show a higher infection rate than the susceptible parental lines Rainer (64.92%), Midas (73.50%) and Pannonikus (64.50%). All resistant parental lines have an infection rate of less than 1%.

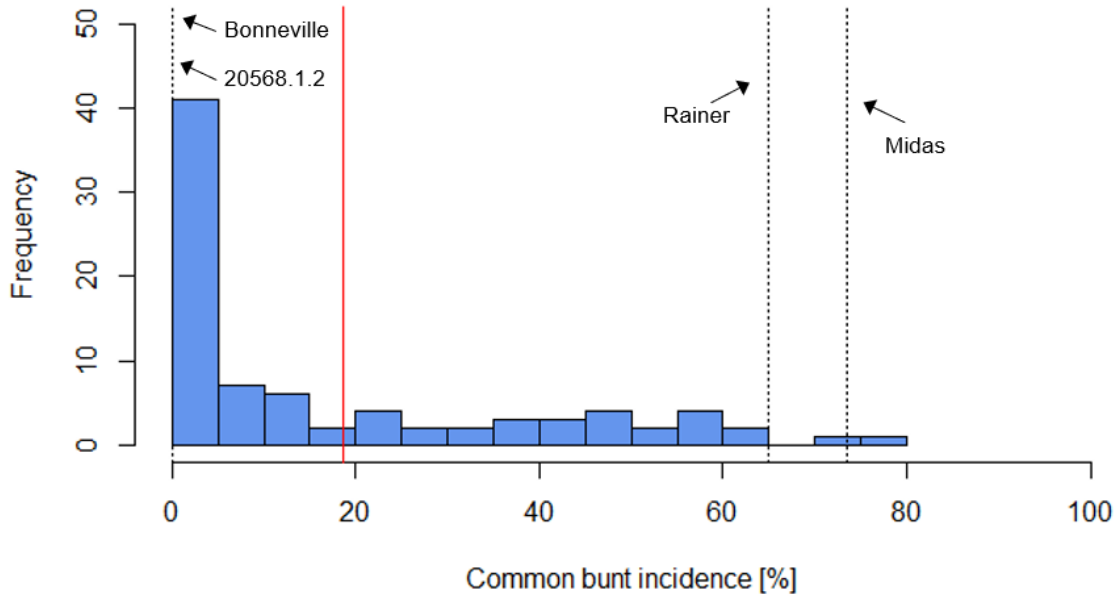

Figure 3-3 Frequency distribution for common bunt incidence (%) for mapping population Bonneville; including the least significant difference (LSD) of 5% (red line); and the common bunt incidence for the parental lines (dotted lines)

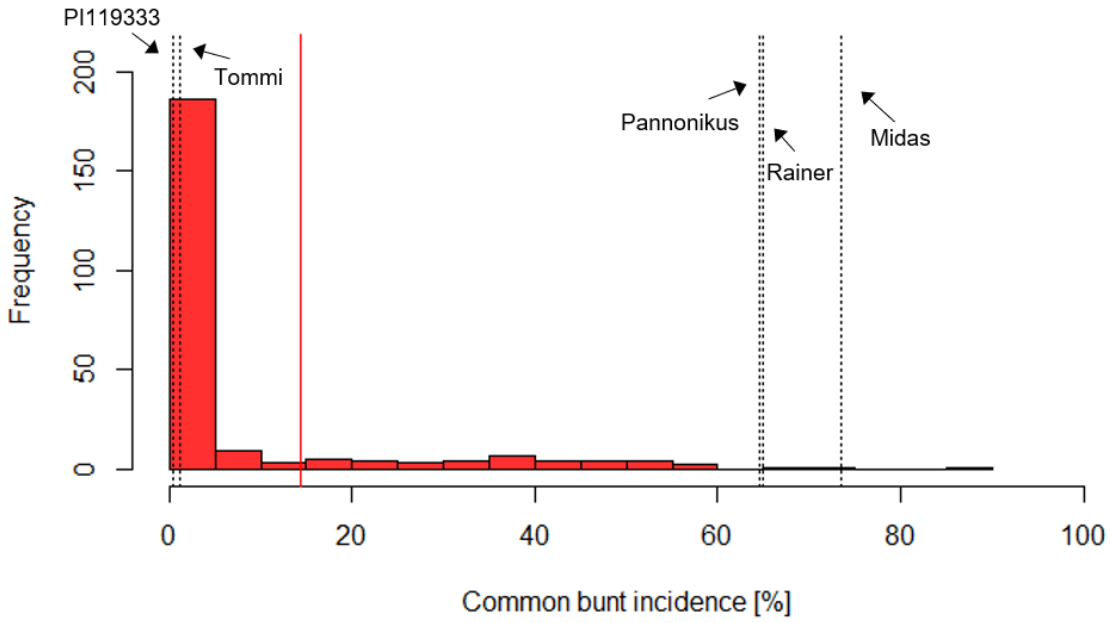

Figure 3-4 Frequency distribution for common bunt incidence (%) for mapping population PI119333; including the least significant difference (LSD) of 5% (red line); and the common bunt incidence for the parental lines (dotted lines)

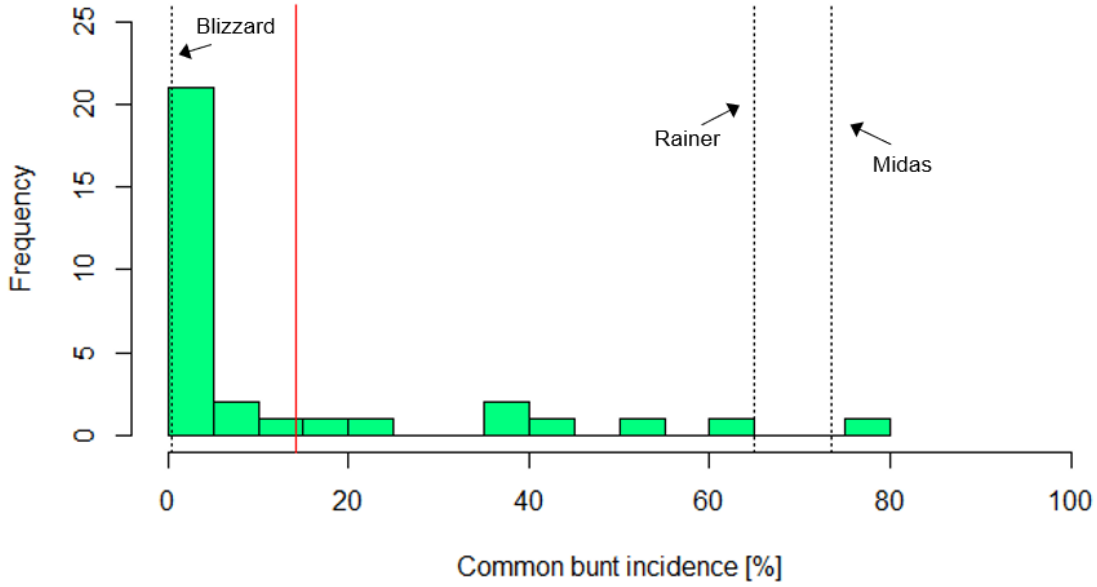

Figure 3-5 Frequency distribution for common bunt incidence (%) for mapping population Blizzard; including the least significant difference (LSD) of 5% (red line); and the common bunt incidence for the parental lines (dotted lines)

The critical value for Population 1 Bonneville is a common bunt incidence of 18.8%. 67% of the lines generated from Bonneville lie beyond this value and can be classified as resistant. For Population 2 PI119333 the critical value is 14.5% which classifies 83% of the recombinant inbred lines as resistant. In Population 3 Blizzard the critical value lies at a common bunt incidence of 14.8%. This classifies 75% of the lines as resistant. In Figure

3-6 the distribution of resistant and susceptible genotypes based on the least significant difference value of 5% is shown for each mapping population.

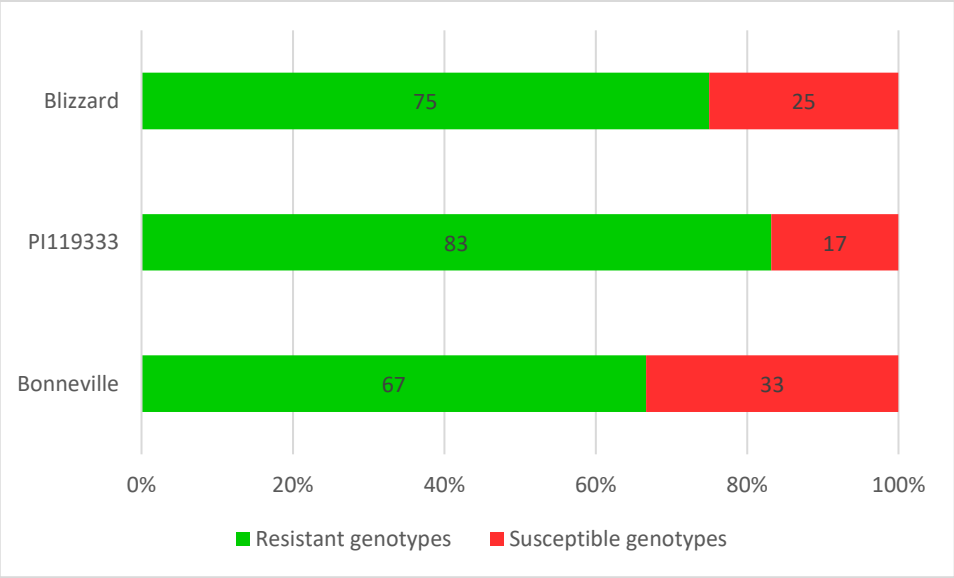

Figure 3-6 Distribution (%) of resistant genotypes and susceptible genotypes for the mapping populations Blizzard, PI119333 and Bonneville

3.2.2 Segregation pattern

The segregation pattern of the three mapping populations was tested with the Chi^2 test. It was tested whether the distribution follows a 1:1 ratio of resistant to susceptible lines i.e. one major resistance gene is present, a 3:1 ratio for two genes and 7:1 ratio for three independent common bunt resistance genes.

The cross Midas/Bonneville//Rainer shows a 1:1 distribution leading to the assumption that one resistance gene is present. For the cross Rainer/Bonneville//20568.1.2 the p-values for a 3:1 and 7:1 segregation are significant meaning that two or even more resistant genes are present. The same result was obtained for population 2, specifically for the cross PI119333/Rainer. For PI119333/Tommi and PI119333/Pannonikus most likely three independently segregating genes are present and for PI119333/Midas the highest p-value was observed for a ratio of 3:1 indicating two genes. In population 3 Blizzard for the lines of Rainer/Blizzard//Midas the p-values of a 1:1 and 3:1 segregation are significant (Table 3-6).

In the crosses Bonneville/Rainer and Blizzard/Rainer no segregation was observed since all lines showed a very low common bunt infection level.

Table 3-6 Segregation pattern (expected and observed) for one (1:1), two (3:1) and three (7:1) independently segregation resistance genes for common bunt, for each cross of the mapping populations Bonneville, PI11933 and Rainer, with Chi² values and p-values (Seg = Segregation, Res = Resistant, Sus = Susceptible)

			Expected		Observed			
Population/ Cross		**Seg**	**Res**	**Sus**	**Res**	**Sus**	**Chi²**	**p-value**
Population 1: Bonneville	Rainer/ Bonneville// 20568.1.2 (n=28)	1:1	14	14	23	5	11.571	<0.001
		3:1	21	7	23	5	0.7619	0.3827
		7:1	24.5	3.5	23	5	0.7347	0.3914
	Midas/ Bonneville// Rainer (n=44)	1:1	22	22	21	23	0.0909	0.763
		3:1	33	11	21	23	17.455	<0.001
		7:1	38.5	5.5	21	23	63.636	<0.001
Population 2: PI119333	PI119333/ Rainer (n=86)	1:1	43	43	71	15	36.465	<0.001
		3:1	64.5	21.5	71	15	2.6202	0.1055
		7:1	15.25	10.75	71	15	1.9203	0.1658
	PI119333/ Tommi (n=84)	1:1	42	42	71	13	40.048	<0.001
		3:1	63	21	71	13	4.0635	0.0438
		7:1	73.5	10.5	71	13	0.6803	0.4095
	PI119333/ Pannonikus (n=39)	1:1	19.5	19.5	34	5	21.564	<0.001
		3:1	29.25	9.75	34	5	3.0855	0.0799
		7:1	34.125	4.875	34	5	0.0037	0.9517
	PI119333/ Midas (n=29)	1:1	14.5	14.5	22	7	7.7586	0.0053
		3:1	21.75	7.25	22	7	0.0115	0.9146
		7:1	25.375	3.625	22	7	3.5911	0.0581
Population 3: Blizzard	Rainer/ Blizzard// Midas (n=20)	1:1	10	10	12	8	0.8	0.3711
		3:1	15	5	12	8	2.4	0.1213
		7:1	17.5	2.5	12	8	13.829	<0.001

3.3 Other evaluated traits

An analysis of variance has been performed for the traits date of heading (DH), plant height (PH), lodging (LOD), awns (AW), fusarium head blight (FHB) and leaf health (LH) for each population. The results are shown in Appendix 6 to Appendix 40. Significant differences ($p < 0.001$) between genotypes were found for the trait DH for all populations, for PH in population 1 and 2, for LOD in population 2, for AW in all populations and for FHB in population 2.

Looking at the parental lines there is a clear difference in plant height between the exotic lines and the adapted ones, with a mean of 110 cm the plants of Bonneville and Blizzard are much taller than the adapted cultivars which have a mean height of about 80cm. With an average of 116.20cm population PI11933 possesses the tallest plants, also leading to a problem with lodging. The population 2 generated from PI119333 also reveals this tendency for tall plants and lodging. Despite that it has the lowest values for the trait FHB and leaf health. For the date of heading all populations have similar means.

Table 3-7 General statistic distribution parameters for the traits date of heading (DH), plant height (PH), lodging (LOD), awns (AW), fusarium head blight (FHB) and leaf health (LH). Data are shown for the parental lines Bonneville, PI119333, Blizzard, Rainer, Midas, Tommi, 20568.1.2 and Pannonikus.

Trait	Line	Mean	Minimum	Maximum	Median
DH	Bonneville	31.50	30.00	33.00	31.50
	PI119333	31.75	28.00	41.00	29.00
	Blizzard	29.00	29.00	29.00	29.00
	Rainer	31.67	29.00	42.00	30.00
	Midas	31.10	26.00	38.00	30.50
	Tommi	38.00	37.00	40.00	37.00
	20568.1.2	24.50	24.00	25.00	24.50
	Pannonikus	30.75	28.00	33.00	31.00
PH	Bonneville	110.00	105.00	115.00	110.00
	PI119333	116.20	90.00	150.00	112.50
	Blizzard	110.00	110.00	110.00	110.00
	Rainer	82.50	70.00	90.00	80.00
	Midas	80.50	70.00	90.00	80.00
	Tommi	76.25	70.00	80.00	77.50
	20568.1.2	80.00	75.00	85.00	80.00
	Pannonikus	82.50	80.00	90.00	80.00
LOD	Bonneville	1.00	1.00	1.00	1.00
	PI119333	3.00	1.00	7.00	3.00
	Blizzard	1.00	1.00	1.00	1.00
	Rainer	1.00	1.00	1.00	1.00
	Midas	1.00	1.00	1.00	1.00
	Tommi	1.00	1.00	1.00	1.00
	20568.1.2	1.00	1.00	1.00	1.00
	Pannonikus	1.00	1.00	1.00	1.00
AW	Bonneville	1.00	1.00	1.00	1.00
	PI119333	1.00	1.00	1.00	1.00

	Blizzard	1.00	1.00	1.00	1.00
	Rainer	0.00	0.00	0.00	0.00
	Midas	1.00	1.00	1.00	1.00
	Tommi	0.00	0.00	0.00	0.00
	20568.1.2	1.00	1.00	1.00	1.00
	Pannonikus	1.00	1.00	1.00	1.00
FHB	Bonneville	3.00	3.00	3.00	3.00
	PI119333	4.50	3.00	6.00	4.50
	Blizzard	3.50	3.00	4.00	3.50
	Rainer	4.75	3.00	6.00	5.00
	Midas	4.30	4.00	6.00	4.00
	Tommi	2.50	2.00	3.00	2.50
	20568.1.2	3.00	3.00	3.00	3.00
	Pannonikus	3.25	3.00	4.00	3.00
LH	Bonneville	6.00	4.00	8.00	6.00
	PI119333	4.50	4.00	6.00	4.75
	Blizzard	5.50	5.00	6.00	5.50
	Rainer	4.17	1.00	8.00	3.50
	Midas	3.40	1.00	7.00	3.00
	Tommi	2.25	1.00	3.00	2.50
	20568.1.2	3.00	1.00	5.00	3.00
	Pannonikus	3.25	2.00	5.00	3.00

Table 3-8 General statistic distribution parameters for the traits date of heading (DH), plant height (PH), lodging (LOD), awns (AW), fusarium head blight (FHB) and leaf health (LH). Data are shown for all recombinant inbred lines and for each mapping population (Population 1 Bonneville, Population 2 PI119333, Population 3 Blizzard)

Trait	Population	Mean	Minimum	Maximum	Median
DH	All populations	30.97	21.00	41.85	30.50
	Population 1	31.09	21.00	41.87	31.00
	Population 2	31.00	25.00	40.50	30.50
	Population 3	30.47	24.50	37.50	29.50
PH	All populations	100.80	49.70	130.00	102.50
	Population 1	91.67	50.00	127.50	90.00
	Population 2	105.30	70.00	130.00	107.50
	Population 3	92.66	80.00	112.50	92.50
LOD	All populations	2.10	1.00	8.00	1.00
	Population 1	1.00	1.00	1.00	1.00

	Population 2	2.64	1.00	8.00	1.00
	Population 3	1.13	1.00	3.00	1.00
AW	All populations	0.67	0.00	1.00	1.00
	Population 1	0.60	0.00	1.00	0.88
	Population 2	0.71	0.00	1.00	1.00
	Population 3	0.61	0.00	1.00	0.88
FHB	All populations	4.04	2.00	8.00	4.00
	Population 1	3.79	2.50	6.00	3.50
	Population 2	4.17	2.00	8.00	4.00
	Population 3	3.83	2.50	6.50	3.50
LH	All populations	4.08	1.50	7.50	4.00
	Population 1	3.31	1.50	6.50	3.00
	Population 2	4.41	2.00	7.50	4.50
	Population 3	3.66	1.50	7.50	3.25

Differences between the mapping populations Bonneville, PI119333 and Blizzard for the trait date of heading, plant height, lodging, leaf health and fusarium head blight are visualized in Figure 3-7 to Figure 3-11. Frequency distribution for these traits are shown in Appendix 23 to Appendix 27.

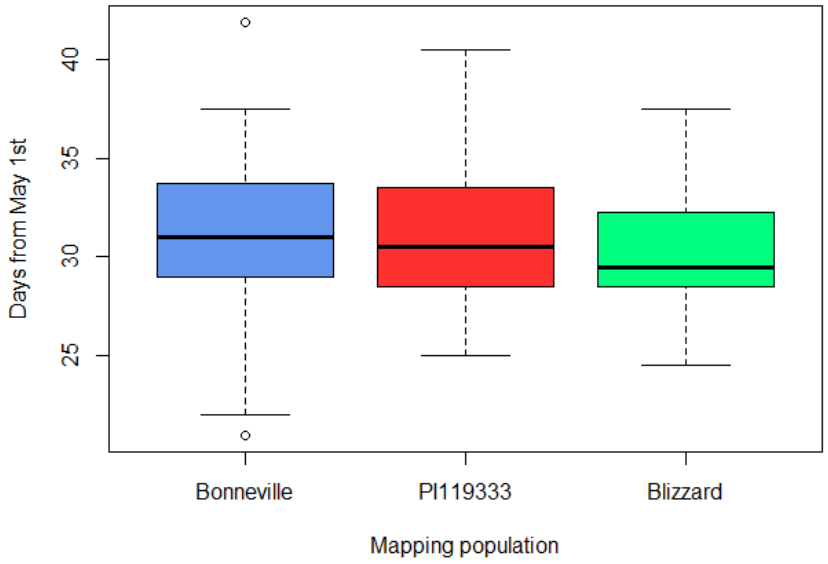

Figure 3-7 Boxplot for the trait date of heading (DH) for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

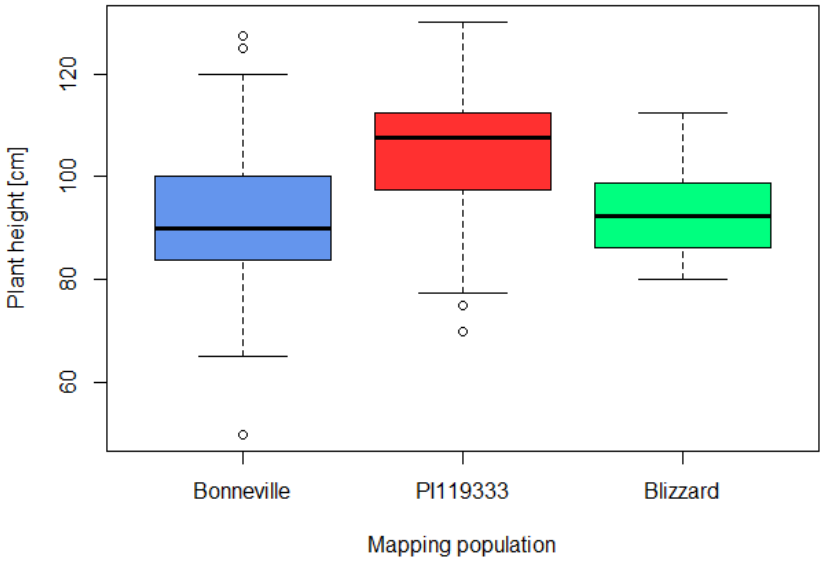

Figure 3-8 Boxplot for the trait plant height (PH) for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

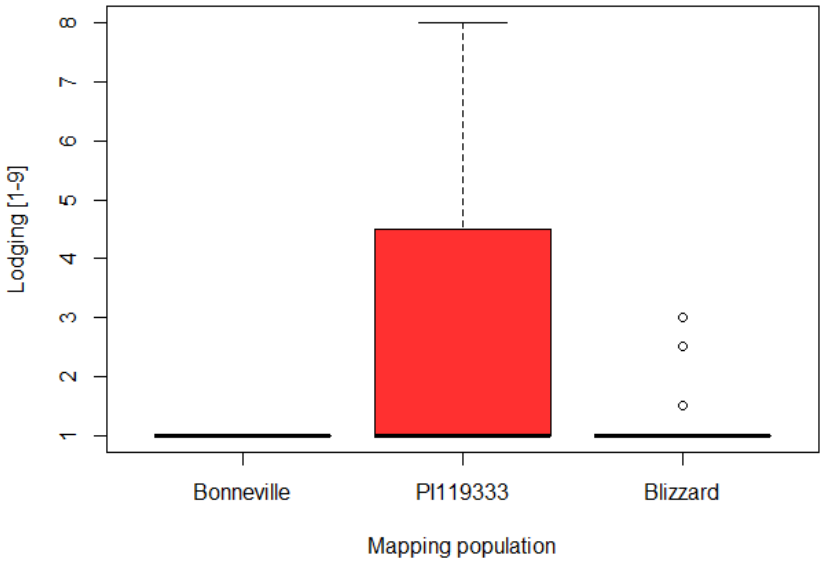

Figure 3-9 Boxplot for the trait lodging (LOD) for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

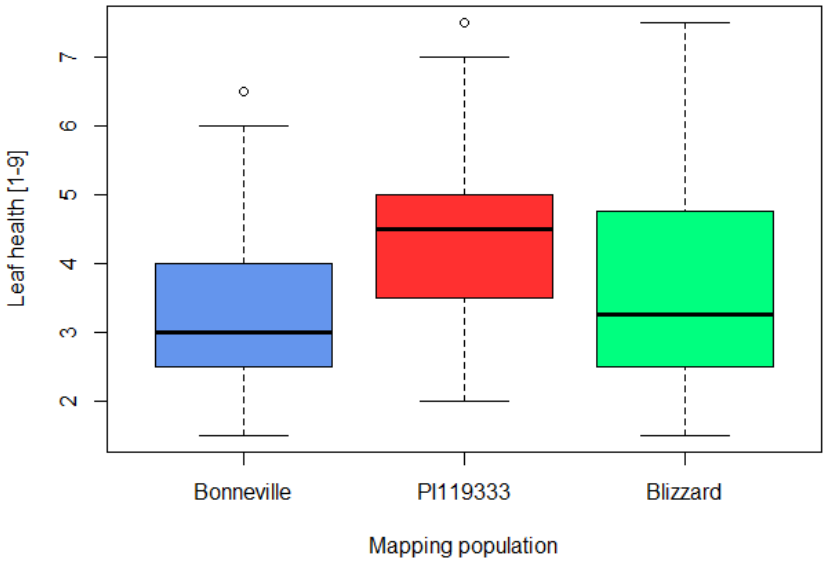

Figure 3-10 Boxplot for the trait leaf health (LH) for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

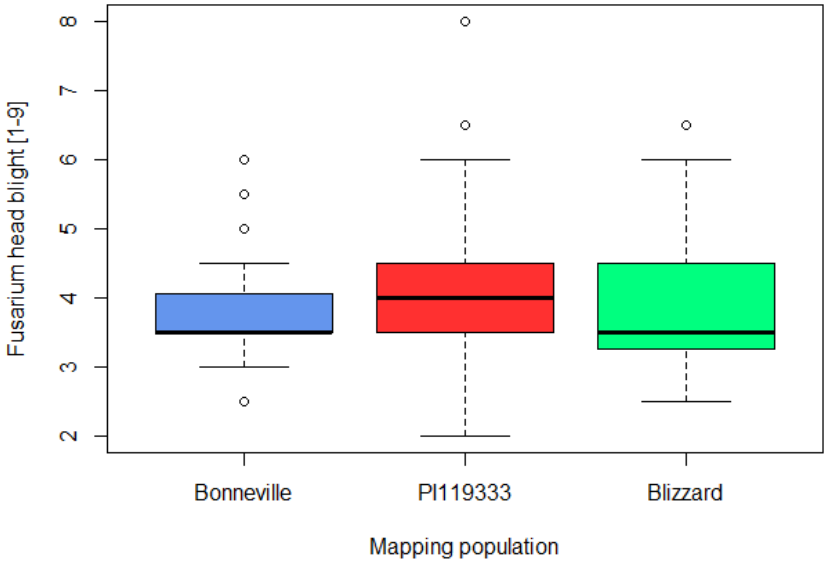

Figure 3-11 Boxplot for the trait fusarium head blight (FHB) for the mapping populations Bonneville, PI119333 and Blizzard; dots representing the outliers, bold horizontal lines representing the median

The heritabilities for the traits date of heading, plant height, awns and fusarium head blight are summarized in Table 3-9. For the date of heading it is similar in each population ranging from 0.74 to 0.80. For fusarium head blight the values were also similar with 0.63 to 0.71. For the plant height and awns the heritabilities varied more between the mapping populations with 0.60 to 0.89 (PH) and 0.54 to 0.79 (AW).

Table 3-9 Heritability (h^2) for date of heading (DH), plant height (PH), awns (AW) and fusarium head blight (FHB) of the mapping population Bonneville (Population 1), PI119333 (Population 2) and Blizzard (Population 3)

	h^2			
Population	**DH**	**PH**	**AW**	**FHB**
All populations	0.77	0.82	0.96	0.69
Population 1	0.77	0.89	0.95	0.63
Population 2	0.80	0.72	0.92	0.71
Population 3	0.74	0.60	0.83	0.63

3.4 Correlation between different traits

In each mapping population all different trait combinations were tested for correlations. The results are shown in Table 3-10, Table 3-11 and Table 3-12.

In general, the significant correlations ($p<0.05$) only have a low correlation coefficient. In all mapping populations the traits plant height and common bunt incidence show a slightly negative correlation (Population 1 r = -0.277, Population 2 r = -0.138, Population 3 r = -0.479). In population 2 the date of heading correlates with all other traits, but especially high with fusarium head blight (r = -0.689). A similar but not as high correlation between those two traits is also visible in Population 3 (r = -0.419). For population 2 also a high positive correlation between plant height and lodging is visible (r = 0.435). Lodging and fusarium head blight are correlated in Population 2 (r = 0.245) and Population 3 (r = 0.364). In Population 3 common bunt incidences are correlated with the date of heading (r = 0.394) and the general leaf health (r = 3.61).

Table 3-10 Pearson correlation coefficient (r) for all trait combinations for Population 1 Bonneville (CB = common bunt, DH = date of heading, PH = plant height, AW = awns, FHB = fusarium head blight, LH = leaf health)

	CB	**DH**	**PH**	**LOD**	**AW**	**FHB**	**LH**
CB		ns	-0.277*	ns	ns	ns	ns
DH			ns	ns	-0.220*	ns	-0.270*
PH				ns	ns	ns	ns
LOD					ns	ns	ns
AW						ns	0.283**
FHB							ns
LH							

Significant codes: <0.001 = ***, <0.01 = **, <0.05 = *, ns = no significant linear correlation

Table 3-11 Pearson correlation coefficient (r) for all trait combinations for Population 2 PI119333 (CB = common bunt, DH = date of heading, PH = plant height, AW = awns, FHB = fusarium head blight, LH = leaf health)

	CB	**DH**	**PH**	**LOD**	**AW**	**FHB**	**LH**
CB		ns	-0.138*	ns	ns	ns	0.145*
DH			-0.189**	-0.232***	-0.160*	-0.689***	-0.198**
PH				0.435***	ns	ns	ns
LOD					ns	0.245***	ns
AW						ns	ns
FHB							0.242***
LH							

Significant codes: <0.001 = ***, <0.01 = **, <0.05 = *, ns = no significant linear correlation

Table 3-12 Pearson correlation coefficient (r) for all trait combinations for Population 3 Blizzard (CB = common bunt, DH = date of heading, PH = plant height, AW = awns, FHB = fusarium head blight, LH = leaf health)

	CB	**DH**	**PH**	**LOD**	**AW**	**FHB**	**LH**
CB		0.394*	-0.479**	ns	ns	ns	0.361*
DH			-0.420*	ns	ns	-0.419*	ns
PH				ns	ns	ns	ns
LOD					ns	0.364*	ns
AW						ns	ns
FHB							ns
LH							

Significant codes: <0.001 = ***, <0.01 = **, <0.05 = *, ns = no significant linear correlation

3.5 Marker – trait association analysis

All three mapping populations were analyzed with the four SSR markers *Xgwm374, Xgwm264, Xbarc83* and *Xcfa2129*. Since the markers *Xgwm374* and *Xgwm264* did not show a polymorphism for population 2 (PI119333) the lines of this mapping population were not included in the genotypic analysis with these two markers. The genotypic scorings of the different markers for all RILs are shown in Appendix 38 for population 1 Bonneville, Appendix 39 for population 2 PI119333 and Appendix 40 for population 3 Blizzard. Frequency distributions of these markers for common bunt incidence is illustrated in Appendix 28 to Appendix 37.

3.5.1 Markers indicative for resistance genes on chromosome 1B

Both markers *Xgwm374* and *Xgwm264* were polymorphic for mapping populations Bonneville and Blizzard but not for PI119333. The analysis of variance showed that the marker *Xgwm374* is significantly ($p<0.001$) associated with common bunt in Population 1 Bonneville and explained 18.56% of the phenotypic variation of the disease resistance (Table 3-13). For population 3 Blizzard the coefficient of determination (R^2) was similar with 16.54%, although its effect was not statistically significant in this population ($p>0.05$) (Table 3-14), most likely due to the comparatively small sample size. The distribution for common bunt incidence of lines carrying the resistant or susceptible allele according to marker *Xgwm374* is graphically shown in Figure 3-14.

Marker *Xgwm264* was also significant for Population 1 Bonneville ($p<0.001$) but not for Population 3 Blizzard ($p>0.05$) (Table 3-15 and Table 3-16). According to the coefficient of determination this marker explained 16.63% of the phenotypic variance for common bunt in Population 1 Bonneville and 10.95% in Population 3 Blizzard. Figure 3-15 shows the distribution of lines carrying the resistant or susceptible allele according to marker *Xgwm264.*

An example of the scoring for markers *Xgwm364* and *Xgwm264* is shown in Figure 3-12 and Figure 3-13.

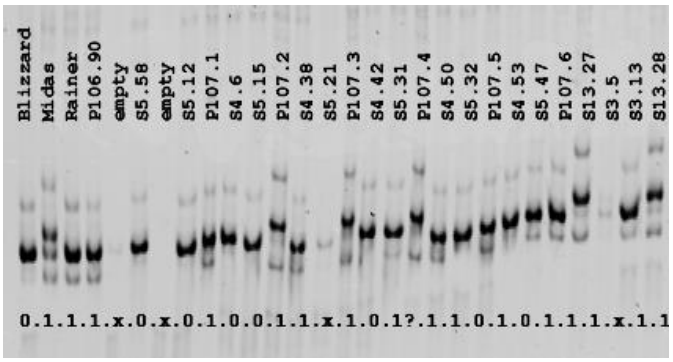

Figure 3-12 PCR-products amplified by marker Xgwm374 on a polyacrylamide gel (0 = resistant, 1 = susceptible, x = missing)

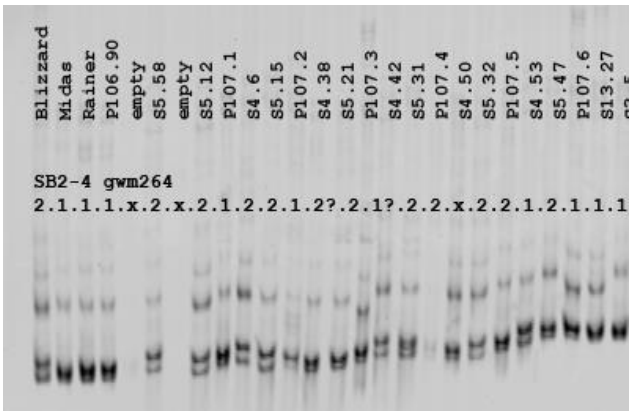

Figure 3-13 PCR-products amplified by marker Xgwm264 on a polyacrylamide gel (1 = resistant, 2 = susceptible, x = missing)

Table 3-13 Results analysis of variance and coefficient of determination (R^2) for marker Xgwm374 for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	6061.4	6061.4	16.177	0.0001422
Residuals	71	26603.5	374.7		
R^2	0.1856				

Table 3-14 Results analysis of variance and coefficient of determination (R^2) for marker Xgwm374 for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	1729.6	1729.56	2.9737	0.1052
Residuals	15	8724.4	581.63		
R^2	0.1654				

Table 3-15 Results analysis of variance and coefficient of determination (R^2) for marker Xgwm264 for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	6397	6396.6	15.759	0.000157
Residuals	79	32066	405.9		
R^2	0.1663				

Table 3-16 Results analysis of variance and coefficient of determination (R^2) for marker Xgwm264 for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	998.7	998.67	3.0743	0.09179
Residuals	25	8121.1	324.85		
R^2	0.1095				

Table 3-17 Number of lines per marker class (1 = resistant, 2 = susceptible, NA = not available) for the Populations Bonneville and Blizzard.

Markers	***Xgwm374***			***Xgwm264***		
Marker scoring	**1**	**2**	**NA**	**1**	**2**	**NA**
Population 1 (Bonneville)	30	47	14	33	52	6
Population 3 (Blizzard)	5	15	15	11	19	5

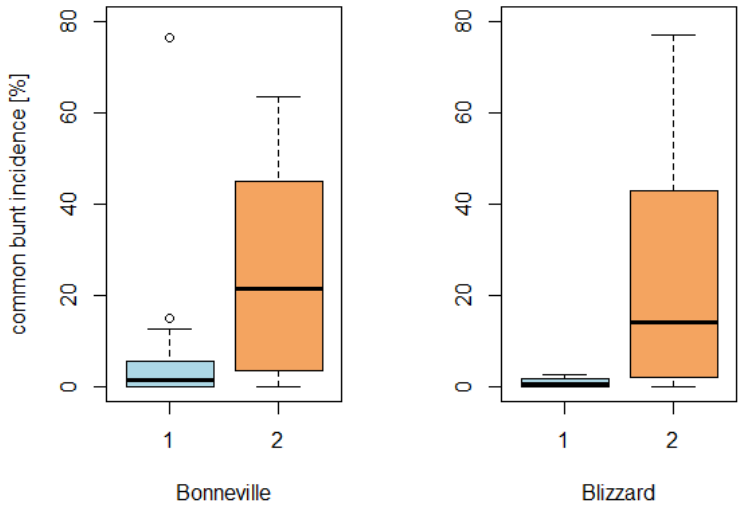

Figure 3-14 Boxplot of common bunt incidence (%) for marker Xgwm374 in population 1 Bonneville and population 3 Blizzard. Lines carrying the allele from the resistant parent are depicted in blue (1), lines carrying the allele from the susceptible parent in orange (2).

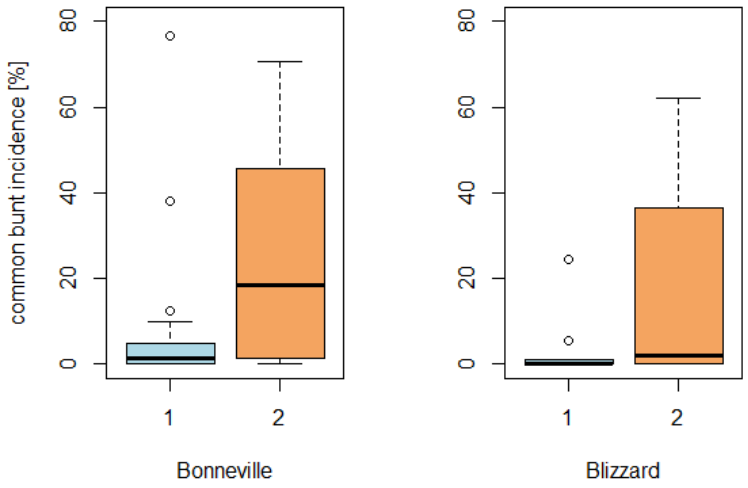

Figure 3-15 Boxplot of common bunt incidence (%) for marker Xgwm264 in population 1 Bonneville and population 3 Blizzard. Lines carrying the allele from the resistant parent are depicted in blue (1), lines carrying the allele from the susceptible parent in orange (2).

3.5.2 Markers indicative for resistance genes on chromosome 1A

The markers *Xbarc83* and *Xcfa2129* showed polymorphism in all three mapping populations. *Xbarc83* was significant for population 1 Bonneville and Population 3 Blizzard ($p<0.05$) and explained 9.79% and 15.87% of the phenotypic variance for common bunt. For Population 2 PI11933 the marker *Xbarc83* was not significantly different. The distribution of lines carrying the resistant or susceptible allele according to marker *Xbarc83* is shown graphically in Figure 3-18 for all three mapping populations.

Also, for marker *Xcfa2129* the analysis of variance showed a significance for mapping population 1 Bonneville and population 3 Blizzard ($p<0.05$) but not for population 2 PI119333 ($p>0.05$). Marker *Xcfa2129* explains 14.15% of the phenotypic variance for common bunt in population 1 Bonneville and 17.56% in population 3 Blizzard. The distribution of lines carrying the resistant or susceptible allele according to marker *Xcfa2129* is shown in Figure 3-19 for all three mapping populations.

An example of the scoring for markers *Xbarc83* and *Xcfa2129* is shown in Figure 3-16 and Figure 3-17.

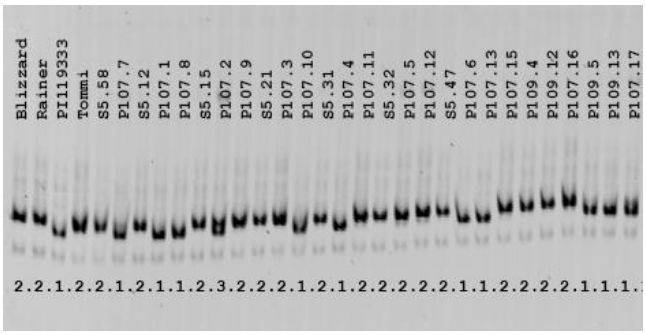

Figure 3-16 PCR-products amplified by marker Xbarc83 on a polyacrylamide gel (1 = resistant, 2 = susceptible, 3 = heterozygous)

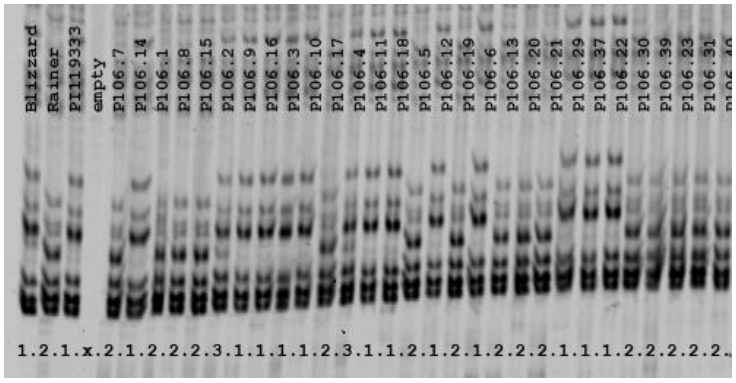

Figure 3-17 PCR-products amplified by marker Xcfa2129 on a polyacrylamide gel (1 = resistant, 2 = susceptible, 3 = heterozygous, x = missing)

Table 3-18 Results analysis of variance and coefficient of determination (R^2) for marker Xbarc83 for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	3571	3571.5	7.3779	0.008365
Residuals	68	32917	484.1		
R^2	0.09788				

Table 3-19 Results analysis of variance and coefficient of determination (R^2) for marker Xbarc83 for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	107	106.56	0.3966	0.5295
Residuals	211	56697	268.71		
R^2	0.001876				

Table 3-20 Results analysis of variance and coefficient of determination (R^2) for marker Xbarc83 for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	1895.2	1895.20	4.7154	0.03959
Residuals	25	10048	401.92		
R^2	0.1587				

Table 3-21 Results analysis of variance and coefficient of determination (R^2) for marker Xcfa2129 for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	5440	5440.1	12.856	0.0005843
Residuals	78	33005	423.1		
R^2	0.1415				

Table 3-22 Results analysis of variance and coefficient of determination (R^2) for marker Xcfa2129 for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	51	51.034	0.1965	0.6579
Residuals	228	59200	259.651		
R^2	0.00086				

Table 3-23 Results analysis of variance and coefficient of determination (R^2) for marker Xcfa2129 for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Marker	1	2390.9	2390.89	6.1789	0.01894
Residuals	29	11221.4	386.94		
R^2	0.1756				

Table 3-24 Number of lines per marker class (1 = resistant, 2 = susceptible, NA = not available) for the Populations Bonneville, PI119333 and Blizzard.

Markers	***Xbarc83***			***Xcfa2129***		
Marker scoring	**1**	**2**	**NA**	**1**	**2**	**NA**
Population 1 (Bonneville)	13	61	17	25	58	8
Population 2 (PI119333)	112	106	27	119	116	10
Population 3 (Blizzard)	11	19	5	15	19	1

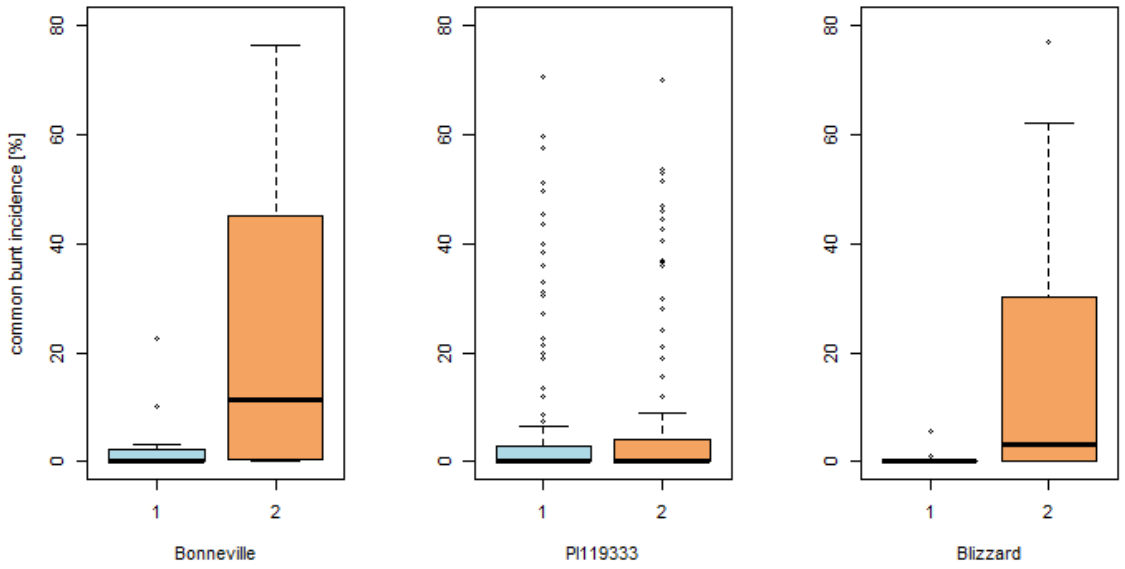

Figure 3-18 Boxplot of common bunt incidence (%) for marker Xbarc83 in population 1 Bonneville, population 2 PI119333 and population 3 Blizzard. Lines carrying the allele from the resistant parent are depicted in blue (1), lines carrying the allele from the susceptible parent in orange (2).

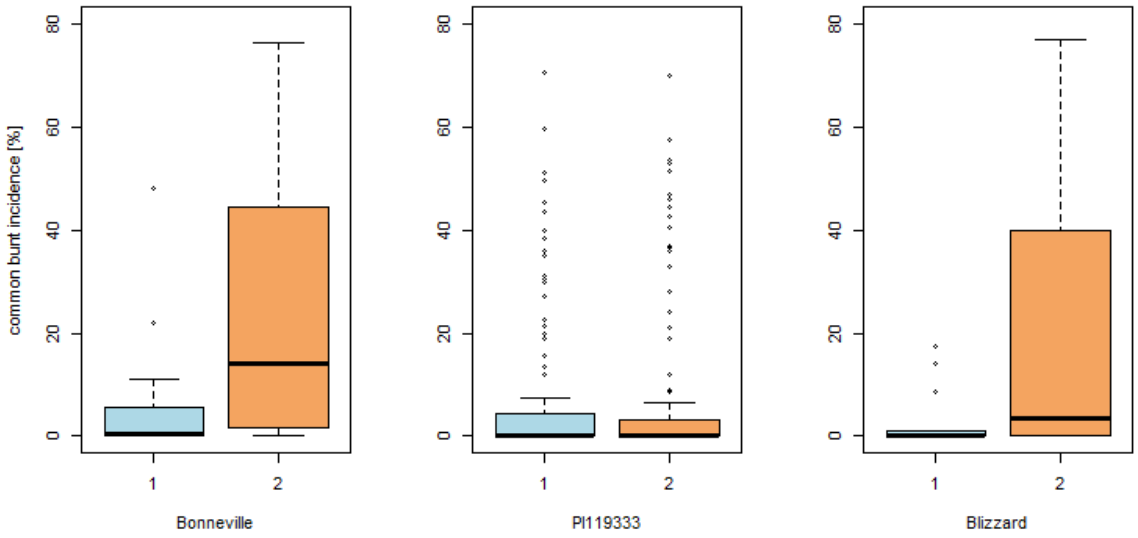

Figure 3-19 Boxplot of common bunt incidence (%) for marker Xcfa2129 in population 1 Bonneville, population 2 PI119333 and population 3 Blizzard. Lines carrying the allele from the resistant parent are depicted in blue (1), lines carrying the allele from the susceptible parent in orange (2).

4 Discussion

The aim of this master thesis was the evaluation of three different mapping populations with regard to their number of segregating resistance genes and the identification of potential breeding relevant lines. Multiple microsatellite markers were tested for their associations with common bunt resistance and their suitability for a marker-assisted selection.

Thirteen RIL populations were developed at the BOKU Department of Agrobiotechnology, Tulln, Austria, from crosses between three exotic resistant lines and five susceptible but in Austria adapted wheat cultivars. The half-sibs among the lines could be classified into three mapping populations according to their common bunt resistant parent. After artificial inoculation of seeds with common bunt teliospores the populations were phenotypically evaluated for their common bunt resistance as well as other agronomic traits. A marker-trait association analysis was performed with marker suggested to be associated with the resistance loci present in the parental lines. The applied markers *Xgwm374* and *Xgwm264* were identified by Wang et al. (2009) to be located on chromosome 1BS and reported to be closely linked to the resistance locus of the cultivar Blizzard. Furthermore, the markers *Xbarc83* and *Xcfa2129* were reported by Chen et al. (2016) to be located on chromosome 1A and also linked to a common bunt resistance locus.

4.1 Phenotyping of the mapping populations for common bunt resistance

The collected field data for common bunt infection was of good quality as seen by the high correlation between replication one and two as well as the high heritabilities. Heritabilities of 0.91 for population 1, 0.89 for population 2 and 0.94 for population 3 were estimated for common bunt incidence. Although it is difficult to evaluate common bunt disease incidence with high reproducibility (Fofana et al. 2007), the results of this study need to be validated in further trials since only one year and one location had been considered in the analysis and some studies noticed a strong genotype-by-environment interaction and emphasized the importance of multi-year testing (Gaudet and Puchalski 1989). These interactions can be ascribed to the annual weather and variable soil temperatures at the seeding date of different locations and years (Wächter et al. 2007). For a high germination rate of teliospores and thus an optimal infection, the soil temperature and moisture content are of particular importance (Matanguihan et al. 2011). In November 2017, the mean monthly temperature was 5.4°C and therefore just in the optimal range of 5°C to 10°C. For all the other evaluated traits beside common bunt incidence, lower heritablities were found: Medium values were found for plant height (0.60 to 0.89), date of heading (0.74 to 0.80) and fusarium head blight

(0.63 to 0.71). Lower heritabilities are probably caused by differences between replication 1 and 2. These can be due to environmental differences along the field test side, unequal disease spreading or different seeding dates.

The three exotic resistant lines used in this trial were Blizzard, PI119333 and Bonneville. These lines were previously described by Goates and Bockelman (2012) and Wang et al. (2009) as effective source of resistance against common bunt, but still possess poor agronomic characteristics under Central European conditions. Huber and Bürstmayr (2006) tested 98 winter wheat genotypes for their resistance against common bunt and dwarf bunt. Thereby Bonneville and PI119333 were identified as highly resistant against *T. caries,* and the underlying genes are promising candidates for introgression into adapted Austrian wheat germplasm. This observation could be verified in the study at hand, where the three mentioned lines showed good common bunt resistance: Bonneville was free of infection and PI119333 and Blizzard only showed a common bunt incidence of 0.5%. To integrate the resistance genes of Bonneville, PI119333 and Blizzard into adapted material, they were crossed with the Austrian wheat cultivars Rainer, Midas, Tommi, Pannonikus and the fusarium resistant pre-breeding line 20568.1.2. Tommi and 20568.1.2 showed low infection levels of 1.25% and 0%. For Tommi, the results are confirmed by the study of Huber and Bürstmayr (2006), who described this cultivar as resistant to *T.caries* whereas all other cultivars were highly susceptible to common bunt. Infection levels ranged from 64.5% for Pannonikus and 64.9% for Rainer, to 73.5% for Midas.

The average common bunt infection differed between the mapping populations: The highest mean was found for population 1 (Bonneville) with 17.51%, followed by population 3 (Blizzard) with 12.31% and the lowest average common bunt incidence of 7.37% was observed for population 2 (PI119333) with a large variation of infection levels within each of these populations. Looking at all populations, common bunt incidence ranged from 0% infection i.e. complete resistance, to high susceptibility with up to 86%. The lowest infection levels with <1% were found in the lines derived from the cross Bonneville/Rainer and Blizzard/Rainer, while lines generated from crosses with the cultivar Midas showed the highest common bunt incidence. The least significant difference to differentiate resistant from susceptible lines was estimated as 18.8%, 14.5% and 14.8% common bunt incidence for the mapping population Bonneville, PI119333 and Blizzard respectively i.e. lines with common bunt incidence above this value were classified as susceptible and the others as being resistant. Employing these thresholds, 67% of the genotypes were classified as

resistant in populations with the parent Bonneville and 75% in the population derived from Blizzard. The highest number of resistant lines was found in Population PI119333 with 83%.

To analyze the genetic basis of common bunt resistance the segregation patterns of the RILs were compared to expected segregation results using the Chi^2-test. It was tested for one, two or three independently segregating resistance genes. The distribution of the phenotypic values in the mapping populations with the resistant parent PI119333 and Bonneville indicated the presence of multiple genes. Hence different hypothesis concerning the segregation pattern were tested to clarify the underlying genetic architecture of common bunt resistance in these populations. In all populations, the ration of resistant to susceptible lines was close to 7:1, indicating the presence of three independent resistance genes. Only in the crosses Midas/Bonneville//Rainer and Rainer/Blizzard//Midas a segregation of 1:1 was observed indicating just one segregating gene. Wang et al. (2009) reported one major resistance gene in Blizzard which can express a complete qualitative type of resistance under optimal conditions, but under suboptimal conditions the resistance can appear as a continuously distributed quantitative trait. With the study at hand the presence of one resistance gene in the line Blizzard can be approved whereas for the lines Bonneville and PI119333 the presence of multiple resistance genes is verified.

4.2 Correlations

4.2.1 Correlation of common bunt incidence with other traits

Common bunt infection not only causes replacement of kernels by bunt balls but also affects agronomic traits of the wheat plants specifically infection with *T.caries* and *T.laevis* can lead to a slightly reduction in plant height (Goates 1996; Dumalasová and Bartos 2008; Gaudet et al. 1991). A negative correlation between common bunt incidence and plant height was accordingly found in all tested populations in this study. Gaudet et al. (1991) reported that plants with shorter culms were preferably infected by the bunt fungus, which seemed to be the major factor in the susceptibility of most cultivars. Ganeva et al. (2014) and Mourad et al. (2018) observed on the other hand a positive correlation between plant height and disease incidence. However, Singh et al. (2016) found two QTLs for plant height on chromosome 4B and 6D, which were co-located to common bunt resistance QTL and contribute to reduced plant height. In the present study in the mapping population derived from Blizzard a positive correlation between common bunt incidence and date of heading was observed, verifying the findings of Ganeva et al. (2014) and Mourad et al. (2018) who found that infected genotypes had significantly later heading dates. In population 2 and 3

common bunt incidence is positive correlated with leaf health, while for all other traits no significant correlation with the level of common bunt infection was found.

4.2.2 Correlation between other traits

A high positive correlation of r=0.435 was found between plant height and lodging in the population generated from PI119333 that generally comprised the tallest plants. This result is also confirmed by other studies showing that short plant are more tolerant to lodging (Navabi et al. 2006), which can though also vary among tall genotypes as shown by the study of Navabi et al. (2006). Since lodging often results in yield losses it is considered an important factor in many plant breeding programs (Tripathi et al. 2003), which has been addressed by the use of the semi-dwarfing *Rht* genes aside from the selection for other features like filled/hollow stems (Navabi et al. 2006). For the trait FHB incidence and date of heading a negative correlation was observed, which was especially high in population 2 with r=-0.689, on the contrary to other studies reporting less FHB infection in early-flowering lines. This deviation probably is caused by involved environmental effects and due to scoring of the trait FHB at the same day for all plants and thus leading to less infection for late-flowering lines where the infection is not yet that far evolved (Steiner et al. 2004). Furthermore, in population 2 and 3 plant height was negatively correlated with date of heading.

4.3 Genetic markers for common bunt resistance

Markers associated with common bunt resistance genes can speed up the development of resistant cultivars by marker-assisted selection among parents or in early generations of variety development (Matanguihan et al. 2011). To be feasible a marker used for marker-assisted selection should at least explain between 10% to 20% of phenotypic variance in a mapping population. A perfect marker would ideally have no recombination to the gene of interest (Miedaner and Korzun 2012). Four different microsatellite markers were tested in the study at hand, which were already reported to be associated with QTLs for common bunt resistance. Somers et al. (2004) mapped microsatellite markers from different research groups, creating a high density map of wheat containing over one thousand microsatellite loci. The markers *Xcfa2129* and *Xbarc83* were thereby mapped on chromosome 1A and markers *Xgwm374* and *Xgwm264* were mapped on chromosome arm 1BS (Somers et al. 2004). *Xgwm374* and *Xgwm264* were already further tested on progeny of the cultivar Blizzard and Wang et al. (2009) reported them to be significantly linked to the resistance locus regarding common bunt resistance. They calculated the distance between the bunt resistance locus and the overlapping markers with 3.9cM. Also markers *Xcfa2129* and

Xbarc83 were already mentioned by Chen et al. (2016) to be possibly associated with bunt resistance loci.

All four markers worked well on the RIL populations generated from Bonneville and Blizzard as the results show significant and polymorphic results for both populations. The makers *Xgwm374, Xgwm264, Xbarc83* and *Xcfa2129* explained 18.6%, 16.6%, 9.8% and 14.2% of the phenotypic variation for common bunt in the population Bonneville. These high values indicate that these markers are most likely tightly linked to a bunt resistance gene. Since all four markers, which are located on two different chromosomes, show association with the resistance loci present in Bonneville more than one gene must be govern the resistance in this population, which also agrees with the found segregation pattern. Thus, we can say that at least one resistance gene on chromosome 1A and one on 1B must be present.

Similar results were achieved for the population Blizzard. Makers *Xbarc83* and *Xcfa2129* explained 15.9% and 17.6%. Markers *Xgwm374* and *Xgwm264* who would explain 16.5% and 11% were not significant in this population probably due to the small sample size. Since the segregation pattern of the population Blizzard indicates a single resistance gene and only the two markers on chromosome 1A are significant, the presence of one common bunt resistance gene on 1A is probable.

Different results were obtained for the mapping population generated from PI119333. Marker *Xbarc83* and Xcfa2129 amplified polymorphic fragments for the RIL populations but the tested marker-trait associations were not significant in this population. Hence, PI119333 carries no resistance genes close to these loci and does not possess the same resistance as Bonneville and Blizzard.

5 Conclusion

The introgression of common bunt resistance genes into adapted wheat cultivars is of major importance for a sustainable and chemical free wheat cultivation. With this research we could show that by introgressing resistance genes from exotic lines into adapted wheat lines it is possible to generate novel and highly breeding relevant germplasm. The lines developed from the exotic resistant parental lines Bonneville, Blizzard and PI119333 were highly resistant against common bunt, and especially the lines descending from PI119333 comprise a high number of resistant genotypes (83%). The generated lines can now be used for further resistance breeding and for developing new adapted wheat cultivars for an organic cultivation in Austria. A significant correlation was found between common bunt incidence and plant height since the exotic resistant lines comprise very tall plants with a tendency for lodging. Hence, in further breeding the loss of these unwanted traits is necessary.

Furthermore, the major next step would be the identification of the causal genes which govern the resistance against common bunt. In the study at hand we can see that for the line Bonneville resistance loci on chromosome 1A and 1B are present and for Blizzard at least one resistance gene exists on chromosome 1A. These resistant genes are candidates for implementation in practical breeding programs in Austria. The segregation pattern also indicates the presence of multiple resistance genes in the line PI119333 but they are located in a different chromosomal region than in Bonneville and Blizzard.

The markers *Xgwm374, Xgwm264, Xbarc83* and *Xcfa2129* can be associated with the resistance loci present in Bonneville and they explain up to 19% of the phenotypic variance for common bunt incidence. For Blizzard the markers *Xbarc83* and *Xcfa2129* were significantly linked to the resistance loci, hence they can be further used for detection of resistant lines. In future the more precisely mapping and the finding of appropriate and closer markers for the genes of interest will be very important for an efficient identification of resistant cultivars. Nowadays microsatellite markers are going to be replaced by KASP markers and thus the development of such for the common bunt resistance loci would be recommended since they are more user-friendly with a higher throughput.

Although this study was only conducted in one year and on one location and thus needs to be validated in further trials, it shows promising results in combating plant diseases in accordance with organic farming guidelines.

6 List of Tables

7 List of Figures

8 References

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9 Appendix

Appendix 1 Pearson's correlation coefficient (r) and p-value for correlation of replication 1 and replication 2 for the traits common bunt incidence (CB), date of heading (DH), lodging (LOD), plant height (PH), awns (AW), fusarium head blight (FHB) and leaf health (LH) for the mapping populations Bonneville, PI119333 and Blizzard

	Trait	r	p-value
Population 1 Bonneville	CB	0.834	2.3e-23
	DH	0.726	2.7e-14
	LOD	1	0
	PH	0.790	1.5e-19
	AW	0.915	6.2e-35
	FHB	0.463	7.2e-06
	LH	0.032	0.7694
Population 2 PI119333	CB	0.809	1.3e-62
	DH	0.732	5.6e-45
	LOD	0.707	2.2e-41
	PH	0.574	1.4e-24
	AW	0.849	8.7e-75
	FHB	0.556	6.3e-23
	LH	0.089	0.1482
Population 3 Blizzard	CB	0.906	1.1e-12
	DH	0.702	7.7e-06
	LOD	1	0
	PH	0.430	0.0141
	AW	0.717	3.8e-06
	FHB	0.400	0.0235
	LH	0.340	0.0567

Appendix 2 General statistic distribution parameters for common bunt incidence (%) for the parental lines Bonneville, PI119333, Rainer, Midas, Tommi, 20568.1.2 and Pannonikus

Line	Mean	Minimum	Maximum	Median
Bonneville (n=2)	0.00	0.00	0.00	0.00
PI119333 (n=4)	0.50	0.00	2.00	0.00
Blizzard (n=2)	0.50	0.00	1.00	0.50
Rainer (n=12)	64.92	49.00	97.00	59.00
Midas (n=10)	73.50	49.00	91.00	76.00
Tommi (n=4)	1.25	0.00	3.00	1.00
20568.1.2 (n=2)	0.00	0.00	0.00	0.00
Pannonikus (n=4)	64.50	58.00	74.00	63.00

9.1 Least square means for parental lines and mapping populations

Appendix 3 Least square means of Population 1 Bonneville for common bunt incidence (%) (CB), date of heading (DH), lodging (LOD), plant height (PH), awns (AW), fusarium head blight (FHB) and leaf health (LH)

Line	Cross	DH	LOD	PH	AW	FHB	LH	CB
20568.1.2		24.50	1.00	80.00	0.50	3.00	3.00	0.00
Bonneville		31.50	1.00	110.00	1.00	3.00	6.00	0.00
Midas		31.10	1.00	80.50	0.80	4.30	3.40	73.50
Rainer		30.00	1.00	82.50	0.00	4.75	4.17	64.92
P101.111	Bonneville/Rainer	29.00	1.00	97.50	0.00	6.00	3.00	0.00
P101.30	Bonneville/Rainer	28.25	1.00	105.00	1.00	3.25	3.50	0.00
P101.31	Bonneville/Rainer	27.00	1.00	105.00	1.00	6.00	5.00	0.00
P101.34	Bonneville/Rainer	34.00	1.00	125.00	0.50	3.00	4.00	0.00
P101.53	Bonneville/Rainer	29.00	1.00	100.00	1.00	4.50	4.50	0.00
P101.65	Bonneville/Rainer	35.50	1.00	110.00	0.00	4.00	2.50	0.00
P101.8	Bonneville/Rainer	31.00	1.00	100.00	1.00	3.50	4.00	0.00
P101.81	Bonneville/Rainer	32.15	1.00	92.50	0.00	3.50	3.50	0.50
P101.83	Bonneville/Rainer	31.50	1.00	95.00	0.25	5.00	2.00	0.00
P101.87	Bonneville/Rainer	28.50	1.00	127.50	0.75	3.00	4.00	10.00
S1.1	Rainer/Bonneville//20568.1.2	23.00	1.00	95.00	1.00	3.00	3.00	3.00
S1.10	Rainer/Bonneville//20568.1.2	24.00	1.00	77.50	1.00	3.50	4.00	9.50
S1.11	Rainer/Bonneville//20568.1.2	21.00	1.00	67.50	1.00	4.50	6.50	14.00
S1.12	Rainer/Bonneville//20568.1.2	29.00	1.00	70.00	0.50	3.00	5.00	11.50
S1.13	Rainer/Bonneville//20568.1.2	36.15	1.00	85.00	0.25	4.00	3.00	50.00
S1.14	Rainer/Bonneville//20568.1.2	33.00	1.00	95.00	1.00	4.00	3.00	12.50
S1.15	Rainer/Bonneville//20568.1.2	33.00	1.00	117.50	1.00	3.00	2.50	1.50
S1.16	Rainer/Bonneville//20568.1.2	28.50	1.00	102.50	1.00	3.00	4.50	0.00
S1.17	Rainer/Bonneville//20568.1.2	30.50	1.00	82.50	1.00	5.00	3.00	0.00
S1.18	Rainer/Bonneville//20568.1.2	21.00	1.00	120.00	0.00	4.00	4.50	0.00
S1.19	Rainer/Bonneville//20568.1.2	22.50	1.00	82.50	0.50	3.00	2.50	0.50
S1.2	Rainer/Bonneville//20568.1.2	22.00	1.00	75.00	1.00	4.50	5.50	0.00
S1.20	Rainer/Bonneville//20568.1.2	36.00	1.00	85.00	0.50	4.50	4.00	15.00
S1.21	Rainer/Bonneville//20568.1.2	36.15	1.00	90.00	1.00	3.00	2.50	7.00
S1.22	Rainer/Bonneville//20568.1.2	25.00	1.00	90.00	1.00	3.50	3.50	76.50
S1.23	Rainer/Bonneville//20568.1.2	27.50	1.00	82.50	1.00	5.50	6.00	4.00
S1.24	Rainer/Bonneville//20568.1.2	28.00	1.00	85.00	1.00	4.50	4.00	0.00
S1.25	Rainer/Bonneville//20568.1.2	33.00	1.00	90.00	0.00	5.00	5.50	38.00
S1.26	Rainer/Bonneville//20568.1.2	28.50	1.00	90.00	1.00	3.50	3.00	1.00
S1.27	Rainer/Bonneville//20568.1.2	35.00	1.00	90.00	0.00	4.50	3.00	4.00
S1.28	Rainer/Bonneville//20568.1.2	25.50	1.00	87.50	1.00	3.00	5.00	35.50
S1.3	Rainer/Bonneville//20568.1.2	37.00	1.00	95.00	1.00	4.00	4.00	5.50
S1.4	Rainer/Bonneville//20568.1.2	32.50	1.00	100.00	1.00	3.50	2.50	7.50
S1.5	Rainer/Bonneville//20568.1.2	29.00	1.00	105.00	0.50	3.50	3.00	18.50
S1.6	Rainer/Bonneville//20568.1.2	32.50	1.00	90.00	0.50	4.00	3.00	33.00
S1.7	Rainer/Bonneville//20568.1.2	29.50	1.00	107.50	1.00	4.00	3.50	15.00
S1.8	Rainer/Bonneville//20568.1.2	32.00	1.00	97.50	1.00	3.50	2.50	7.50
S1.9	Rainer/Bonneville//20568.1.2	30.00	1.00	90.00	0.00	3.50	4.50	1.00

S2.1	Midas/Bonneville//Rainer	28.00	1.00	110.00	0.00	4.00	2.50	3.00
S2.10	Midas/Bonneville//Rainer	31.00	1.00	82.50	0.50	4.50	3.00	44.00
S2.11	Midas/Bonneville//Rainer	29.00	1.00	92.50	0.00	3.50	1.50	1.50
S2.12	Midas/Bonneville//Rainer	34.00	1.00	97.50	1.00	4.00	5.00	0.00
S2.13	Midas/Bonneville//Rainer	29.00	1.00	102.50	1.00	2.50	1.50	0.00
S2.14	Midas/Bonneville//Rainer	33.00	1.00	85.00	0.00	4.00	2.50	0.00
S2.15	Midas/Bonneville//Rainer	27.50	1.00	75.00	1.00	2.50	3.00	24.00
S2.16	Midas/Bonneville//Rainer	32.50	1.00	92.50	1.00	4.50	2.00	39.50
S2.17	Midas/Bonneville//Rainer	31.00	1.00	82.50	0.00	3.50	1.50	8.50
S2.18	Midas/Bonneville//Rainer	33.00	1.00	85.00	0.50	3.50	1.50	0.00
S2.2	Midas/Bonneville//Rainer	25.50	1.00	97.50	1.00	3.00	2.00	46.00
S2.20	Midas/Bonneville//Rainer	32.50	1.00	87.50	1.00	4.50	2.50	63.00
S2.21	Midas/Bonneville//Rainer	37.00	1.00	92.50	1.00	3.50	3.00	45.00
S2.22	Midas/Bonneville//Rainer	29.50	1.00	90.00	1.00	4.00	3.00	22.50
S2.23	Midas/Bonneville//Rainer	29.50	1.00	100.00	0.50	4.50	2.50	18.50
S2.24	Midas/Bonneville//Rainer	36.50	1.00	87.50	0.50	3.50	2.00	3.50
S2.25	Midas/Bonneville//Rainer	37.00	1.00	77.50	0.00	4.00	2.00	3.50
S2.26	Midas/Bonneville//Rainer	29.00	1.00	90.00	0.25	3.50	5.00	0.50
S2.27	Midas/Bonneville//Rainer	29.50	1.00	75.00	1.00	3.50	2.50	22.00
S2.28	Midas/Bonneville//Rainer	32.00	1.00	97.50	0.00	4.00	3.00	3.50
S2.29	Midas/Bonneville//Rainer	37.00	1.00	92.50	0.00	3.50	4.00	2.00
S2.3	Midas/Bonneville//Rainer	30.50	1.00	90.00	0.00	3.50	3.00	21.50
S2.30	Midas/Bonneville//Rainer	30.50	1.00	102.50	0.00	3.00	2.50	70.50
S2.31	Midas/Bonneville//Rainer	33.00	1.00	82.50	1.00	3.50	2.00	48.00
S2.32	Midas/Bonneville//Rainer	37.50	1.00	85.00	0.50	4.00	2.50	63.50
S2.33	Midas/Bonneville//Rainer	36.15	1.00	97.50	0.00	3.00	2.50	0.50
S2.34	Midas/Bonneville//Rainer	31.00	1.00	70.00	1.00	4.00	4.00	60.00
S2.35	Midas/Bonneville//Rainer	41.85	1.00	90.00	1.00	2.50	6.00	3.50
S2.36	Midas/Bonneville//Rainer	33.50	1.00	65.00	1.00	3.00	4.00	42.50
S2.37	Midas/Bonneville//Rainer	34.00	1.00	92.50	0.00	3.50	1.50	52.00
S2.38	Midas/Bonneville//Rainer	35.00	1.00	95.00	0.00	4.00	2.00	34.50
S2.4	Midas/Bonneville//Rainer	32.50	1.00	85.00	0.50	2.50	2.50	51.50
S2.40	Midas/Bonneville//Rainer	28.50	1.00	87.50	1.00	3.50	6.00	56.50
S2.42	Midas/Bonneville//Rainer	37.00	1.00	80.00	1.00	3.50	2.50	1.50
S2.43	Midas/Bonneville//Rainer	35.50	1.00	97.50	0.50	3.00	2.50	29.00
S2.44	Midas/Bonneville//Rainer	36.15	1.00	80.00	0.00	4.50	2.50	56.50
S2.45	Midas/Bonneville//Rainer	29.50	1.00	49.70	0.01	4.06	2.93	26.96
S2.46	Midas/Bonneville//Rainer	30.00	1.00	80.00	0.00	4.00	2.50	1.50
S2.47	Midas/Bonneville//Rainer	33.00	1.00	105.00	0.00	4.00	2.00	2.50
S2.5	Midas/Bonneville//Rainer	29.50	1.00	75.00	1.00	3.00	4.00	1.50
S2.6	Midas/Bonneville//Rainer	33.00	1.00	80.00	1.00	4.00	4.00	45.50
S2.7	Midas/Bonneville//Rainer	29.50	1.00	92.50	0.00	4.50	2.50	59.00
S2.8	Midas/Bonneville//Rainer	30.00	1.00	107.50	1.00	4.00	3.50	0.00
S2.9	Midas/Bonneville//Rainer	31.50	1.00	105.00	0.00	3.50	5.50	11.00
S7.20	Bonneville/Rainer	28.50	1.00	105.00	1.00	4.50	3.50	0.00
S7.4	Bonneville/Rainer	38.38	1.00	105.00	1.00	4.50	4.00	0.00

Appendix 4 Least square means of Population 2 PI119333 for common bunt incidence (%) (CB), date of heading (DH), lodging (LOD), plant height (PH), awns (AW), fusarium head blight (FHB) and leaf health (LH)

Line	Cross	DH	LOD	PH	AW	FHB	LH	CB
Midas		31.10	1.00	80.50	0.80	4.30	3.40	73.50
Pannonikus		31.75	1.00	82.50	0.75	3.25	3.25	64.50
PI119333		31.67	3.50	116.25	0.50	4.50	4.75	0.50
Rainer		30.00	1.00	82.50	0.00	4.75	4.17	64.92
Tommi			1.00	76.25	0.00	2.50	2.25	1.25
P106.1	PI119333/Rainer	28.50	2.00	105.00	0.00	5.00	3.50	0.00
P106.10	PI119333/Rainer	27.00	1.00	107.50	1.00	4.50	3.50	7.50
P106.11	PI119333/Rainer	30.50	4.00	97.50	1.00	6.00	6.00	0.00
P106.12	PI119333/Rainer	27.50	7.50	105.00	1.00	4.00	2.50	0.00
P106.13	PI119333/Rainer	34.00	2.50	102.50	1.00	6.00	3.50	0.00
P106.14	PI119333/Rainer	27.50	5.00	95.00	1.00	4.00	5.50	20.00
P106.15	PI119333/Rainer	30.00	4.50	112.50	1.00	5.00	5.50	0.00
P106.16	PI119333/Rainer	28.50	6.00	111.25	0.00	4.25	3.50	0.00
P106.17	PI119333/Rainer	28.50	1.00	102.50	0.00	5.00	6.00	0.00
P106.18	PI119333/Rainer	28.00	5.00	107.50	0.00	5.50	5.00	0.00
P106.19	PI119333/Rainer	28.50	8.00	102.50	1.00	4.50	4.50	0.00
P106.2	PI119333/Rainer	34.00	2.50	97.50	0.75	4.00	3.50	2.50
P106.20	PI119333/Rainer	28.00	2.00	110.00	0.00	4.50	4.00	44.50
P106.21	PI119333/Rainer	28.50	7.00	107.50	1.00	5.00	4.00	1.00
P106.22	PI119333/Rainer	27.50	2.00	105.00	1.00	5.50	4.00	0.00
P106.23	PI119333/Rainer	29.50	1.00	97.50	0.00	5.50	4.50	0.00
P106.24	PI119333/Rainer	28.00	6.25	115.00	1.00	4.25	5.75	0.25
P106.25	PI119333/Rainer	30.50	5.00	117.50	1.00	4.50	4.00	12.00
P106.26	PI119333/Rainer	25.00	1.00	80.00	1.00	4.00	4.00	0.00
P106.27	PI119333/Rainer	27.50	6.50	110.00	1.00	6.50	4.50	0.00
P106.28	PI119333/Rainer	30.00	5.50	123.75	0.75	5.50	2.75	0.00
P106.29	PI119333/Rainer	28.00	1.00	112.50	0.50	5.00	4.00	1.50
P106.3	PI119333/Rainer	27.50	1.00	107.50	1.00	5.00	4.00	0.00
P106.30	PI119333/Rainer	25.00	1.00	112.50	0.25	4.75	6.00	0.00
P106.31	PI119333/Rainer	32.00	5.50	105.00	0.00	8.00	5.50	8.50
P106.32	PI119333/Rainer	29.00	5.00	107.50	0.00	5.00	6.50	46.00
P106.33	PI119333/Rainer	27.00	4.50	127.50	1.00	5.00	3.50	13.50
P106.34	PI119333/Rainer	31.50	1.00	125.00	0.00	5.00	6.50	0.00
P106.35	PI119333/Rainer	31.00	5.50	112.50	0.50	4.50	5.50	45.50
P106.36	PI119333/Rainer	28.50	7.00	107.50	1.00	4.00	4.50	0.00
P106.37	PI119333/Rainer	28.50	7.00	130.00	1.00	5.00	3.50	0.00
P106.39	PI119333/Rainer	28.50	7.00	100.00	0.00	4.00	5.00	0.00
P106.4	PI119333/Rainer	34.00	7.50	102.50	1.00	5.50	5.00	0.00
P106.40	PI119333/Rainer	36.00	6.50	120.00	0.00	3.50	6.00	0.50
P106.41	PI119333/Rainer	29.50	7.00	110.00	0.00	4.00	6.50	37.00
P106.42	PI119333/Rainer	28.00	4.50	105.00	0.00	4.50	3.50	1.50
P106.43	PI119333/Rainer	29.50	3.00	100.00	0.00	4.50	5.00	3.00
P106.44	PI119333/Rainer	28.50	6.00	105.00	0.50	5.50	5.00	22.50
P106.45	PI119333/Rainer	31.00	1.00	97.50	1.00	6.50	3.00	40.50
P106.46	PI119333/Rainer	25.00	5.00	112.50	1.00	4.00	3.50	27.00

P106.48	PI119333/Rainer	28.50	1.00	97.50	1.00	6.50	3.50	4.50
P106.49	PI119333/Rainer	33.50	1.00	107.50	1.00	4.00	5.00	0.00
P106.5	PI119333/Rainer	29.50	1.00	122.50	0.00	3.50	4.00	1.50
P106.50	PI119333/Rainer	26.75	3.50	107.50	0.50	4.00	4.00	0.00
P106.51	PI119333/Rainer	33.50	5.00	113.75	1.00	5.50	3.75	0.00
P106.52	PI119333/Rainer	30.00	1.50	107.50	0.50	3.50	4.50	3.00
P106.53	PI119333/Rainer	28.50	1.00	110.00	0.50	4.50	5.50	0.00
P106.54	PI119333/Rainer	36.00	1.00	105.00	1.00	4.50	5.50	0.00
P106.55	PI119333/Rainer	28.50	3.50	122.50	0.50	5.00	3.00	0.00
P106.56	PI119333/Rainer	32.15	2.50	110.00	0.00	4.50	4.50	0.00
P106.57	PI119333/Rainer	30.00	3.00	107.50	1.00	3.00	3.00	0.00
P106.58	PI119333/Rainer	28.50	6.50	112.50	0.50	4.50	4.00	0.00
P106.59	PI119333/Rainer	28.00	5.50	105.00	0.75	4.50	6.50	36.00
P106.6	PI119333/Rainer	28.50	6.50	97.50	1.00	5.00	3.50	1.50
P106.60	PI119333/Rainer	37.50	1.00	102.50	0.00	5.00	5.00	0.50
P106.61	PI119333/Rainer	25.50	1.00	120.00	0.50	4.00	4.50	1.00
P106.62	PI119333/Rainer	31.50	1.00	100.00	1.00	6.00	3.50	0.00
P106.63	PI119333/Rainer	34.00	6.00	120.00	1.00	4.00	5.50	0.00
P106.64	PI119333/Rainer	29.00	4.50	120.00	1.00	3.50	6.00	49.50
P106.65	PI119333/Rainer	28.00	7.50	115.00	1.00	6.00	3.50	0.00
P106.66	PI119333/Rainer	28.50	1.00	102.50	0.50	5.50	4.00	31.00
P106.67	PI119333/Rainer	29.50	5.50	110.00	0.00	4.50	3.50	0.00
P106.68	PI119333/Rainer	26.25	1.00	107.50	0.00	4.00	3.50	0.00
P106.69	PI119333/Rainer	27.50	1.00	116.25	0.00	5.00	4.50	0.00
P106.7	PI119333/Rainer	29.15	4.50	118.75	0.00	4.50	3.50	0.00
P106.70	PI119333/Rainer	28.00	6.50	100.00	0.50	5.00	4.93	2.00
P106.71	PI119333/Rainer	28.00	3.50	105.00	1.00	4.00	4.00	0.00
P106.72	PI119333/Rainer	31.75	1.00	105.00	0.50	4.50	5.00	0.00
P106.73	PI119333/Rainer	30.50	1.00	95.00	0.00	3.75	2.50	0.00
P106.74	PI119333/Rainer	28.00	1.00	110.00	1.00	5.00	6.00	0.00
P106.75	PI119333/Rainer	28.50	5.00	110.00	0.00	6.00	7.00	36.50
P106.76	PI119333/Rainer	29.50	1.00	105.00	1.00	4.50	6.00	40.00
P106.77	PI119333/Rainer	31.00	3.50	107.50	0.00	4.50	3.50	0.00
P106.78	PI119333/Rainer	30.00	2.50	122.50	0.00	3.50	3.50	0.00
P106.79	PI119333/Rainer	35.00	2.50	110.00	0.00	4.50	5.00	0.00
P106.8	PI119333/Rainer	37.00	3.50	117.50	0.00	4.00	5.00	21.00
P106.80	PI119333/Rainer	34.50	1.00	100.00	0.00	3.50	3.50	0.50
P106.81	PI119333/Rainer	27.50	5.50	111.25	0.25	3.75	4.00	0.00
P106.82	PI119333/Rainer	27.50	7.50	120.00	0.00	5.50	4.50	3.50
P106.83	PI119333/Rainer	31.00	2.50	110.00	0.00	6.50	7.50	0.00
P106.84	PI119333/Rainer	28.00	6.50	110.00	1.00	3.50	5.00	5.50
P106.85	PI119333/Rainer	29.00	1.00	110.00	1.00	5.50	5.00	0.00
P106.86	PI119333/Rainer	29.00	6.00	125.00	1.00	4.00	4.00	3.50
P106.87	PI119333/Rainer	28.50	3.00	105.00	1.00	4.00	5.00	0.00
P106.9	PI119333/Rainer	34.50	6.00	127.50	1.00	5.00	4.00	5.50
P106.90	PI119333/Rainer	34.00	6.00	117.50	0.00	3.50	3.00	15.50
P107.1	PI119333/Tommi	40.50	1.00	70.00	0.50	4.00	3.50	21.50
P107.10	PI119333/Tommi	36.00	1.00	87.50	1.00	3.00	3.00	0.00
P107.11	PI119333/Tommi	37.00	1.00	120.00	1.00	2.50	2.50	0.50

P107.12	PI119333/Tommi	29.50	3.50	110.00	1.00	3.50	5.00	0.00
P107.13	PI119333/Tommi	33.00	1.00	92.50	1.00	4.00	4.50	1.50
P107.15	PI119333/Tommi	31.50	2.50	95.00	0.00	4.00	4.00	28.00
P107.16	PI119333/Tommi	34.00	1.00	117.50	1.00	3.50	4.50	0.00
P107.17	PI119333/Tommi	32.00	1.00	115.00	1.00	3.00	5.50	0.00
P107.18	PI119333/Tommi	33.50	2.50	75.00	0.50	4.00	3.00	0.00
P107.19	PI119333/Tommi	33.00	1.00	107.50	0.50	3.50	3.50	1.50
P107.2	PI119333/Tommi	36.50	1.00	82.50	1.00	3.50	4.00	35.00
P107.20	PI119333/Tommi	28.50	3.50	122.50	0.00	3.00	5.00	0.00
P107.3	PI119333/Tommi	33.50	1.00	92.50	0.50	3.00	4.00	0.50
P107.4	PI119333/Tommi	34.00	1.00	107.50	0.00	4.00	4.50	0.00
P107.5	PI119333/Tommi	28.50	1.00	102.50	0.00	3.50	2.50	19.00
P107.6	PI119333/Tommi	28.50	1.00	95.00	1.00	4.50	4.00	0.00
P107.7	PI119333/Tommi	31.50	1.00	110.00	1.00	6.00	5.00	0.00
P107.8	PI119333/Tommi	32.00	2.00	82.50	1.00	3.00	5.00	0.00
P107.9	PI119333/Tommi	29.50	1.00	105.00	0.00	4.50	4.50	0.00
P109.1	PI119333/Tommi	35.50	4.50	112.50	1.00	4.00	7.00	0.00
P109.10	PI119333/Tommi	28.50	1.00	95.00	1.00	3.00	3.00	0.00
P109.11	PI119333/Tommi	31.00	1.00	102.50	1.00	4.00	5.00	85.50
P109.12	PI119333/Tommi	28.00	1.00	92.50	1.00	3.50	3.00	0.00
P109.13	PI119333/Tommi	31.00	1.00	82.50	1.00	4.00	4.00	1.50
P109.14	PI119333/Tommi	31.50	3.50	117.50	0.75	4.50	5.00	0.00
P109.15	PI119333/Tommi	30.50	1.00	92.50	1.00	4.00	5.00	0.00
P109.16	PI119333/Tommi	27.15	1.00	110.00	1.00	3.50	4.50	0.00
P109.17	PI119333/Tommi	35.00	6.50	110.00	1.00	5.00	5.50	0.50
P109.18	PI119333/Tommi	38.00	4.50	105.00	0.50	4.00	4.00	47.00
P109.19	PI119333/Tommi	28.50	1.00	97.50	0.50	3.50	5.50	53.50
P109.2	PI119333/Tommi	32.00	1.00	97.50	1.00	4.00	3.50	0.00
P109.20	PI119333/Tommi	31.50	2.00	87.50	1.00	4.00	6.00	36.00
P109.21	PI119333/Tommi	30.00	1.00	97.50	1.00	4.00	4.50	0.00
P109.22	PI119333/Tommi	25.00	1.00	100.00	1.00	4.00	4.50	0.00
P109.23	PI119333/Tommi	30.50	1.00	110.00	1.00	4.50	4.50	4.50
P109.24	PI119333/Tommi	31.50	2.50	107.50	1.00	4.00	4.50	0.50
P109.25	PI119333/Tommi	27.50	1.00	115.00	1.00	4.00	4.50	2.50
P109.26	PI119333/Tommi	25.50	1.00	92.50	0.00	5.00	7.00	33.00
P109.27	PI119333/Tommi	30.00	1.50	100.00	1.00	5.00	5.50	0.50
P109.29	PI119333/Tommi	30.50	1.00	92.50	1.00	3.50	3.50	0.00
P109.4	PI119333/Tommi	32.50	1.00	95.00	1.00	4.50	3.00	42.50
P109.5	PI119333/Tommi	35.50	1.00	95.00	0.00	3.00	4.50	3.00
P109.6	PI119333/Tommi	30.00	6.00	97.50	1.00	3.50	4.00	0.00
P109.7	PI119333/Tommi	31.50	1.00	97.50	1.00	3.50	5.50	2.50
P109.8	PI119333/Tommi	31.00	1.00	105.00	1.00	4.00	4.00	19.00
P109.9	PI119333/Tommi	30.75	1.00	85.00	1.00	4.50	5.00	0.50
S12.1	PI119333/Pannonikus	27.50	1.00	107.50	1.00	4.00	3.00	1.50
S12.10	PI119333/Pannonikus	29.00	1.00	90.00	1.00	6.00	5.50	0.00
S12.11	PI119333/Pannonikus	33.00	5.50	117.50	1.00	4.50	5.00	1.00
S12.12	PI119333/Pannonikus	34.50	3.00	110.00	1.00	3.00	3.50	6.00
S12.13	PI119333/Pannonikus	31.00	1.00	130.00	1.00	2.50	4.00	0.50
S12.14	PI119333/Pannonikus	28.50	1.00	110.00	1.00	3.50	5.50	38.50

S12.15	PI119333/Pannonikus	37.85	1.00	105.00	1.00	4.00	5.00	0.00
S12.16	PI119333/Pannonikus	31.00	1.00	107.50	0.75	3.00	3.50	2.50
S12.17	PI119333/Pannonikus	28.50	1.00	107.50	0.75	3.50	4.00	12.00
S12.18	PI119333/Pannonikus	29.50	5.50	105.00	1.00	5.50	3.00	0.00
S12.19	PI119333/Pannonikus	30.50	1.00	100.00	1.00	6.00	5.50	0.00
S12.2	PI119333/Pannonikus	31.50	3.50	117.50	1.00	3.50	3.00	0.00
S12.20	PI119333/Pannonikus	33.00	4.00	110.00	1.00	3.00	6.50	0.50
S12.21	PI119333/Pannonikus	28.50	6.00	120.00	1.00	3.00	4.00	0.00
S12.22	PI119333/Pannonikus	31.00	2.50	100.00	1.00	5.00	6.00	0.00
S12.23	PI119333/Pannonikus	39.50	4.00	107.50	1.00	4.50	5.00	40.00
S12.24	PI119333/Pannonikus	34.50	1.00	100.00	1.00	3.00	3.00	0.50
S12.25	PI119333/Pannonikus	30.00	1.00	115.00	1.00	4.00	5.00	0.00
S12.26	PI119333/Pannonikus	31.00	1.00	105.00	1.00	4.50	4.00	0.50
S12.27	PI119333/Pannonikus	31.50	1.00	100.00	0.00	4.00	5.00	30.00
S12.28	PI119333/Pannonikus	32.50	5.00	112.50	1.00	3.50	6.00	0.50
S12.29	PI119333/Pannonikus	33.00	2.00	107.50	1.00	3.50	4.50	0.00
S12.3	PI119333/Pannonikus	37.00	1.00	107.50	1.00	3.00	5.50	0.00
S12.30	PI119333/Pannonikus	33.00	1.00	115.00	1.00	2.00	2.50	0.00
S12.31	PI119333/Pannonikus	33.50	1.00	102.50	0.75	4.00	4.00	0.00
S12.32	PI119333/Pannonikus	28.00	1.00	97.50	1.00	3.50	4.50	0.00
S12.34	PI119333/Pannonikus	30.50	2.00	97.50	0.75	3.50	3.50	51.50
S12.35	PI119333/Pannonikus	30.50	3.50	105.00	1.00	4.50	5.50	0.00
S12.36	PI119333/Pannonikus	31.00	1.00	92.50	1.00	5.50	4.50	51.00
S12.37	PI119333/Pannonikus	36.00	3.50	117.50	1.00	3.50	4.50	0.00
S12.38	PI119333/Pannonikus	29.50	1.50	95.00	1.00	3.00	2.50	0.50
S12.39	PI119333/Pannonikus	29.50	1.00	97.50	1.00	4.50	4.00	0.50
S12.4	PI119333/Pannonikus	32.00	1.00	112.50	1.00	3.00	6.00	0.00
S12.40	PI119333/Pannonikus	29.00	1.00	100.00	1.00	3.50	5.00	0.00
S12.5	PI119333/Pannonikus	27.00	1.00	95.00	1.00	5.00	5.00	0.00
S12.6	PI119333/Pannonikus	29.00	1.00	110.00	1.00	5.50	2.50	0.00
S12.7	PI119333/Pannonikus	31.00	6.00	120.00	1.00	4.50	5.00	0.00
S12.8	PI119333/Pannonikus	29.00	1.00	105.00	1.00	3.00	3.50	0.00
S12.9	PI119333/Pannonikus	26.50	1.00	105.00	1.00	5.00	4.50	6.50
S13.1	PI119333/Midas	32.50	1.00	110.00	1.00	5.50	4.50	1.50
S13.10	PI119333/Midas	29.50	1.00	102.50	0.75	3.00	4.50	9.00
S13.11	PI119333/Midas	25.50	5.50	117.50	1.00	4.00	5.50	0.00
S13.12	PI119333/Midas	31.15	2.50	112.50	1.00	6.50	6.00	4.00
S13.13	PI119333/Midas	31.00	3.50	110.00	1.00	4.00	4.50	6.50
S13.15	PI119333/Midas	28.00	1.00	95.00	1.00	3.50	4.00	0.00
S13.16	PI119333/Midas	29.50	6.50	115.00	1.00	4.50	4.00	0.00
S13.17	PI119333/Midas	26.50	5.50	122.50	1.00	4.50	3.00	43.50
S13.18	PI119333/Midas	28.50	1.00	100.00	1.00	6.00	4.50	0.50
S13.19	PI119333/Midas	30.50	5.00	125.00	1.00	3.50	5.00	0.00
S13.2	PI119333/Midas	28.50	3.00	105.00	1.00	4.50	4.50	0.50
S13.20	PI119333/Midas	34.00	5.00	130.00	1.00	4.50	4.50	0.00
S13.21	PI119333/Midas	27.00	3.00	112.50	1.00	3.50	4.00	0.00
S13.22	PI119333/Midas	29.50	1.00	92.50	1.00	4.50	4.50	24.00
S13.23	PI119333/Midas	27.50	1.00	95.00	1.00	3.50	2.50	53.00
S13.24	PI119333/Midas	28.50	1.00	110.00	1.00	4.00	5.00	0.00

S13.25	PI119333/Midas	30.50	1.00	100.00	1.00	4.50	2.50	6.50
S13.26	PI119333/Midas	32.00	1.00	117.50	1.00	3.50	4.00	0.00
S13.27	PI119333/Midas	32.00	1.00	105.00	0.75	4.50	4.50	1.00
S13.28	PI119333/Midas	29.50	2.00	100.00	1.00	4.00	6.00	70.00
S13.29	PI119333/Midas	26.50	1.00	100.00	1.00	4.00	4.50	0.00
S13.3	PI119333/Midas	34.00	5.00	110.00	1.00	5.00	4.00	1.00
S13.30	PI119333/Midas	31.00	1.00	92.50	1.00	4.00	6.00	57.50
S13.4	PI119333/Midas	30.00	6.50	125.00	1.00	3.50	2.50	0.00
S13.5	PI119333/Midas	29.50	5.50	100.00	1.00	4.50	3.50	70.50
S13.6	PI119333/Midas	29.50	6.00	122.50	1.00	4.00	5.00	0.00
S13.7	PI119333/Midas	27.00	2.00	112.50	1.00	4.50	5.50	0.00
S13.8	PI119333/Midas	30.00	6.00	115.00	1.00	5.00	6.00	4.00
S13.9	PI119333/Midas	36.00	1.00	100.00	1.00	4.00	5.50	59.50
S14.1	PI119333/Tommi	40.50	1.00	92.50	1.00	3.00	5.00	0.00
S14.10	PI119333/Tommi	33.50	1.00	85.00	0.00	3.00	3.00	0.00
S14.11	PI119333/Tommi	33.50	4.50	112.50	1.00	3.50	4.00	0.00
S14.12	PI119333/Tommi	30.00	1.00	107.50	1.00	3.00	5.00	0.00
S14.13	PI119333/Tommi	29.50	1.00	97.50	0.00	6.00	5.50	0.50
S14.14	PI119333/Tommi	35.50	1.00	95.00	1.00	4.00	5.00	0.00
S14.15	PI119333/Tommi	38.00	1.00	115.00	0.00	3.00	5.00	0.00
S14.16	PI119333/Tommi	29.00	1.00	85.00	1.00	2.50	4.00	0.50
S14.17	PI119333/Tommi	39.50	6.50	110.00	0.00	4.00	5.00	0.00
S14.18	PI119333/Tommi	34.00	1.00	80.00	1.00	3.50	6.00	30.50
S14.19	PI119333/Tommi	37.00	1.00	80.00	0.00	3.00	4.00	0.00
S14.2	PI119333/Tommi	31.50	1.00	90.00	0.00	4.50	6.00	0.00
S14.20	PI119333/Tommi	36.00	5.50	115.00	0.00	4.00	5.00	0.00
S14.21	PI119333/Tommi	31.00	1.00	90.00	1.00	3.50	4.00	0.00
S14.22	PI119333/Tommi	33.50	1.00	112.50	0.25	4.00	4.50	0.00
S14.23	PI119333/Tommi	35.50	1.00	85.00	0.00	3.00	4.00	0.00
S14.24	PI119333/Tommi	38.00	1.00	107.50	1.00	2.50	2.00	0.00
S14.25	PI119333/Tommi	34.50	1.00	120.00	0.00	3.50	3.50	1.00
S14.26	PI119333/Tommi	31.50	1.00	107.50	1.00	2.50	4.00	2.00
S14.27	PI119333/Tommi	36.00	1.00	110.00	1.00	4.00	3.50	0.00
S14.28	PI119333/Tommi	33.50	1.00	100.00	1.00	3.50	4.00	0.00
S14.29	PI119333/Tommi	31.50	1.00	110.00	0.75	3.50	4.50	0.00
S14.3	PI119333/Tommi	33.50	1.00	112.50	0.75	3.50	5.50	0.00
S14.30	PI119333/Tommi	29.50	1.00	102.50	0.50	3.00	4.00	1.50
S14.31	PI119333/Tommi	36.00	1.00	105.00	1.00	3.50	7.00	0.00
S14.32	PI119333/Tommi	33.50	1.00	87.50	1.00	2.50	5.50	0.00
S14.33	PI119333/Tommi	32.00	3.00	107.50	0.50	3.00	2.50	0.00
S14.34	PI119333/Tommi	33.00	2.00	107.50	1.00	3.50	2.50	0.00
S14.35	PI119333/Tommi	37.00	2.50	95.00	1.00	3.50	4.00	0.00
S14.36	PI119333/Tommi	37.00	1.00	105.00	0.00	4.00	3.00	0.00
S14.37	PI119333/Tommi	30.50	1.00	87.50	0.00	3.00	2.50	19.00
S14.38	PI119333/Tommi	33.50	1.00	90.00	1.00	3.50	4.00	4.00
S14.4	PI119333/Tommi	40.00	1.00	92.50	0.00	2.50	2.50	0.00
S14.5	PI119333/Tommi	33.50	1.00	77.50	0.00	3.50	4.50	0.00
S14.6	PI119333/Tommi	33.00	1.00	80.00	0.00	4.00	4.50	0.00
S14.7	PI119333/Tommi	30.50	2.00	100.00	0.00	3.00	3.00	0.00

S14.8	PI119333/Tommi	34.00	3.50	110.00	0.75	5.00	4.00	0.00
S14.9	PI119333/Tommi	34.00	1.50	110.00	1.00	3.00	2.50	0.00

Appendix 5 Least square means of Population 3 Blizzard for common bunt incidence (%) (CB), date of heading (DH), lodging (LOD), plant height (PH), awns (AW), fusarium head blight (FHB) and leaf health (LH)

Line	Cross	DH	LOD	PH	AW	FHB	LH	CB
Blizzard		29.00	1.00	110.00	1.00	3.50	5.50	0.50
Midas		31.10	1.00	80.50	0.80	4.30	3.40	73.50
Rainer		30.00	1.00	82.50	0.00	4.75	4.17	64.92
S3.1	Rainer/Blizzard//Midas	33.00	1.00	92.50	0.50	3.50	2.50	0.00
S3.10	Rainer/Blizzard//Midas	37.50	1.00	85.00	0.50	3.00	3.00	36.50
S3.11	Rainer/Blizzard//Midas	34.00	1.00	87.50	0.50	3.50	2.50	0.00
S3.12	Rainer/Blizzard//Midas	29.00	1.00	92.50	0.50	2.50	2.00	0.00
S3.13	Rainer/Blizzard//Midas	33.00	1.00	100.00	1.00	3.50	4.50	14.00
S3.14	Rainer/Blizzard//Midas	30.00	1.00	90.00	0.00	3.50	3.00	43.00
S3.15	Rainer/Blizzard//Midas	37.00	1.00	95.00	1.00	4.00	4.50	8.50
S3.16	Rainer/Blizzard//Midas	29.50	1.00	80.00	0.75	5.00	7.50	53.00
S3.17	Rainer/Blizzard//Midas	29.50	1.00	90.00	0.00	4.00	2.00	2.00
S3.18	Rainer/Blizzard//Midas	31.00	1.00	82.50	0.25	3.50	4.00	40.00
S3.19	Rainer/Blizzard//Midas	29.50	1.00	90.00	0.50	3.00	5.50	2.50
S3.2	Rainer/Blizzard//Midas	31.00	1.00	95.00	1.00	4.00	3.50	1.00
S3.20	Rainer/Blizzard//Midas	30.50	1.00	80.00	1.00	3.00	2.50	3.50
S3.3	Rainer/Blizzard//Midas	37.00	1.00	82.50	1.00	3.50	6.50	77.00
S3.4	Rainer/Blizzard//Midas	29.50	1.00	80.00	0.00	3.50	2.00	62.00
S3.5	Rainer/Blizzard//Midas	29.00	1.00	95.00	1.00	3.00	3.00	1.50
S3.6	Rainer/Blizzard//Midas	28.50	1.00	85.00	1.00	3.00	2.50	0.00
S3.7	Rainer/Blizzard//Midas	30.00	1.00	85.00	1.00	3.50	4.00	0.00
S3.8	Rainer/Blizzard//Midas	28.50	1.00	92.50	1.00	3.00	3.00	17.50
S3.9	Rainer/Blizzard//Midas	26.50	1.00	105.00	0.00	4.50	6.00	24.50
S4.38	Blizzard/Rainer	31.00	1.00	112.50	0.00	4.50	2.50	0.00
S4.42	Blizzard/Rainer	34.00	1.00	107.50	0.00	4.50	1.50	0.00
S4.50	Blizzard/Rainer	28.50	1.00	92.50	0.00	3.50	3.50	0.00
S4.53	Blizzard/Rainer	25.00	1.00	100.00	1.00	4.50	4.00	1.00
S4.6	Blizzard/Rainer	28.00	1.00	90.00	1.00	5.00	5.00	0.00
S5.12	Blizzard/Rainer	28.00	1.00	100.00	0.00	4.50	5.50	0.00
S5.15	Blizzard/Rainer	34.50	1.00	105.00	1.00	6.00	5.00	5.50
S5.21	Blizzard/Rainer	28.50	2.50	95.00	1.00	3.50	5.00	0.00
S5.31	Blizzard/Rainer	28.50	1.00	97.50	1.00	3.50	3.50	0.50
S5.32	Blizzard/Rainer	29.50	1.00	100.00	0.00	3.50	2.50	0.00
S5.47	Blizzard/Rainer	24.50	1.50	92.50	1.00	3.00	2.00	0.50
S5.58	Blizzard/Rainer	29.50	3.00	87.50	1.00	6.50	3.00	0.00

9.2 ANOVA results

Appendix 6 Results analysis of variance and coefficient of determination (R^2) for the trait date of heading for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	82	2500.91	30.499	4.9569	6.896e-12
Replication	1	212.33	212.333	34.5102	9.881e-08
Residuals	78	479.92	6.153		
R^2	0.8497				

Appendix 7 Results analysis of variance and coefficient of determination (R^2) for the trait date of heading for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	5081.5	21.44	5.0822	<2.2e-16
Replication	1	695.0	695.04	164.7472	<2.2e-16
Residuals	250	1054.7	4.22		
R^2	0.8456				

Appendix 8 Results analysis of variance and coefficient of determination (R^2) for the trait date of heading for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	625.94	20.192	3.9182	0.0001266
Replication	1	42.25	42.250	8.1987	0.0074534
Residuals	31	159.75	5.153		
R^2	0.8071				

Appendix 9 Results analysis of variance and coefficient of determination (R^2) for the trait plant height for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	83	26900.1	324.10	9.0874	<2e-16
Replication	1	29.2	29.17	0.8178	0.3684
Residuals	84	2995.8	35.66		
R^2	0.8999				

Appendix 10 Results analysis of variance and coefficient of determination (R^2) for the trait plant height for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	59062	249.21	3.7214	<2e-16
Replication	1	349	348.63	5.2061	0.02333
Residuals	255	17076			
R^2	0.7767				

Appendix 11 Results analysis of variance and coefficient of determination (R^2) for the trait plant height for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	4248.4	137.046	2.5083	0.00626
Replication	1	156.2	156.250	2.8598	0.10085
Residuals	31	1693.7	54.637		
R^2	0.7223				

Appendix 12 Results analysis of variance and coefficient of determination (R^2) for the trait lodging for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	2221.86	9.3750	5.8152	<2.2e-16
Replication	1	20.65	20.6498	12.8088	0.0004129
Residuals	255	411.10	1.6122		
R^2	0.8451				

Appendix 13 Results analysis of variance and coefficient of determination (R^2) for the trait lodging for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	12	0.3817	1.0000	0.5000
Replication	1	1	1.000	2.5833	0.1181
Residuals	31	12	0.3871		
R^2	0.52				

Appendix 14 Results analysis of variance and coefficient of determination (R^2) for the trait awns for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	83	32.438	0.39082	22.6964	<2e-16
Replication	1	0.054	0.05357	3.1111	0.0814
Residuals	84	1.446	0.01722		
R^2	0.9574				

Appendix 15 Results analysis of variance and coefficient of determination (R^2) for the trait awns for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	87.524	0.36930	12.7769	<2e-16
Replication	1	0.005	0.00455	0.1576	0.6917
Residuals	255	7.370	0.02890		
R^2	0.9223				

Appendix 16 Results analysis of variance and coefficient of determination (R^2) for the trait awns for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	11.984	0.38659	5.9922	1.54e-6
Replication	1	0.250	0.25000	3.8750	0.05801
Residuals	31	2.000	0.06452		
R^2	0.8595				

Appendix 17 Results analysis of variance and coefficient of determination (R^2) for the trait fusarium head blight for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	83	87.149	1.04999	2.7054	4.298e-6
Replication	1	3.149	3.14881	8.1132	0.005524
Residuals	84	32.601	0.38811		
R^2	0.7347				

Appendix 18 Results analysis of variance and coefficient of determination (R^2) for the trait fusarium head blight for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	428.35	1.80740	3.4893	<2e-16
Replication	1	1.17	1.16599	2.2511	0.1348
Residuals	255	132.08	0.51798		
R^2	0.7648				

Appendix 19 Results analysis of variance and coefficient of determination (R^2) for the trait fusarium head blight for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	49.609	1.60030	2.1124	0.02057
Replication	1	0.016	0.01563	0.0206	0.88673
Residuals	31	23.484	0.75756		
R^2	0.6788				

Appendix 20 Results analysis of variance and coefficient of determination (R^2) for the trait leaf health for mapping population Bonneville

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	83	234.746	2.8283	0.9029	0.67890
Replication	1	14.881	14.8810	4.7507	0.03209
Residuals	84	263.119	3.1324		
R^2	0.4868				

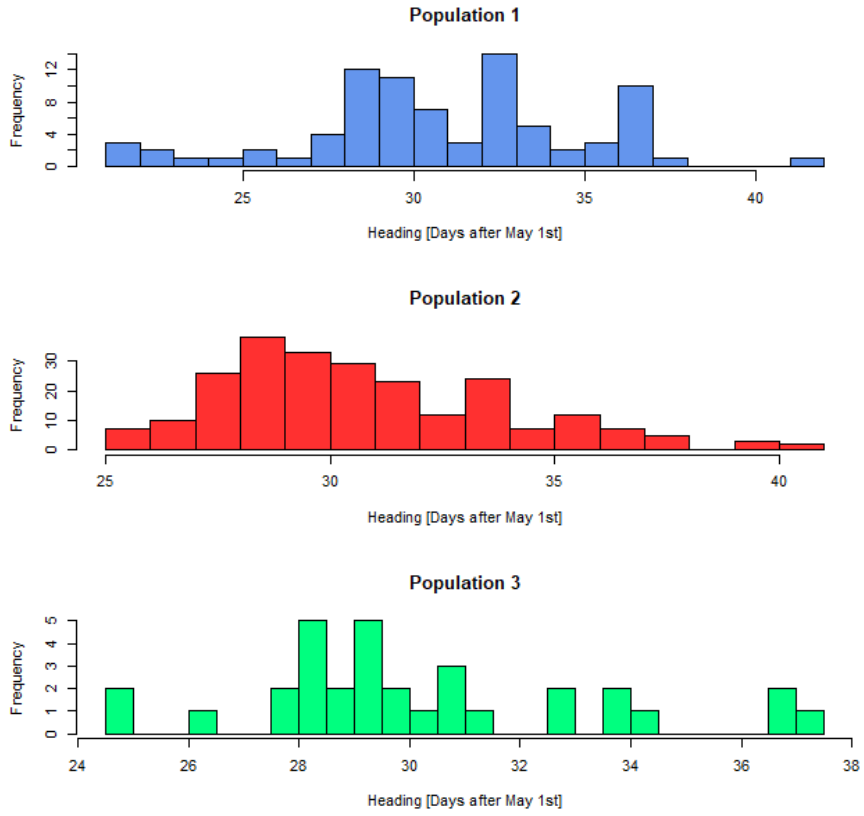
Appendix 21 Results analysis of variance and coefficient of determination (R^2) for the trait leaf health for mapping population PI119333

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	237	573.83	2.4212	0.8159	0.9437
Replication	1	2.49	2.4898	0.8390	
Residuals	254	753.76	2.9676		
R^2	0.4333				

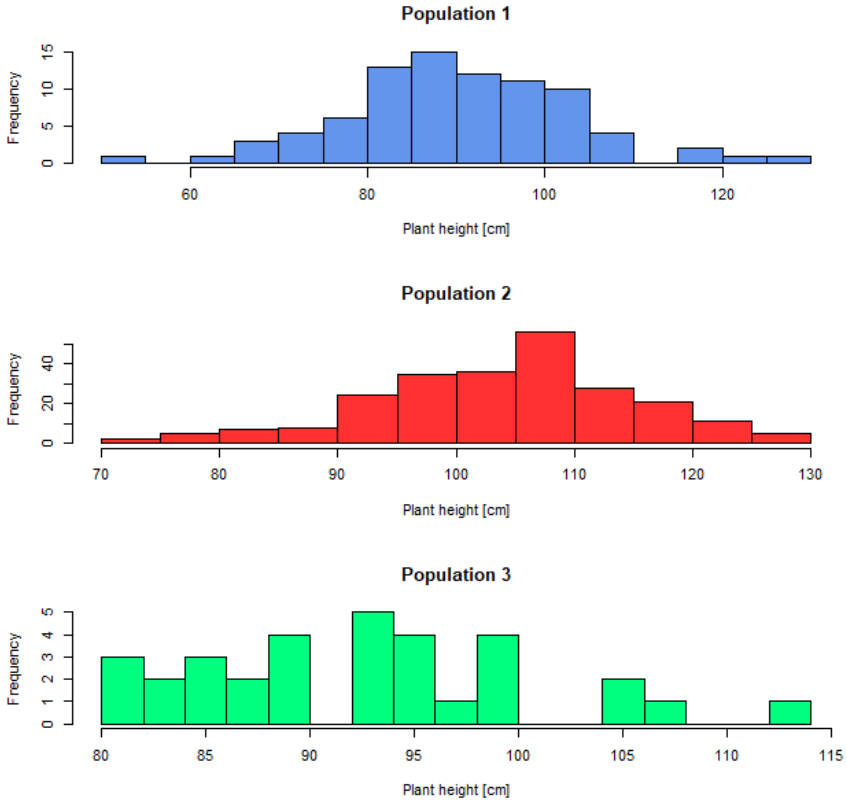
Appendix 22 Results analysis of variance and coefficient of determination (R^2) for the trait leaf health for mapping population Blizzard

	Df	Sum Sq	Mean Sq	F value	P value
Genotype	31	136.437	4.4012	1.6255	0.09093
Replication	1	0.062	0.0625	0.0231	0.88023
Residuals	31	83.938	2.7077		
R^2	0.6192				

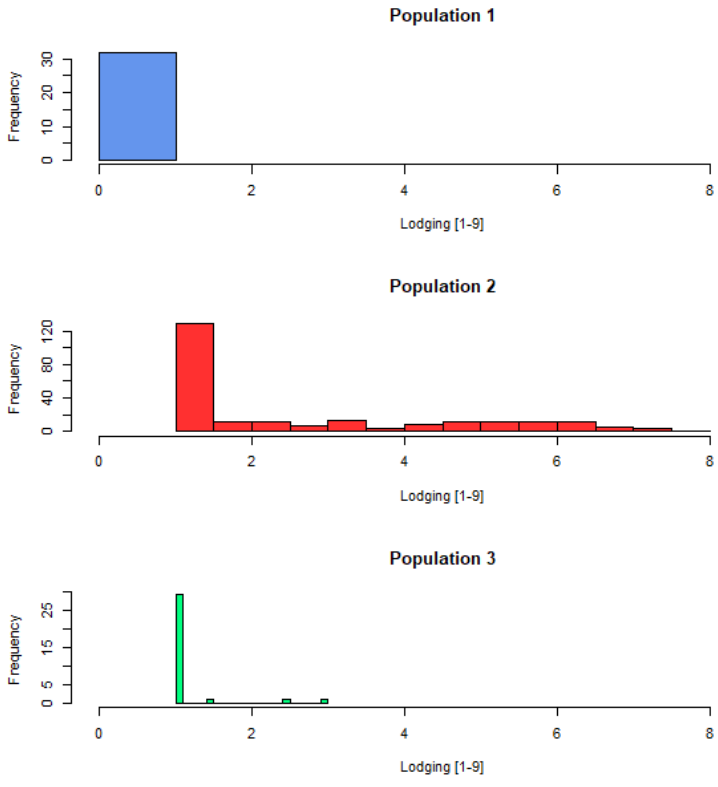
9.3 Frequency distributions



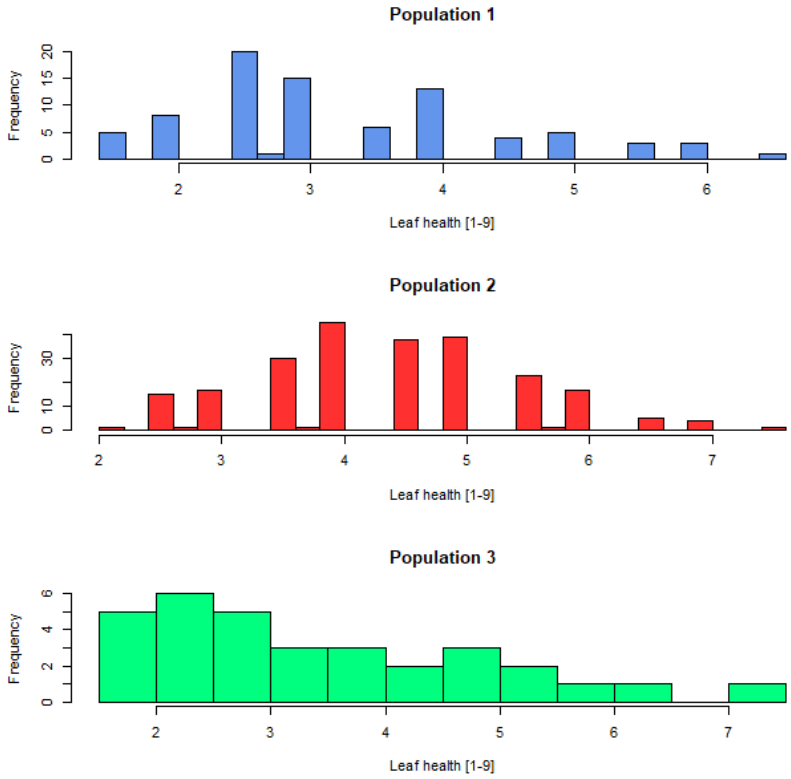
Appendix 23 Frequency distribution of the trait date of heading (DH) for mapping population 1 Bonneville, population 2 PI119333 and population 3 Blizzard



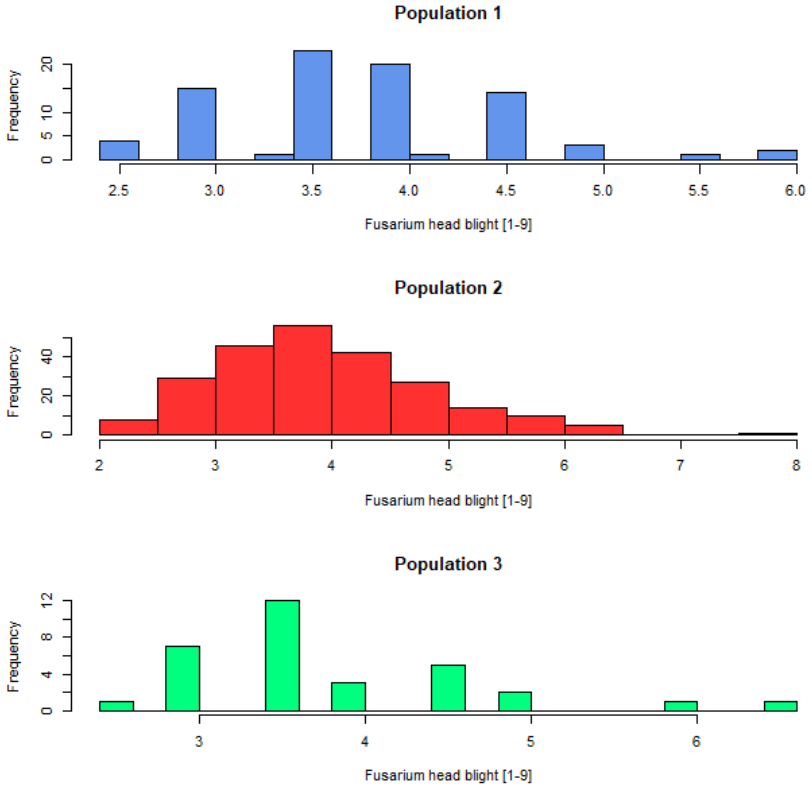
Appendix 24 Frequency distribution of the trait plant height (PH) for mapping population 1 Bonneville, population 2 PI119333 and population 3 Blizzard



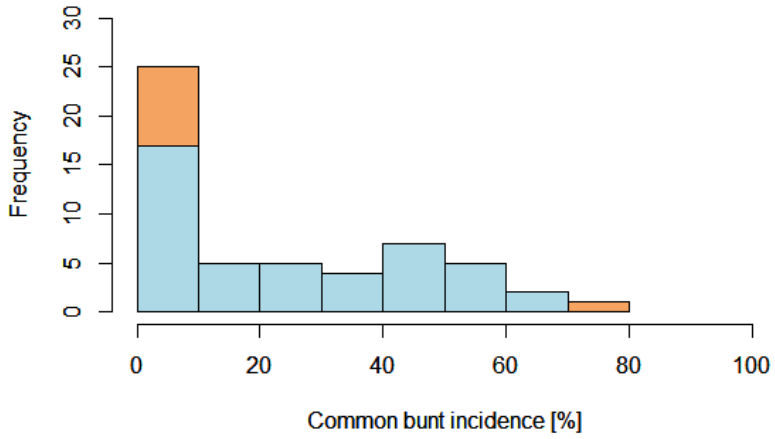
Appendix 25 Frequency distribution of the trait lodging (LOD) for mapping population 1 Bonneville, population 2 PI119333 and population 3 Blizzard



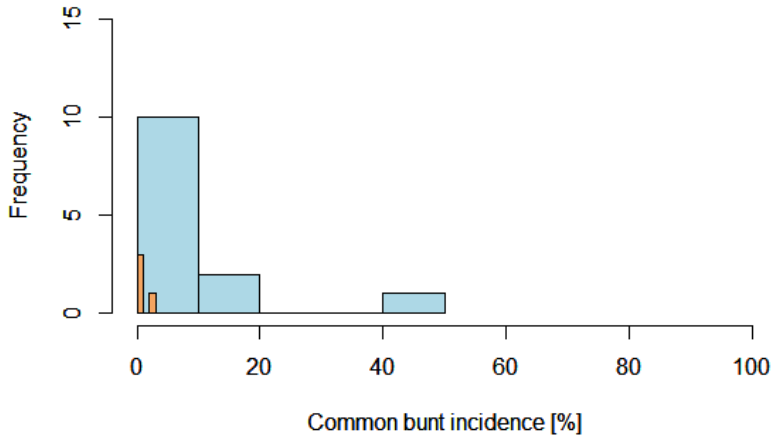
Appendix 26 Frequency distribution of the trait leaf health (LH) for mapping population 1 Bonneville, population 2 PI119333 and population 3 Blizzard



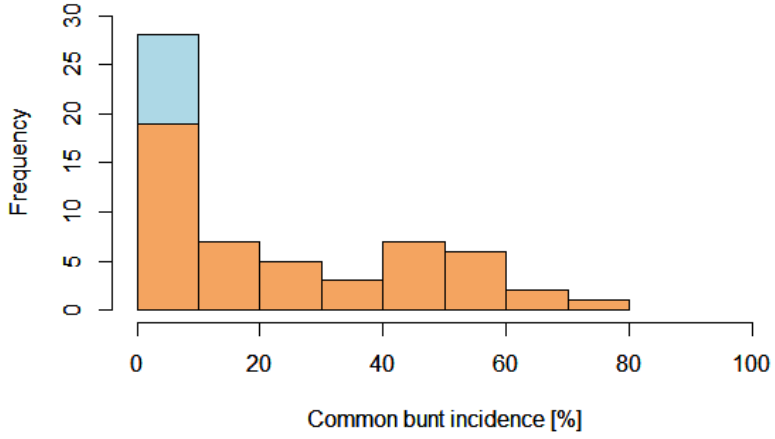
Appendix 27 Frequency distribution of the trait fusarium head blight (FHB) for mapping population 1 Bonneville, population 2 PI119333 and population 3 Blizzard



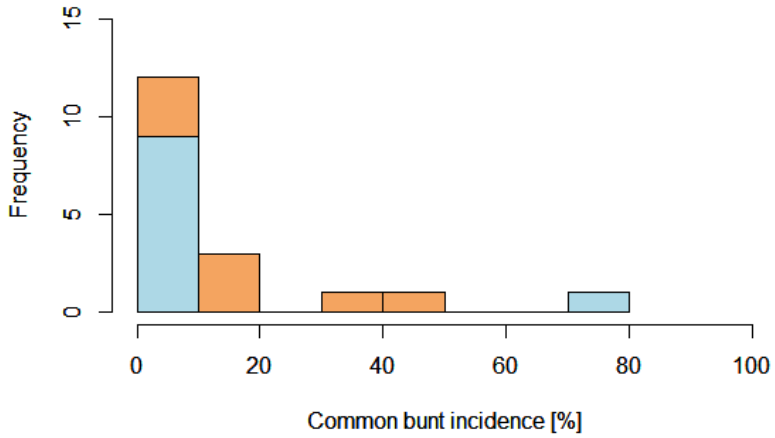
Appendix 28 Frequency distribution of common bunt incidence (%) in mapping population 1 Bonneville for the marker Xgwm374. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



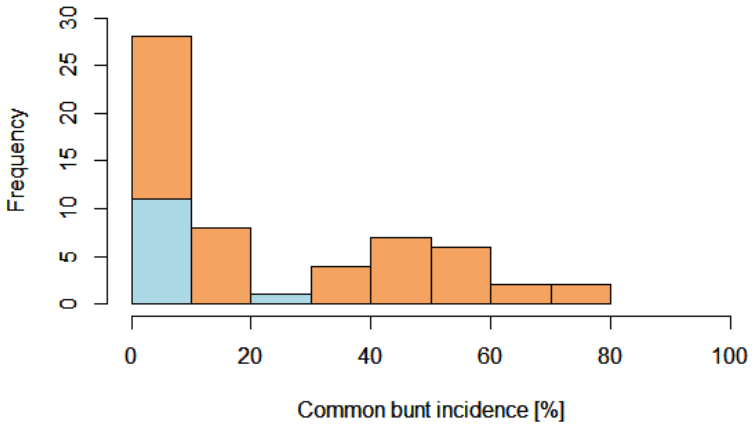
Appendix 29 Frequency distribution of common bunt incidence (%) in mapping population 3 Blizzard for the marker Xgwm374. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



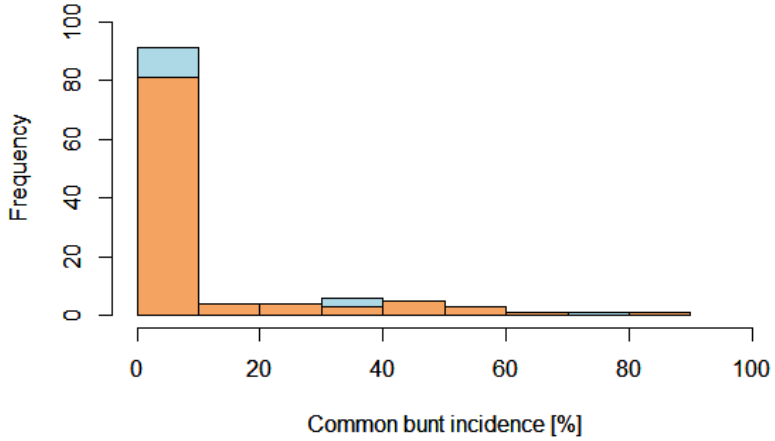
Appendix 30 Frequency distribution of common bunt incidence (%) in mapping population 1 Bonneville for the marker Xgwm264. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



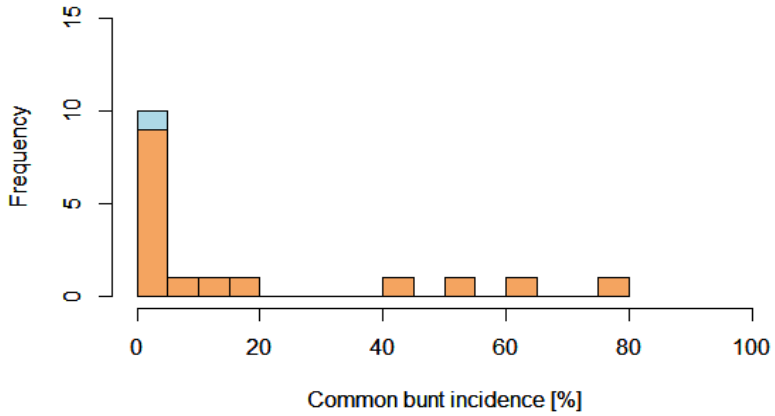
Appendix 31 Frequency distribution of common bunt incidence (%) in mapping population 3 Blizzard for the marker Xgwm264. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



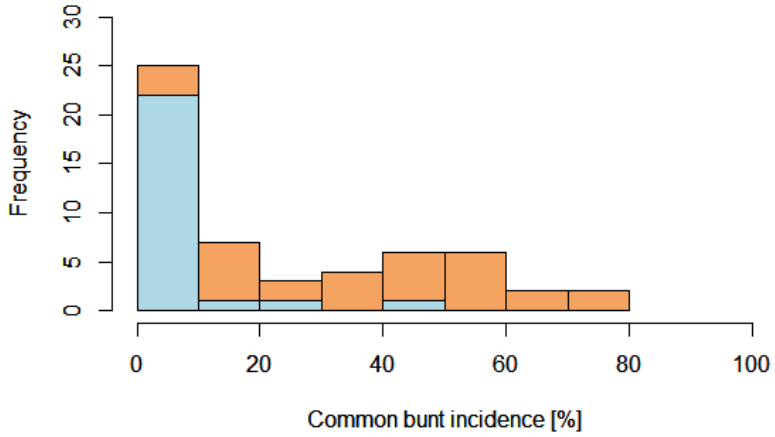
Appendix 32 Frequency distribution of common bunt incidence (%) in mapping population 1 Bonneville for the marker Xbarc83. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



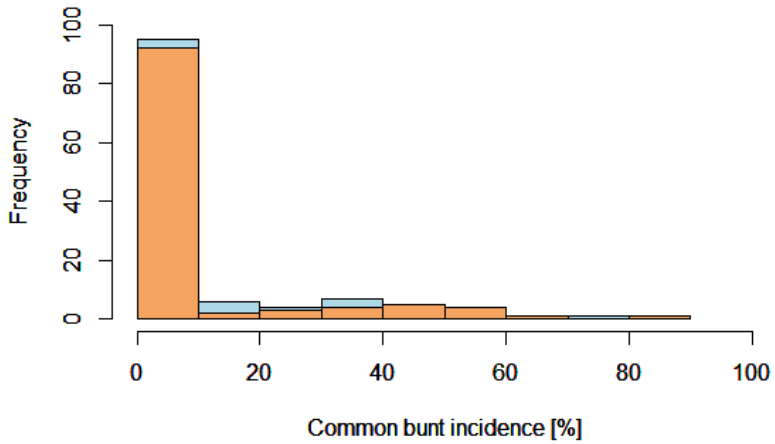
Appendix 33 Frequency distribution of common bunt incidence (%) in mapping population 2 PI119333 for the marker Xbarc83. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



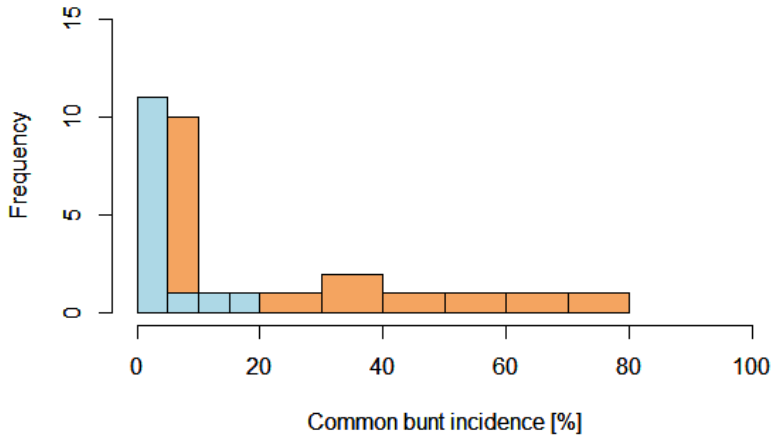
Appendix 34 Frequency distribution of common bunt incidence (%) in mapping population 3 Blizzard for the marker Xbarc83. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



Appendix 35 Frequency distribution of common bunt incidence (%) in mapping population 1 Bonneville for the marker Xcfa2129. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



Appendix 36 Frequency distribution of common bunt incidence (%) in mapping population 2 PI119333 for the marker Xcfa2129. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.



Appendix 37 Frequency distribution of common bunt incidence (%) in mapping population 3 Blizzard for the marker Xcfa2129. Lines carrying the allele from the resistant parent are depicted in blue, lines carrying the allele from the susceptible parent in orange.

9.4 Genotypic scoring

Appendix 38 Genotypic scoring of Population 1 (Bonneville) with the SSR markers Xgwm374, Xgwm264, Xbarc83 and Xcfa2129 (1=resistant, 2=susceptible, 3=heterozygote, NA=not available)

Line	Cross	Xgwm374	Xgwm264	Xbarc83	Xcfa2129
Bonneville	NA	1	1	1	NA
20568.1.2	NA	1	1	2	2
Midas	NA	2	2	2	2
Rainer	NA	2	2	2	2
P101.111	Bonneville/Rainer	1	1	2	2
P101.30	Bonneville/Rainer	1	1	2	1
P101.31	Bonneville/Rainer	2	2	1	1
P101.34	Bonneville/Rainer	2	2	1	1
P101.53	Bonneville/Rainer	1	1	1	1
P101.65	Bonneville/Rainer	NA	2	1	1
P101.8	Bonneville/Rainer	2	2	1	1
P101.81	Bonneville/Rainer	1	1	1	1
P101.83	Bonneville/Rainer	2	2	2	2
P101.87	Bonneville/Rainer	1	1	1	1
S1.1	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.10	Rainer/Bonneville//20568.1.2	2	2	2	1
S1.11	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.12	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.13	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.14	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.15	Rainer/Bonneville//20568.1.2	1	2	1	2
S1.16	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.17	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.18	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.19	Rainer/Bonneville//20568.1.2	1	1	2	2

S1.2	Rainer/Bonneville//20568.1.2	2	2	2	1
S1.20	Rainer/Bonneville//20568.1.2	1	2	2	2
S1.21	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.22	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.23	Rainer/Bonneville//20568.1.2	NA	1	2	2
S1.24	Rainer/Bonneville//20568.1.2	1	1	2	1
S1.25	Rainer/Bonneville//20568.1.2	2	1	2	2
S1.26	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.27	Rainer/Bonneville//20568.1.2	1	1	3	1
S1.28	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.3	Rainer/Bonneville//20568.1.2	2	1	3	1
S1.4	Rainer/Bonneville//20568.1.2	NA	2	3	1
S1.5	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.6	Rainer/Bonneville//20568.1.2	2	2	2	2
S1.7	Rainer/Bonneville//20568.1.2	NA	2	2	2
S1.8	Rainer/Bonneville//20568.1.2	1	1	2	2
S1.9	Rainer/Bonneville//20568.1.2	NA	2	3	2
S2.1	Midas/Bonneville//Rainer	1	2	1	1
S2.10	Midas/Bonneville//Rainer	2	2	2	2
S2.11	Midas/Bonneville//Rainer	1	1	3	1
S2.12	Midas/Bonneville//Rainer	1	1	2	2
S2.13	Midas/Bonneville//Rainer	2	2	1	1
S2.14	Midas/Bonneville//Rainer	1	1	1	1
S2.15	Midas/Bonneville//Rainer	2	2	3	2
S2.16	Midas/Bonneville//Rainer	2	2	2	2
S2.17	Midas/Bonneville//Rainer	1	1	2	2
S2.18	Midas/Bonneville//Rainer	2	2	2	2
S2.19	Midas/Bonneville//Rainer	NA	NA	NA	NA
S2.2	Midas/Bonneville//Rainer	2	2	2	2
S2.20	Midas/Bonneville//Rainer	2	2	2	2
S2.21	Midas/Bonneville//Rainer	2	2	2	2
S2.22	Midas/Bonneville//Rainer	2	2	1	2
S2.23	Midas/Bonneville//Rainer	2	2	2	2
S2.24	Midas/Bonneville//Rainer	2	1	2	2
S2.25	Midas/Bonneville//Rainer	2	2	2	2
S2.26	Midas/Bonneville//Rainer	2	2	2	2
S2.27	Midas/Bonneville//Rainer	2	2	3	1
S2.28	Midas/Bonneville//Rainer	2	2	2	2
S2.29	Midas/Bonneville//Rainer	1	1	2	2
S2.3	Midas/Bonneville//Rainer	2	2	NA	NA
S2.30	Midas/Bonneville//Rainer	NA	2	2	2
S2.31	Midas/Bonneville//Rainer	2	2	2	1
S2.32	Midas/Bonneville//Rainer	2	2	2	2
S2.33	Midas/Bonneville//Rainer	1	1	2	2
S2.34	Midas/Bonneville//Rainer	NA	2	2	2
S2.35	Midas/Bonneville//Rainer	2	2	2	1
S2.36	Midas/Bonneville//Rainer	2	2	2	2
S2.37	Midas/Bonneville//Rainer	2	2	2	2
S2.38	Midas/Bonneville//Rainer	NA	NA	NA	NA

S2.39	Midas/Bonneville//Rainer	NA	NA	NA	NA
S2.4	Midas/Bonneville//Rainer	2	2	2	2
S2.40	Midas/Bonneville//Rainer	2	2	2	2
S2.41	Midas/Bonneville//Rainer	NA	NA	NA	NA
S2.42	Midas/Bonneville//Rainer	1	1	3	1
S2.43	Midas/Bonneville//Rainer	2	2	2	2
S2.44	Midas/Bonneville//Rainer	2	2	2	2
S2.45	Midas/Bonneville//Rainer	NA	NA	NA	NA
S2.46	Midas/Bonneville//Rainer	2	2	3	2
S2.47	Midas/Bonneville//Rainer	NA	NA	NA	NA
S2.5	Midas/Bonneville//Rainer	2	1	2	2
S2.6	Midas/Bonneville//Rainer	2	2	2	2
S2.7	Midas/Bonneville//Rainer	2	2	2	2
S2.8	Midas/Bonneville//Rainer	1	1	2	1
S2.9	Midas/Bonneville//Rainer	2	2	2	1
S7.20	Bonneville/Rainer	NA	1	2	1
S7.4	Bonneville/Rainer	1	1	3	2

Appendix 39 Genotypic scoring of Population 2 (PI119333) with the SSR markers Xgwm374, Xgwm264, Xbarc83 and Xcfa2129 (1=resistant, 2=susceptible, 3=heterozygote, NA=not available)

Line	Cross	Xgwm374	Xgwm264	Xbarc83	Xcfa2129
PI119333	NA	NA	NA	1	1
Midas	NA	2	2	2	2
Pannonikus	NA	NA	NA	2	2
Rainer	NA	2	2	2	2
Tommi	NA	NA	NA	2	2
P106.1	PI119333/Rainer	NA	NA	2	2
P106.10	PI119333/Rainer	NA	NA	1	1
P106.11	PI119333/Rainer	NA	NA	1	1
P106.12	PI119333/Rainer	NA	NA	2	1
P106.13	PI119333/Rainer	NA	NA	2	2
P106.14	PI119333/Rainer	NA	NA	1	1
P106.15	PI119333/Rainer	NA	NA	2	2
P106.16	PI119333/Rainer	NA	NA	1	1
P106.17	PI119333/Rainer	NA	NA	1	2
P106.18	PI119333/Rainer	NA	NA	1	1
P106.19	PI119333/Rainer	NA	NA	2	2
P106.2	PI119333/Rainer	NA	NA	2	3
P106.20	PI119333/Rainer	NA	NA	2	2
P106.21	PI119333/Rainer	NA	NA	2	2
P106.22	PI119333/Rainer	NA	NA	1	1
P106.23	PI119333/Rainer	NA	NA	2	2
P106.24	PI119333/Rainer	NA	NA	1	1
P106.25	PI119333/Rainer	NA	NA	1	1
P106.26	PI119333/Rainer	NA	NA	2	2
P106.27	PI119333/Rainer	NA	NA	2	2
P106.28	PI119333/Rainer	NA	NA	2	2
P106.29	PI119333/Rainer	NA	NA	1	1

P106.3	PI119333/Rainer	NA	NA	1	1
P106.30	PI119333/Rainer	NA	NA	1	2
P106.31	PI119333/Rainer	NA	NA	1	2
P106.32	PI119333/Rainer	NA	NA	2	2
P106.33	PI119333/Rainer	NA	NA	1	1
P106.34	PI119333/Rainer	NA	NA	1	1
P106.35	PI119333/Rainer	NA	NA	1	1
P106.36	PI119333/Rainer	NA	NA	1	1
P106.37	PI119333/Rainer	NA	NA	1	1
P106.39	PI119333/Rainer	NA	NA	2	2
P106.4	PI119333/Rainer	NA	NA	3	3
P106.40	PI119333/Rainer	NA	NA	2	2
P106.41	PI119333/Rainer	NA	NA	2	2
P106.42	PI119333/Rainer	NA	NA	1	1
P106.43	PI119333/Rainer	NA	NA	3	2
P106.44	PI119333/Rainer	NA	NA	1	1
P106.45	PI119333/Rainer	NA	NA	2	2
P106.46	PI119333/Rainer	NA	NA	1	1
P106.48	PI119333/Rainer	NA	NA	1	1
P106.49	PI119333/Rainer	NA	NA	1	1
P106.5	PI119333/Rainer	NA	NA	2	2
P106.50	PI119333/Rainer	NA	NA	2	2
P106.51	PI119333/Rainer	NA	NA	2	2
P106.52	PI119333/Rainer	NA	NA	3	2
P106.53	PI119333/Rainer	NA	NA	1	1
P106.54	PI119333/Rainer	NA	NA	1	1
P106.55	PI119333/Rainer	NA	NA	1	1
P106.56	PI119333/Rainer	NA	NA	3	3
P106.57	PI119333/Rainer	NA	NA	2	2
P106.58	PI119333/Rainer	NA	NA	1	1
P106.59	PI119333/Rainer	NA	NA	1	1
P106.6	PI119333/Rainer	NA	NA	1	1
P106.60	PI119333/Rainer	NA	NA	1	1
P106.61	PI119333/Rainer	NA	NA	2	2
P106.62	PI119333/Rainer	NA	NA	2	2
P106.63	PI119333/Rainer	NA	NA	2	2
P106.64	PI119333/Rainer	NA	NA	1	1
P106.65	PI119333/Rainer	NA	NA	2	2
P106.66	PI119333/Rainer	NA	NA	1	1
P106.67	PI119333/Rainer	NA	NA	2	2
P106.68	PI119333/Rainer	NA	NA	1	1
P106.69	PI119333/Rainer	NA	NA	1	NA
P106.7	PI119333/Rainer	NA	NA	NA	2
P106.70	PI119333/Rainer	NA	NA	2	2
P106.71	PI119333/Rainer	NA	NA	1	1
P106.72	PI119333/Rainer	NA	NA	1	1
P106.73	PI119333/Rainer	NA	NA	3	3
P106.74	PI119333/Rainer	NA	NA	1	1
P106.75	PI119333/Rainer	NA	NA	2	2

P106.76	PI119333/Rainer	NA	NA	3	1
P106.77	PI119333/Rainer	NA	NA	2	2
P106.78	PI119333/Rainer	NA	NA	1	1
P106.79	PI119333/Rainer	NA	NA	3	3
P106.8	PI119333/Rainer	NA	NA	2	2
P106.80	PI119333/Rainer	NA	NA	1	1
P106.81	PI119333/Rainer	NA	NA	1	2
P106.82	PI119333/Rainer	NA	NA	2	2
P106.83	PI119333/Rainer	NA	NA	3	2
P106.84	PI119333/Rainer	NA	NA	1	1
P106.85	PI119333/Rainer	NA	NA	1	1
P106.86	PI119333/Rainer	NA	NA	3	3
P106.87	PI119333/Rainer	NA	NA	1	1
P106.9	PI119333/Rainer	NA	NA	2	1
P106.90	PI119333/Rainer	2	2	2	1
P107.1	PI119333/Tommi	2	2	1	1
P107.10	PI119333/Tommi	NA	NA	1	2
P107.11	PI119333/Tommi	NA	NA	2	2
P107.12	PI119333/Tommi	NA	NA	2	2
P107.13	PI119333/Tommi	NA	NA	1	1
P107.15	PI119333/Tommi	NA	NA	2	2
P107.16	PI119333/Tommi	NA	NA	2	1
P107.17	PI119333/Tommi	NA	NA	1	1
P107.18	PI119333/Tommi	NA	NA	2	2
P107.19	PI119333/Tommi	NA	NA	2	2
P107.2	PI119333/Tommi	2	2	3	1
P107.20	PI119333/Tommi	NA	NA	1	2
P107.3	PI119333/Tommi	2	2	2	2
P107.4	PI119333/Tommi	2	NA	1	1
P107.5	PI119333/Tommi	2	2	2	2
P107.6	PI119333/Tommi	2	2	1	1
P107.7	PI119333/Tommi	NA	NA	1	1
P107.8	PI119333/Tommi	NA	NA	1	2
P107.9	PI119333/Tommi	NA	NA	2	2
P109.1	PI119333/Tommi	NA	NA	2	2
P109.10	PI119333/Tommi	NA	NA	1	1
P109.11	PI119333/Tommi	NA	NA	2	2
P109.12	PI119333/Tommi	NA	NA	2	2
P109.13	PI119333/Tommi	NA	NA	1	1
P109.14	PI119333/Tommi	NA	NA	1	1
P109.15	PI119333/Tommi	NA	NA	1	1
P109.16	PI119333/Tommi	NA	NA	1	1
P109.17	PI119333/Tommi	NA	NA	1	1
P109.18	PI119333/Tommi	NA	NA	2	2
P109.19	PI119333/Tommi	NA	NA	2	2
P109.2	PI119333/Tommi	NA	NA	2	2
P109.20	PI119333/Tommi	NA	NA	2	2
P109.21	PI119333/Tommi	NA	NA	2	2
P109.22	PI119333/Tommi	NA	NA	2	2

P109.23	PI119333/Tommi	NA	NA	2	1
P109.24	PI119333/Tommi	NA	NA	3	2
P109.25	PI119333/Tommi	NA	NA	2	2
P109.26	PI119333/Tommi	NA	NA	1	2
P109.27	PI119333/Tommi	NA	NA	1	1
P109.29	PI119333/Tommi	NA	NA	1	1
P109.4	PI119333/Tommi	NA	NA	2	2
P109.5	PI119333/Tommi	NA	NA	1	1
P109.6	PI119333/Tommi	NA	NA	1	1
P109.7	PI119333/Tommi	NA	NA	3	1
P109.8	PI119333/Tommi	NA	NA	1	1
P109.9	PI119333/Tommi	NA	NA	2	2
S12.1	PI119333/Pannonikus	NA	NA	3	1
S12.10	PI119333/Pannonikus	NA	NA	2	1
S12.11	PI119333/Pannonikus	NA	NA	2	2
S12.12	PI119333/Pannonikus	NA	NA	1	1
S12.13	PI119333/Pannonikus	NA	NA	1	1
S12.14	PI119333/Pannonikus	NA	NA	1	1
S12.15	PI119333/Pannonikus	NA	NA	2	1
S12.16	PI119333/Pannonikus	NA	NA	1	1
S12.17	PI119333/Pannonikus	NA	NA	2	2
S12.18	PI119333/Pannonikus	NA	NA	2	2
S12.19	PI119333/Pannonikus	NA	NA	2	2
S12.2	PI119333/Pannonikus	NA	NA	NA	1
S12.20	PI119333/Pannonikus	NA	NA	2	1
S12.21	PI119333/Pannonikus	NA	NA	1	2
S12.22	PI119333/Pannonikus	NA	NA	1	1
S12.23	PI119333/Pannonikus	NA	NA	1	1
S12.24	PI119333/Pannonikus	NA	NA	1	1
S12.25	PI119333/Pannonikus	NA	NA	3	2
S12.26	PI119333/Pannonikus	NA	NA	2	2
S12.27	PI119333/Pannonikus	NA	NA	2	1
S12.28	PI119333/Pannonikus	NA	NA	1	1
S12.29	PI119333/Pannonikus	NA	NA	2	2
S12.3	PI119333/Pannonikus	NA	NA	1	1
S12.30	PI119333/Pannonikus	NA	NA	2	2
S12.31	PI119333/Pannonikus	NA	NA	2	2
S12.32	PI119333/Pannonikus	NA	NA	1	1
S12.33	PI119333/Pannonikus	NA	NA	NA	NA
S12.34	PI119333/Pannonikus	NA	NA	2	2
S12.35	PI119333/Pannonikus	NA	NA	1	1
S12.36	PI119333/Pannonikus	NA	NA	1	1
S12.37	PI119333/Pannonikus	NA	NA	2	1
S12.38	PI119333/Pannonikus	NA	NA	2	1
S12.39	PI119333/Pannonikus	NA	NA	1	1
S12.4	PI119333/Pannonikus	NA	NA	1	1
S12.40	PI119333/Pannonikus	NA	NA	2	2
S12.5	PI119333/Pannonikus	NA	NA	2	2
S12.6	PI119333/Pannonikus	NA	NA	1	1

S12.7	PI119333/Pannonikus	NA	NA	2	2
S12.8	PI119333/Pannonikus	NA	NA	2	1
S12.9	PI119333/Pannonikus	NA	NA	3	2
S13.1	PI119333/Midas	NA	NA	2	2
S13.10	PI119333/Midas	NA	NA	2	2
S13.11	PI119333/Midas	NA	NA	1	1
S13.12	PI119333/Midas	NA	NA	1	1
S13.13	PI119333/Midas	NA	NA	1	1
S13.14	PI119333/Midas	NA	NA	NA	NA
S13.15	PI119333/Midas	NA	NA	3	2
S13.16	PI119333/Midas	NA	NA	2	2
S13.17	PI119333/Midas	NA	NA	1	1
S13.18	PI119333/Midas	NA	NA	3	1
S13.19	PI119333/Midas	NA	NA	2	2
S13.2	PI119333/Midas	NA	NA	1	1
S13.20	PI119333/Midas	NA	NA	2	2
S13.21	PI119333/Midas	NA	NA	1	1
S13.22	PI119333/Midas	NA	NA	2	2
S13.23	PI119333/Midas	NA	NA	2	2
S13.24	PI119333/Midas	NA	NA	2	2
S13.25	PI119333/Midas	NA	NA	2	2
S13.26	PI119333/Midas	NA	NA	1	1
S13.27	PI119333/Midas	2	2	3	1
S13.28	PI119333/Midas	2	2	2	2
S13.29	PI119333/Midas	2	2	1	2
S13.3	PI119333/Midas	NA	NA	1	1
S13.30	PI119333/Midas	2	2	1	2
S13.4	PI119333/Midas	NA	NA	2	2
S13.5	PI119333/Midas	NA	NA	1	1
S13.6	PI119333/Midas	NA	NA	1	1
S13.7	PI119333/Midas	NA	NA	1	1
S13.8	PI119333/Midas	NA	NA	2	2
S13.9	PI119333/Midas	NA	NA	1	1
S14.1	PI119333/Tommi	NA	NA	3	2
S14.10	PI119333/Tommi	NA	NA	1	1
S14.11	PI119333/Tommi	NA	NA	3	1
S14.12	PI119333/Tommi	NA	NA	2	2
S14.13	PI119333/Tommi	NA	NA	1	1
S14.14	PI119333/Tommi	NA	NA	1	1
S14.15	PI119333/Tommi	NA	NA	1	1
S14.16	PI119333/Tommi	NA	NA	1	2
S14.17	PI119333/Tommi	NA	NA	2	2
S14.18	PI119333/Tommi	NA	NA	1	1
S14.19	PI119333/Tommi	NA	NA	1	1
S14.2	PI119333/Tommi	NA	NA	2	2
S14.20	PI119333/Tommi	NA	NA	1	1
S14.21	PI119333/Tommi	NA	NA	1	2
S14.22	PI119333/Tommi	NA	NA	1	2
S14.23	PI119333/Tommi	NA	NA	2	2

S14.24	PI119333/Tommi	NA	NA	2	1
S14.25	PI119333/Tommi	NA	NA	2	2
S14.26	PI119333/Tommi	NA	NA	2	2
S14.27	PI119333/Tommi	NA	NA	2	1
S14.28	PI119333/Tommi	NA	NA	1	1
S14.29	PI119333/Tommi	NA	NA	2	2
S14.3	PI119333/Tommi	NA	NA	1	1
S14.30	PI119333/Tommi	NA	NA	1	1
S14.31	PI119333/Tommi	NA	NA	1	NA
S14.32	PI119333/Tommi	NA	NA	2	2
S14.33	PI119333/Tommi	NA	NA	2	2
S14.34	PI119333/Tommi	NA	NA	2	2
S14.35	PI119333/Tommi	NA	NA	3	2
S14.36	PI119333/Tommi	NA	NA	1	2
S14.37	PI119333/Tommi	NA	NA	2	1
S14.38	PI119333/Tommi	NA	NA	3	2
S14.4	PI119333/Tommi	NA	NA	3	2
S14.5	PI119333/Tommi	NA	NA	1	1
S14.6	PI119333/Tommi	NA	NA	2	2
S14.7	PI119333/Tommi	NA	NA	2	1
S14.8	PI119333/Tommi	NA	NA	2	2
S14.9	PI119333/Tommi	NA	NA	1	1

Appendix 40 Genotypic scoring of Population 3 (Blizzard) with the SSR markers Xgwm374, Xgwm264, Xbarc83 and Xcfa2129 (1=resistant, 2=susceptible, 3=heterozygote, NA=not available)

Line	Cross	Xgwm374	Xgwm264	Xbarc83	Xcfa2129
Blizzard	NA	1	1	2	1
Midas	NA	2	2	2	2
Rainer	NA	2	2	2	2
S3.1	Rainer/Blizzard//Midas	1	1	2	1
S3.10	Rainer/Blizzard//Midas	NA	2	NA	2
S3.11	Rainer/Blizzard//Midas	1	NA	2	1
S3.12	Rainer/Blizzard//Midas	2	2	2	1
S3.13	Rainer/Blizzard//Midas	2	NA	2	1
S3.14	Rainer/Blizzard//Midas	2	2	2	2
S3.15	Rainer/Blizzard//Midas	2	2	2	1
S3.16	Rainer/Blizzard//Midas	2	2	2	2
S3.17	Rainer/Blizzard//Midas	2	2	NA	2
S3.18	Rainer/Blizzard//Midas	2	2	NA	2
S3.19	Rainer/Blizzard//Midas	1	NA	2	2
S3.2	Rainer/Blizzard//Midas	1	1	NA	1
S3.20	Rainer/Blizzard//Midas	2	2	2	2
S3.3	Rainer/Blizzard//Midas	2	NA	2	2
S3.4	Rainer/Blizzard//Midas	2	2	2	2
S3.5	Rainer/Blizzard//Midas	NA	2	2	2
S3.6	Rainer/Blizzard//Midas	2	2	2	2
S3.7	Rainer/Blizzard//Midas	2	2	2	2
S3.8	Rainer/Blizzard//Midas	2	2	2	1
S3.9	Rainer/Blizzard//Midas	NA	1	NA	2

S4.38	Blizzard/Rainer	NA	2	1	2
S4.42	Blizzard/Rainer	NA	1	1	2
S4.50	Blizzard/Rainer	NA	2	1	1
S4.53	Blizzard/Rainer	NA	1	1	3
S4.6	Blizzard/Rainer	NA	1	1	1
S5.12	Blizzard/Rainer	NA	1	1	1
S5.15	Blizzard/Rainer	NA	1	1	2
S5.21	Blizzard/Rainer	NA	NA	1	1
S5.31	Blizzard/Rainer	NA	2	1	1
S5.32	Blizzard/Rainer	NA	1	1	2
S5.47	Blizzard/Rainer	NA	2	2	1
S5.58	Blizzard/Rainer	NA	1	1	1

9.5 R-script

9.5.1 Phenotypic analysis

```
#Phenotypic Analysis Preparation
library(sommer)
setwd("E:/Statistik neu")

mydata <- read.table ("Phänotypisierung_neu.csv",header=T, sep=";",dec=",")
summary(mydata)
str(mydata)

mydata$WH <- as.factor(as.character(mydata$WH))

#split into single Populations
Bonneville <- droplevels(mydata[which(mydata$Rsource %in% "Bonneville"),])
PI119333 <- droplevels(mydata[which(mydata$Rsource %in% "PI119333"),])
Blizzard <- droplevels(mydata[which(mydata$Rsource %in% "Blizzard"),])

########################################
##Population 1 Bonnevillle
model <- mmer2(fixed = CB~1,random=~Linie + WH,data=Bonneville, silent=F)
plot(model)
model$var.comp

vg <- model$var.comp$Linie
ve <- model$var.comp$units
h2 <- vg/(vg + ve/2)
h2
sqrt(h2)

model <- mmer2(fixed=CB~-1 +Linie,random=~WH,data=Bonneville,silent=F)
lsmeans_P1 <- data.frame(Linie=gsub("Linie","",rownames(model$beta.hat)),CB=model$beta.hat)
summary(lsmeans_P1)
summary(mydata)
hist(lsmeans_P1$CB)

write.table(lsmeans_P1,"lsmeans_CB_P1.csv",col.names=T,row.names=F,sep=";")

#LSD = t-value * s.e.d
#s.e.d. = sqrt(2ve/r)
#model_lm <- lm(CB~1 + Linie + WH,data=mydata)
#anova(model_lm)
sed <- sqrt(2*ve/2)
lsd <- 1.96*sed
hist(lsmeans_P1$CB, col="cornflowerblue", xlab="Common bunt incidence [%]", ylim=c(0,50),
xlim=c(0,100), breaks=20)
abline(v=lsd,col="red")

model_lm <- lm(CB~1 + Linie + WH,data=Bonneville)
anova(model_lm)
summary(model_lm)
```

```
#segregation pattern
t.value1 <- abs(qt(p=0.05/2,df=nrow(lsmeans_P1)))
lsd1 <- t.value1*sed
lsmeans_P1_res <- droplevels(lsmeans_P1[which(lsmeans_P1$CB<as.numeric(lsd1)),])
lsmeans_P1_sus <- droplevels(lsmeans_P1[which(lsmeans_P1$CB>=as.numeric(lsd1)),])

chisq.test(x=c(12,0),p=c(0.5,0.5))
chisq.test(x=c(12,0),p=c(0.75,0.25))
chisq.test(x=c(12,0),p=c(7/8,1/8))

##########Correlation between replications
setwd("E:/Statistik neu")
WH1 <- read.table ("WH1.csv",header=T, sep=";",dec=",")
WH2 <- read.table ("WH2.csv",header=T, sep=";",dec=",")

data <- droplevels(merge(WH1,WH2,by.x="Linie",by.y="Linie",all.x=F,all.y=F))
str(data)

cor(data[,-1])
plot(data$WH1,data$WH2)

################
mydata <- read.table ("Phänotypisierung_neu.csv",header=T, sep=";",dec=",")
summary(mydata)
str(mydata)

mydata$WH <- as.factor(as.character(mydata$WH))
mydata$AW <- as.numeric(as.numeric(mydata$AW))

WH1 <- droplevels(mydata[which(mydata$WH %in% "1"),])
WH2 <- droplevels(mydata[which(mydata$WH %in% "2"),])

data <- droplevels(merge(WH1,WH2,by.x="Linie",by.y="Linie",all.x=F,all.y=F))
str(data)

CB<-cor.test(data$CB.x, data$CB.y)
CB$estimate
CB$p.value

plot(data$CB.x, data$CB.y,
    xlab="Replication 1", ylab="Replication 2", main="",
    xlim=c(0,100), ylim=c(0,100))
abline(lm(data$CB.x~data$CB.y), col="red")

######################
Pop1_WH1 <- droplevels(WH1[which(WH1$Rsource %in% "Bonneville"),])
Pop1_WH2 <- droplevels(WH2[which(WH2$Rsource %in% "Bonneville"),])

Pop2_WH1 <- droplevels(WH1[which(WH1$Rsource %in% "PI119333"),])
Pop2_WH2 <- droplevels(WH2[which(WH2$Rsource %in% "PI119333"),])

Pop3_WH1 <- droplevels(WH1[which(WH1$Rsource %in% "Blizzard"),])
Pop3_WH2 <- droplevels(WH2[which(WH2$Rsource %in% "Blizzard"),])
```

```
#####################Pop1
data <- droplevels(merge(Pop1_WH1,Pop1_WH2,by.x="Linie",by.y="Linie",all.x=F,all.y=F))

CB<-cor.test(data$CB.x, data$CB.y)
CB$estimate
CB$p.value

plot(data$CB.x, data$CB.y,
    xlab="Replication 1", ylab="Replication 2", main="Population 1: Bonneville",
    xlim=c(0,100), ylim=c(0,100))
abline(lm(data$CB.x~data$CB.y), col="red")

DH<-cor.test(data$DH.x, data$DH.y)
DH$estimate
DH$p.value

LOD<-cor.test(data$LOD.x, data$LOD.y)
LOD$estimate
LOD$p.value

PH<-cor.test(data$WUH.x, data$WUH.y)
PH$estimate
PH$p.value

CN<-cor.test(data$cn.x, data$cn.y)
CN$estimate
CN$p.value

AW<-cor.test(data$AW.x, data$AW.y)
AW$estimate
AW$p.value

FHB<-cor.test(data$FHB.x, data$FHB.y)
FHB$estimate
FHB$p.value

LH<-cor.test(data$LH.x, data$LH.y)
LH$estimate
LH$p.value
```

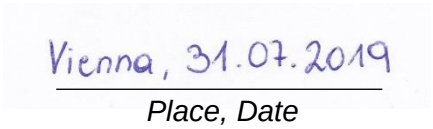
9.5.2 Genotypic analysis

```
#Genotypic analysis
#Preparation
library(sommer)
setwd("E:/Statistik neu")

mydata_CB <- read.table("lsmeans_CB.csv", header=T, sep=";",dec=".")
marker <- read.table("Genotypisierung_neu.csv", header=T, sep=";", dec=".")
marker <- droplevels(marker[!duplicated(marker$Linie),])
str(mydata_CB)
str(marker)

mydata_CB <- droplevels(merge(mydata_CB,marker,by.x = "Linie",by.y = "Linie",all.x =
FALSE,all.y = FALSE))
mydata_CB$barc83_2 <- mydata_CB$barc83
mydata_CB$barc83_2[which(mydata_CB$barc83_2==3)] <- NA
mydata_CB$cfa2129_2 <- mydata_CB$cfa2129
mydata_CB$cfa2129_2[which(mydata_CB$cfa2129_2==3)] <- NA

mydata_CB$gwm374 <- as.factor(as.character(mydata_CB$gwm374))
mydata_CB$gwm264 <- as.factor(as.character(mydata_CB$gwm264))
mydata_CB$barc83 <- as.factor(as.character(mydata_CB$barc83))
mydata_CB$cfa2129 <- as.factor(as.character(mydata_CB$cfa2129))
mydata_CB$barc83_2 <- as.factor(as.character(mydata_CB$barc83_2))
mydata_CB$cfa2129_2 <- as.factor(as.character(mydata_CB$cfa2129_2))
str(mydata)

##Varianzanalyse populations
###Pop1 Bonneville
#gwm374
lsmeans_Pop1m <- droplevels(mydata_CB[which(mydata_CB$Rsource %in% "Bonneville"),])
summary(lsmeans_Pop1m)
hist(lsmeans_Pop1m$CB)

#gwm374
model <- lm(CB~gwm374,data=lsmeans_Pop1m)
anova(model)
boxplot(CB~gwm374,data=lsmeans_Pop1m)
summary(model)

#barc83_2
model <- lm(CB~barc83_2,data=lsmeans_Pop1m)
anova(model)
boxplot(CB~barc83_2,data=lsmeans_Pop1m)
summary(model)

#gwm264
model <- lm(CB~gwm264,data=lsmeans_Pop1m)
anova(model)
boxplot(CB~gwm264,data=lsmeans_Pop1m)
summary(model)

#cfa2192_2
```

```
model <- lm(CB~cfa2129_2,data=lsmeans_Pop1m)
anova(model)
boxplot(CB~cfa2129_2,data=lsmeans_Pop1m)
summary(model)

model <- lm(CB~gwm374*cfa2129_2,data=lsmeans_Pop1m)
anova(model)

###Pop2 PI119333
lsmeans_Pop2m <- droplevels(mydata_CB[which(mydata_CB$Rsource %in% "PI119333"),])
summary(lsmeans_Pop2m)
hist(lsmeans_Pop2m$CB)

#barc83_2
model <- lm(CB~barc83_2,data=lsmeans_Pop2m)
anova(model)
boxplot(CB~barc83_2,data=lsmeans_Pop2m)
summary(model)

#cfa2129_2
model <- lm(CB~cfa2129_2,data=lsmeans_Pop2m)
anova(model)
boxplot(CB~cfa2129_2,data=lsmeans_Pop2m)
summary(model)

###Pop3 Blizzard
lsmeans_Pop3m <- droplevels(mydata_CB[which(mydata_CB$Rsource %in% "Blizzard"),])
summary(lsmeans_Pop3m)
hist(lsmeans_Pop3m$CB)

#gwm374
model <- lm(CB~gwm374,data=lsmeans_Pop3m)
anova(model)
boxplot(CB~gwm374,data=lsmeans_Pop3m)
summary(model)

#gwm264
model <- lm(CB~gwm264,data=lsmeans_Pop3m)
anova(model)
boxplot(CB~gwm264,data=lsmeans_Pop3m)
summary(model)

#barc83_2
model <- lm(CB~barc83_2,data=lsmeans_Pop3m)
anova(model)
boxplot(CB~barc83_2,data=lsmeans_Pop3m)
summary(model)

#cfa2129_2
model <- lm(CB~cfa2129_2,data=lsmeans_Pop3m)
anova(model)
boxplot(CB~cfa2129_2,data=lsmeans_Pop3m)
summary(model)
```

Affidavit

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.



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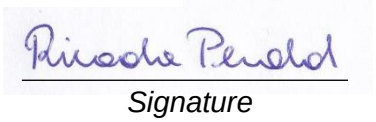

Signature