BOKU
University of Natural Resources and Life Sciences Vienna
Department of Agrobiotechnology Tulln
IFA Tulln
Institute for Biotechnology in Plant Production

Diploma thesis

The association of the semi-dwarf genes *Rht-B1* and *Rht-D1* with Fusarium head blight resistance

By

TABI TATAW James
Matriculation number 9740376

Field of study
Phytomedecin

Branch of study
Crop production

Supervisor:
Univ.-Prof. Dipl.-Ing. Dr. Hermann Bürstmayr
Vienna, January 2011

DEDICATION

This book is dedicated to my beloved son

TABI TATAW James jr.

ACKNOWLEDGMENTS

I am sincerely grateful to my supervisor Univ.-Prof. Dipl.-Ing. Dr. Hermann Bürstmayr for giving me the opportunity to carry out this research at the institute of biotechnology in plant production . He actually provided the necessary datasets and was especially helpful in coordinating and correcting the entire project despite of his busy time schedule.

I am also grateful to the all the entire staffs at the institute of biotechnology in plant production especially Dipl.-Ing Maria Bürstmayr and for their assistance in the laboratory experiments and Ing. Matthias Fidesser for information on the field experiments.

Thanks goes also to my family, friends and well wishers who supported me morally, financially, and socially during my entire studies. Thanks also to the African catholic community in Vienna, the catholic charismatic renewal in Vienna, the Cameroonian association in Vienna, and the Cameroonian association in Linz,

The following people have been of great help, in one way or another to me during my lifetime, Mrs AkO Frida Ashu (my beloved mother), Chief Natribo-Tataw J.K, Mrs Ntaribo-Tataw Clara, Mrs Ntaribo- Tataw Mary, Miss Ashu Linda, Tabi Annete, Tabi Susan Ndakor, Tabi George, Bih Quinta Tem, Ebot Paul Campling, Kossiwa Kougbany, Axel Schopf, Epossi Mary, Ntaribo Tataw Jackson, Yvonne Tem, Ntaribo-Tataw Emmanuel, Ntaribo-Tataw Joseph Bakwa, Mrs Tupa Brenda, and Gathien Marie-Laure, Christian Gschlad, Kuya Sandjong Suzarine L., Bitouh Daniel, Amanm Emem, Walter Linkberger and Tabi Tataw Elsie Frida

ABSTRACT

Fusarium head blight (FHB) is a fungal disease which causes losses to farmers in many wheat production regions. The fungus induces shrivelling of the seeds directly affecting yield as well as losses in quality due to the production of mycotoxins. The contamination of the grains with mycotoxins is hazardous to humans as well as to animals. In the wheat gene pool large quantitative variation for resistance to Fusarium head blight is evident. In this study the association of the stem shortening genes *Rht-B1* and *Rht-D1* with resistance to Fusarium Head Blight (FHB) was examined.

Two double haploid populations derived from crosses between the parents *Herman*×*Mulan* and *Herman*×*Nord010044* were artificially inoculated with *Fusarium culmorum* in a field experiment. The traits flowering date, plant height and FHB severity were visually assessed in both populations. The doubled haploid lines were examined at the molecular level using diagnostic PCR markers for genes *Rht-B1* and *Rht-D1*.

The dwarfing genes were not associated with flowering date in these populations. The lines which carried the 'semi-dwarf' alleles *Rht-B1b* or *Rht-D1b* both showed on average significantly reduced plant height (as expected), but also higher FHB severity than the plants with the 'tall' *Rht-B1a* or *Rht-D1a* alleles. However, there was large variation in FHB severity within each group for specific *Rht*-alleles. Therefore, the selection of moderately resistant wheat lines carrying one 'semi-dwarf' allele, either *Rht-D1b* or *Rht-B1b* appears feasible. On the other hand, selection of double-dwarf lines (*Rht-B1b* and *Rht-D1b* combined) with a good level of FHB resistance seems almost impossible.

ZUSAMMENFASSUNG

Die Ährenfusariose ist eine Krankheit an Weizen und anderen Getreidearten, welche in vielen Regionen der Erde zur bedeutenden Ertrags- und Qualitätseinbußen führen kann. Ein Befall der Ähre führt zur Ausbildung von Kümmerkörnern, zu Ertragsreduktion und bewirkt, dass das Erntegut mit Pilzgiften (Mykotoxinen) belastet wird. Fusarium-Pilzgifte sind für Mensch und Tier schädlich. Im Genpool des Weizens gibt es große genetische Variabilität für das Merkmal Resistenz gegen Ährenfusariose. In der vorliegenden Arbeit wurde der Zusammenhang zwischen den Halmverkürzungsgenen *Rht-B1* und *Rht-D1* und der Resistenz gegenüber Ährenfusariose untersucht.

Zwei doppelhaploide Populationen aus den Kreuzungen *Herman*×*Mulan* und *Herman*×*Nord010044* wurden in einem inokulierten Feldversuch auf Ährenfusarioseresistenz überprüft. Zusätzlich wurde die Pflanzenlänge und das Blühdatum der Linien erhoben. Die Populationen wurden mittels diagnostischer PCR Marker für die Halmverkürzungsgene *Rht-B1* und *Rht-D1* molekulargenetisch analysiert.

Die Halmverkürzungsgene hatten keinen Einfluss auf das Merkmal Blühdatum. Linien welche die halmverkürzenden Allele *Rht-B1b* bzw. *Rht-D1b* trugen zeigten wie erwartet signifikant verringerte Wuchshöhe, gleichzeitig allerdings signifikant erhöhten Fusariumbefall im Vergleich zu den Linien mit den nicht-halmverkürzenden Allelen (*Rht-B1a* bzw. *Rht-D1a*). Die Variation für Fusariumbefall innerhalb der jeweiligen Gruppe war groß, sodass die Selektion von fusariumresistenten Zuchtlinien mit mittlerer Pflanzenlänge welche entweder das Allel *Rht-B1b* oder *Rht-D1b* aufweisen möglich erscheint.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Problem description

Wheat is one of the most highly demanding crops in the world, and the steady increase in world population means also that the demand for wheat will increase proportionately. Wheat breeders throughout the world are working to increase the production of wheat by reducing plant height, thus making the plants resistance to lodging in extensive agriculture. Wheat belongs to the plant family *Poaceae* (*Gramineae* = grasses), whose production began about 7000 before Christ in the Euro-Asia region. There are various varieties of wheat grown around the world, of which *Triticum aestivum* is one of the most important, which alone account for over 25% of the world crop production and supplies about 55% of the world's carbohydrate. Wheat have a polyploidy genome making it a model system for cytogenetic. The wheat genomes have been intensely studied of recent and genetic maps for all its chromosomes are available (Gupta et al. 1999).

The *Rht* dwarfing genes are responsible for controlling plant height. According to Cho et al. (1993) the short varieties originated from Korea as early as the third and fourth centuries. They were afterward introduced to Japan from there they were transferred through long and complex geohistorical pathways to Europe, America and the rest of the world (Borojevic & Borojevic 2005). Ninety percent of the semi-dwarf wheat varieties grown worldwide have the semi-dwarf phenotype controlled by three major genes *Rht-B1 (formerly Rht1), Rht-D1 (formerly Rht2)* and *Rht8*. The proportion of the major genes was later explained by Tosovic-Maric et al. (2008), by evaluating the *Rht* genes in hexaploid wheat of 172 genotype originating from over 20 different countries, using STSs und SSR markers. They found out that the *Rht-B1b, Rht-D1b* and the *Rht8* were in a ratio of 40 %, 22% and 62% respectively. The highest difference in allele frequency was determined in the *Rht8* genotypes. The *Rht-B1b* was dominantly present in foreign (varieties outside Serbia) germplasm (57%), while the *Rht-D1b* was almost the same in both the domestic and foreign varieties with 22.6% and 21.2% respectively. Their findings were starting points for the use of marker Assisted Selection (MAS) for high yielding wheat genotype in Serbia and the Mediterranean region. In a similar way, Yamada (1990) carried out a similar research in order to study the distribution of the Rht genes in wheat in Japan. By analysing the GA insensitive *Rht* genes in *Triticum aestivum* he identified 18 *Rht* genes in Japanese modern varieties and landraces. Only 6

varieties out of 12 modern varieties tested carried the *Rht1 (Rht-B1b)* allele. The remaining 6 carried the *Rht2 (Rht-D1b)* allele. No varieties tested carried both the *Rht-B1b* and *RhtD1b* alleles combined. All 6 land races tested carried the *Rht-D1b* gene only. In his study the geographical distribution of the *Rht* genotypes in Japanese modern varieties was clearly localised.

Paveley et al. (2002) in an experiment aimed at reducing or eliminating the use of pesticide and to minimised unit cost in wheat cultivars, found out that this can be attend by improving and exploiting the "self defence" against wheat disease . Three of the self defence mechanism includes disease escape, resistance and tolerance. They found out that the dwarfing gene affects the mechanism of disease escape. In an experiment with isogenic lines of wheat infected of *septoria* with equal levels of resistance, but which vary for single dwarfing gene, which control internode length and stem extension rate. They found that higher levels of dwarfism from *Rht3 (Rht-B1c)* and *Rht12*, caused increased spores transfer and consequently early epidemics. The *Rht2 (Rht-D1b)* dwarfing genes reduced height to an agronomical acceptable extent, while maintaining the escape benefits of the tall *Rht-B1a* (old name *rht1)* "wild type" which is to escape inhabited spores to be transferred to the upper canopy.

Fusarium head blight (FHB) is a fungal disease that mostly affects wheat, corn and barley, including other crops. It was first discovered in England in the year 1884 where because of the chalky, lifeless appearance of the infected kernels, it was called wheat scab, and later tombstone disease (Clear & Patrick 2010). FHB can cause significant yield losses due to floret sterility and reduced grain weight as well as quality reductions due to the production of mycotoxins. The occurrence of such natural contaminants in wheat (and other cereals) is of great concern because their presence in feeds and foods is often associated with chronic or acute mycotoxicoses in livestock and could threaten human health (Visconti & Pascale 2010). The control of FHB with the use of management strategies like, crop rotation, tillage and the application of fungicide produces only limited results (Paul et al. 2005). The most effective strategy for controlling FHB in wheat is through the development of resistant cultivars. Resistance to FHB exhibits quantitative variation and its inheritance involves several loci on different chromosomes (Kolb et al. 2001). Genotype x environment interaction complicates the phenotypic evaluation of FHB resistance and makes screening of FHB resistance laborious, time consuming and costly (Rudd et al. 2001).

Triticum aestivum, is hexaploid, which means it has three genomes (A, B, and D), these being derived from its three wild diploid ancestors. Each genome contains 14 chromosomes so that bread wheat has 42 chromosomes in total. The A, B, and D genomes are very similar to each other and are described as homoeologous. *Rht-B1b* and *Rht-D1b* are corresponding (homoeologous) genes on the B and D genomes, respectively. The aim of this study is to find out the association of the semi-dwarf genes *Rht-B1b* and *Rht-D1* with FHB, using different molecular markers. The correlation of the semi-dwarfing genes with plant height, date of anthesis and FHB severity will be examined.

1.2. The *Rht* genes and plant height

The use of fertilizers and pesticides on wheat leads to an increase in plant height as well as grain yield. However, the heavier grains caused the plants to become unstable and prone to lodging (falling over) in high winds and rainfall. Borlaug (1968) introduced dwarfing genes into wheat, giving the plants a stronger, shorter stem that resisted lodging. Plant height is thus an important factor associated with the *Rht* gene.

Zaccai et al. (1987) studying in Jerusalem, the relationship between plant height and the grain protein content in tetraploid wheat (*Tritium turgidium*) in an F2 cross between a high protein line and a semi dwarf cultivar segregating for the dwarfing gene *Rht1 (Rht-B1b),* found out that the grain of plants homozygous for *Rht1 (Rht-B1b)* had on average 1.2% less protein than the grains of plants not homozygous for the *rht1 (Rht-B1a)* genes.

Flintham et al. (1997) comparing the *Rht-B1b, Rht-D1b* and *Rht-B1c* for gibberellins insensitive dwarfism in a hexaploid wheat (*Titricum astivum*) over the tall controls observed yield advantages of shorter plants over taller plants in several trials with mean grain yield ranging from 200 to 760 g $m^{-2.}$ The Ear yield also increased due to the present of the *Rht* gene. They explained that the *Rht* genes achieve these by accelerating the loss of cell wall extensibility. The *Rht-B1b* and *Rht-D1b* had similar effect on plant height with 86% and 83% respectively when compared to the tall controls, while their combined effect reduced the plant height to 58%. The *Rht-B1c* caused further dwarfism of 50% and its combination with the *Rht-D1b* allele produced a more severe dwarfism of 41%

Seefeldt et al. (1999) using near isolines of Nugaines winter *Triticum aestivum* that differed in height with and without *Aegilops cylindrica* to determine the effect of plant height on

competition against *Ae. cylindrica*, found out that the isolines had either the reduced height gene *Rht1 (Rht-B1b), Rht2 (Rht-D1b), Rht1(Rht-B1b)* plus *Rht2(Rht-D1b)*, or neither *Rht* genes had an averaged 79, 77, 51, and 101 cm tall, respectively, when grown with or without competition from *Ae. cylindrica*. Plants with fewer reduced height genes had the faster rates of height and weight gain, which are important traits for enhanced competitiveness. When growing in competition with *Ae. cylindrica*, the shortest isoline allowed the greatest amount of *Ae. cylindrica* seed production but did not have the lowest *T. aestivum* yield. However, when compared to the *A. cylindrica*-free control, the shortest isoline had the greatest percent yield loss. The tallest isoline reduced *Ae. cylindrica* seed production the most, and *T. aestivum* yield reduction due to *Ae. cylindrica* on a percent basis that was the least when averaged over 2 years. When competing against *Ae. cylindrica,* the tallest isoline did not always have the largest yield and yield parameters, and the shortest isoline did not always have the smallest yield and yield parameters. There is a cost to the *T. aestivum* plant to produce extra stem biomass that may reduce yield potential of taller plants and reduce the advantage gained by being taller than the surrounding weeds.

Singha et al. (2001) analyzing the *Rht1 (Rht-B1b)* and *Rht 2 (Rht-D1b)* dwarfing genes for plant height, kernel weight, coleoptile length and grain yield, in bread wheat (*Triticum aestivum*) and durum wheat (*T. turgidium*) found out that a number of bread wheat cultivars showed relatively small height increases following the removal of the *Rht* gene and substantially greater increases in coleoptile length. The grain yield and plant height were positively correlated among the semi - dwarf *Rht* genes. Coleoptile length correlated highly with seed weight among cultivars without the *Rht* genes compared to cultivars with the *Rht*-genes. The tall durum wheat tended to be taller than their bread wheat counterpart, indicating the absence of minor genes for reduced height. Also the durum wheat regardless of stature produced longer coleoptile length than bread wheat at higher temperatures.

Knopf et al. (2008) found out that wheat varieties with the *Rht-D1b* were shorter and produces higher yield than varieties without this allele, but more susceptible to Fusarium Head Blight (FHB) one of the most destructive wheat disease caused by *Fusarium graminearum*, *Fusarium culmorum* and other species.

On the other side, analyzing near –isogenic lines carrying *Rht1 (Rht-B1b), Rht2 (Rht-D1b)* or *Rht3 (Rht-B1c)* in a genetic background of spring and winter wheat varieties, Youssefian et al. (1992) found out that these dwarfing genes have no effect on the number of organs, or on the timing of developmental events, when characters like leaf and spikelet primordium initiation, leaf

emergence and stem internodes extension were studied. Also Katashi et al. (2004) found out that the *Rht* genes in wheat limit the ability of the roots to penetrate compacted soil layers

Although the studies of Youssefian et al. (1992) and Katashi et al. (2004) portrait a negative impact of the *Rht* gene with plant yield, the majority of researches showed clearly that the *Rht* genes positive affects plant yield by reducing plant height.

Most of these reduced height *(Rht)* genes are dominant or semi-dominant, indicating that they actively inhibit growth through a so-called gain-of-function mutation. The identity and function of most of these genes are not known, but some have been found to prevent the action of gibberellins. The *Rht-B1b* and *Rht-D1b* are used in many commercial wheat varieties. Some varieties containing both of these dwarfing genes have an additive effect on growth.

The *Rht-B1b* and *Rht-D1b* dwarfing genes were derived from *Norin 10*, which was a semi-dwarf variety bred in Japan and released in 1935 (Gale & Youssefian 1985) while the wild-type *Rht-B1a* and *Rht-D1a*, were isolated a few years ago (Peng et al. 1999) These wild-type gene encode DELLA proteins, which are components of the GA signal transduction pathway. DELLA proteins act as repressors of plant growth, although the mechanism by which they do this is not well understood.

1.3. The association of *Rht* genes with Fusarium head blight resistance

Looking for a QTL is one of the major activities of modern-day plant breeders. Several FHB resistance loci have been found (e.g. Shen et al. 2003; Zhou et al. 2003, Buerstmayr et al. 2003, Mesfin et al. 2003, Steiner at al. 2004, Srinivasachary et al. 2009, Miedaner & Voss 2008, Yu & Bai 2009, Yu et al 2010).

In a Chinese source *Sumai 3* (spring wheat), a major quantitative trait loci (QTL) on chromosome 3BS explained up to 50% of the phenotypic variation and seems to be primarily associated with Type II resistance to FHB, which is the resistance to fungal spread after infection (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001*). Qfhs.ndsu-3BS*, re-designated as *Fhb 1* has been identified and verified by several research groups in several wheat backgrounds and environments. In the same region on chromosome 3BS between the markers *Xgwm493* and *Xgwm389*, three other fungal resistance genes/QTLs have been localized*: Sr2* (durable stem rust resistance), *Stb2* (*Septoria tritici* blotch) and *QSng.sfr.-3BS* (*Stagonospora* blotch) (Uphaus et al., 2005). Additionally, QTLs for FHB resistance on chromosomes 6B (Waldron et al., 1999), 2A and

2B (Zhou et al., 2002) with minor influence have been reported.

Buerstmayr et al. (2003) found two QTLs on chromosomes 3B *(Qfhs.ndsu-3BS)* and 5A *(Qfhs.ifa-5A)* for FHB severity in a DH wheat cultivar situated in Tulln and Hohenheim. The QTL (*Qfhs.ndsu-3BS*) appeared to be associated mainly with resistance to fungal spread, while the (QTL *Qfhs.ifa-5A*) was associated mainly to fungal penetration (Type I resistance to FHB). These two major QTL operating together with unknown numbers of minor genes controls the FHB resistance. The selection of these QTL by breeder is useful for the production of resistant wheat cultivars. In the same manner Häberle et al. (2008) found further QTL (*Qfhs.lfl-1Bl, Qfhs.lfl-6AL. Qfhs-lfl-7BS*) mapping to clusters of loci involved in FHB resistance. Their important for a good FHB resistance is highlighted by the fact that the most resistant genotypes carried at least one of the mentioned resistance alleles.

Srinivasachary et al. (2009) after crossing the wheat species *Soissons (Rht-B1b; Rht-D1a)* with *Orvantis (Rht-B1a; Rht-D1b)* doubled haploid population in three trials found out that *Soissions* contributed a single major FHB QTL linked to the *Rht-D1* locus. The *Rht-B1b* contributed by *Soissons* conferred no negative effect on FHB resistance; it even conferred a minor positive effect on one trial. In another experiment with the two other species, near iosogenic lines, the *Rht-B1b* and *Rht-D1b* significantly decreased the resistance to initial infection while *Rht-D1b* had no effect on the resistance to spread of the fungus within the spike. This showed that the semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight and may be a significant consideration in plant breeding where resistance to FHB is important.

In a trial to map QTL for resistance to FHB, Draeger et al (2007) were able to detect ten QTL or associated trait resistance to FHB, in a DH population, from a cross between the Swiss FHB resistance variety *Arina* and the UK FHB susceptible variety *Riband*, in five trials across different years and location. They found out that the most stable and significant QTL was located on chromosome 4D were also the *Rht-D1* locus for height was located. This association was attributed to linkage of deleterious genes to the *Rht-D1b* allele rather than differences in height. They equally found out that the FHB resistance of *Arina* was conferred by several genes of moderate effect making it difficult to exploit in marker assisted selection (MAS) breeding programmes.

Miedaner & Voss (2008) found in two British cultivars that the *Rht-B1b* and *Rht-D1b* significantly increased the mean FHB rating by 35 and 52% respectively. Although the *Rht* alleles increased FHB subceptibility, they found out that these negative effects can be largely counteracted

by a more resistant genetic background.

Segregation for FHB resistance in three European winter population enabled Holzapfel et al. (2008) to identify resistance loci in well adapted germplasm. They found a total of 18 genomic regions repeatedly associated with FHB in populations obtained from crosses of resistant cultivars *Apache, History* and *Romanus* with susceptible semi-dwarf cultivars *Biscay, Rubens* and *Pirat* respectively. They found out that the semi- dwarfing allele *Rht-D1b* was a major source for FHB susceptibility in European winter wheat. The semi-dwarf parent contributed resistance alleles of major effects compensating the negative effects of *Rht-D1b* on FHB reactions. They attributed high level of resistance of the cultivars *Romanus* and *History* to the several minor resistance QTL interacting with the environment and the absence of the *Rht-D1b* allele.

To find out the genetic basis why the variety *Spark* is more resistance than most other UK winter varieties, Srinivasachary et al. (2008) crossed *Spark* with the FHB subceptible variety *Rialto* to identify QTL associated with resistance. A total of nine QTL for FHB resistance and four QTL for plant height were identified. One of the identified QTL locus coincided with the *Rht-D1* locus and accounted for up to 51% of phenotypic variance. The *Rht-D1b* allele associated with FHB susceptibility but had no effect on plant height. They found out further that lines carrying the *Rht-D1b* allele are compromised in resistance to initial infection (type I resistance), but unaffected in resistance to spread within spike (type II resistance). This finding was again confirmed by Srinivasachary et al. (2009) in a cross between the resistance variety *Soissons (Rht-B1b; Rht-D1a)* and the variety *Orvantis (Rht-B1a; Rht-D1a). Rht-B1b* and *Rht-D1b* significantly decreased type I resistance under high disease pressure while *Rht-B1b* significantly increase type II resistance. *Rht-D1b* had no effect on type II resistance.

Yu & Bai (2009) crossing a long coleoptile Chinese landrace with a short US coleoptile wheat cultivar was able to find six significant QTL on 1B, 3D, 4DS, 4DL 5AS, and 5B for the coleoptile length. Four of them on *3D, 4DS, 4DL* and *5AS*, showed pleiotropic effects on plant height *(Rht)*. One major QTL for long coleoptile was mapped on the locus *Rht-D1 (Rht2)* for reduced height *(Rht)* on chromosome 4DS and explained up to 65% of phenotypic variation for coleoptile length. Another major QTL was located on 4DL and explained up to 33% of phenotypic variation for coleoptile length. The height allele *Rht-D1a* from a Chinese genotype appeared to have an epistatic effect on the chromosome 4DL QTL for long coleoptile. The *Rht- D1a* explained a major portion of genetic variation in the Chinese genotype, and its combination with gibberellic acid-insensitive *Rht* genes for reduced height from other sources was able to select for long

coleoptiles.

By genotyping two F2 population, *Renan (Rht-B1b)*Camp-Rémy* and *Rendez-Vous (Rht-D1b)* Roazon,* Sourdille et al. (1996) was able to identify that the RFLP loci, *Xpsr 114-4B* was linked to the dwarfing gene *Rht-B1* and on chromosome 4BS and also the RFLP loci *Xglk578* was linked to the dwarfing gene *Rht-D1* on the chromosome 4DS in *Triticum aestivum.*

These researches show that FHB resistance QTL have steadily been discovered throughout the past decade and the search for further QTL continues.

1.4 The association of Rht genes with plant morphology

Developmental genes often have pleiotropic effects on plant morphology, development and biomechanics. The vegetative morphological and physiological characters of the *Rht1 (Rht-B1b), Rht2 (Rht-D1b)* and *Rht3 (Rht-B1c)* in near –isogenic lines of wheat were investigated by King et al. (1983). They found out that the *Rht* genes affect leaf size with a resultant decrease in leaf area of the whole plant. The *Rht3 (Rht-B1c)* gene reduced leaf area in young plants by as much as 30 percent. It equally altered the flag leaf dimensions and stomata distribution, but had no effect on its area and the net CO_2 exchange. Dominick et al.1996 equally found a negative correlation for *Rht*-dosage with respect to the breaking strength, because *Rht* plants had a greater transverse dimension and equal amounts of principal fiber strand compared to wild type. Transverse size variables such as blade transverse area and blade width were positively related to the breaking stress of leaves, as well as the total area of the principal fiber strand. This was explained by Gent & Kiyomoto, (1997) that the *Rht1 (Rht-B1b), Rht2 (Rht-D1b* modulates the morphology und physiology of wheat in a manner that involves the compensation among several physiological processes, such as decrease in leaf area but increase in photosynthesis per unit area, so that the biomass accumulation remains unaltered. The *Rht* genes equally increase leaf permeability to water vapour, but the plant water status changes to minimize differences in water use efficiency. Semi dwarf wheat has greater harvest index at maturity than their tall counterparts probably due to less competition for carbohydrate during stem elongation. The greater grain number per spike of the semi dwarf wheat at maturity is not completely compensated by the tall wheat greater weight per kernel at maturity. It seems the reduction in plant height retards canopy closure and efficient the interception of solar

radiation resulting in lower biomass and yield of the dwarf wheat. The compensation that leads to greater yield appears to fail in the *Rht1 (Rht-B1b), Rht2 (Rh-D1b* or *Rht3 (Rht-B1c)* dwarf wheat. Here specific photosynthesise does not completely compensate for decreased area per leaf. The *Rht* genes have insignificant effects in the rate of development or winter hardiness however drought stress reduces yield and harvest index of wheat more than tall wheat. The effect of *Rht* genes on the physiology appears to be similar in winter, spring and durum wheat.

In an effort to evaluate the influence of *Rht* on the mechanics of soil penetration by an organ, using various *Rht* dosage (Rht 0 2 4) among coleoptiles in wheat seedlings, Karl & Dominick (1990) found out that the *Rht* genes reduces tissue elastic modulus E, increases the second moment of area I, and decreases the slenderness ratio (l/r) of coleoptiles. *Rht* is biomechanically disadvantageous to seedling establishment. *Rht* affects a variety of mechanical features whose influence is dependent upon the stage of seedling growth and the degree of soil compaction.

Investigating the effect of various reducing height genes on the early stage of development of a plant using a combination of near isogenic, recombinant, mutant and wild type genes, Ellis et al. (2004) were able to prove that Gibberellin insensitivity caused by *Rht-B1b* or *Rht-D1b* was associated with reduced leaf elongation rate and coleoptile length. The case was also the same for the dwarfing gens *Rht1 (Rht-B1b)* and *Rht7*. However for the dwarfing gene *Rht8* no effect was found regarding the coleoptile length, leaf elongation or the response to the plant hormone Gibberellin, indicating that these dwarfing genes may act later in wheat development to reduced plant height and increase the harvest index, without affecting early growth. They found another *Rht* gene in cultivars with durum background which reduced the coleoptile lengths and leaf elongation rates, but had a greater response to Gibberellin than tall varieties.

Based on previous finding that the *Rht* genes affect the plant hormone gibberellic acid (GA3) in the metabolic pathway (Spielmeyer et al. 2004), Hoops (2006) working on the hypothesis that these genes will also dwarf or affect the root character as well as shoot character, and this may in turn affect the nutrient uptake and grain yield in these wheat lines, carried out a project to determine the effect of the stem dwarfing alleles *(Rht1 (Rht-B1b), Rht2 (Rht-D1b), Rht3 (Rht-B1c)* and the tall alleles *rht)* on components of root system in near isogenic lines of two wheat cultivar, *Maringa* and *Nainari 60* . The expected result was that the lines with the wild-type tall alleles should have a greater and longer root system than the dwarf *Rht3 (Rht-B1c)/Rht3 (Rht-B1c)* lines, and the semi-dwarf genotypes to be intermediate. It was expected that the 45 day old *Maringa* isogenic lines was to have a larger root system than the *Nainari 60* isogenic series , if root character

reflect shoot character at this early stage of plant growth. The length of the longest root may be similar in each line but the total length of all seminal roots longer than 30 cm may be significantly different among lines. It was also expected that genotypes with a larger root system should take up more water and fertilizer than those with small root systems. The real results however showed that there was no effect on the two varieties in the number of root more that 30 cm, longest root and the total root length. These three parameters all had a similar value. There was no evidence that the semi-dwarfing genes *Rht1 (Rht-B1b)* and *Rht2 (Rht-D1b)* in both varieties reduced root traits. There were small differences in the biomass of different lines. However these differences do not show a decreased biomass in the dwarf lines. The dwarfing genes *Rht3 (Rht-B1c)* consistently reduced shallow and deep root biomass only in the *Maringa* lines.

1.5. The association of *Rht* genes with yield

High plant yield is economically the greatest aspect in agriculture. In the past higher wheat grain yield was obtained through the use on much fertiliser and pesticides. Nowadays most commercial wheat in temperate and sub tropical regions, higher yield are obtained by introducing the dwarfing genes which increases the harvest index by accumulating a greater proportion of the products of photosynthesis in the grain than in the leaves (Evans 1998).

Pinthus & Levy (1983) carried out a study in an F5 cultivar, uniform in height and in heading date, of three crosses between the semi dwarf spring wheat cultivars *Triticum aestivum* differing in grain weight and in the *Rht* gene. The result showed that no differences in grain weight were recorded between the two semi dwarf genotype *(Rht1 (Rht-B1b)* and *Rht2 (Rht-D1b)*. The tall genotype *Rht1 (Rht-B1b)* was significantly higher in grain weight than the two semi dwarf genotypes. These effects were independent of differences in height, heading date or number of grain per spike.

Balyan & Lohia (1998) evaluating the dwarfing genes *(Rht)* and the tall genes *(rht)* in a wheat cultivar in India, under an irrigated (non-stress) environment and a rain-fed (water stress) environment, showed that the *Rht1 (Rht-B1b)* and *Rht2 (Rht-D1b)* dwarfing genes have a negative effect on grain yield under water stress conditions. The declined in grain yield was caused by reduced number of lighter tillers, reduction in the number of floret per spike, reduction in the number of grain per spike, reduced grain weight and early maturity. They concluded that the poor

biological and grain yield of the *Rht* dwarfing genes under water stress should come from their greater sensitivity to factor associated with diminished soil moisture content. They noted variability for stress susceptibility index, and its negative association with grain yield of the dwarfing genes under water stress conditions which led them to suggest that it is possible to select *Rht* containing semi dwarf lines with high yield potential and greater tolerance to water stress conditions.

Ellis et al. (2005) demonstrated that the dwarfing genes *Rht-B1b* and *Rht-D1b* could be replaced by other dwarfing genes for bread wheat improvement. They found the linked markers *Rht5* on chromosome 3BS, *Rht12* on chromosome 5AL and *Rht13*on chromosome 7BS, which produces height differences of until 43cm indicting their linkage to the height reducing genes. Further, *Rht4* on chromosome 2BL was found accounting for up till 30% of variance in height, *Rht9* on chromosome 5AL which produced only small but significant height effect, and *Rht8* on chromosome 2DS. The height-reducing effect of these dwarfing genes was repeatable across a range of environments, validating their application for higher wheat yield.

Drouyer et al. (2008), carrying out a glasshouse experiment with a set of near - isogenic line (NILs) dwarfing genotype *Rht-B1b, Rht13* and the tall genotype with the objective to determine how the *Rht13* backcross lines may have produced equivalent yields but lower levels of screening (small grains) in multi-environment yield trials compared to sister lines carrying *Rht-B1b* or *Rht-D1b*. The *Rht13* grew at a similar rate compared to the tall genotype until the late stem elongation, both faster than the *Rht-B1b* NIL. The growth of the late stem elongation to the anthesis, growth of the penultimate internodes were strongly reduced with the *Rht13* compared with both the *Rht-B1b* and the Tall NIL, however the total water soluble carbohydrate content were similar. Ear length was reduced in the *Rht13* NIL compared to both the *Rht-B1b* and the tall NILs suggesting a possible reduction in grain number that may contribute to the lower screenings observed in the field. Further results from field trial and phytotron experiment are still to reveal if differences in biomass accumulation and partitioning are associated with the reduced screenings and equivalent yield of lines carrying the *Rht13, Rht-B1b* or *Rht-D1b.*

The above mentioned researches clearly portray increases in wheat yield attributed to the *Rht* genes.

1.6. Fusarium head blight (FHB) in cereals

Fusarium head blight is a disease which affects wheat together with other cereals such as maize. It also affects some grasses. This disease cause losses in grain yield and quality, however the most severe threat of FHB is the contamination of harvested grain with mycotoxins. The control of this disease through chemical and agronomical methods is either not available or not feasible. The cultivation of genetically resistant cultivar is the most cost- effective method to control the disease. FHB is caused by fungal species in the genus *Fusarium*. The two most common species causing FHB are *Fusarium graminearum* and *Fusarium culmorum.* On barley, two other Fusarium species, *Fusarium poae* and *Fusarium avenaceum*, also may cause kernel blight. In this research the plants were artificially inoculated with *Fusarium culmorum*.

Fusaruim culmorum is a plant pathogen which affects many monocots and dicots, including cereals and grasses causing diseases like head blight, seedling blight, foot rot, ear blight stalk rot, common root rot etc. It is classified under the *Fungi* kingdom to the phylum *Ascomycota* under the class *Sordariomycetes*, and to the Order *Hypocreales*, to the family *Nectriaceae* and to the genus *Fusarium* and to the species *F. culmorum.*

The pathogen is dominant in cooler areas like north, central and Western Europe. The fungus reproduces asexually by means of conidia, which form the main mode of dispersal. Colonies of *F. culmorum* grow rapidly on PDA. Aerial mycelium is whitish to yellow, tan or pale orange, but become brown to dark brown to red-brown with age. Under alternating conditions of light and temperature, rings of spore masses may be formed by some isolates. Microconidia are absent but the Macroconidia are usually abundant. Germination of macroconidia of *F. culmorum* can occur over a wide range of temperatures (5-35◦C) with an incubation period of about 40 days Orange to brown coloured Sporodochia are relatively common. Claymydospores about 9-14 µm in diameter, found singly in clumps or chains on the hyphae and macroconidia are usually abundant and are form relatively quickly, requiring 3-5 weeks on carnation leaf agar (CLA).

Head blight is by far the most serious concern of *Fusarium* infection on pre-harvest wheat and other small grain cereals. The first symptoms of Fusarium head blight include a tan or brown discoloration at the base of a floret within the spikelets of the head. Afterward the diseased spikelets become light tan or bleached in appearance. The infection may be limited to one spikelet, but if the fungus invades the rachis the entire head may develop symptoms of the disease. The base of the infected spikelets and portions of the rachis often develop a dark brown colour. With favourable weather condition the pathogen reproduces, by producing small orange clusters of spores or black

reproductive structures called perithecia on the surface of the glumes. Infected kernels are often shrivelled, white, and chalky in appearance. There are also cases were the diseased kernels may be coloured red or Pink. In addition to wheat, *F. culmorum* can also cause diseases in corn, and grasses commonly grown for forage. This is important for it to complete its life cycle since the fungus can survive and reproduce in the crop residues, and from there transferred by wind or rain to the developing wheat or barley, where its life cycle can continue.

The significance of *F. culmorum* in wheat production is attributed to both head blight and mycotoxin contamination of the grain harvested from infected ears. According to Wagacha & Muthomi (2006)**,** ear infection mainly occurs during anthesis and is favoured by wet weather or high humidity and warm temperatures. The major mycotoxins produced by *F. culmorum* are deoxynivalenol, nivalenol and zearalenone, which are potential health hazard for both humans and animals. The mycotoxin deoxynivalenol is believed to play a role in disease development. Studying the effect of the disease head blight caused by the mycotoxin of *F. culmorum* on the content and weight of wheat kernels, Snijders & Perkowski (1990), found high correlation between the mycotoxin deoxynivalenol and the yield content, simultaneously high correlation were also found between the toxin and the kernel weight reduction, which led them to conclude that infection of highly pathogenic strain reduced yield in terms of kernel number. The detail role of mycotoxins is still unclear, but they may enable fungi to occupy a particular niche, or assist in excluding other competitors from the same niche. The ecological strategies used by *F. culmorum* to occupy and dominate in the grain niche are not understood. Generally fungi can use a combative, stress or ruderal strategies

Available options of managing FHB include use of fungicides, cultural practices, resistant cultivars and biological agents. However, no wheat cultivar is completely resistant to FHB while fungicides are at most 70% effective against natural infection.

1.7. DNA electrophoresis

DNA electrophoresis is a technique use to separate DNA fragment based on is size. The DNA fragments to be analysed are place on a gel. An electric field is then applied through the gel, which make the negatively charge DNA, due its net negatively charge phosphate backbone, to migrate toward the anode, with a positive potential. The fragments are separated based on their different

mobility through the gel. Longer molecules migrate slower due to their larger molecular weight than smaller fragments which end up closer to the anode. After a period of time the voltage is removed and the fragment gradient is analyzed. The voltage and the run time can be adjusted for the separation of fragment with similar sizes.

Larger DNA fragments are usually cut to smaller fragment by restriction enzymes. PCR amplified samples usually have enzymes present that affects the mobility of molecules through the gel. These enzymes should be removed from the fragments before being placed in the wells.

The different fragments are usually visualized using a fluorescent dye, such as ethidium bromide or SYBR-safe, which shows up as bands. Since ethidium bromide is not visible in natural light, the DNA is usually mixed with a negatively charge, visual, and co- sedimentary loading buffer before being placed in the wells. Fragment sizes are reported in nucleotides, base pairs or kb, depending on whether single or double stranded DNA has been separated. Fragment size determination is done by comparing it to a DNA ladder whose DNA fragment length is known.

The gel used for the separation of DNA fragment in this project was the agarose gel, which usually good for relatively long DNA molecules. Polyacrylamide gel is used for high resolution of short DNA fragment such as DNA sequencing. The pulsed field gel and alkaline gel electrophoresis are used to assess DNA damage. Very long DNA segments could be detected using also the pulsed-field gel electrophoresis. In this process an electrical field is used that constantly undergoes subtle changes in direction to keep very long strands oriented correctly as they move through the agarose. Gels are usually been run in a slab, however there are also capillary electrophoresis for high throughput electrophoresis, whose results are displaced on a trace view called electropherogram. Measurements and analysis are usually done with specialized software.

1.8. Microsatellite markers

In this project the molecular markers used were microsatellite markers which are based on a polymerase chain reaction (PCR). PCR is a process which enables million of DNA fragment are produced in vitro from just a single DNA fragment in just about two hours. Nowadays thermocyclers are used. A master mixed solution composing of the four nucleotides, the DNA fragments as matrix, a heat stable DNA polymerase called *Taq* polymerase extracted from heat

resistant bacteria *Thermus aquaticus* and two specific primers (Short DNA Fragments containing sequences complementary to the target region with a DNA polymerase) is made. They are put into tubes and paced in the thermo-cycler. Here three major processes, denaturisation, annealing and extension are involved. All the processes relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. These thermal cycling steps are necessary first to physically separate the two strands at high temperature and at low temperature each strand is used as a template by DNA polymerase to selectively amplify the target DNA. The Polymerase binds to the primer template hybrid and begins DNA synthesizing by adding dNTPs that are complementary to the template in a 5`to 3`direction condensing the 5`phosphate group of the dNTPs with the 3`hydroxyl group of the extending DNA strand. The newly made double stranded DNA together with the old strands will then be used a template for the next cycle. In this manner several DNA fragments are made. After 25 cycles, 2^{25} copies of the original fragments are amplified in vitro. (Linnert 1997)

Microsatellites are sections of DNA composed of repeats of short motifs (e.g. CA, GTG, and TGCT etc) arranged in tandem. They are found in genomes and distributed through out all the chromosomes (Linnert 1997). According to Gill & Gill (1994), many wheat genes have up till 80% of repetitive DNA sequences. The sequence surrounding the repeat region is usually conserved, allowing PCR primers to be designed so that the repeat region and a short flanking sequence can be amplified. Individuals may differ in the number of repeats present, meaning that the length of the PCR product varies. Products can be scored using various forms of high resolution electrophoresis (polyacrylamide or semi automated sequencers). Simple Sequence Repeats (SSR) is stretches of 1 to 6 nucleotides units repeated in tandem and randomly spread in eucaryotic genomes. SSR are very polymorphic due to the high mutation rate affecting the number of repeat units. The high mutation rate of microsatellites is attributed to relatively high rates of error during DNA replication (slippage) and during recombination (unequal crossover). Such length-polymorphisms can be easily detected on high resolution gels (e. g. sequencing gels), by running PCR amplified fragments obtained using a unique pair of primers flanking the repeat (Weber & May 1989). After gel electrophoresis and amplification, bands are obtained which shows polymorphism based on the number of repeated sequences (Linert 1997).

Microsatellites are not suitable as Maker for single genes since they occur in large number and distributed throughout the entire genome so that when hybridised they so many fragments will be

produced. The major disadvantage of microsatellite is the enormous job involve in its production. Many preliminary steps like cloning, sequencing and the synthesis of Oligonucleotides are necessary in order to produce the specific locus marker (Paredes –Lopez 1999).

Used as molecular markers, they are co-dominant, often highly polymorphic and relatively easy to score. The banding patterns produced by microsatellite from different genotype are so unique that can be used as to identify an organism and are also called genetic fingerprint. In plant breeding as well as in human medicine and other domains of research they are used to proof the relationships between individuals as well as to differentiate between homozygote and heterozygote. The use of microsatellite has increase in recent time, because of the modern PCR technique, which enables the automatic production of microsatellite and also on the fact that there exist already many publish primers for the microsatellite of different organism, which can be used without again producing the primer.

Plaschke et al. (1995) discovered that the most microsatellite come from B- genome. They are found mostly near the telomeres, near to the centromeres and the nucleolus. Mostly found are the dinucleotides repetition (eg ACn or GAn TAn), however there exist also the tri and tetra-nucleotides.

2. MATERTIALS AND METHODS

2.1. The plant materials

The plants used in this experiment originated from three parents (*Herman, Mulan* and *Nord01044/01*).The first population derived from the cross between *Herman* Mulan* and the second population were crosses between *Herman*Nord01044/01*. The Plant materials were two double haploid (DH) lines. The parents *Herman* having the allele *Rht-B1b*, *Rht-D1a* and *Mulan* having the alleles *Rht-B1a*, *Rht-D1a* were varieties from Germany donated by a German company called NORDSAAT, having progenies of 59 lines. The parents *Nord 01044/01* with the alleles *Rht-B1a*, *Rht-D1b* were breeding lines from Germany which were crossed with *Herman* (*Rht-B1b*, *Rht-D1a*) to make the second population having progenies of 99 lines. The parent *Nord 01044/01* was not available any more and therefore not included in the experiments. Other lines used for marker analysis as control lines *were Skalmeje, Toras, Phlillip, Capo, Courtot, Triticum macha, line 6408* and *Chinese Spring*

2.2. Field experiments

The field experiment was carried out in the year 2009. They were carried out by the staff of the institute of IFA-Tulln and the field data were given to me for analyses.

2.2.1. The experiment site

The site of this experiment is located at the Department for Agrobiotechnology-Tulln in Austria, coordinates: 16°03’ east, 48°19’north. This area lies at an elevation of 174 m above sea level, with the soil type was meadow–czernosem.

2.2.2. Experimental layout

The plot for this experiment had a length of about 0, 9 m and a width of about 0, 5 m with about

17cm row spacing. The first replication was done on the 27 October 2008 and the second replication was done on the 18 November 2008. The sowing density was 5g of seeds for a plot of double row (90 cm long) ~ 180kg/ha. For the seed treatment Celest Extra 050 FS (2ml/kg) and Gaucho FS 600 rot (O, 58ml/kg) were used.
The fertilizer used were
KAS (27% N) 170kg/ha applied on the 9th April 2009,
DC 45 (15% P, 30 % K) 180kg/ha applied on the 20th April 2009,
KAS (27% N) 110 Kg/ha on the 24th April 2009,
KAS (27% N) 100 kg/ha applied on the 18th May 2009.

The control of undergrowth was done with the herbicide Platform S (750g/ha) and Express SX (25g/ha), on the 7th May 2009.

2.3. Fusarium resistance testing

Spores isolated from *F. culmorum* were used for inoculations. Macroconidia of the *F. culmorum* isolate "IFA 104"were prepared as described by Snijders and Van Eeuwijk (1991) and Buerstmayr et al. (2000, 2002). A mixture of wheat and oat kernels (3:1) was soaked overnight in water and then autoclaved and inoculated. The mixture was then incubated for 2 weeks at 25°C followed by 3 weeks at 5°C in the dark, leading to production of macroconidia. Macroconidia were washed off the colonized grains with deionised water.
The concentration of the conidia was calculated with a Bürker-türk counting cambers under the microscope. The concentration of the conidia was 25.000 conidia/ml was produced and stored at -80°C for the inoculation procedure (Buerstmayr at al. 2002).

The plants were artificially inoculated between 23rd May and 10th June 2009. They were inoculated at a time when about 50 % of the ears were flowering. This was seen when the anthers appear from

the middle of the ears. Using a motor driven back-pack sprayer, 50 ml of inoculums was sprayed on the heads. Neighbouring plots were protected by a plastic shield. Inoculations were carried out in the evenings on alternate days. An automated mist-irrigation system, switched by leaf-wetness measurement, maintained humidity and kept the plants wet for 20 h after inoculation.

After two days the same procedure was repeated. The inoculation was done at the evening at about 5 pm to give the fungi optimum temperatures and humidity.

2.4. Scoring of data

2.4.1. FHB severity (AUDPC)

For scoring we assumed an average head-size of 20 spikelets per spike as the basis for estimating FHB severity; e.g. an average of one infected spikelet per spike was rated as 5% FHB severity. Disease symptoms were recorded on the 10, 14, 18, 22, 26 and 30 days after inoculation. In each plot the percentage of visually infected spikelets was estimated according to a linear scale 0 to 100% infected spikelets on a whole plot basis. The area under the disease progress curve (AUDPC) was calculated for each entry as an integrated measure for disease severity i.e. combined resistance to fungal penetration and spread. The AUDPC was calculated for each plot and was used for further statistical analysis as described by Buerstmayr et al. (2000)

2.4.2. Plant height

In order to study the relationship between the grown height and the disease intensity the plant height was measures, on the 1st July 2009 A graduated measuring rot was to measure the plant height from the earth surface to the apex of the ears.

2.4.3. Flowering date (= date of anthesis)

The flowering date was measured for each genotype. The scored days to flowering from the first of May until flowering were registered.

2.5. Molecular genetic analyses

The variation on the DNA level was estimated using two molecular marker techniques: diagnostic markers for the *Rht*-genes and one microsatellite marker. These were carried out on all the progenies of the two experimented populations.

2.5.1. Collection of leaf samples, drying and grinding of the samples

Leaves samples were harvested with a scissor from the pots where they plants were planted. From each genotype about 5 healthy leaves were cut and placed in little paper bags. They were named and numbered.

The leaf samples were dried in a dryer at 35°C for about two days, after which they were cut with a scissor and transferred to 2 ml Eppendorf tubes.

3 to 5 sterile glass balls were added to the tubes and placed in a Mixer Mill (Retsch, Germany) in which by shaking them at very high speed for about 10 minutes the leaves were grinded to powder forms. The samples were then centrifuged and ready for DNA extraction.

2.5.2. DNA Extraction

Genomic DNA was isolated from young leaves using the CTAB extraction method described by Saghai-Maroof et al. (1984). 750 µl of CTAB- buffer (2% cetrylmethylammonium bromide, 100 mM Tris pH8, 20 mM EDTA, 14 M NaCl, 2% PVP 40) was added to the grinded tissues of each genotype and incubated at 60°C for 60-90 minutes into a water bath with gentle rocking. To avoid clumping of dry tissues at the bottom of the tube, the dry tissues were first distributed along the sides of the tube, before adding the buffer. The samples were allowed to cool down at room temperature for about 4-5 minutes after which 300 µl of chloroform/isoamyl alcohol (24/1) were added to each well. They were rocked gently to mix by inversion and then centrifuge for 10 minutes at RCF 3500g. The top aqueous layer which contained the DNA was pipetted off into new 2.0ml tubes. To extract the maximum amount of DNA, 300 µl of chloroform/isoamyl alcohol (24/1) were added again to each well. After a gentle 5 minutes shake, the samples were centrifuged for 10

minutes at RCF 3500g. The top aqueous layer was again pipetted off and placed in new stripes tubes. 400 µl of isopropyl alcohol was added to each tube in order to precipitate DNA. The samples were centrifuged for 8 minutes at RCF 400g. DNA-pellet stuck was precipitated at the bottom of the tubes and the liquid was carefully poured off.

100 µl of WASH 1 (76% EtOH, 0.2 M NaOAc) was poured to each tube. The DNA-pellet stuck remained in WASH 1 for 30 minutes after which the tubes were closed and centrifuged at RCF 400g for 8 minutes, immediately afterward the liquid was carefully poured off. The DNA was again briefly rinsed in 100 µl of WASH 2 (76% EtOH, 10mM NH_4OAc). The samples were centrifuged at RCF 400g for 8 minutes and the remaining liquid poured off. The pellet was dried over night and the next morning 100 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH8) was added to the samples. The tubes were gently shacked for one day at room temperature in order to dissolve the DNA-pellet.

A quality check was done on an agarose gel. The DNA concentrations in the samples were measured by means of a Pharmacia Gene Quant UV Photometer which evaluated the optical density at a wavelength of 260nm. A standard concentration of 100ng/ul in the samples was obtained by adding appropriate amount to TE Puffer. The plates containing the DNA were stored at -4°C.

2.6. Allele specific DNA markers for *Rht* genes

Four different primer combinations were used for the molecular genetic analyses. They were either located on chromosome 4B or 4D The primer sequences listed in Table 1 were obtained from Paul Nicholson, John Innes Centre Norwich, UK, based on the paper from Ellis et al. (2005) with minor modifications.

Table 1: The primer sequences and the annealing temperature of the dwarfing genes

	Type	Forward primer	Sequence	T_m	Reverse primer	Sequence	T_m
4B	Rht1 mutant (RhtB1b)	NH-BF.2	TCTCCTCCCTCCCCACCCCAAC	75.1 °C	MR1	CATCCCCATGGCCATCTCGAGCTA	75.3 °C
4B	Rht1 wildtype (RhtB1a)	NH-BF.2	TCTCCTCCCTCCCCACCCCAAC	75.1 °C	WR1.2	CCATGGCCATCTCGAGCTGC	72.5 °C
4D	Rht2 mutant (RhtD1b)	DF	CGCGCAATTATTGGCCAGAGATAC	70.7 °C	MR2	CCCCATGGCCATCTCGAGCTGCTA	77.1 °C
4D	Rht2 wildtype (RhtD1a)	DF	CGCGCAATTATTGGCCAGAGATAC	70.7 °C	WR2.4	CCATGGCCATCTCGAGCTGCAC	75.1 °C

2.6.1. Primer combination for each of four *Rht* alleles

1 *Rht-B1b* (dwarf)
Forward, NH-BF.2 (TCTCCTCCCTCCCCACCCCAAC)
Reverse, MR1 (CATCCCCATGGCCATCTCGAGCTA

2 *Rht-B1a* (tall)
Forward, NH-BF.2 (TCTCCTCCCTCCCCACCCCAAC)
Reverse, WR1.2 (CCATGGCCATCTCGAGCTGC)

3 *Rht-D1a* (tall)
Forward, DF (CGCGCAATTATTGGCCAGAGATAG)
Reverse, WR2.4 (CCCCATGGCCATCTCGAGCTGCTA)

4 *Rht-D1b* (dwarf)
Forward, DF (CGCGCAATTATTGGCCAGAGATAG)
Reverse, MR2 (CCATGGCCATCTCGAGCTGCAC)

2.7. Electrophoresis

The four different primer combinations were first tested for polymorphism an agarose gel, firstly on the parents and later on the genotypes of the mapping populations. The agarose gel was prepared as follows.

1.2g of agarose was measured and placed inside a reaction glass. To it 100ml TAE was added and stirred. It was heated inside a microwave oven until it began boiling. It was then allowed to cool down to about 60°C at room temperature, or in a water bath. The solution was stirred or

swirled while cooling. 10µl ethidium bromide was added. (ethidium bromide is highly mutagenic and for the entire preparation gloves were used. Afterward for subsequent preparations ethidium bromide was replaced by SYBR Safe). The solution was stirred to disperse the ethidium bromide, and then poured into the gel rack. Combs were inserted at one side of the gel, about 5-10mm from the end of the gel. The gel was allowed to cool down and solidify. The combs were carefully removed. Wells or slots were then created in the gel. 10% TAE was then poured to cover gel and the slots inside the rack.

After the gel has been prepared, a micropipette was used to inject about 2.5µl of stained DNA into the wells. The lids of the electrophoresis chambers were closed and current was applied. It was then allowed to run for 10 minutes at 30 volts and later for 15minutes for 80 volts. The DNA move toward the positive anode due it's the negatively charged phosphate backbone. The electrophoresis chambers containing the stained DNA with ethidium bromide was then transferred into a photogram device where the stained DNA was made visible under ultraviolet light. The pictures were then stored by means of a computer attached to the device for analysing.

2.8. PCR test of allele specific marker for the *Rht*-genes

.Since the mutant and wild-type sequences differ only by a single nucleotide, high stringency PCR conditions are required to prevent non-specific annealing. A number of negative controls always include.

The conditions of the PCR were as follows for 10 µl reaction: 5 µl of Go-Taq (Promega, containing 2X TAQ polymerase, 2X reaction buffer, 400µM of each dNTP, 3mM $MgCl_2$) 0.4 µl each of forward and reverse primers (concentration 10 µM) and 2 µl of template DNA (50 ng/µl). A hot start PCR was performed to avoid unspecific annealing. The amplification was carried out on 96-well Eppendorf Mastercycler running the following program: 2 minutes at 94 °C ; 35 cycles of 30 seconds at 94 °C, 30 seconds at 63 °C, 30 seconds at 72 °C ; and a final step of 5 minutes at 72 °C. PCR products were then separated and visualised with agarose gel electrophoresis as described

above. Presence of a PCR product was scored as indicative for the presence of the specific *Rht*-allele under investigation.

The PCR Product was approximately 400bp in size, visualised on 1.5% agarose TAE gel (1:20 000 dilution of Ethidium Bromide)

2.9. PCR analysis of microsatellite (SSR) markers

To assess the association of the *Rht* gene of the genotypes, at the DNA Level, on chromosome 4B and 4D of the two populations, in addition to the *Rht*-gene markers a microsatellite marker analysis was done. These regions are suspected to be associated with FHB resistance in wheat**.**

Microsatellite was performed using flouresecnt detection on a LI-COR 4200 DNA-dye sequencing system. For this method a forward primers with an M-13 tailed fluorescent primer was added to the PCR reaction. The reaction mix for the M13-tailed SSRs contained 0.02 µl forward primer (10µM, with M13-tail at the 5' end: CCCAGTCACGACGTTG), 0.18 µl M13-primer (with a fluorochrome, IRD700 or IRD800, at the 5' end) 0.2 µl reverse primer (10µM), 1 µl 10 X PCR buffer including 15mM $MgCl_2$, 1 µl dNTP-mix (2mM each dNTP), 0.1 µl Taq polymerase (5 units/µl) and 2 µl of template DNA for a 10 µl reaction. The PCR Programme for the M13-tailed primer was 94°C for 2 minutes and then 30 cycles of 94°C for 1 minute, 0.5°C s^{-1} to 51°C, 51°C for 30s, 0.5°C s^{-1} to72°C and 72°C for 1 minute, followed by 72°C for 5 minutes. PCR were performed on a 384 well Eppendorf Mastercycler

The electrophoresis was performed on LI-COR 4200 IR^2DNA analyzer (LI-COR Inc. Lincoln, Nebraska, USA). 5 µl of formamide tracking dye (95% formamide deionised, 0.5 mM EDTA, 0.1 mg/ml new fuchsin red) were added and the samples were denatured for 3 minutes at 95 °C. Eventually, 0.8 µl was loaded on 7% polyacrylamide gel using a 64-well shark tooth comb. The gel was cast in plates with 0.25 mm thick spacer. Electrophoresis was carried out at 40 W on constant power and at temperature of 48 °C. Gel image was then collected on a personal computer and picture was scored visually.

Markers were search which map near to the *Rht* genes on the Graingenes database (www.wheat.pw.usda.gov/GG2/index.shtml) which was all on chromosome 4B and were available

in the laboratory. The SSR markers *Barc20, Gwm213, Gwm540, Gwm 285, Gwm511, Gwm574, Gwm617, and Gwm710* were screened for polymorphism between the parental lines.
The SSR marker *Barc20* with the sequence below was selected for further analysis on the populations (5'GCGATCCACACTTTGCCTCTTTTACA3';
5'GCGATGTCGGTTTTCAGCCTTTT 3')

2. Statistical analyses

The statistical programme SAS/STAT 9.1 was applied (SAS Institute Inc. 2004) was used to study the FHB resistance trial, and also the relationship between the *Rht* alleles and the FHB severity.

As an integrated measure for FHB severity the area under the disease progress curve (AUDPC) was calculated using the formula below.

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

AUDPC …. Area under the disease progress curve

y_i …….Rating in percentage of the i observation

x_i …….Day of the i observation

n……...Total number of observations

Analysis of variance using the procedure GLM and analysis of correlation using the procedure CORR were employed. Means and standard deviations of means were calculated using the MEANS procedure.

For each marker, inside every population, the mean value of all the lines possessing the alternative *Rht*-alleles were compared. In case the difference was significant it is concluded that the respective *Rht*-gene had an influence on the trait under investigation. The coefficient of determination allows an estimation of the proportion of the phenotypic variation explained by this marker locus in a specific population.

The statistical programme SPSS was also used to construct the histogram and the box plot for the two populations and also to show the relationship between the various marker and FHB severity

3. RESULTS

3.1. Results of the field experiments

Each of the grown lines were artificially inoculated with *Fusarium culmorum* during flowering The lines all reacted to the inoculums and showed different symptoms. Figure 1 shows the FHB spread of the parents (*Herman*, *Mulan*) together with the mean value of the two populations. *Herman* had the highest infection rate on the 30 day after the first inoculation. The infection rate of the parent *Mulan* was clearly below that of *Herman* at all the days after the first inoculation. The average FHB spread values of the two populations lied between the two parents *Herman* and *Mulan,* but very closed to *Mulan*. The mean FHB spread increased from the first day of inoculation to the 30day after inoculation for all of the genotype.

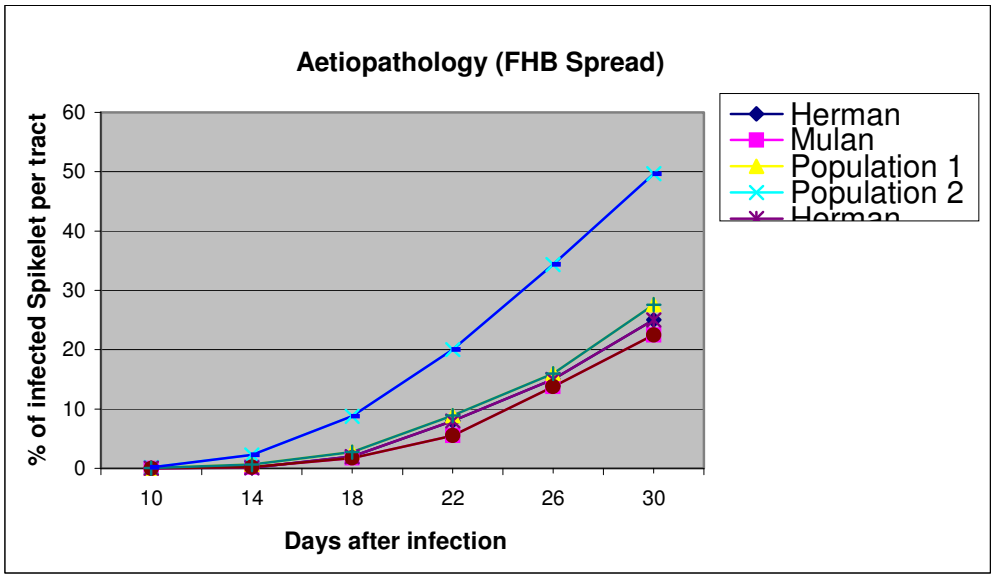


Figure 1: Showing the course of disease for the parents and the mean value of the two populations

From the disease progress curve the AUDPC was calculated. The mean value of the AUDPC for both populations together with the plant height and flowering date are shown on Tables 2 and 3. The values of the parent *Nord01044/01* are missing. The average value of the progenies from the second population doubled that of the first population, while the flowering dates in both populations were almost similar (Table 2 and 3)

Table 2: Mean values of the parents and the population and trait variation in population 1

	HERMAN	MULAN	PROGENIES	RANGE	
				LOWEST	HIGHEST
AUDPC	200,8	174,9	223,5	26,2	661,2
PLANT HEIGHT	87,5	85,0	87,9	75,0	100,0
FLOWERING DATE	34,0	30,5	32,4	29,0	38

Table 3: Mean values of the parents and the population and trait variation in population 2

	HERMAN	NORD01044/01	PROGENIES	RANGE	
				LOWEST	HIGHEST
AUDPC	200,8	-	461,3	29,8	1088,5
PLANT HEIGHT	87,5	-	82,6	55,0	112,5
FLOWERING DATE	34,0	-	32,5	28,0	37,0

- = missing values

3.2. FHB Severity

Values for FHB Severities are shown (in the Tables 4 and 5) below as the Area under the disease progress curve. The P-value of the blocks and the lines for the two populations are shown on table 4 and 5. The repetitions of the inoculations were significant in all the populations. There were very significant differences in the FHB severity among the genotypes (P<0.0001)

Table 4: Analysis of variance for FHB severity measured by AUDPC in population 1

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	1090700,44	1090700,44	58,99	<0,0001
LINES	59	3156154,58	53494,145	2,89	<0,0001
ERROR	61	112782,544	18489,714		

Table 5: Analysis of variance for FHB severity measured by AUDPC in population 2

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	2540325,62	2540325,62	92,69	0<0001
LINES	99	14319788,9	144644,33	5,28	0<0001
ERROR	99	2713317,79	27407,25		

3.3. Plant height

Significant increased in plant heights was observed from lines and blocks of genotypes of both populations 1and 2, as shown on Table 6 and 7 below.

Table 6: Analysis of variance for plant height (in cm) in population 1

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	138,52459	138,52459	10,1	0,0023
LINES	59	4477,45902	75,889136	5,53	<0,0001
ERROR	61	836,47541	13,712712		

Table 7: Analysis of variance for plant height (in cm) in population 2

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	300,125	300,125	21,42	<0,0001
LINES	99	35211,375	355,67045	25,38	<0,0001
ERROR	99	1387,375	14,01389		

3.4. Flowering date

The date at which the plants flowered had a significant effect if the plants were having the *Rht-B1* gene or the *Rht-D1* genes. Both the blocks and the lines of population 1 and 2 had p-values <0, 0001 (Table 8 and 9)

Table 8: Analysis of variance for flowering date (in days after May 1st) in population 1

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	2199,37705	2199,37705	908,82	<0,0001
LINES	59	439,131148	7,442901	3,08	<0,0001
ERROR	61	147,622951	2,420048		

Table 9: Analysis of variance for flowering date (in days after May 1st) in population 2

SOURCE	DF	SS	MS	F-value	P-value
BLOCKS	1	2352,98	2352,98	1206,84	<0,0001
LINES	99	925,98	9,353333	4,8	<0,0001
ERROR	99	193,02	1,949697		

3.5. Histograms of the population *Herman*Mulan* and *Herman*Nord01044/01*

The frequency of distribution of the flowering dates are shown on Figure 2. The mean flowering date of both populations was on the 32 day.

The frequency of distribution of the plant height is shown of Figure 3. Population 1

genotypes with plant height ranging from 75 to 100 and population 2 hat genotypes with plant height ranging from 55cm to 112,5 cm. The plant height varied enormously in the second population. Lines with the lowest and highest plant height were found on population 2.

In Figure 4 all the lines of the genotypes of population 1 showed lower values of disease severity than the parent. In population 2 some of the lines showed lower infections than the parent *Herman*. Certain lines were more infected than the parents. The healthiest line AUDPC with 29,8 and the most infected 1088,5 were found on population 2. Averagely population 2 was mostly infected than population 1

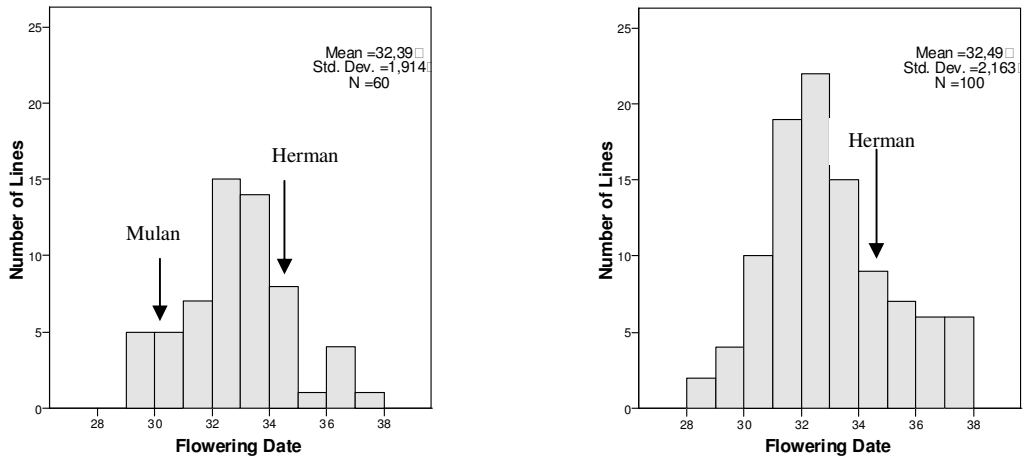


Figure 2: Histogram of the trait flowering date (days after May 1st) in populations 1 (left) and 2 (right)

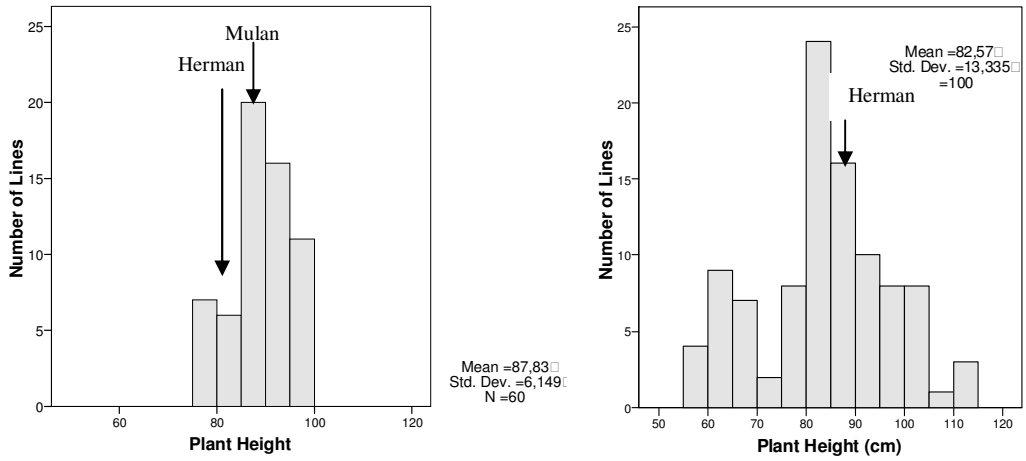


Figure 3: Histogram of the trait plant height (cm) for population 1 (left) and population 2 (right)

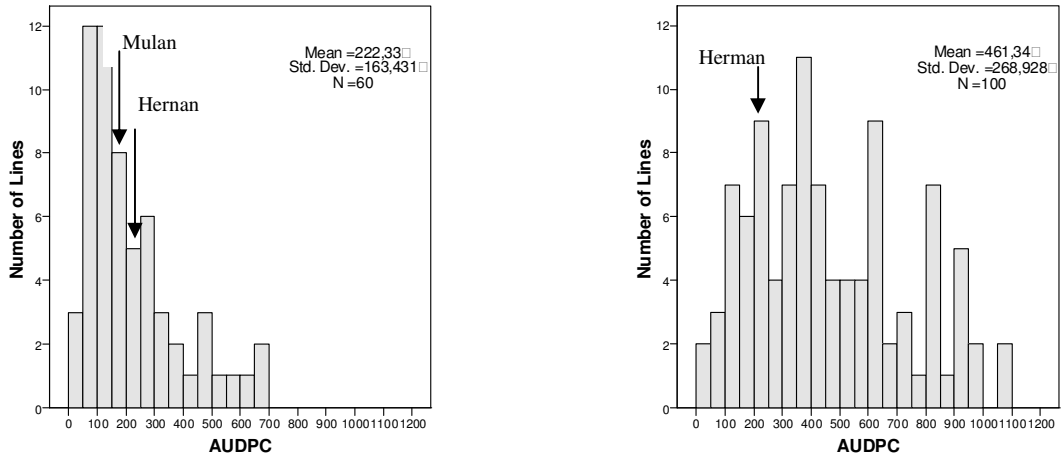


Figure 4: Histogram of the trait Fusarium head blight severity (AUDPC) for the populations 1 (*Herman*Mulan*, left) and 2 (*Herman*Nord01044/01,* right).

3.6. Statistical Correlations

Positive correlations where found between the plant height and the FHB severity. Population 2 had the correlation coefficient r =- 0.82 (Figure 6). Population 1 with a lower sampling size also showed a positive correlation of r = 0.-68 (Figure 5). In both populations no correlation was found between the traits the flowering date and the disease severity as well as between the trait flowering date and the plant height.

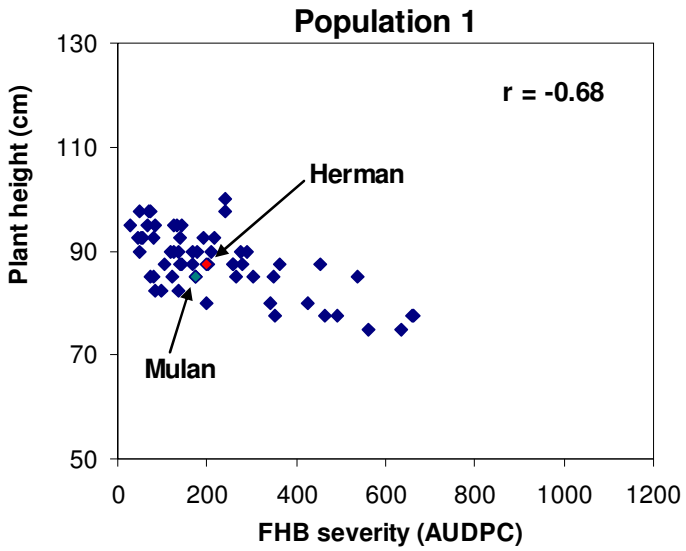


Figure 5: Scatter-plot for the traits plant height and FHB severity (AUDPC) in population 1

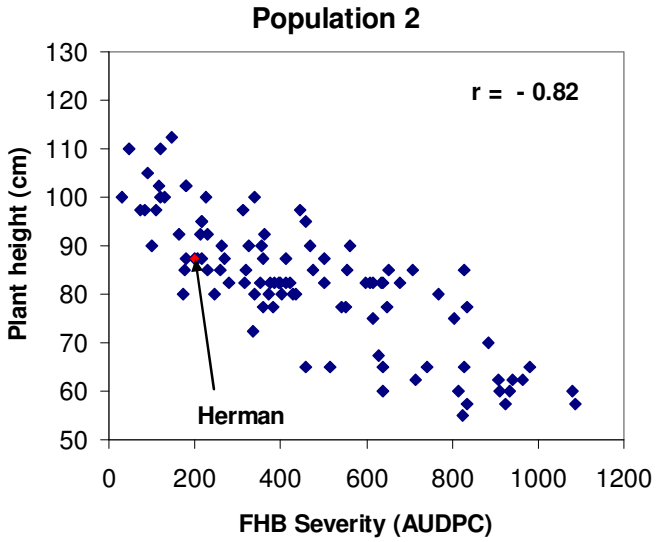


Figure 6: Scatter-plot for the traits plant height and FHB severity (AUDPC) in population 2

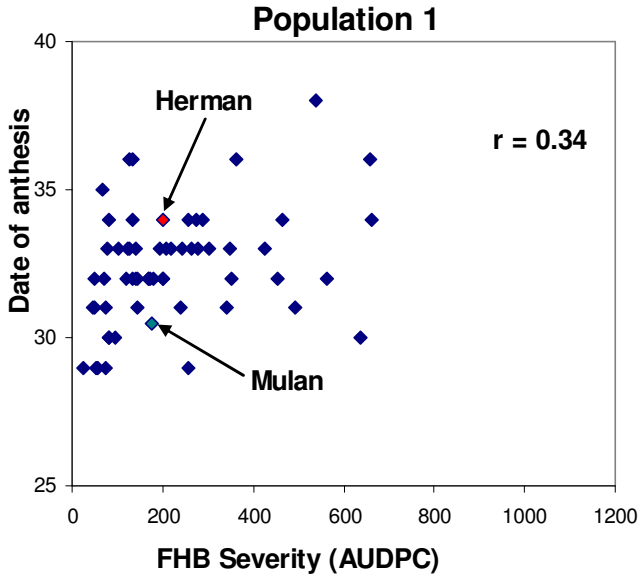


Figure 7: Scatter-plot for the traits flowering date and FHB severity (AUDPC) in population 1

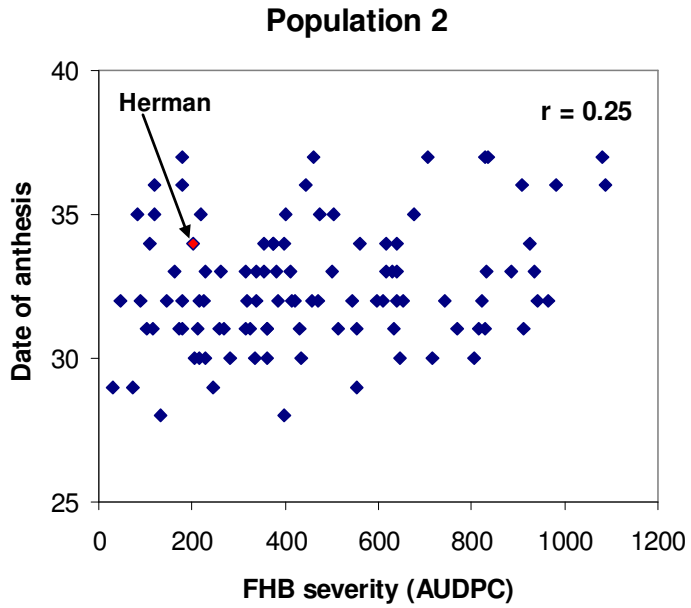


Figure 8: Scatter-plot for the traits flowering date and the FHB severity (AUDPC) in population 2

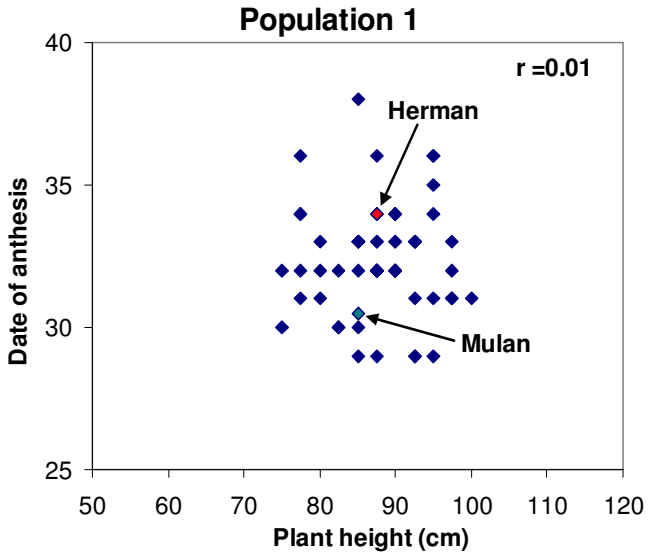


Figure 9: Scatter-plot for the traits flowering date and the plant height in population 1

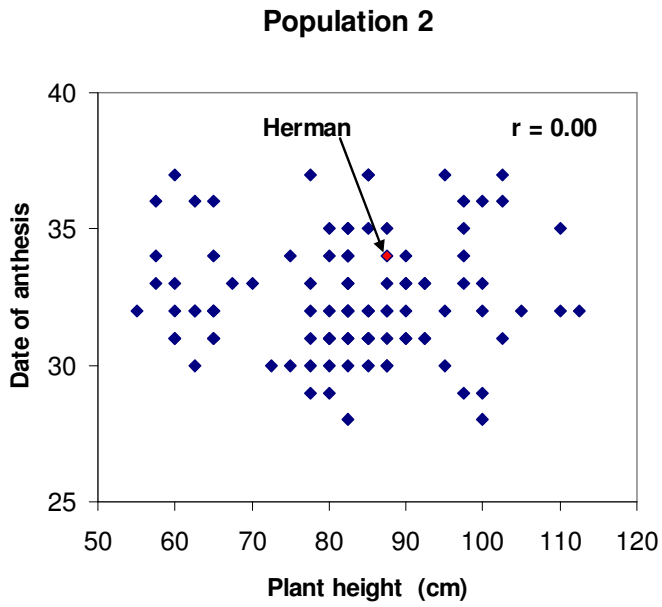
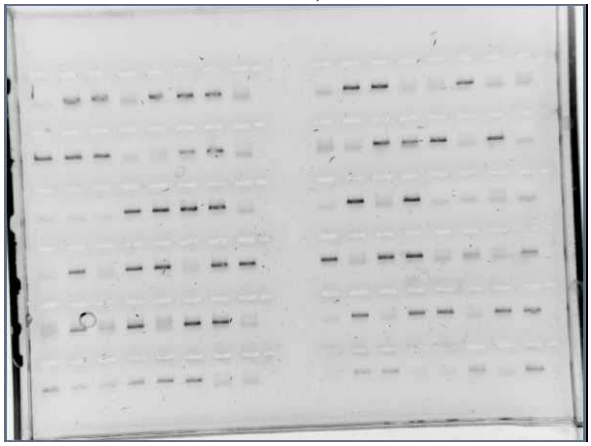


Figure 10: Scatter-plot for the traits flowering date and the plant height in population 2

3.7. Electrophoresis of the markers

The bands for the various genotyped were produced on the gel. Figure 11 shows two out of the many bandings patterns produced by the genotyped. One can clearly see that the banding pattern obtained with the PCR markers for *Rht-B1b* (left) 1 is a mirror to that obtained with the marker for *Rht-B1a* (right). The gel lanes on which a band is visible with the marker for *Rht-B1b* (left) have the missing band with marker for *Rht-B1a* (right) and vice versa. Each the four primers combinations were first tested on the parents and later on all the lines of the two populations.

PCR marker for *Rht-B1b*, Plate 1+2



PCR marker for *Rht-B1a*, Plate 1+2

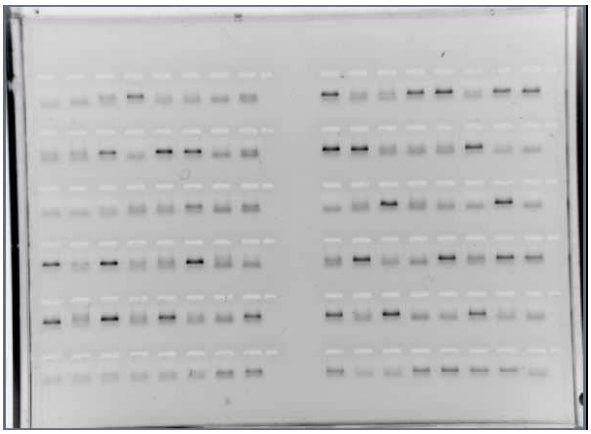


Figure 11: Examples of electropherograms for the allele specific marker for *Rht-B1b* (left) and *Rht-B1a* (right). Presence of a clear band indicates presence of the respective allele.

Population 1 (*Herman*Mulan*) segregated for the *Rht-B1* gene only and was homozygous for the allele *Rht-D1a*. Population 2 (*Herman*Nord01044/01*) segregated at both *Rht*-loci (i.e. *Rht-B1* and *Rht-D1*). Table 10 below shows the amplification of other species including the parents of the genotypes. The varieties *Herman Courtot* and *6408* possessed the semi-dwarf allele *Rht-B1b* allele while the cultivars, *Skalmeje, Toras, and Courtot* had the *Rht-D1b* semi-dwarf allele. *Mulan*. *T. macha* and *Chinese Spring* had at both loci the tall alleles, while *Courtot* hat at both *Rht* loci the short alleles.

Table 10: *Rht*-alleles found in the parental lines and several control lines.

Parents	***Rht-B1a***	***Rht-B1b***	***Rht-D1a***	***Rht-D1b***
Herman	-	+	+	-
Mulan	+	-	+	-
Skalmeje	+	-	-	+
Toras	+	-	-	+
Courtot	-	+	-	+
T.macha	+	-	+	-
Chinese Spring	+	-	+	-
6408	-	+	+	-

- = missing PCR fragment, + = present PCR fragment

3.8. Marker-trait analysis

The marker *Rht-B1* showed in population 1 a significant association with the traits plant height (Table 12) and FHB severity (Table 13) but no significant association with the trait flowering date (Table 11). In population 2 neither *Rht-B1* nor *Rht-D1* were significantly associated with flowering date (Table 14). Moth genes showed a significant association with plant height (Table 15) and with FHB severity (Table 16). For the trait plant height the interaction between *Rht-B1 and Rht-D1* was significant (Table 15) but nor for the trait AUDPC (Table 16).

Table 11: Analysis of variance for the marker *Rht-B1* in population 1 for the trait flowering date

Source	**DF**	**SS**	**MS**	**F-Value**	**P- Value**
Rht-B1	1	3,523	3,523	0,94	0,3371
Error	51	191,344	3,752		

Table 12: Analysis of variance for the marker *Rht-B1* in population 1 for the trait plant height

Source	**DF**	**SS**	**MS**	**F-Value**	**P- Value**
Rht-B1	1	679,287	679,287	23,41	<.0001
Error	51	1479,911	29,018		

Table 13: Analysis of variance for the marker *Rht-B1* in population 1 for the trait AUDPC

Source	DF	SS	MS	F-Value	P- Value
Rht-B1	1	301949,019	301949,019	12,75	0,0008
Error	51	1207533,762	23677,133		

Table 14: Analysis of variance for the markers *Rht-B1* and *Rht-D1* in population 2 for the trait flowering date

Source	DF	SS	MS	F-Value	P-Value
Rht-D1	1	1,281	1,281	0,29	0,5907
Rht-B1	1	1,806	1,806	0,41	0,5233
*Rht-D1-*Rht-B1*	1	0,000	0,000	0	0,9956
Error	91	400,350	43994466,000		

Table 15: Analysis of variance for the markers *Rht-B1* and *Rht-D1* in population 2 for the trait plant height

Source	DF	SS	MS	F-Value	P-Value
Rht-D1	1	6794,729	6794,729	122,53	<.0001
Rht-B1	1	5516,345	5516,345	99,48	<.0001
*Rht-D1*Rht-B1*	1	243,156	243,156	4,38	0,039
Error	91	5046,147	55,452		

Table 16: Analysis of variance for the markers *Rht-B1* and *Rht-D1* in population2 for the trait AUDPC

Source	DF	SS	MS	F-Value	P-Value
Rht-D1	1	1776087,646	1776087,646	42,41	<.0001
Rht-B1	1	186166,488	186166,488	28,32	<.0001
*Rht-D1*Rht-B1*	1	91642,887	91642,887	2,19	0,1425
Error	91	3810838,766	41877,349		

In population 1 which segregated only for the *Rht-B1* genes, lines with the *Rht-B1b* allele doubled lines with the *Rht-B1a* allele (Table 17). The *Rht-B1b* had an average height of 85 cm in population 1 and 74 cm in population 2 while it was 93 cm and 90 cm for the *Rht-B1a* allele in population 1 and 2, respectively (Table 17 and Table 19).

The mean FHB disease severity of lines with the *Rht-B1b* allele over doubled those with the with the *Rht-B1a* allele in population 1 (Table 17). In population 2 were the number of lines with the *Rht-B1*a and *Rht-B1b* were almost equal, the *Rht-B1b* caused a more disease severity than *Rht-B1a.*

The *Rht-D1* gene which segregated only in population 2 had almost the same number of lines with the *Rht-D1b* and *Rht-D1a* alleles. Here plants with the *Rht-B1a* allele were averagely taller than those with the *Rht-D1b* allele. The taller plants were less diseased than the shorter plants (Table 18).

Table 17: Mean values and standard deviations of the groups of lines with the alleles *Rht-B1a* or *Rht-B1b* for the traits flowering date, plant height and AUDPC in population 1.

Flowering date			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	35	32,6	1,9
Rht-B1a	18	32,1	2,1
Plant height			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	35	85,4	5,7
Rht-B1a	18	92,9	4,8
AUDPC			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	35	281,4	180,3
Rht-B1a	18	122,0	77,5

Table 18: Mean values and standard deviations of the groups of lines with the alleles *Rht-D1a* or *Rht-D1b* for the traits flowering date, plant height and AUDPC in population 2.

Flowering date			
Allele	Number of lines	Mean	Std Dev
Rht-D1b	46	32,4	1,7
Rht-D1a	40	32,7	2,3
Plant height			
Allele	Number of lines	Mean	Std Dev
Rht-D1b	46	74,0	11,4
Rht-D1a	46	90,3	10,6
AUDPC			
Allele	Number of lines	Mean	Std Dev
Rht-D1b	46	602,5	249,7
Rht-D1a	40	333,7	211,0

Table 19: Mean values and standard deviations of the groups of lines with the alleles *Rht-B1a* or *Rht-B1b* for the traits flowering date, plant height and AUDPC in population 2.

Flowering date			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	44	32,5	2,0
Rht-B1a	42	32,5	2,0
Plant height			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	44	74,1	12,6
Rht-B1a	42	89,3	10,1
AUDPC			
Allele	Number of lines	Mean	Std Dev
Rht-B1b	44	578,4	282,5
Rht-B1a	42	371,8	206,1

3.9. Box-plot for the marker trait association in population (1) *Herman*Mulan* and population (2) *Herman*Nord01044/01*

Figure 12 shows the variation of the trait AUDPC for plants with the alleles *Rht-D1b and Rht-D1a* in both populations. The mean value for plants with the *Rht-D1a* alleles were higher in Population 2 than in Population 1, however these two values were both lower than the average value of plants with *Rht-D1b* alleles in population 2 (Figure 12). Box plot showing the mean value of the *Rht-B1b* and the *Rht-B1a* alleles for the two populations based on the AUDPC is shown on figure 13. The *Rht-B1b* and *Rht-B1a* alleles were both higher in population 2 than in population 1.

The variation of the *Rht-D1b* and *Rht-D1a* alleles on both populations based on plant height is illustrated on figure 14. Plants with the *Rht-Dla* alleles had almost the same height on both population 1and 2, which were all higher than plants in population2 with the *Rht-D1b* allele. Whereas the distribution and mean value of the alleles *Rht-B1b* and *Rht-B1a* for both populations based on plant height shown on figure 15 illustrates that the *Rht-B1a* and *Rht-B1b* alleles each showed higher mean values in population 1 than in population 2 population.

The mean values for the flowering dates of the alleles *Rht-D1b, Rht-B1b, Rht-B1a,* and *Rht-D1a* for plants with the *Rht-D1B1* genes is shown on Figure 16. These alleles combination had no effect on flowering date.

Plants having the allele's combination *Rht-D1b&Rht-B1b* had the lowest plant height while plants having the *Rht-D1a&Rht-B1a* had the highest plant height (Figure 17), while plants of population 2 with the alleles *Rht-D1b&Rht-B1b* had the highest FHB disease severity (Figure 18).

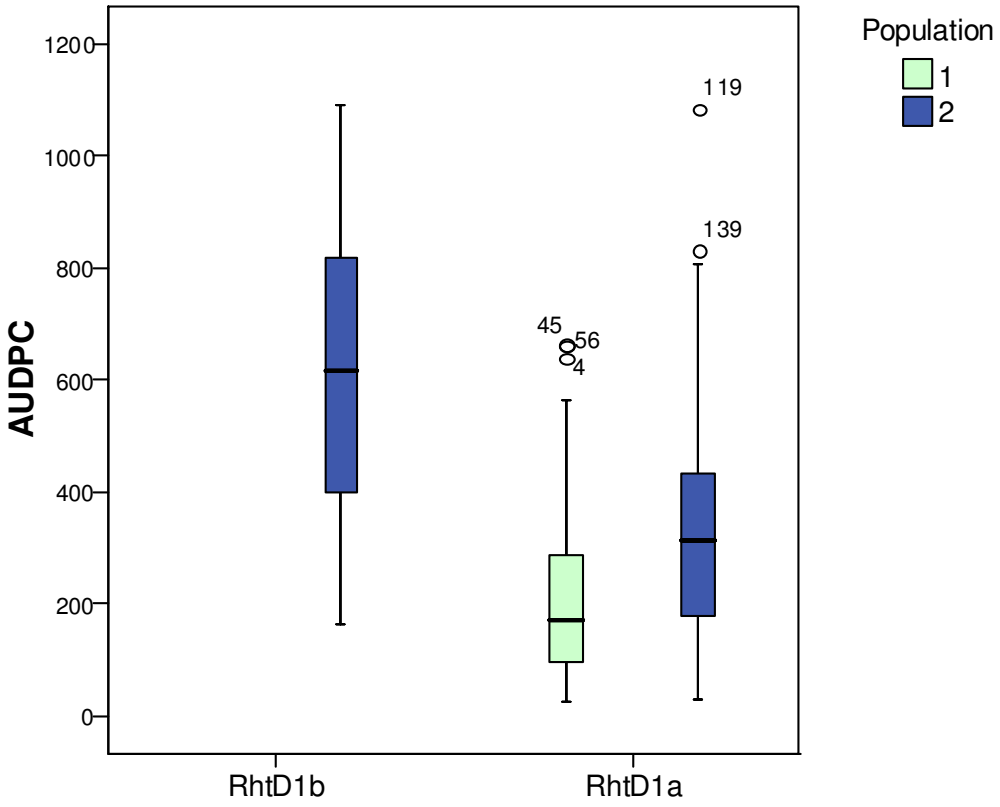


Figure 12: Box-plots showing the variation of the trait AUDPC among the groups of lines carrying either *Rht-D1a* or *Rht-D1b* alleles in the two populations.

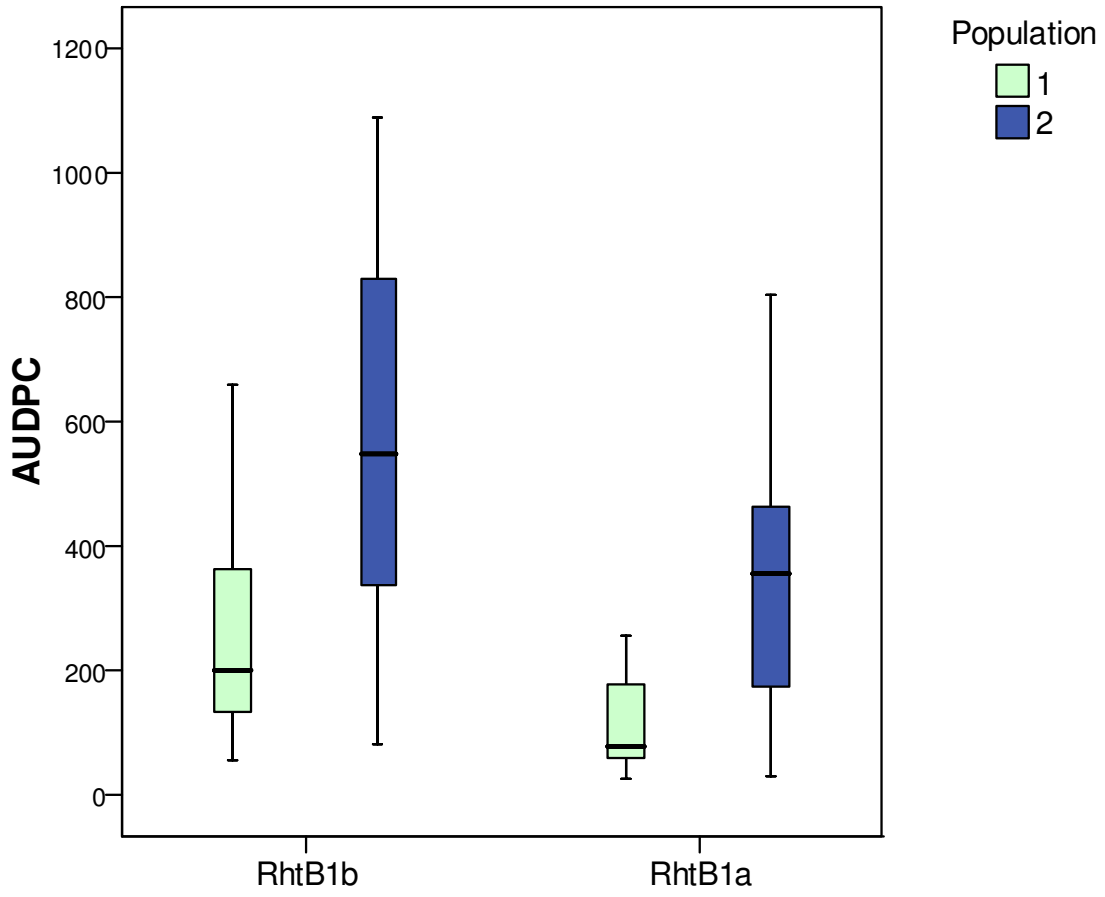


Figure 13: Box-plots showing the variation of the trait AUDPC among the groups of lines carrying either *Rht-B1b* or *Rht-B1a* alleles in the two populations

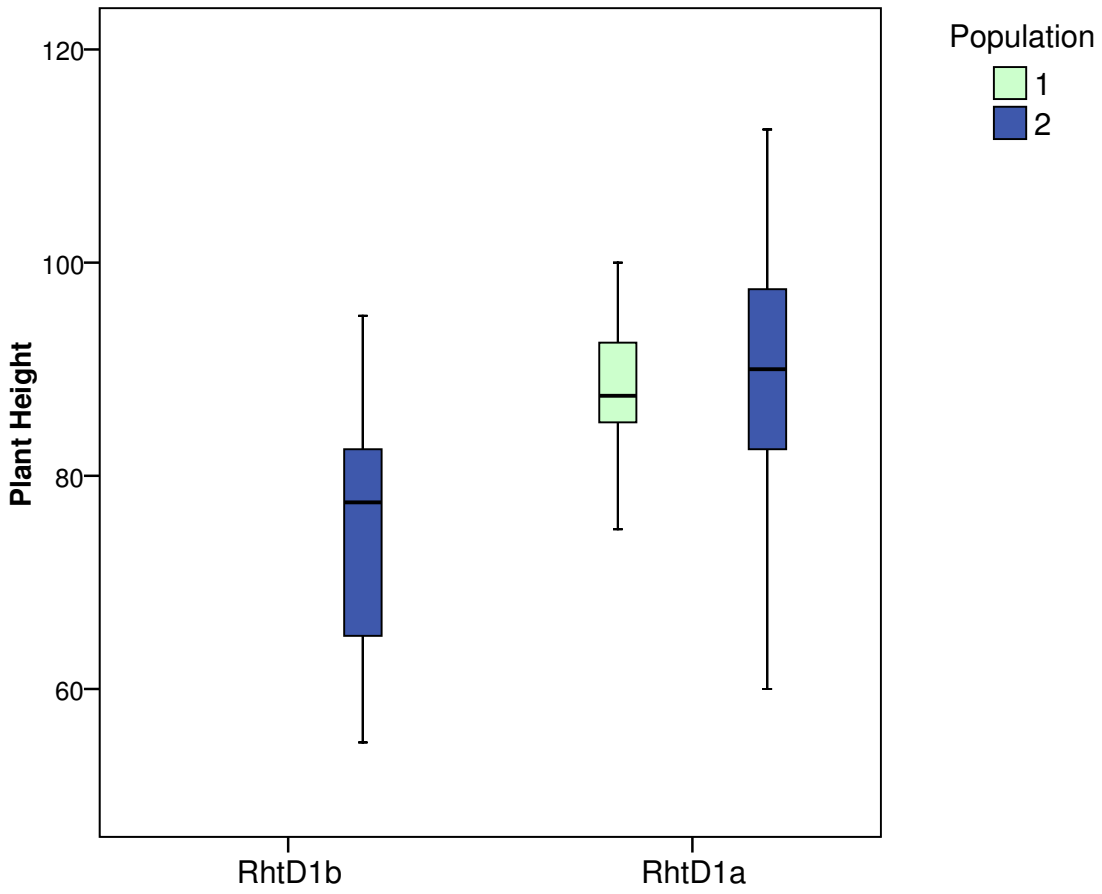


Figure 14: Box-plots showing the variation of the trait plant height (cm) among the groups of lines carrying either *Rht-D1b* or *Rht-D1a* alleles in the two populations

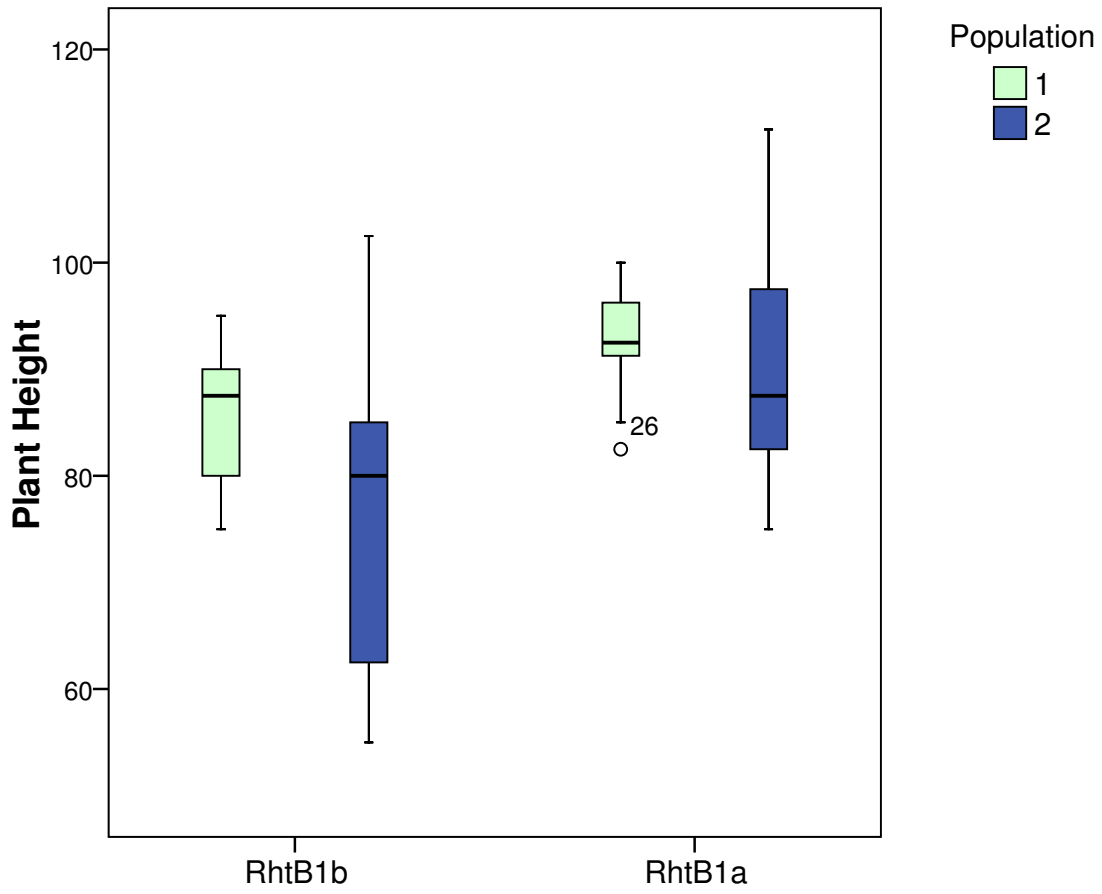


Figure 15: Box-plots showing the variation of the trait plant height (cm) among the groups of lines carrying either *Rht-B1b* or *Rht-B1a* alleles in the two populations.

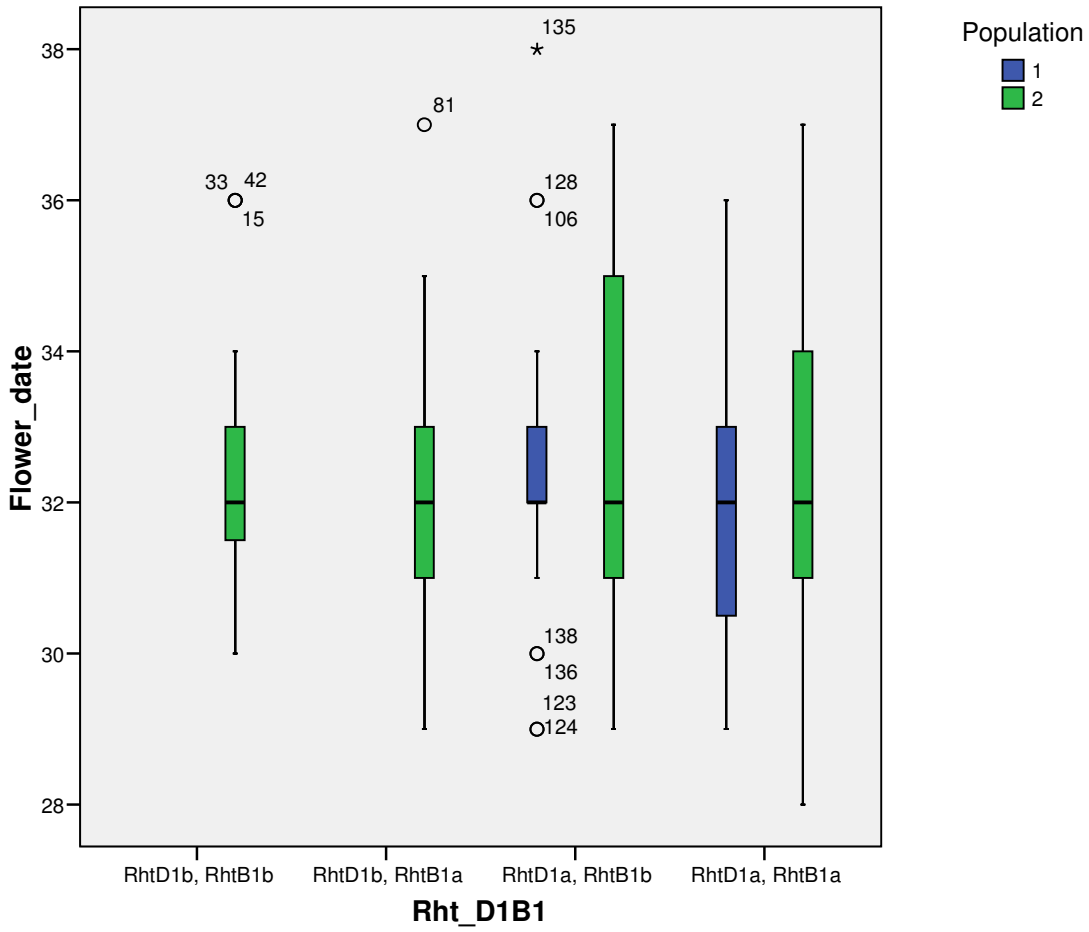


Figure 16: Box-plots for the trait flowering date showing the variation among groups of lines carrying the allele combinations *Rht-D1b & Rht-B1b; Rht-D1b & Rht-B1a, Rht-D1b & Rht-B1b,* or *Rht-D1a & Rth-B1a* in populations 1 and 2.

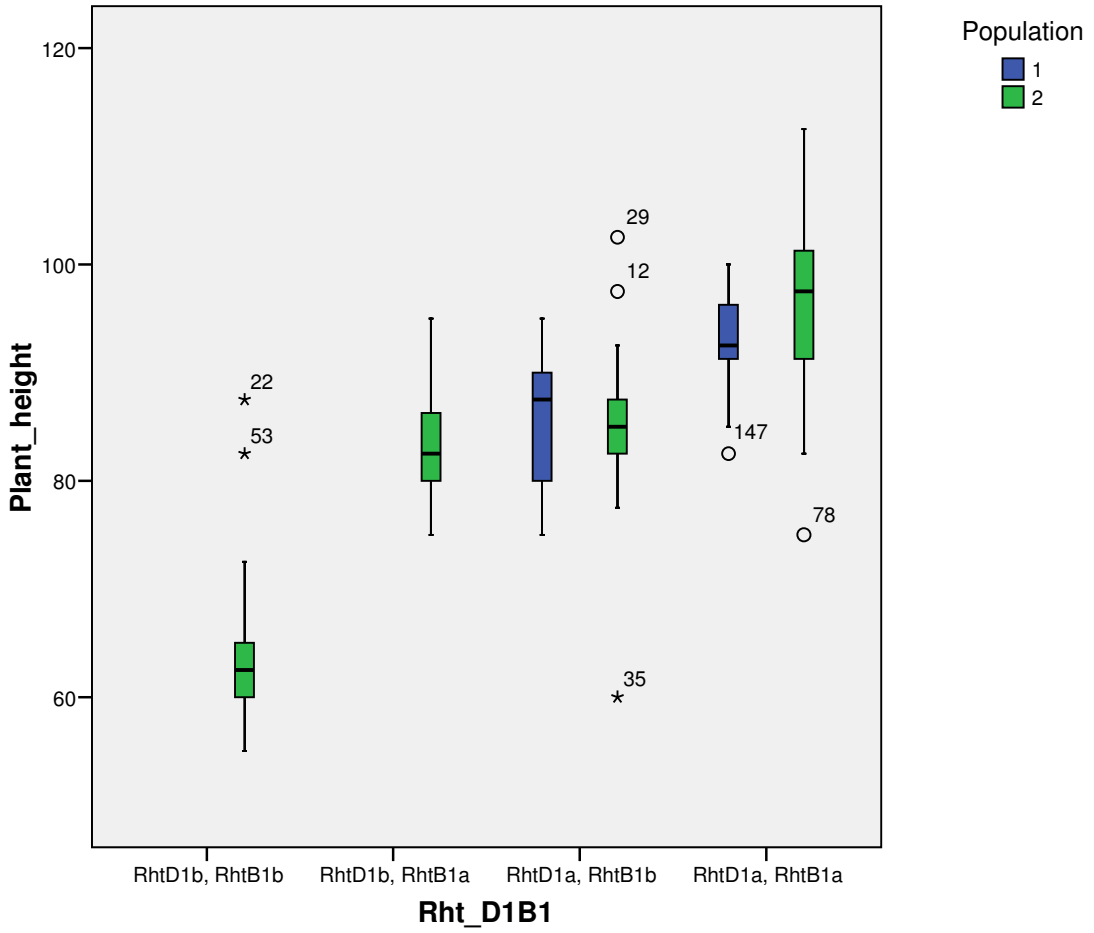
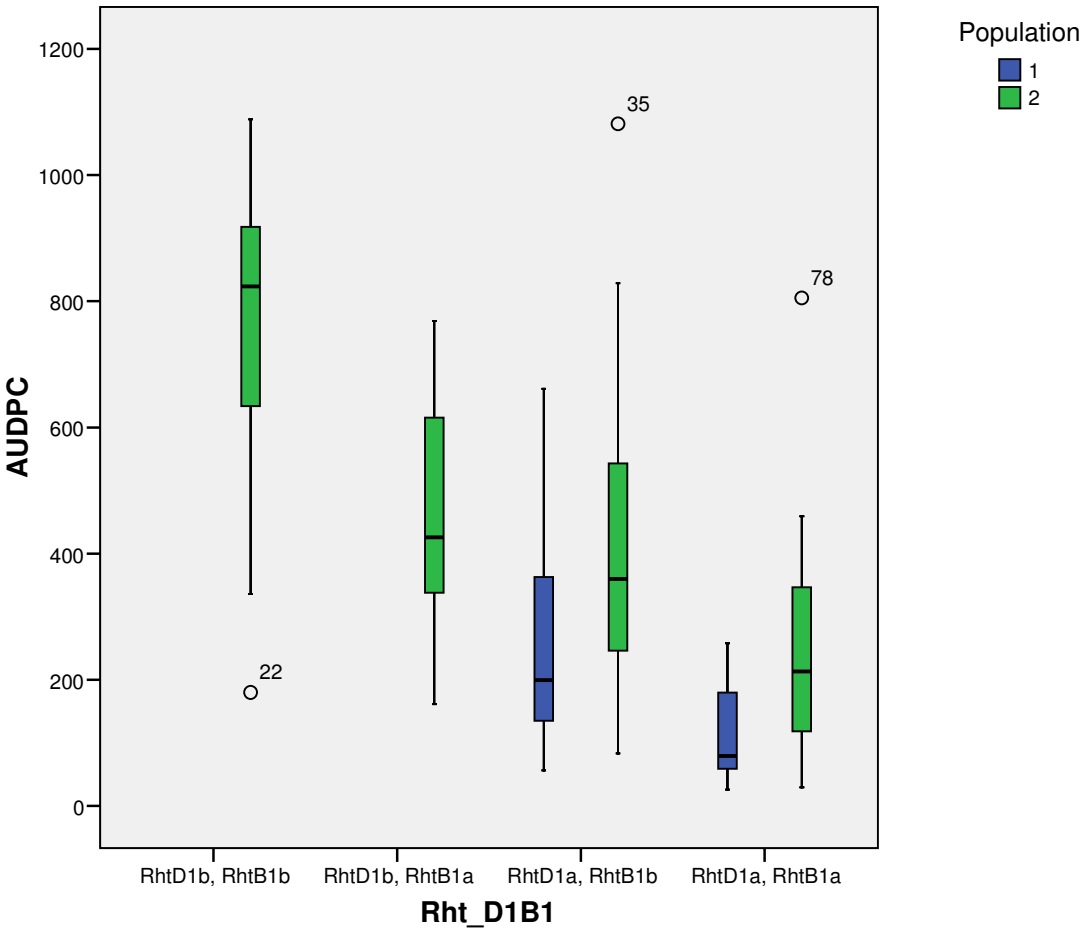


Figure 17: Box-plots for the trait Plant height showing the variation among groups of lines carrying the allele combinations *Rht-D1b & Rht-B1b; Rht-D1b & Rht-B1a, Rht-D1b & Rht-B1b,* or *Rht-D1a & Rth-B1a* in populations 1 and 2



.

Figure 18: Box-plots for the trait AUDPC showing the variation among groups of lines carrying the allele combinations *Rht-D1b & Rht-B1b; Rht-D1b & Rht-B1a, Rht-D1b & Rht-B1b,* or *Rht-D1a & Rth-B1a* in populations 1 and 2

The SSR marker *Barc20* was also analysed on the population, but it did not have any significant association with Fusarium resistance, therefore the results with *Barc20* are not further shown or discussed in this thesis.

Figure 20: Part of the field experiment showing genotypes with different plant height.

The difference in plant height among the genotypes is caused by the different dwarfing genes. The shortest group of line (left) shows genotypes with the two dwarfing genes i.e. *Rht-B1b, Rht-D1b* (full dwarf). The middle line represents genotypes with only one of the dwarfing alleles (semi-dwarf), i.e. either *Rht-B1b* or *Rht-D1b* while the line on the right represents genotypes having only the wild type tall alleles (*Rht-B1a, Rht-D1a*)

Figure 21: Part of the field experiment showing the difference in FHB susceptibility among the genotypes

The picture above (Figure 21) shows three DH lines in the field experiment taken about three weeks after inoculation with *Fusarium culmorum*. The difference in FHB severity among the different lines of genotypes can be clearly seen. The line on the left (full dwarf) had the two dwarfing alleles *Rht-B1b, Rht-D1b* and was the most susceptible to FHB. The line on the right had the two wild type alleles *Rht-B1a, Rht-D1a* and was the least susceptible to FHB. The middle line represents plants with just one of the dwarfing alleles *Rht-B1b,* or *Rht-D1b*, whose degree of FHB severity lies between the tall and the very short genotypes.

4. DISCUSSION

Plant breeder during the 1960s developed new cereal varieties with shorter stems than before, which resulted to better yields, since dwarf plants use more of their energy for filling the grains than in growing taller. In addiction the shorter plants were more stable against winds and high precipitations, since they were less likely to fall over, which also increases the overall harvest yield. This huge increase in grain after the 1960s, resulting from the introduction of new varieties of wheat and rice helped to prevent starvation throughout the world was termed *"The Green Revolution"*. This development was a major factor in maintaining per capita food supplies worldwide in the late-twentieth century, despite a doubling in the world population during this time (Evans 1998).

Higher yields were obtained in the past through the use of much fertilisers and pesticides, which were economically ecologically and toxicologically not attractive to humans and the environment. Borlaug (1968) introduced dwarfing genes into wheat, giving the plants a stronger, shorter stem that resisted lodging. He developed high yielding wheat varieties suitable for growing in subtropical and tropical climates. His findings were recognised in 1978 and he was awarded the Nobel Peace Prize.

An unexpected additional benefit from these the dwarfing genes its improvement of the harvesting index, which is the proportion of plant weight in the grain. This means that the greater proportion of photosynthesis products accumulate in the grains rather than the leaves. Due to the introduction of the dwarfing genes modern day wheat varieties have a harvest index of over 50 percent. The disadvantage of shorter varieties resulting from competition with weeds is overcome with nowadays by the extensive use of herbicides (Evans 1998).

GM-assisted plant breeding could provide a key to a second Green Revolution needed to provide enough food to support an ever increasing world population (Peng et al.1993. Peng et al.1997).

There are many genes associated with the semi-dwarf growth habit in wheat (Ellis et al. 2005). They are known as reduced height (*Rht*) genes, and many of them are dominant or semidominant, indicating that they actively inhibit growth through a so-called gain-of-function mutation. The

identity and function of most of these genes are not known, but some have been found to prevent the action of gibberellins. Two genes in particular, *Rht-B1b* and *Rht-D1b*, are used in many commercial wheat varieties—some varieties containing both of these dwarfing gene—which have an additive effect on growth. The effects of these two genes on plant height are compared in Figure 17. Bread wheat, *Triticum aestivum*, is hexaploid, which means it has three genomes (A, B, and D), these being derived from its three wild diploid ancestors. Each genome contains 14 chromosomes so that bread wheat has 42 chromosomes in total. The A, B, and D genomes are very similar to each other and are described as homoeologous. *Rht-B1b* and *Rht-D1b*, which were formerly called *Rht1* and *Rht2*, are corresponding (homoeologous) genes on the B and D genomes, respectively.

The dwarfing genes are responsible for high yield wheat cultivars. Wheat plants which are smaller in height are more stable than their taller counterparts since they can resist abiotic damages from strong winds rain fall or other forms of precipitation. Plant breeders all over the words are working hard then to produce wheat cultivars which are of smaller height and high yield. The identification and selection of the *Rht* genes is of great importance in breeding programmes.

Fusarium culmorum causes great losses to wheat farmer all over the world. The fungus productions on mycotoxins are hazardous to humans as well as to animals**.** **(**Prelusky et al.1994)**.** Besides wheat it also infects maize and other cereals, causing major epidemics in Europe, Asia, North and South America. As a result of which there are many researches to produce resistant species (Trail et al. 2001)

Investigating associations and impacts of these dwarfing genes *Rht-B1* and *Rht-D1* in a *Fusarium culmorum* inoculated wheat cultivar, using molecular marker will help in the production of resistance wheat species, which will lead to an increase in quality and quantity of wheat production.

4.1. Plant height

The plant height plays an important role if the plant is to be infected or not (Mesterharzy 1995). Plants with lower plant height are more liable to be infected by released spores since the spores can easily reached the heads of shorter plants than their tall counterparts. The heads of taller plants are held at higher distances to debris which are the primary source of infection. Planting dwarf plants

can also affect the micro-climate on the soil surface as well as the production of perithecia and ascospores. (Adolf 1998).

In this experiment, even though the plant height varied enormously between 55- 115 cm, a positive correlation was found between the plant height and the AUDPC. Steiner et al. (2003) also found that the plant height significantly correlated with FHB incidence and severity. Taller lines tended to be less diseased than shorter genotypes. Prat (2010) equally found significant negative correlation between plant height and FHB resistance confirming that short genotypes tend to be more susceptible. This phenomenon appears to be a common feature reported in several studies (Mesterhazy 1995, Buerstmayr et al. 2000, Buerstmayr et al. 2003. Ellis et al. 2005, Knopf et al. 2008). This was however contrary to the findings of Lengauer (2002) of which for almost the same plant heights found no correlation with the AUDPC.

Population one with just about 50 % of correlation with disease severity could be attributed to artificial spray inoculation which minimizes the confounding effect of plant height. Despite the mist irrigation for humidity control, the heads of taller plants may dry faster and could therefore be exposed to less humidity and wetness than those of short plants, leading to a bias in the FHB resistance measurement.

4.2. FHB severity

In this experiment the genotypes were artificially inoculated at anthesis with a macroconidia spore suspension which led to the development of FHB of all the genotypes under investigation. Disease symptoms were recorded on the 10, 14, 18, 22, 26 and 30 days after inoculation. The results were plotted on a curve. The area under this curve (AUDPC) was calculated for each entry as an integrated measure for disease severity.

FHB disease severity correlated very much with the plant height in all the two investigated populations. Both the lines and the blocks of populations 1 and 2 had significant positive value ($p < 0.0001$). This result was in contradiction to previous results obtained by Lengauer (2002) who obtained for all the four investigated populations no significant correlation.

Plants with the *Rht-D1* genes were lower in height than those with the *Rht-B1* genes but were more infected with *Fusarium culmorum* than plants with the *Rht-B1* genes. This finding was in accordance with the findings of Flintham et al. (1997), who also observed a more severe dwarfism for plants with the *Rht-D1* genes. The results were again confirmed by Srinivasachary et al. (2008) that the *Rht-D1b* allele was associated with FHB susceptibility.

4.3. The marker analysis for *Rht*-genes

The molecular markers used for predicting the presence of specific *Rht* alleles gave consistent and reproducible results. The allele specific primers for *Rht-B1* produced distinct bands which were easy to score. The primers for *Rht-D1* were not so easy to score. These had a tendency toward false positive bands and needed to be replicated in order to achieve reliable results.

4. Association of *Rht* alleles with Fusarium resistance

In the population 1, which segregated for *Rht-B1* only lines with the tall allele (Rht-B1a) were significantly less compared to lines with the *Rht-B1b* allele. In population two which segregated for two *Rht* genes both were associated with FHB resistance and in both cases the short allele increased susceptibility. Lines with both semi-dwarf alleles combined (full dwarfs) were the most susceptible group (See Fig. 23). My findings are in agreement with Draeger et al. (2007), Holzapfel et al. (2008), Miedaner & Voss (2008), Voss et al. (2008), Srinivasachary et al. (2008) but in some disagreement with Srinivasachary et al. (2009). Draeger et al. (2007), Holzapfel et al. (2008), Voss et al. (2008) and Srinivasachary et al. (2008) could clearly show that *Rht-D1* is strongly associated with FHB resistance. In all cases lines with the *Rht-D1b* allele were more susceptible than lines with the *Rht-D1a* allele. Srinivasachary et al. (2009) reported that only *Rht-D1* was associated with FHB resistance but not *Rht-B1* in a Orvantis x Soisson doubled haploid population, while in my population both *Rht*-genes were significantly associated with FHB resistance in the Hermann x Nord01044/01 population.

5. CONCLUSIONS

Plants with the *Rht-B1* genes were on average taller than plants with the *Rht-D1* genes. Likewise lines with *Rht-D1b* had a slightly higher mean FHB severity than plants with *Rht-B1b* (Figure 18). The *Rht-B1* and the *Rht-D1* genes had no effect on the date of anthesis. The alleles *Rht-B1b* and *Rht-D1b* had about the same effect on reducing plant height. Significant negative correlation between plant height and disease severity of r=-0.68 and r=-0.82 were found for population 1 and 2, respectively. The lines which carried the alleles *Rht-B1b* or *Rht-D1b* both expressed on average more FHB severity than plants with the *Rht-B1a* or the *Rht-D1a* alleles. Double dwarf lines (possessing *Rht-B1b* plus *Rht-D1b* combined, in population 2) were very short in plant height and even more FHB susceptible than lines with a single semi-dwarf allele either *Rht-B1b* or *Rht-D1b* (Figure 18). Despite that the variation in FHB severity was large within each group for the specific *Rht*-alleles (see Figure 18). Therefore, the selection of breeding lines with medium to short straw length, carrying either *Rht-B1b* or *Rht-D1b* and acceptable FHB resistance appears difficult but feasible (Figure 18).

6. ABBREVIATIONS

ANOVA : Analysis of variance

AUDPC : Area under disease progress curve

bp : base pairs

BME: ß-mercaptoethanol

CLA: Carnation Leaf Agar

CORR : Correlation analysis

CTAB : Mixed alkyltrimetyl-ammonium bromide

DELLA: DELLA Proteins

DF : Degree of Freedom

DH : Double Haploid

DNA : Deoxyribonucleic acid

dNTP: deoxyribonucleotide triphosphate.

DON : Deoxynivalenol

EDTA : Ethylenediaminetetraacetic acid

EtOH : Ethanol

FAM : 6-carboxy-fluorescine

FHB : Fusarium head blight

F-Value : how big the difference in variances (named after Fischer)

GA : Gibberellins Acid

GLM : General linear model

HEX : Hexachloro-6-carboxy-fluorescine

IRD700/800 : Infrared dyes 700/800

LSD : Least significant difference

MAS : Marker assisted selection

$MgCl_2$: Magnesiumchlorid

MS: Mean Squares

NaCl : Sodium chloride

NaOAc : sodium acetate

NH_4OAc: Ammonium acetate

NIL : Near Isogenic lines

PCR : Polymerase chain reaction

PDA: Potato Dextrose Agar P-Value : the probability of accepting the null hypothesis

QTL : Quantitative trait locus

RCF : Relative centrifugal force

RFLP : Restriction fragment length polymorphism

RIL : Recombinant inbred line

Rpm: Rotation per minute

SNP : Single nucleotide polymorphism

SS : Sum of Squares

SSR : Simple sequence repeat

Std Dev : Standard Deviation

STS : Sequence Tagged Site

TAE : Tris-acetate-EDTA

TAQ: *Thermus aquaticus*

TE : Tris-EDTA

UV : Ultraviolet

7. INDEX OF FIGURES

8. INDEX OF TABLES

9. LITERATURE

Adolf B. (1998). Epidemiologie und Nachweis von Getreidefusariosen: Untersuchungen an Weizen und Gerste: Herbert Utz Verlag München: 3-9.

Anderson, J.A., Stack, R. W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Mitchell Fetch, J., Song, Q.J., Cregan, P.B., Frohberg, R.C. (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations: Theor. Appl. Genet. 102: 1164-1168.

Bai, G., Kolb, F.L., Shaner, G., Dornier, L.L. (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat: Phytopathology 89: 343-347

Balyan, H.S. and Lohia, R.S. (1998). Pleiotropic effects of Rht dwarfing genes on grain yield and its component traits in wheat under rainfed environment: Indian Journal of Genetics 58(2): 169-176

Borlaug, N.E. (1968). Wheat breeding and its impact on world food supply: In, K.W. Finlay and Shepherd K.W. (eds.). Proc. 3rd. Intern. Wheat Genet. Symp. Canberra, Australia. Butterworths Australian Academy of Sci.: 1-36

Borojevic K. and Borojevic K. (2002). The Transfer and History of "Reduced Height Genes" (Rht) in Wheat from Japan to Europe: Journal of Heditory 2002 Volume 96, Issue4: 455-459

Buerstmayr H., Steiner B., Lemmens M. and Ruckenbauer P. (2000). Resistance to Fusarium head blight in two winter wheat crosses: heritability and trait associations. Crop Sci. 40: 1012-1018

Buerstmayr H., Lemmens M., Hartl L., Doldi L., Steiner B., Stierschneider M. Ruckenbauer P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance): Theor. Appl. Genet. 104: 84-91

Buerstmayr H., Steiner B., Hartl L., Griesser M., Angerer N., Lengauer D., Miedaner T., Schneider

B. Lemmens M., (2003). Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread: Theor. Appl. Genet. 107: 503-508.

Clear R and Patrick S. (2010). Fusarium head blight in western Canada: Mycology, Grain Research Laboratory, Canadian Grain Commission, found on http://www.hylandseeds.com/file/fhbwc-foc-eng.pdf

Cho C., Kyu H., and Lee S. (1993). Origin, dissemination and utilization of semi dwarf genes in Korea: 7th Int. Wheat Genetic Symp. (Miller TE, Koebner RMD, eds.). Bath: Bath Press: 223–231.

Diminick J. P., and Karl J. N. (1996). Effect of RHT Dosage on the Breaking Strength of Wheat Deedling Leaves: American Journal of Botany 83 (5): 567-572.

Draeger R., Gosman N., Steed A., Chandler E., Thomsett M., Srinivasachary, Schondelmaier J., Buerstmayr H., Lemmens M., Schmolke M., Mesterhazy A., Nicholson P. (2007). Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina: Theor. Appl. Genet. 115: 617-625

Drouyer G. J.P., Bonnett D.G., Ellis M.H., Sirault X.R.R. (2008). Unravelling the effects of GA-responsive dwarfing gene Rht13 on yield and grain size: 11th International Wheat Genetics Symposium 2008. Band 2. UDC 575.22.: 633:11

Ellis M. H., Rebetzke G. J., Chandler P. A., Bonnett D., Spielmeyer W., Richards R. A. (2004). The effect of different height reducing genes on the early growth of wheat: Functional Plant Biology 31(6): 583–589

Ellis M. H., Rebetzke G. J., Azanza F., Richards R. A., and Spielmeyer W. (2005). Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat: Theor. Appl. Genet. 111: 423–430.

Evans L. T. (1998). Feeding the Ten Billion: Plant and Population Growth: Cambridge University Press, Cambridge, UK.
(http://www.cambridge.org/uk/catalogue/catalogue.asp?isbn=9780521646857)

Flintham J. E., Börner A., Worland A. J., Gale M. D. (1997). Optimizing wheat grain yield effects of Rht (gibberellin-insensitive) dwarfing genes: The Journal of Agricultural Sci. (97), 128:1: Cambridge University Press: 11-25

Gale, M. D., and Youssefian, S. (1985). Dwarfing genes in wheat: In *Progress in Plant Breeding*, Russell G. E. (ed.), Butterworths, London: 1–35.

Gent M. P. N. and. Kiyomoto R. K. (1997). Physiological and Agronomic Consequences of Rht Genes in Wheat: Journal of Crop Production, Volume 1, Issue 1 October 1997: 27 – 46

Gill K. S. and Gill B. S. (1994). Mapping in the realm of polyploidy, the wheat model: Bioessays 16: 841-946
Gupta P. K., Varshney R.K., Sharma P.K. and Ramesh B. (1999). Molecular markers and their applications in wheat breeding: Plant Breeding 118: 369-390.

Häberle J., Holzapfel J., und Hartl L. (2008). Die Genetik der Fusariumresistenz in europäischem Winterweizen: SAAT GUT Osterreich 59. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute: 5-8

Holzapfel J, Voss H.H, Miedaner T, Korzun V., Häberle J., Schweizer G., Mohler V., Zimmermann G., Hartl L. (2008). Inheritance of resistance to Fusarium head blight in three European winter wheat populations: Theor. Appl. Genet. 117: 1119-1128

Hoops A. (2006). Effect of Reduced Height Genes (Rht) on Components of the Root System in Bread Wheat: Thesis at the University of California, College of Natural and Agricultural Sciences, Botany & Plant Sci.:1-4

Karl J. N. and Dominick J.P.,JR.(1990). Biomechanical and Morphometric Differences in Triticum aestivum Seedlings Differing in Rht Gene-Dosage: Annals of Botany 65: 365-377.

Katashi K., Yutaka J., Kazuto I., Nobuyoshi W., Akira Y., Ismahane E. and Miloudi M.N. (2004). Effect of semi-dwarf genes on the root penetration ability of wheat: Proceedings of the 4th International Crop Science Congress. Brisbane, Australia, 26 Sep – 1 Oct 2004 | ISBN 1 920842 20 9 www.cropscience.org.au.

King R.W., Gale M. D. and Quarrie S. A. (1983). Effects of NORIN 10 and Tom Thumb Dwarfing Genes on Morphology, Physiology and Abscisic Acid Production in Wheat: Annals of Botany 51: 201-208

Knopf C., Becker H., Ebmeyer E., Korzun V. (2008). Occurrence of three dwarfing Rht genes in German winter wheat varieties: Cereal Research Communications 36(4): 553–560.

Kolb F.L., Bai G-H., Muehlbauer G.J., Anderson J.A., Smith K.P. and Fedak G. (2001). Host and plant resistance genes for Fusarium head blight mapping and manipulation with molecular markers: Crop Sci. 41: 611-619

Lengauer D. (2002). Verifizierung von DNA Markern für Ährenfusariosereistenz in vier Winterweizenpopulation: Diplomarbeit an der Universität für Bodenkultur Wien, Institut für Planzenbau und Planzenzüchtung

Linnert G. (1997) in Odenbach W. (Hrsg) Biologische Grundlagen der Pflanzenzüchtung: Parey Buchverlag Berlin. DNA-Marker in der Pflanzenzüchtung: 324-324

Miedaner, T., and Voss H.H. (2008). Effect of Dwarfing Rht Genes on Fusarium Head Blight Resistance in Two Sets of Near-Isogenic Lines of Wheat and Check Cultivars: Crop Sci 48: 2115-2122.

Mesfin A., Smith K.P., Dill-Macky R., Evans C.K., Waugh R., Gustus C.D. and Muehlbauer G.J. (2003). Quantitative Trait Loci for Fusarium head blight resistance in barley detected in a two-

rowed by six-rowed population: Crop Sci. 43: 307-318.

Mesterhazy A. (1995). Types and components of resistance to Fusarium head blight of wheat: Plant Breeding 114: 377-386.

Parades-Lopez O. (1999). Molecular biotechnology for plant food production: Technomic Publishing Company, Inc., Lancaster: 275-303

Paul P.A., El-Allaf M., Lipps P.E., Madden L.V., (2005). Relationship between incidence and severity of Fusarium head blight on winter wheat in Ohio: Phytopathology 95: 1049-1060.

Paveley N., Parker S., Foulkes J., Syvester-Bradley R., Snape J., Farrar J., Lovell D., Neumann S., Ellerbrook C., Greenhous J. (2002). Improving and exploiting 'self-defence' against wheat diseases: HGCA conference 2002, Agronomic intelligence, the basis for profitable production, http://www.hgca.com/publications/documents/cropresearch/RD_conference_2002_paper_9.pdf

Peng J. B., Chen X.Z., Berger U.V., Vassilev P.M., Tsukaguchi H., Brown E.M., Hediger M.A. (1999). Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption: J Biol. Chem. 274: 22739–22746

Peng J., and Harberd N.P. (1993). Derivative alleles of the Arabidopsis gibberellin-insensitive gai mutation confer a wild-type phenotype: Plant Cell: 351-360

Peng J. and Harberd N.P. (1997). Gibberellin deficiency and response mutations suppress the stem elongation phenotype of phytochrome deficient mutants of Arabidopsis: Plant Physiology: 1049-1057

Pinthus J. and Levy A. A. (1983). The relationship between the Rht1 and Rht2 dwarfing genes and grain weight in Triticum aestivum L. spring wheat Theor. Appl. Genet. 66: 153-157

Plaschke J., Ganal M.W., Roeder M.S. (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers: Theor. Appl. Genet. 91: 1001-1007

Prat N. (2010). Characterization of FHB resistance in three spring wheat populations: Thesis am Insitite für Planzenbau und Planzenzüchtung Universität für Bodenkultur Wien

Prelusky D.B., Rotter B.A., Rotter R.G. (1994). Toxicology of mycotoxins: In; Miller J.D., Trenholm H.L. (eds), Mycotoxins in grain, compounds other than Aflatoxin. Eagan Press, St. Paul: 359-403.

Randy C. and Susan P. (2010). Fusarium head blight in western Canada: Grain Research Laboratory, Canadian Grain Commission Cereal Chemistry Revised January 25, 2010 (History): 1-6

Rudd J.C., Horsley R.D., McKendry A.L.,Elias E.M. (2001). Host plant resistance genes for Fusarium head blight: sources, mechanisms and utility in conventional breeding systems: Crop Sci. 41: 620-627.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A., Allard R.W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics: Proc. Natl. Acad. Sci. USA 81: 8014-8018.

SAS Institute Inc (2008) SAS/STAT® 9.2 User's Guide. Cary, NC.
Savelkoul P.H.M., Aarts H.J.M., De Haas J., Dijkshoorn L., Dum B., Otsen M., Rademaker J.L.W., Schouls L. and Lenstra J.A., (1999). Amlified-fragment length polymorphism analysis: the state of an art. Journal of Clinical Microbiology 37: 3083-3091.

Seefeldt S. S., Ogg A. G., Hou Y. S. (1999). Near –isogenic lines for Triticum Aestivum height and crop competitiveness: Weed Sci. 47: 316-320

Shen, X., Ittu M., Ohm, H. W. (2003) Quantitative trait loci conditioning resistance to Fusarium head blight in wheat: Crop Sci. 43: 850-857.

Singha R. P., Huerta-Espinob J., Crossaa J., Van Ginkela M. (2001). Coleoptile length variation of near-isogenic Rht lines of modern CIMMYT bread and durum wheats: Field Crops Research Volume 70, Issue 3: 167-176

Snijders C.H.A. and Perkowski J. (1990). Effects of head blight caused by Fusarium culmorum on toxin content and weight of kernels: Phytopathology 80: 566-570

Snijders C.H.A. and Van Eeuwijk F.A. (1991). Genotyp*strain interactions to Fusarium head blight caused by Fusarium culmorum in winter wheat: Theor. Appl. Genet. 81: 239-244.

Sourdille P., Perretant M.R., Charmet G., Leroy P., Gautier M.F., Joudrier P., Nelson J.C., Sorrells M.E.Bernard M. (1996). Linkage between RFLP markers and genes affecting kernel hardness in wheat: Theor. Appl. Genet. 93: 580-586.

Spielmeyer W., Ellis M., Robertson M., Shahjahan A., Lenton J.R., Chandler P.M. (2004) .Isolation of gibberellin metabolic pathway genes from barley and comparative mapping in barley, wheat and rice: Theor. Appl. Genet.. 109:847-855

Srinivasachary, Gosman N., Steed A., Simmonds J., Leverington-Waite M., Wang Y., Snape J., Nicholson P. (2008). Susceptibility to Fusarium head blight is associated with the Rht-D1b semi-dwarfing allele in wheat: Theor. Appl. Genet. 116: 1145-1153

Srinivasachary, Gosman N., Steed A., Hollins T.W., Bayles R., Jennings P., Nicholson P. (2009). Semi-dwarfing Rht-B1 and Rht-D1 loci of wheat differ significantly in their influence on resistance to Fusarium head blight: Theor. Appl. Genet. 118: 695-702

Steiner B. (2003). Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana: Dissertation zur Erlangung des Doktorgrades an der Universität für Bodenkultur.

Steiner B., Lemmens M., Griesser M., Scholtz U., Schondelmaier J., Buerstmayr H. (2004). Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana: Theor. Appl. Genet. 109: 215-224.

Tosovic-Maric B., Kobiljski B., Obrehti D., and Vapa L., (2008). Evaluation of Wheat Rht Genes using Molecukar Markers: Genetika, Vol.40, No. 1: 31-38.

Trail F., Xu J.R., San Miguel P., Gattoor I., Kristler C. (2001). Expressed sequence tags from developmental stages of *Gibberella zeae*: Fusarium Head Blight Forun 8-10 Dec. Cicinnati: 37

Uphaus J., Shen X., Goodwin S., Buechley G., Breeden J., and Ohm H. (2005). Developing multiple disease resistance linkage block on wheat chromosome 3 BS In: Canty, S. M. Boring , T., Wardwell, J., Siler, L., and Ward, R. W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, Wisconsin. East Lansing: Michigan State University: 94.

Visconti A., and Pascale M. (2010). An Overview on Fusarium Mycotoxins in the Durum Wheat Pasta Production Chain: Cereal Chemistry, volume 87: 21-27

Voss H.H., Holzapfel J., Hartl L., Korzun V., Rabenstein F., Ebmeyer E., Coester H., Kempf H., Miedaner T. (2008). Effect of the Rht-D1 dwarfing locus on Fusarium head blight rating in three segregating populations of winter wheat: Plant Breed. 127: 333-339

Wagacha J.M. and Muthomi J.W. (2006). *Fusarium culmorum*: Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat: Crop Protection Volume 26: 877-885

Waldron B.L., Moreno-Sevilla B., Anderson J.A., Stack R. W., Frohberg R.C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat: Crop Sci. 39: 805-811.

Weber J.L., and May P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction: American journal of human genetics (Am J Hum Genet.) 44(3): 388-96.

Yamada T. (1990). Classification of GA response, Rht genes and culm length in Japanese varieties and landraces of wheat: Euphytica 50: 22 1-239

Youssefian S., Kirby E. J. M., and Gale M. D. (1992). Pleiotropic effects of the GA-insensitive Rht dwarfing genes in wheat. 1. Effects on development of the ear, stem and leaves: Field Crops Research Volume 28: 179-190.

Yu G. T., Franckowiak J. D., Neate S. M., Zhang B., and Horsley R. D. (2010). A native QTL for Fusarium head blight resistance in North American barley (Hordeum vulgare L.) independent of height, maturity, and spike type loci: Genome, Volume 53, Number 2:111-118

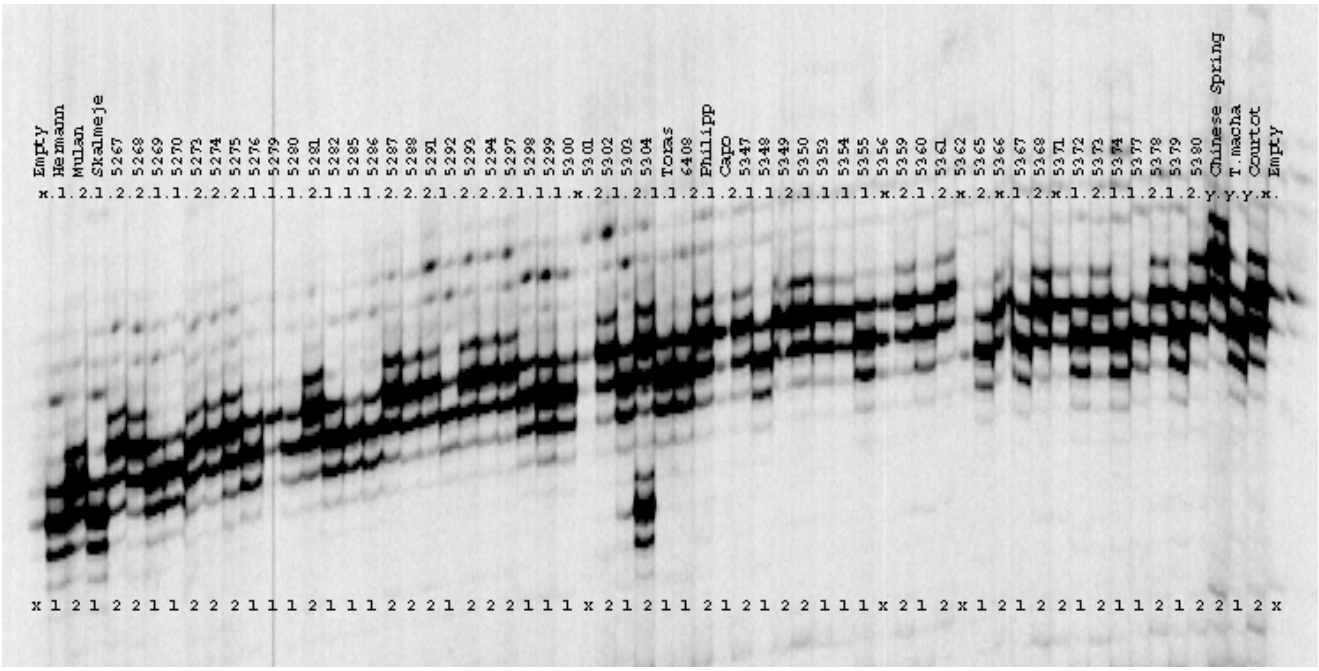
Yu J.B., and Bai G.H. (2009). Mapping Quantitative Trait Loci for Long Coleoptile in Chinese Wheat Landrace Wangshuibai : Crop Sci. 50: 43-50

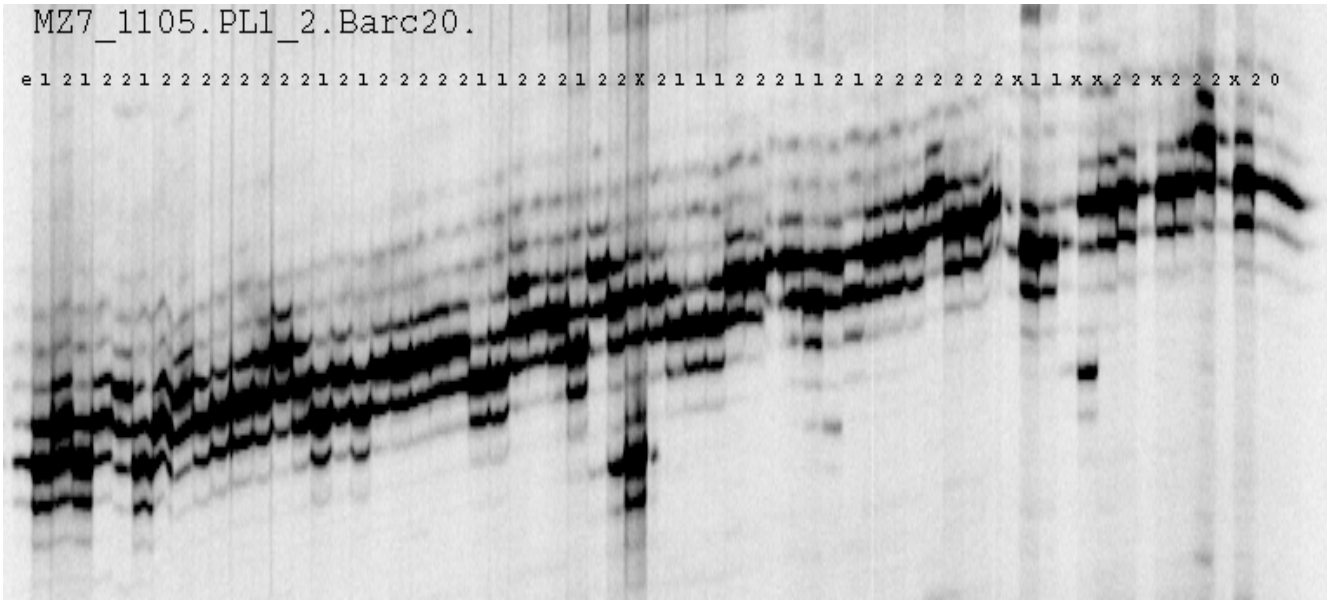
Zaccai M., Pinthus M. J., and Levy A. A., (1987). The effect of the Rht1 gene on grain protein content in tetraploid wheat Triticum turgidum: Journal of Cereal Sci. Volume 6, Issue 1: 27-32

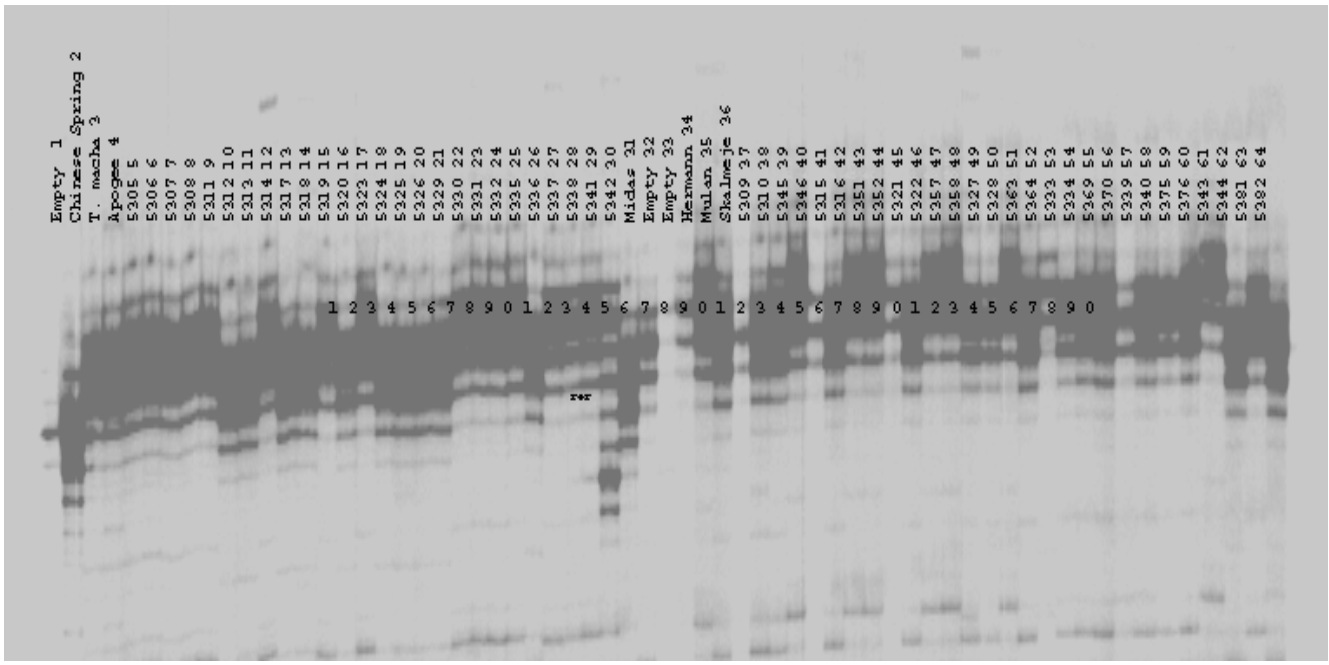
Zhou W.C., Kolb F. L., Bai G. H., Shaner G., Domier L. L. (2002). Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers: Genome 45: 719-727.

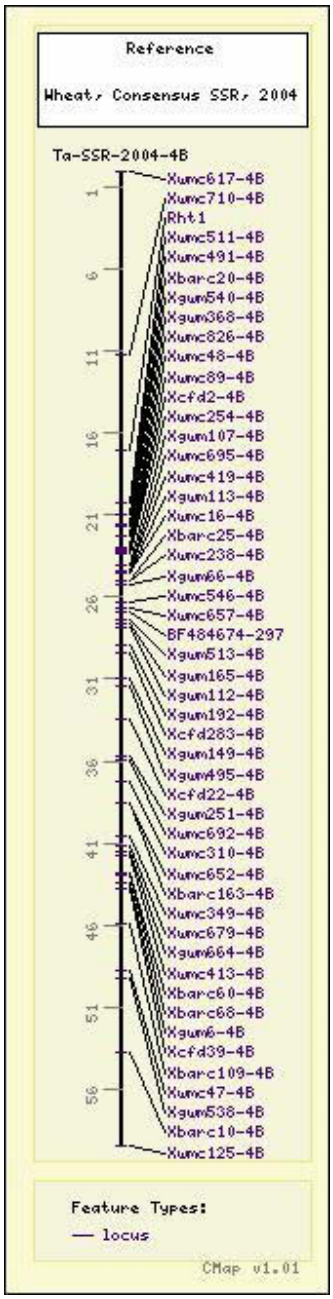
Zhou W.C., Kolb F.L., Bai G.H., Domier L.L., Boze L.K., Smith N.J. (2003). Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat: Plant Breed 122: 40-46.

10. APPENDIX


Empty
Hermann
Mulan
Skalmeje
5267
5268
5269
5270
5273
5274
5275
5276
5279
5280
5281
5282
5285
5286
5287
5288
5291
5292
5293
5294
5297
5298
5299
5300
5301
5302
5303
5304
Tosas
6408
Philipp
Capo
5347
5348
5349
5350
5353
5354
5355
5356
5359
5360
5361
5362
5365
5366
5367
5368
5371
5372
5373
5374
5377
5378
5379
5380
Chinese Spring
T.macha
Courtot
Empty


MZ7_1105.PL1_2.Barc20.
e 1 2 1 2 2 1 2 2 2 2 2 2 2 2 2 1 2 1 2 2 2 2 2 1 1 2 2 2 1 2 2 X 2 1 1 1 2 2 2 1 1 2 1 2 2 2 2 2 2 2 2 x 1 1 x x 2 2 x 2 2 2 x 2 0


Empty 1
Chinese Spring 2
T. macha 3
Apogee 4
5305 5
5306 6
5307 7
5308 8
5311 9
5312 10
5313 11
5314 12
5317 13
5318 14
5319 15
5320 16
5323 17
5324 18
5325 19
5326 20
5329 21
5330 22
5331 23
5332 24
5335 25
5336 26
5337 27
5338 28
5341 29
5342 30
Midas 31
Empty 32
Empty 33
Herman 34
Mulan 35
Skalmeje 36
5309 37
5310 38
5345 39
5346 40
5315 41
5316 42
5351 43
5352 44
5321 45
5322 46
5357 47
5358 48
5327 49
5328 50
5363 51
5364 52
5333 53
5334 54
5369 55
5370 56
5339 57
5340 58
5375 59
5376 60
5343 61
5344 62
5381 63
5382 64
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0



The locus of Barc20 on chromosome 4B