Lehrstuhl für Pflanzenbau und Pflanzenzüchtung
der Technischen Universität München
in Freising-Weihenstephan

Localization and characterization of quantitative trait loci for fusarium head blight resistance in wheat by means of molecular markers

Mahmoud El-Zaabalawy Mahmoud El-Badawy

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Agrarwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. G. Forkmann

Prüfer der Dissertation: 1. Univ.-Prof. Dr. G. Wenzel
2. Univ.-Prof. Dr.G. Müller-Starck

Die Dissertation wurde am 05.07.2001 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 22.10.2001 angenommen.

Contents

Liste of tables

1 Introduction

Wheat is a cereal grass of the *Gramineae* (*Poaceae*) family and of the genus *Triticum* and it is an edible grain, one of the oldest and most important of the cereal crops. Though grown under a wide range of climates and soils, wheat is best adapted to temperate regions with rainfall between 30 and 90 cm (12 and 36 inches). Winter and spring wheat are the two major types of the crop, with the severity of the winter determining whether a winter or spring type is cultivated. Winter wheat is always sown in the fall; spring wheat is generally sown in the spring but can be sown in the fall where winters are mild. Therefore, today wheat is grown all over the world, with different varieties sown according to the various climates. For example, in Canada, the harsh winters require a fast growing grain, with wheat sown and matured in about 90 days. By comparison, UK wheat is harvested in August; having been planted the previous September. Different varieties again are required to cope with the dry sun-baked lands of northern India. Accordingly, more of the world's farmland is devoted to wheat than to any other food crop; in the late 20th century about 570,000,000 acres (230,000,000 hectares) were sown annually, with a total production of almost 600,000,000 metric tons. The world's largest producer is China, with an estimated annual yield of almost 114,400,066 metric tons (FAO, 1999). Other leading producers are; India, the United States, France, Russia, Canada, Germany, Turkey, Kazakstan, Ukraine and Pakistan.

The greatest portion of the wheat flour produced is used for breadmaking. Wheat grown in dry climates is generally hard type, having protein content of 11-15 percent and strong gluten (elastic protein). The hard type produces flour best suited for breadmaking. The wheat of humid areas is softer, with protein content of about 8-10 percent and weak gluten. The softer type of wheat produces flour suitable for cakes, crackers, cookies, and pastries and household flours. Durum wheat semolina (from the endosperm) is used for making pastas, or alimentary pastes.

Although most wheat is grown for human food, and about 10 percent is retained for seed, industry for production of starch, paste, malt, dextrose, gluten, alcohol, and other products use small quantities. Inferior and surplus wheat and various milling by-products are used for livestock feeds. The composition of the wheat grain, a major source of energy in the human diet, varies somewhat with differences in climate and soil. On an average, the kernel contains 12 percent water, 70

percent carbohydrates, 12 percent protein, 2 percent fat, 1.8 percent minerals, and 2.2 percent crude fibbers. A pound of wheat contains about 1,500 calories (100 grams contains about 330 calories). Thiamin, riboflavin, niacin, and small amounts of vitamin A are present, but the milling processes remove the bran and germ, where these vitamins are found in the greatest abundance.

1.1 Fusarium head blight of wheat

1.1.1 Fusarium head blight (FHB) pathogens

Several fungal, bacterial and viral diseases, which lead to reduction in either or yield and quality attack it. Wheat Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* and *Fusarium culmorum*. FHB, is one of the most destructive diseases world-wide (Bai and Shaner, 1994; Miedaner, 1997). Many of species of Fusarium can cause wheat FHB and the symptoms caused by different species are almost the same. *F. graminearum and F. clumorum* are the principal pathogens responsible for head blight in many countries (Clear and Abramson, 1986; Schroeder and Christensen, 1963; Sutton, 1982; Wang et al., 1982 and Wiese, 1987). There are related species may also contribute to the head blight but generally less important. This fungus can survive as mycelium, ascospores, macroconidia and chlamydospores. Ascospores are the propagules of the sexual stage and in soil, the macroconidia or mycelium may be transformed into chlamydospores (Reis, 1990). *F. graminearum* survives between wheat crops in living or dead host tissues. Ascospores, macroconidia, chlamydospores and hyphal fragments all can serve as inoculum (Zhu and Fan, 1989). Ascospores and macroconidia are the principal inoculum because aerial dispersal is necessary for the fungus to reach the infection site (Sutton, 1982). Crop residues such as debris of wheat, corn or rice on the soil surface are the most important source of inoculum. In the areas where wheat is planted after rice, rice stubble is a major source of inoculum for wheat head blight (Zhu and Fan, 1989). Also, wheat planted after corn often has significantly more head blight than wheat planted after other crops. Therefore, reduced tillage for soil conservation increases the amount of inoculum that infects wheat (Teich and Hamilton, 1985).

Fig 1: Phenotypic severity of FHB (*Fusarium culmorum*) disease in wheat

The infection initiates by airborne ascospores, which are deposited on wheat spikelets, and consequently, they germinate inside them. The fungus may also infect by direct penetration of the glum, palea or rachila. Soon after infection dark brown spots appear on the infected spikelets and later the entire spikeletes become blighted (Bennet, 1931). If the weather is favorable, aerial mycelium spreads externally from the spikelets to another. If the fungus spreads internally, brownish chlorotic symptoms extend up and down and the entire spike die. Visible pink mold appears on the spike when it is humid. Therefore, infected florets often fail to produce grain (Wiese, 1987).

In nature, wheat head infection can occur any time after the beginning of the flowering. Generally, it is most susceptible to infection at the flowering stage, however some cultivars are most susceptible at the milk or soft dough stages (Schroeder and Christensen, 1963). Anthesis is the greatest period for susceptibility; therefore, the fungus is limited to one infection cycle per season (Strange and Smith, 1987). The incubation period is as short as 2-3 days in the greenhouse or laboratory and 4-5 days in the field (Xiao et al., 1989). Primary infection may occur on several florets in the field, therefore, the dark brown symptoms usually extend into the rachis. The pathogen mycelium invades parenchymatous tissue as well as vascular tissue (Schroeder and Christensen, 1963). Thus, the clogging of

vascular tissue in the rachis can cause the head to ripen prematurely. If the heads invade extensively at early stages, kernels may fail to develop entirely (Schroeder and Christensen, 1963). The optimum temperature for infection and development is 25˚C, little or no infection occurred at 15 ˚C and incidence increases as temperature increases from 20 to 30 ˚C. Also, the moist period required for infection ranges from 36 to 72 hours (Anderson, 1948).

1.1.2 Economic loss by FHB

FHB significantly reduces wheat grain yield and quality. Yield reduction results from shriveled grains, which may be light enough to be expelled from the combine with the chaff. In addition, the infected grains that are not eliminated with the chaff have reduced test weight because they are light and shriveled. Also, it reduces seed germination and causes seedling blight and poor stand when it is used as seed (Bai and Shanner, 1994).

FHB infection have been reported worldwide from wherever cereal crops are grown. Damage due to FHB in the USA was estimated at more than one billion dollars in 1993 and 500 million in 1994. Epidemics in China are most common and severe in Yangtze River Valley and can affect more than seven million hectares of wheat. It is estimated that in China up to 2.5 million tons of grain may be lost to FHB in epidemic years. In Uruguay, the damage caused by FHB under field conditions appears more severe than the actual losses (diaz de Ackerman and Kohli, 1996). Yield losses caused by FHB during the epidemics years of 1990, 1991 and 1993 ranged between 0.5-31% (diaz de Ackerman and Kohli, 1996). An average of 54% infected heads between 1990-1993 was responsible for only a 10% yield loss. In Argentina, the worst outbreaks occurred in 1978, 1985 and 1993 where yield losses varied among zones but were estimated to average between 20-30% (Galich, 1996).

1.1.3 Toxin production by FHB pathogens

FHB causes additional loss for agriculture because of the potent mycotoxins produced by the fungus. The mycotoxins produced by Fusarium graminearum in infected grains are detrimental to livestock and are a safety concern in human foods. The two most important mycotoxin produced by Fusarium graminearum are the estrogenic toxin zearalenone (ZEA) and the trichothecene deoxynivalenol

(DON), a vomitoxin (Tuite et al., 1990). Grain with one or both toxins may be graded down or rejected entirely in commerce.

1.1.4 Control of FHB

There are various cultural control practices for reducing the disease spread have been experienced. Crop rotation coupled with plowing to bury infested crop residues and weed hosts can be effective. Also, appropriate methods of land preparation, good crop husbandry, timely harvest, proper storage and other practices help reduce disease by reducing primary inoculation. However, because of the ubiquitous nature and wide host range of F. graminearuma adequate control by these methods is not possible (Reis, 1990).

Another way to control FHB disease is fungicide treatment. Seed-treatment fungicides reduce the spread of seed-borne inoculum and increase seedling vigor. Although, foliar application of fungicide at anthesis might provide some protection, there are many problems against this application. Cost of treatment and the difficulty of determining the optimum time of application also make this means of control less attractive to farmers. Even if a fungicide reduces direct yield loss, it may not reduce mycotoxin contamination to a tolerable level (Martin and Johnston, 1982).

1.1.5 Breeding for resistance to FHB

Hence, the use of resistance in wheat cultivars has many advantages. First, is the economic saving of the costs of pesticide application. Second, is the advantage of reducing the environmental pollution. Therefore, breeding for durable resistance against this fungal disease in wheat is the most economical and effective mean of reducing yield mycotoxin contamination causes by this fungus. The methodology applied has two steps. The first involves the identification of resistance sources. In the second, is to incorporate resistance into genotypes with good agronomic traits. Therefore, considerable effort has been devoted to finding sources of resistance that can be used in breeding programs. Considerable progress in the search for resistance has been made in different places in the world e.g. USA, China, Canada. Unfortunately, most of resistant cultivars could had other undesirable agronomic traits, therefore, they could not be used directly in commercial production. Some of them were used as parents in breeding pro-

grams but resistance was difficult to incorporate into elite lines. Sumai 3 was reported to have high combining ability for both FHB resistance and yield traits and has been widely used in wheat breeding programs with some success (Wang et al., 1989; Zhaung and Li, 1993).

1.1.6 Resistance types

Resistance to FHB was divided into three types by (Schroeder and Christensen, 1963) : resistance to primary infection (penetration resistance, type I), resistance to spread of hyphae within a wheat spike (spread resistance, type II) and resistance to the accumulation of DON within host tissue (biochemical resistance, type III). However, five types of resistance to FHB have been proposed by Wang and Miller (1988) and Mesterhazy (1995): (1) resistance to initial infection, (2) resistance to spread of infection, (3) resistance to kernel infection, (4) tolerance and (5) resistance to toxins accumulation. Nevertheless, only spread in the head and initial infection types of resistance can be used in the search for resistant germplasm and screening breeding lines (Bai and Shanner, 1994).

1.1.7 Inheritance of FHB resistance

Many investigators considered FHB resistance to be quantitatively inherited and controlled by minor genes (Yu, 1982; Chen, 1983; Liao and Yu, 1985; Wu, 1986; Snijders, 1990; Yu, 1990) but others provide evidence of oligogenic control (Li and Yu, 1988; Bai and Xiao, 1989; Bai et al., 1990; Van Ginkel et al., 1996 and Yao et al., 1997).

Using of generation mean analysis to study the resistance in crosses between resistant and susceptible cultivars indicated that resistance is controlled by additive genetic effects but nonadditive effects might also be significant (Chen, 1983; Bai et al., 1989; Snijders, 1990; Bai et al., 1993). Within the nonadditive components, dominance appears to be the most important (Bai et al., 1990; Snijders, 1990). Epistatic effects were considered significant in the study by Bai et al. (1993) but Zhuang and Li (1993) considered these effects were not significant. Bai et al. (1989); Chen (1983) and Liao and Yu (1985) found high estimate values for hertability.

Also, these analyses indicated that many resistant cultivars possess genes for both resistance and susceptibility. Chen (1989) proposed one dominant gene and

some minor modifiers for FHB resistance in Sumai 3 cultivar, Zhou et al. (1987) estimated two and Bai et al. (1989) three major resistance genes. Bai (1995) confirmed three genes with major effects on FHB resistance in Sumai 3 and Ning 7840 (a cultivar descended from Sumai 3). In the Brazilian cultivar Frontana, another important FHB resistance source, Singh et al. (1995) estimated at least three resistance genes and Van Ginkel et al. (1996) reported two in Frontana and two in Ning 7840 with all four genes being different.

At the same time, using cytogenetic analysis (monosomic analysis) resistance genes were assigned to several chromosomes. Yu (1982) showed that Sumai 3 has FHB resistance genes on chromosomes 1B, 2A, 5A, 6D and 7D. Liao and Yu (1985) indicated that Wangshuibai cultivar has resistance genes on chromosomes 4A, 5A, 7A, and 4D. Yu (1990) showed that the genes for resistance to FHB in cultivar PHJZM are located on chromosomes 6D, 7A, 3B, 5B and 6B. Also, he indicated that the cultivar HHDTB has genes for resistance on chromosomes 5D, 1B, 7B and 4D. In addition, in cultivar YGFZ, resistance genes are located on chromosomes 3A and 4D.

Although resistance to FHB is a complicated quantitative trait, resistance to spread within a spike is the main component of resistance and may be controlled by a few major genes (Bai et al., 1990). It is a relatively stable character and less affected by the environment than is resistance to the initial infection (Bai and Shaner, 1996). In the field, some susceptible cultivars may escape FHB because the weather when they are flowering is not conducive to infection and therefore they appear to be resistant. Several investigators concluded that resistance to FHB spread within a spike is controlled by many minor genes (Chen 1983; Liao and Yu, 1985; Snijders, 1990 a, b, d) whereas others concluded that resistance is under the control of few genes (Bai and Xiao, 1989; Bai et al., 1990; Van Ginkel et al., 1996; Yao et al., 1997). Recently, Bai et al. (2000) concluded that most probably one to three genes control FHB resistance.

1.1.8 Breeding strategies for FHB resistance

Because the genes for resistance in different cultivars appear to be on different chromosomes, crosses between these cultivars may yield transgressive progenies with greater resistance than any of the parents. Liu and Wang (1991) reviewed the progress in China toward breeding culivars for FHB resistance. They found that

one tenth of the lines with resistance to FHB were selected from transgressive segregants. Sumai 3, a well known resistant cultivar in China, was derived from a simple cross of two moderately susceptible cultivars, Funo and Taiwanmia. Snijders (1990c) presented evidence for the transgressive segregation of resistance in an F_2 population. Also, Ning 7840 and several other resistant cutivars are derivatives of Sumai 3. Another indigenous cultivar from China, Wabshuibai which is more resistant than Suami 3, was also used as a resistant parent but no resistant cultivars were selected from its progenies because of low combining ability for agronomic traits (Bai et al., 1989). Because highly resistant cultivars usually have undesirable agronomic traits that cannot be entirely eliminate, therefore, more success can be achieved by selecting transgressive segregants from crosses of moderately resistant cultivars with better agronomic characters (Liu and Wang, 1991). Bai et al. (2000) stated that since only a few cultivars have a high degree of resistance and these materials have many other undesired traits, the use of resistance genes from moderately resistant or moderately susceptible in a breeding program may permit combining different resistance genes in a genetic background that results in desired agronomic traits. Therefore, it should be possible to combine resistance genes from different sources into a single cultivar by selecting from transgressive segregants.

To select for quantitative resistance for FHB, we have to distinguish between genetic and environmental variance. The presence of heavy disease pressure is an essential to evaluate the level of resistance, therefore, such an optimal condition is difficult to achieve through artificial inoculation. Wu (1986) proposed an in vitro inoculation technique in which detached wheat spikes were inoculated by placing spores in a central spikelet of the spike and were then cultured in containers with sterile water in a growth chamber with controlled temperature and moisture. With this technique FHB symptoms may be confounded by discoloration due to senescence of the detached spike. In China, some successful breeding programs are equipped to provide light overhead irrigation in breeding nurseries to simulate rainfall after anthesis. To select for resistance in early segregation generation, large test populations are inoculated by scattering FHB wheat kernels in the field before plants reach the booting stage, then spraying plants with water during and after anthesis. To screen more advanced generations of near-

homozygous progenies, spores are injected into a central spikelet under more controlled conditions. Lines that pass this selection test are sent to different FHB epidemic areas for evaluation of resistance and yield potential (Bai and Shaner, 1994).

1.1.9 Marker-assisted selection

Generally, quantitative resistance depends strongly on the environmental effects, which makes the assessment of the disease for both inheritance studies and resistance breeding very difficult. At the same time, FHB is a head disease and direct phenotypic assessment of resistance must be delayed until the main culm of plant reaches the anthesis stage. This precludes use of the tested spike as a parent in the same generation in which the resistance phenotype is assessed. If the tested plant produces tillers, these may be used for making crosses, but in the greenhouse fertile tillers may not always form. Moreover, even in cases in which FHB resistance appears to be controlled by major genes, there is no a nongenetic component of phenotype that can make classification of individual plants in segregating generations uncertain. For these reasons, FHB resistance breeding would be greatly facilitated by marker-select selection.

The use of polymorphic single genes to facilitate the process of plant breeding was proposed early in this century (Sax, 1923). The basic principle is that selection for characters with easily detectable phenotypes can simplify the recovery of genes of interests linked to them and more difficult to score. The first marker loci available were that have an obvious impact on the morphology of the plant genes that affect form, coloration or male sterility among other have been genetically analyzed in many plant species. In some well characterized crops like maize, tomato, pea, barley or wheat, tens or even hundred of such genes have been assigned to different chromosomes (O'Brien, 1993). Major genes responsible for economically important characters are frequent in the plant kingdom (Gottlieb, 1986). Characters like male sterility, self incompatibility and others related to the shape, color and architecture of the whole plant, fruits, flowers or leaves are often of mono or oligogenic nature (Arus and Moreno-Gonzalez, 1993). Marker loci tightly linked to major genes can be used for selection, sometimes more efficiently than direct selection for the target gene. There are three situations in which marker-assist selection will clearly be more favorable: (1) when the selected char-

acter is expressed late in plant development, like fruit and flower features or adult characters in species with a juvenile period. (2) when the expression of the target gene is recessive; (3) when there is a requirement for special operation in order for the gene to be expressed, as in the case of breeding for disease (or) pest resistance (Arus and Mores-Gonozalez, 1993). There are several additional advantages for the use of markers in breeding for disease resistance (Koebner and Martin, 1990), like; (1) selection can be performed without inoculation (errors due to unavailable inoculation, methods are avoided); (2) Breeding for resistance can be done in areas where field inoculation with the pathogen of interest is not allowed for safety reason (3) problems in the recognition of the effects of environmentally unstable resistance genes can be eluded. If one marker is to be used for gene tagging, it is necessary that linkage with target gene be tight (5 recombination units or CM) in order to insure that only a minor fraction of the selected individuals will be recombinants. Alternatively when two flanking markers can be used, it is only required that the interval between them is approximately of 20 since selection for both markers at the same time results n the recovery of the target gene with a probability of at least 99 % (Tanksley, 1983). Selection with markers depends heavily on the quality of the polymorphism used. The most important properties for good quality markers are: (1) easy recognition of all possible phenotypes (Homo - and heterozygotes) from all different alleles; (2) early expression in the development of the plant; (3) no effects on the plant morphology of alternate alleles at the marker loci; (4) Low or null interaction among markers allowing the use of many at the same time in a segregation population (Arus and Moreno-Gonzalez, 1993).

Morphological characteristics such as plant height, spikelet density, spike morphology and awnedness have received attention as possible markers for breeding for FHB resistance (Chen, 1983; Liao and Yu, 1985; Sheng and He, 1989; Snijders, 1990a). Unfortunately, the general properties of morphological markers are not ideal: dominance and late expression, deleterious effects, pleiotropy, epstasis and rare polymorphism are the rule. As a consequence, their use in breeding for FHB resistance has been very limited (Arus and Moreno- Gonzalez, 1993). Also, the association between the trait and FHB resistance is not consistent (Lu et al., 1990; Yu, 1990).

New sources of high quality genetic markers based on the identification of polymorphism in proteins and DNA have been developed during the last three decades. They have been termed molecular markers (Tanksley, 1983) and include isozymes, RFLP, RAPD, AFLP and others. These markers have most or all the requisite properties mentioned above and, for this reason; their potential as tools for plant breeding is much greater than of the morphological genes.

1.2 Molecular-markers

Tanksley (1983) has described five inherit properties of molecular markers that distinguish them from morphological markers. These properties are: (1) genotypes can be determined at the whole plant tissue, cellular levels, (2) a relatively large number of naturally occurring alleles exist at many loci, (3) deleterious effects are not usually associated with different alleles, (4) alleles at most loci are codominant, thus all possible genotypes can be distinguished and (5) few epistatic or pleiotropic effects are produced, thus a very large number of segregation markers can be monitored in a single population.

There are two main types of molecular markers; isozyme markers and DNA markers. Markert and Moller (1959) were first to describe the differing forms of bands that they were able to visualize with specific enzyme stains and they were the first to introduce the term isozyme. Following this discovery further investigations showed that many of these enzymes were developmentally start being tissue specific and generally unaffected by environmental or other factors. Lately, it was found that this type of markers have many limitations and disadvantages (Tanksley, 1983)

The other type is DNA based markers; recombinant DNA technology has provided new more powerful tools for studying genetic variation with a greater resolution than all previous experimental methods, including protein electrophoresis Recombinant DNA technology can be applied to a variety of in vitro techniques which include DNA isolation and production of new combination of heritable material by the splicing of the nucleic acids in vitro (Old and Primrose, 1989). The main advantage of DNA based markers is that they give information of about any kind of sequence in the genome, not only of isozymes or highly expressed non-isozymatic proteins such as storage proteins but also unexpressed sequences.

1.2.1 DNA based markers

Now it is possible to gain information about the whole genome and any of its components, surpassing the limitations of protein electrophoresis, which only gives information on translated sequences. DNA based markers have overcome main limitations of protein electrophoresis since the detection of variation is not limited to coding regions and all categories of mutational events can, in principle be detected.

1.2.1.1 Restriction fragment length polymorphisms RFLP

Originally, there was only one major type of molecular marker, termed Restriction Fragment Length Polymorphism or RFLP; however recently many types have been proposed such as Randomly Amplified Polymorphic DNA or RAPD and Amplified Fragment Length Polymorphism or AFLP. Also. RFLP was the first such DNA markers to be utilized. They produced by restriction enzyme digestion. Differences in the length of a restriction fragments due to insertion or deletion and changes in the number of fragments together with the appearance of new ones indicate the loss or grain of restriction sites for such an enzyme, caused by base substitution or by insertion or deletion whose and points fall within the site (P$\bar{e}$rez de la Vega, 1993).

In its original form, RFLP analysis consisted of DNA isolation from a suitable set of plants digestion of the DNA with a restriction enzyme, separation of the restriction fragments by agarose gel electrophoresis, transfer of the separated restriction fragments to a filter by Southern blotting, detection of individual restriction fragments by nucleic acid hybridization with a radioactively labeled cloned probe, and scoring of RFLPS by direct observation of autoradiograms (Helentjaris et al., 1985; Landry and Michelmore, 1987; Tanksley et al., 1988).

In spite of the short period during which RFLP analysis has been used, its importance in the assessment of plant diversity, population characterization and plant breeding has already been stressed (Helentjaris et al., 1985; Beckmann and Soller, 1986). While the limited sample of individuals assayed in DNA analysis is the advantage is that the number of loci that can be detected is many orders of magnitude greater than for isozymes. More than 1000 RFLP markers have been mapped on the tomato genome (Tanksley, 1993). Another advantage is that the

variation in DNA sequences is several times higher than protein sequences. An additional advantage of eukaryotic organisms is that not only the nuclear genome but also organelle genomes can be analyzed by RFLP (Palmer, 1987). But analysis is limited by the relatively large amount of DNA required for restriction digestion, southern blotting and hybridization plus the requirement for radioactive and autoradiography. These factors make conventional RFLP analysis relatively slow and expensive. Further, due to low frequency of RFLP in wheat, this approach has been relatively less useful in this crop. This is sometimes attributed to polyploid nature, high proportion of repetitive DNA and large genome size in wheat. Despite these difficulties, sufficient applications of RFLP were practiced in wheat. These purposes included, genome mapping (Devos and Gale, 1993), variety identification (Gupta et al., 1998) and marker aided selection (Gale et al., 1995).

1.2.2 PCR-based markers

The development of new methods to perform analysis with molecular markers has been the focus of many recent studies, and most of these are based on PCR amplification of genomic DNA (Kochert, 1994).

Polymerase chain reactions (PCR has been considered the most revolutionary modern techniques of molecular biology in 1980 s. PCR is a powerful extremely sensitive technique with applications in many fields such as molecular biology diagnostics, population genetics and forensic analysis. Recombinant DNA techniques have revolutionized genetics by permitting the isolation and characterization of genes, allowing the detailed study of their function and expression during development processes, or as a response to environmental factors. More of the cloning methods involved can be accelerated and sometimes even circumvented by using PCR, and novel applications of the technique now permit studies that were not possible before. The idea of PCR is a simple process in which a specific segment of DNA is synthesized repeatedly, resulting in the production of large amounts of a single DNA sequence starting from a minute quantity of template. (Saiki et al.,1985). The process depends on primer sequences of DNA which match flanking sequences at both ends of targeted sequence. Through repeated denaturing, annealing and synthesized steps, the intervening sequence is synthesized in a 2^n amplification.

1.2.2.1 Random amplified polymorphic DNA (RAPD) marker

The RAPD technique is a variation of the polymerase chain reaction (PCR) that has been widely used as a molecular marker since 1990. Two groups developed the RAPD assay. A group at Dupont co. (Wilmington. U) called the new method RAPD (random amplification polymorphic DNA) (Williams et al.,1990) and described its genetic mapping applications. Another group at the California Institute of Biological Research USA (Welsh and McCelland, 1990) focused on genome fingerprinting and collection their assay arbitrary primed polymerase chain reaction (AP PCR). Both these assays are based on the observation that a single short oligo deoxynucleotide of a randomly chosen sequence when mixed with genomic DNA, dNTPs, buffer and thermostable DNA polymerase and subjected to temperature cycling, amplified several DNA fragments (Innis et al., 1990). RAPD assay is a modification of the basic polymerase chain reaction (PCR) technique (Mullis et al., 1987). This assay, unlike the PCR, does not require knowledge of the target DNA sequence, and a single arbitrary primer will support DNA amplification from a genomic template if binding sites on opposite strands of the template exist within a distance that can be traversed by the thermostable equation usually random oligonucleotides (or 10 bases) used as primer to amplify discrete fragments of genomic DNA. The primers are generally of random sequence, contain at least 50% G and C and without internal inverted repeats. The products are easily separated by standard electrophoretic technique and visualized under ultraviolet (UV) illumination of ethidium bromide stained agarose gels. Polymorphism results from changes in either the sequence of the primer binding site (e.g., point mutation) which prevent stable association with the primer or from changes which alter the size or prevent amplification of target DNA (e.g., insertions, deletions inversions. However the polymorphisms between individuals result from sequence differences in one or both of the primer binding sites, and are visibility as presence or absence of a particular RAPD band such polymorphisms in general behave as dominant genetic markers.

RAPDs offers many advantages; (1) non- radioactive detection (2) no prior DNA sequence information for a genome is required (3) universal primers work in any genome (4) very small amounts of genomic DNA are sufficient (5.25 ng) (5) multi-

plex detection of polymorphism (6) experimental simplicity (7) no need for expensive equipment beyond a thermocycler and transilluminator.

Given all of the advantages, one might think that RAPDs are the perfect marker system unfortunately, there are a few disadvantages which limit the utility of RAPDs as genetic markers For instance, because the typical polymorphism observed is a presence or absence of a band, RAPDs detect dominant loci (not - co dominant). There fore it is difficult or impossible to determine the heterozygous condition in an individual. This severely limits the amount of genetic information derived from each individual in a selfed population although they are useful in backcross and recombining inbred populations. RAPD can only efficiently amplify within a certain size range of DNA, and Taq DNA-polymerase will introduce errors. The accumulated mutation are after 20-30 cycles was reported to be as high as 0.3 – 0.8 % (Keohavong and Thilly, 1989; Belyavsky, 1989). Also, some of the minor fragments are unstable which have been suggested to result from non-specific amplification when template/primer homology is not perfect (He et al., 1992). Therefore, some modifications have been introduced to improve the RAPD technique and to overcome many of its limitations.

1.2.2.2 DNA amplification fingerprinting (DAF)

A modification of the RADP assay, named DNA amplification fingerprinting (DAF), has been describe by Caetano-Anoles et al. (1991). The difference from the other procedures is that the PCR products are separated on polyacrylamide urea gels and visualized by silver stain. In the DAF procedure primers as short as five nucleotides, produce complex band patterns ideally suited for genome fingerprinting applications.

1.2.2.2.1 Denaturing gradient gel - electrophoresis (DGGE)

Denaturing polyacrylamide gradient gel - electrophoresis (DGGE) has been used to resolve DNA sequence differences among fragments of similar or identical size (Fisher and Lerman, 1983; Myers et al.,1987). Using the DGGE procedure, single base differences result in altered migration of DNA fragments and thus produce polymorphic DNA fragments. Because of this the DGGE procedure is considered to be highly suitable for self pollinating species.

1.2.2.3 Directed search (amplification of low copy DNA)

In general. Cereals have a high level of repetitive DNA sequences (e.g., about 70% of the DNA sequence in wheat are repetitive). Removal of repetitive DNA sequences before PCR has been reported to produce polymorphic and reproducible DNA fragments (Eastwood et al., 1994). Hydroxylapatite column chromatography is used to enrich low copy DNA sequences (Clark et al., 1992). Once the procedure is standardized, it could be useful to screen marker linked to disease resistance and other traits.

1.2.2.4 Sequence characterized amplified regions (SCARs)

Paran and Michelmore (1993), developed a dependable PCR - based technique called sequence characterized amplified regions (SCARs). In this procedure, the polymorphic DNA fragment is cloned and sequenced.

1.2.2.5 Sequence-tagged site (STSs)

STSs is a short, unique sequence that identified a specific locus and can be amplified by PCR. Each STS is characterized by a pair of PCR primers, that are designed by sequencing an RFLP probe representing a mapped low-copy number sequence. Talbert et al. (1994) showed that PCR can be used to detect polymorphism in wheat with primer sequences derived from the alpha-amylase and gamagladine genes. In another study in wheat, RFLP probe Xbcd1231, linked with Pm4a locus was converted into an STS marker (Liu et al., 1998). Also, Roy et al. (1999) found an STS marker which showed a strong association with preharvest sprouting tolerance in wheat.

1.2.2.6 Microsatellites or Simple Sequence Repeats (SSRs)

Microsatellites, also called simple sequence repeats (SSRs), are tandem repeated arrays of short core sequence. They are present in the vast majority of eukaryotic genomes. The total number of different dinucleotide blocks has been estimated for several species (Ma et al., 1996; Wu and Tanksley, 1993; Morgante and Olivieri, 1993). The number of sites ranged from 10^3 to 10^5 depending on the species and repeat motif. Polymorphism produced by a variable number of tandem repeats has been demonstrated in a large number species. This feature has made microsattelites a very attractive molecular marker for species with a narrow

genetic base such as wheat and barley. This methodology is based on the use of primers complementary to SSRs. Multilocus profiles have been generated using different kinds of oligonucleotide containing simple sequence repeats as single primer (Gupta et al., 1994; Nagoka and Ogihara, 1997) or in combination with arbitrary sequence oligonucleotides (Wu et al., 1994). These studies have shown the reproducibility of the patterns generated the Mendelian inheritance of the polymorphic amplified bands and their usefulness in the investigation of the genetic relationships. Mapping of this kind of marker in maize (Gupta et al., 1994) Arabidobsis (Wu et al., 1995) and barley (Becker and Heun, 1995) has been conducted.

In wheat, Devos et al. (1995) searched sequence database and converted two microsatellite sequences into PCR based markers. Roeder et al. (1995), Ma et al. (1996), and Plaschke et al. (1995) investigated the potential of microsatellite sequences as genetic markers in hexaploid wheat. These markers were genome specific and displayed high levels of variation. More recently, a detailed genetic map of 279 microsatellite loci (Roeder et al., 1998) and another map of 53 loci (Stephenson et al., 1998) have been identified in bread wheat. Therefore, the availability of extensive molecular maps of wheat microsatellites will help in tagging genes of economic importance for marker assisted selection. In wheat, microsatellite markers have been used to tag several genes or QTLs such as; Rht8 (Korzun et al., 1998; Worland et al., 1998), *Rht12*, *Vrn1* (Korzun et al., 1997b), *Pm24* (Huang et al., 2000) a QTL for protein content (Prasad et al., 1999) and a QTL for pre-harvest sprouting tolerance (Roy et al., 1999).

Little is known about the molecular nature of the polymorphism associated with these markers. The primer design allowed the evaluation of two alternatives. Firstly, when 5'-anchored oligonucleotides are used as primers, polymorphism will be produced in the variation of the number of repeats of the core sequence at each locus. Secondly, when 3'-anchored oligonucleotides are used, polymorphism was attributed not to variation at the priming site but to the variation of the inter-repeat sequence. The polymorphism generated in both cases termed random amplified microsatellite polymorphism (RAMP) (Wu et al., 1994). Unanchored, oligonucleotides will produce one or the other kind of product depending upon where they annealed with the SSR sequence. The polymorphism generated termed mi-

crosatellite primed-PCR (MP-PCR) (Weising et al., 1995). This MP-PCR relies on the presence of two microsattelites having the same repeat unit in inverse orientation, separated by an amplifiable distance within the genome, so that the interpret sequences are amplified.

1.2.2.7 Amplification fragment length polymorphism (AFLP)

AFLP is a new powerful DNA marker based on the detection of DNA restriction fragments by PCR amplification (Zabeau and Vos, 1993; Vos et al., 1995). In this technique amplification of restriction fragments is accomplished by the ligation of double-stranded (ds) adapter sequences to the ends of the restriction sites which subsequently serve as binding sites for primer annealing in PCR. In this way, restriction fragments of a particular DNA can be amplified with universal AFLP primers corresponding to the restriction site and a adapter sequence. Adding 1-3 selective bases to these oligonucleotide adapters used as primers can restrict the number of DNA fragments, which are amplified. This marker was originally conceived to allow the construction of very high density DNA marker maps for application in genome research and positional cloning of genes. It is equally suitable for application in genetic analysis, which require more modest DNA marker densities.

The AFLP analysis has been conducted in bread wheat (Ma and Lapitan 1998; Goodwin et al., 1998; Barrett and Kidwell, 1998; Barrett et al., 1998; Koebner et al., 1998). Also, many diagnostic molecular markers for different traits have been identified by AFLPs in bread wheat (Goodwin et al., 1998; Hartl et al., 1998). It is apparent that the AFLP approach is now widely used for developing polymorphic markers. High frequency of identifiable AFLP coupled with high reproducibility makes this technology an attractive tool for identifying polymorphism and for determining linkages by analyzing individuals from a segregating population.

1.3 Marker-assisted breeding for FHB resistance

The use of resistant cultivars is an effective way to control the disease FHB in wheat. Breeding for wheat FHB resistance with traditional methods requires substantial time and effort because resistance is quantitatively controlled and because evaluation of resistance requires a misting system and laborious inoculation and evaluation procedure. Molecular markers are powerful tools that can be used for marker-assisted selection and as landmarks for map-based cloning of resistance

genes. Molecular markers are especially advantageous for FHB resistance trait, which is difficult to score. After a linkage between a QTL and molecular markers has been determined, the QTL can be transferred into different genetic backgrounds by marker-assisted selection (MAS).

The essential requirements for MAS in a plant breeding program are: (a) markers should co-segregate or be closely linked (1cM or less) with the desired trait; (b) an efficient means of screening large populations for the molecular markers should be available; and (c) the screening technique should have high reproducibility across laboratories, be economical to use and should be used friendly. The choice of marker system to be used for detection of DNA polymorphism, depends on the objective of the study, on the needs dictated by the specific application and on the facilities and skills available. So, a comparative account of different technologies may be helpful to choose suitable marker before carrying out the study particularly for wheat breeding. Microsatellite markers are co-dominant and have the high information content of all marker types. However, the very high cost to develop them, restrict their uses in many laboratories. Now, the availability of large number of microsatellite primer pairs (around 1000) will accelerate their uses. Also, locus specificity and high-level of polymorphism make them the markers of choice for practical breeding. For RAPDs, their lack of reproducibility and dominant nature limits their utility in breeding programs. The RFLP technology offers co-dominant markers and is easy and convenient, although, the use of radioactivity is a big limitation. A low level of polymorphism of RAPDs and RFLPs within wheat may be a barrier to the identification of markers that are closely linked to FHB resistance genes. AFLPs provide powerful tools for detection of large number of DNA polymorphism in wheat because they have the potential to generate a large number of polymorphic loci. AFLPs combine the merits of both RFLP and PCR-based multilocus markers. Detection of a virtually unlimited number of restriction fragments in complex genomes is especially useful for plant species with low polymorphism like wheat. Therefore, before using the molecular markers in actual breeding, it is necessary to undertake studies on the marker validation and examining the behavior of markers and the associated polymorphism in different genetic background.

Few results have been published on molecular markers for FHB resistance in wheat. Some of these studies used the RAPD marker method. Bai (1995) and Bai et al. (1995) used bulked segregant analysis with random amplified polymorphic DNA (RAPD) markers in an F_6 recombinant inbred population of Clark (susceptible) / Ning 7840 (resistant). Of 1120 primers, five markers were associated with resistance. These RAPD markers were placed on two linkage groups. Two loci for FHB resistance associated with these RAPD markers were identified using interval mapping. Gilbert et al. (1995) screened 600 RAPDs and found one RAPD band possibly associated with resistance in Chinese cultivars including Sumai 3. Ban (1996) evaluated 501 oligonucleotide primers, 50 displayed polymorphisms between Fukuho-komigo (moderately susceptible) and Oligo Culm highly (highly susceptible), with a total of 65 polymorphic bands. These were used to construct a linkage map containing 17 apparent linkage groups. Subsequently, 110 double haploid lines derived from an F_1 cross (Fukuho-komigo / Oligo Culm) were classified into two groups based on responses to four RAPD markers (OPZ-06 345, OPAG-18 340, OPAF-06 345 and OPW-13 435). Also, he showed that, classifications by the four RAPD markers indicated significant differences in the average FHB severity of the two the groups at the 5% significance levels. Three of these RAPD markers were linked to each other with recombination values of 6.4% and 11.8%. Other researchers (Waldron et al., 1999) used RFLP system to map FHB genes for resistance. They evaluated a population of 112 F_5-derived recombinant lines (RI) wheat lines from the cross Sumai 3 (resistant) and Stoa (moderately susceptible) in two greenhouses experiments. On the basis of RFLP marker analyses, they found that five genomic regions were significantly associated with FHB resistance, three derived from Sumai 3 and two from Stao. They identified regions on chromosomes 3BS from Sumai3 and 2AL from Stao by using interval analysis. The RFLP marker in the 3BS region explained 15.4% of the variation and a multiple regression model consisting of three QTL explained 29.5% of the variation. However, very few studies used the microsatellite system to analyze the resistance to FHB. Procunier et al. (1998) used microsatellite system for identification of FHB resistance genes. They developed pentaploids and D chromosome addition lines in tetraploid AABB genetic background from hexaploid Sumai 3 (FHB resistant) and tetraploid DT486 (FHB susceptible) parents. The selfed F_1

progeny were screened for FHB reaction and for presence of D chromosomes using specific wheat microsatellite primers. They suggested that the Sumai 3 D genome lack the major FHB resistance genes. Their results will accelerate the screening of microsattelite markers linked to FHB resistance genes.

Another study used AFLP marker system to map the QTLs for FHB resistance. AFLPs are efficient for high resolution and chromosome landing. Bai et al. (1999) studied recombinant inbred lines (RILs) derived by single-seed descent from a cross between the resistance wheat cultivar Ning 7840 and the susceptible cultivar Clark. In the greenhouse, F_5, F_6, F_7 and F_{10} families were evaluated for to spread of FHB within a spike. DNA was isolated from both parents and F_9 plants of 133 RILs. They screened a total of 300 combinations of amplified AFLP primers for polymorphism using bulked segregant analysis. 20 pairs of primers revealed at least one polymorphic band between the two contrasting bulks. 11 AFLP markers showed significant association with FHB resistance and an individual marker explained up to 53% of the total variation (R^2). The markers with high R^2 values mapped to a single linkage group. By interval analysis, they identified one major quantitative trait locus of FHB resistance that explaining up to 60% of the genetic variation for FHB resistance. They concluded that, some of these AFLP markers might be useful in marker-assisted breeding to improve resistance to FHB in wheat.

Other studies used more than one marker system to analyze the FHB resistance. Anderson et al. (1998) screened a population of 112 F_5-derived recombinant lines from the cross Sumai 3 (resistant) / Stao (moderately susceptible) for resistance after inoculation with *F. graminearum* conidia. They performed RFLP analysis. Also, because of the difficulties in obtaining linked markers for genomic regions significantly associated with FHB resistance, they performed the AFLP analysis. They found five QTLs were significantly associated with resistance. One QTL on chromosome 3BS which was linked to an AFLP marker explained more than 16% of the variation for FHB resistance in this population. Also, Buerstmayr et al. (1998) started a mapping program in 1997. They produced F_1-derived double haploids from two crosses between two resistant cultivars Frotana and CM82036 and one susceptible cultivar Remus. 250 double haploids population achieved from the cross Remus/CM82036 and 180 population of the cross Re-

mus/Frontana. They tested different types of DNA markers for polymorphism in these populations. They found that 143 RFLP probes detected polymorphism between Remus and CM82036 and 129 between Remus and Frontana. Also, they indicated that 60% of the 41-microsattelite markers, which have been tested, were polymorphic. With AFLP they found 5-15 polymorphic bands per reaction between the parents.

Finally, for a quantitative trait like FHB resistance where there is major genotype X environment interaction, the effect of a QTL in a specific environment is difficult to predict. The different loci may interact differently with different environment. The difficulty may be overcome by identification of more and more QTL linked with independent molecular markers followed by mapping these identified QTLs and subjecting them to interval mapping. Ittu et al. (2000) studied the genetic control of resistance to *Fusarium spp.* using recombinant inbred lines derived from the cross between a susceptible winter wheat, F1054W, with moderately resistant parent, Sincron. They suggested presence of a FHB resistance QTL on chromosome T1BL.1RS and another QTL on chromosome 1D. The effects of these QTLs were cumulative.

Molecular markers are powerful tools that have been used for marker-assisted selection and as landmarks for map-based cloning of genes. Molecular markers associated with QTLs have been reported for many important traits. After a linkage between a QTL and molecular marker has been determined, the QTL can be transferred into genetic background by marker assisted selection. Therefore, the objectives of this study were:

1. Evaluating the inheritance of FHB resistance under different environmental conditions.
2. Determination of the number, chromosome position, and effects of the QTLs conditioning FHB resistance.
3. Identification of RFLP, SSR and AFLP markers associated with the QTLs for FHB resistance.
4. Exploring the potential of marker-assisted selection in improving wheat resistance FHB.

2 Materials and methods

2.1 Plant materials

The FHB mapping population consisted of 180 F_3 families developed from a cross between the variety "Apollo" and the breeding line "Sagvari.Nobeoka Bozu x Mini Mano.Sumai3". These two genotypes were chosen according to their resistance to the disease FHB caused by *Fusarium culmorum*. The parcentage and degrees of resistance of these two genotypes are listed in (Table 1). "Apollo" is a FHB susceptible German winter wheat variety which originally came from a cross between "Maris Beacon x Clement" and "Kronjuwel". On the other hand, the line "Sagvari.Nobeoka Bozu x Mini Mann.Sumai3" was derived from four FHB resistant Asian parents namely; "Sagvari" (Sgv), "Nobeoka Bozu" (NB), "Mini Mann" (MM) and "Sumai3" (Sum3).

2.2 Enzymes and solutions

2.2.1 Enzymes

- Restriction endonucleases

Name	Restriction site	Supplier
EcoRI	5`-G↓AATTC-3`	Pharmacia
BamHI	5`-G↓GATCC-3`	Biolabs
HindIII	5`-A↓AGCTT-3`	Pharmacia
XbaI	5`-T↓CTAGA-3`	Pharmacia
EcoRV	5`-GAT ↓ACT-3`	Biolabs

Remaining enzymes	Supplier
T4-DNA Ligase	Biolabs
Taq-DNA-Polymerase	Qiagen

2.2.2 Basic solutions

- Ampicillin (40mg/ml) in H_2O
- Ammoniumpersulfate (APS)/10%

- Loading buffer for agarose gel electrophoresis
  0.2 M EDTA
  40% (v/v) glycerine
  0.03% (w/v) bromophenolblue

- Loading buffer for polyacrylamidgel electrophoresis
  Formamide Amresco
  Dextran blue Fluka
- Chloroform/Isoamyl alcohol (25/1)
- 0.5 M EDTA (pH 8.0)
- Ethidiumbromide (10 mg/ml)
- Sodium acetate (NaAc) 2M
- NaCl 5 M
- Ammonium acetate (NH_4Ac) 5M

- RNase A (10 mg/ml)

DNase-free RNase A was dissolved in sterile solution of 10 mM Tris-CL (pH 7.5) and 15 mM NaCl for 15 min on 100 °C. After cooling RNase A was tested and stored at –20 °C gelagert.

- 10% SDS
- 20 X SSC

For 1000 ml:	Concentration
175.3 g NaCl	3.0 M NaCl
88.2 g sodium cetrate	0.3 M sodium cetrate

- 1 M Tris (pH7.5)
  50 X TAE buffer

For 1000 ml:		Concentration
242 g Tris	2 M	Tris
57.1 ml cold hydroxy acetate	1 M	HAc
18.6 g EDTA	50 mM	EDTA

- 10X TBE buffer

For 1000 ml:	Concentration
108.0 g Trisbase	0.89 M Trisbase
55.0 g boric acid	0.89 M boric acid
8.3 g EDTA	20 mM EDTA

- TE buffer

For 1000 ml:	Concentration
10 ml 1 M Tris	10 mM Tris-Cl
2 ml 0.5 M EDTA (pH 8.0)	1 mM EDTA

2.2.3 Solutions for DNA isolation

- 1.5 X CTAB

For 500 ml:	Concentration
7.5 g CTAB	1.5% (w/v) CTAB
75 ml 1 M Tris	150 mM Tris-CL
15 ml 0.5 M EDTA (pH 7.5)	1.5 mM EDTA
105 ml 5 M NaCl	105 mM NaCl

- 75% EtOH

2.2.4 Buffer for electrophoresis and DNA blotting

- 1 X TAE
- Transfer buffer

For 5 L		Concentration
175.2 g	NaCl	0.6 M NaCl
80.0 g	NaOH	0.4 M NaOH

2.2.5 Materials for probe labelling and hybridization

- Maleic acid buffer

For 100 ml:	Concentration
10 ml 1 M maleic acid	100 mM malic acid
3 ml 5 M NaCl	50 mM NaCl

- Blocking reagent

10% (w/v) blocking reagent (Boehringer) was dissolved in maleic acid buffer pH 7.5. The solution was autoclaved and stored at -20 °C.

- Prehybridizatin / Hybridization buffer

For 100 ml:	Concentration:
1 ml 10% (w/v) NLS	0.1% (w/v) NLS
10 ml 10% (w/v) blocking reagents	1% (w/v) Blocker
25 ml 20 X SSC	5 X SSC
0.2 ml 10% SDS	0.02% (w/v) SDS

- Sephadex solution

  4 g Sephadex G-50 dissolved in 100 ml TE buffer and autoclaved.

- Sephadex column

A small piece of glass wool was inserted in the bottom of 1000 µl Eppendorf tip and autoclaved. The tip was filled with sephadex solution and was left until it became dry.

- Washing solution I

For 1000 ml:	Concentration:
25 ml 20X SSC	0.5 X SSC
10 ml 10% (w/v) SDS	0.1% (w/v) SDS

- Washing solution II

For 1000 ml:	Concentration:
10 ml 20X SSC	0.2 X SSC
10 ml 10% (w/v)SDS	0.1% (w/v) SDS

- Stripping solution

For 1000 ml:	Concentration:
20 ml 10 M NaOH	0.2 M NaOH
10 ml 10% (w/v) SDS	0.1% (w/v) SDS

2.2.6 Solution for AFLP analysis

- 1 X $TE_{0.1}$ buffer

For 500 ml:	Concentration:
10 ml 1M Tris	20 mM Tris

100 µl 0.5 M EDTA (pH 8.0)	0.1 mM EDTA

2.3 Field experiments

Crosses were made for F_1s and F_2s. In 1998 season, 180 F_3 families were grown in Gruenbach, each family was cultivated in a separate plot, which consisted of three rows (120 kernel per 1.6 m^2). In 1999 season, F_3 families and their parents were grown in five locations namely; Bergen, Obertraubling, Herzogenaurach, Hadmersleben and Gruenbach. Two replications were planted in each location where each family was sown in 6-row plot (250 kernel per 2.6 m^2). Cultivation processes were carried out according to the regular cultivation of each location.

2.3.1 FHB inoculation

F_3 families and their parents of each field trial in each location were artificially inoculated with FHB spores in 1998 and 1999 seasons. The inoculum of *Fusarium clumorum* was a mixture of isolates that originated from Bavaria Inoculation was made within spikes by Conidiospores which produced in mung bean liquid medium as described by Bai and Shaner (1996) with a concentration of 2 X 10^6 spore per ml. Spikes were inoculated when spikes just beginning to flower. Thus, plants were inoculated on two different dates (02/04.06 and 09.06) every year.

2.3.2 FHB scoring

Visual symptoms ranged from dark brown, water-soaked spots on the glumes to bleached spikelets. All of these symptoms were recorded as scabbed spikelets. Scabbed spikelets were counted 20, 30 and 36 days after the second inoculation. Disease severity values were calculated as the percentage of scabbed plants per plot.

2.3.3 Plant height and heading date

Plant height (HT) of each of F_3 family (180 families) was measured. The height of 10 plants per family were measured at Gruenbach in 1998 and 1999 seasons as the distance from the ground to the top of the spike in centimeter. Also, heading date (HD) at Gruenbach in 1998 and 1999 seasons for each F_3 family was

quantified as the number of days from planting to flowering of 50% of plants of each family. Finally, the mean of each trait for each family was calculated.

2.4 DNA isolation

Total genomic DNA was isolated according to the protocol of Saghai-Maroof et al. (1984) with minor modifications. Briefly, 3 - 5 g of leaf tissue per sample (each sample was collected from leaves of ten plants from each F_3 family, 180 family in total) were ground in liquid nitrogen and incubated at 60°C for 30 min with 15 mL of 1.5 x CTAB (cetyltrimethlyammonium bromide) extraction buffer (1.5% (w/v) CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.05 M NaCl and 1.5%-mercaptoethanol) in 50-mL polypropylene tubes. Samples were left to cool down on ice for 5 min and then 15 mL 24:1 chloroform : isoamyl alcohol were added to each sample. Samples were incubated for 30 min by shaking and then centrifuged at 2100 x g for 30 min. The aqueous layer was transferred to a new tube and 20 µl RNase A (10 mg/ml) were added. Samples were incubated for 30 min at room temperature. One volume of cold isopropanol was added to precipitate DNA. After 30 min incubation at 4°C, precipitated DNA was hooked out and placed in a 2-mL reaction tube containing 1 mL of 75% ethanol. After washing twice with 75% ethanol, the DNA pellet was dried thoroughly and dissolved in TE buffer. The DNA samples were diluted and stored at -20°C.

2.5 RFLP analysis

2.5.1 Southern blotting

For RFLP analysis, DNA isolated from the 180 samples was digested with five restriction enzymes; *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI and *Xba*I as described by the manufacturer,s recommendations (Pharmacia Uppsala). For each sample, 10 µg of DNA were digested with 3 units of enzyme for each microgram of DNA at 37 °C for a minimum of 4 h in the buffer provided by the manufacturer. Loading buffer (0.2 M EDTA, 40% v/v glycerine and 0.03% w/v bromophenol blue) was added onto each sample and they were loaded onto 0.8 % agarose gel. Each gel comb contained 22 lanes, therefore, 21 samples were loaded onto each gel with one lane for *HindIII*-digested lambda DNA marker which was used to estimate the fragment sizes. Electrophoresis was carried out in 1 X TAE buffer at 5V/cm for

overnight. DNA was transferred to a biodyne B nylon membrane (Pall, Dreieich,Germany) using alkaline transfer buffer as described by Reed and Mann (1985) for overnight. Each membrane was washed in 2 X SSC buffer is composed of for 2-3 min and subsequently dried at 60 - 80°C for 2h.

2.5.2 Probe labeling

Probes were labeled with radioactive P^{32}-deoxycitidin triphosphate (P^{32}-dCTP) by the random hexamer method (Feinberg and Vogelstein, 1983). Briefly, 50 ng of probe DNA was denatured at 95 °C for 10 min and transferred to ice.

The reaction mixture was composed of :

DNA (50 ng)	5.0
Hexanuleotide primer solution	2.0
10 X Reaction buffer	2.0
Klenow enzyme (2U/µl)	1.0
Nucleotide mix	3.0
P^{32}-dCTP	2.0
H_2O	5.0
Total	20 µl

Each reaction mixture was incubated in an Eppendorf Thermomixer 5436 for 2 h at 37°C. Next, 30 µl of TE buffer was added and applied on a Sephadex-G50 column. Then, 400 µl of TE buffer was added to the column. Finally, another 400 µl of TE buffer was added to elute the labeled DNA probe. The cpm of each probe was measured and the probes which gave values less than 1 X 10^6 cpm were excluded.

2.5.3 Hybridization

Membranes were washed for 20 min at 60°C in washing solution I and rinsed with distilled water. Then, they were prehybridized at 65°C for 2-5 h in hybridization tubes. The labeled probe was denatured at 95°C for 10 min and added to new hybridization solution buffer along with labeled *Hin*dIII-digested lamda DNA. Hybridization was carried out overnight at 65°C in an hybridization oven (Bachofer). Membranes were washed in the washing solution I for 10 min at 50 - 60°C in a waterbath. Membranes which indicated signals more than 60 cpm were washed

twice. They were then blotted between two sheets of Whatmann paper, placed in acetate sheet protectors, loaded into film holder with Kodak XAR-5 photographic film and two intensifying screens and exposed for 5-12 d at -70°C. After exposure, membranes were stripped by immersion in stripe solution for 4-5 min at room temperature and neutralized by immersion in 30 mM Tris pH 7.5.

2.5.4 Clone libraries

Clone libraries used were: WG, wheat genomic libraries obtained from (M.E. Sorrells, Ithaca, USA); KSU, *T. tauschii* genomic clones from (B. S. Gill, Manhattan, Kansas, USA); PSR, wheat genomic clones from (M. D. Gale, Norwich, England); WHS, wheat genomics clones, (Weihenstephan, Germany); and MWG, barley genomic clones and cMWG, barley cDNAs, from (A. Graner, Gruenbach, Germany)

2.5.5 Clones mapping

60 clones were selected based on the RFLP maps of barley, wheat and *Triticum taushii* cited above. They were surveyed for polymorphism with parental DNA digested with each of the five restriction enzymes; *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI and *Xba*I. The clones showed best polymorphism were used in F_3 population analysis. Finally, a set of membranes bearing the 180 samples of the F_3 population that have been digested with the appropriate enzyme was hybridized with the clone insert as described.

2.6 AFLP analysis

The AFLP protocol was carried out as described by Vos et. al (1995) with minor modifications.

2.6.1 Ligation reaction

0.5 µg of genomic DNA was digested with one unit *Mse*I and five units *Eco*RI. 5 pmole *Eco*RI adaptor and 50 pmoles MseI adaptor were ligated with one unit T4 DNA ligase (all enzymes New England Biolabs, Beverly, Mass.) in a buffer containing 10 mM Tris-HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 1mM ATP and 50 ng/µl bovine serum albumine in a total volume of 11 µl for 3h at 37°C.

At the end of the ligation reaction, the DNA samples were diluted with $TE_{0.1}$ buffer to a final volume of 200 µl and stored at -20°C. The sequence of *Eco*RI adaptor was 5'-CTCGTAGACTGCGTACC-3', 3'-CTGACGCATGGTTAA-5' and the sequence of the *Mse*I adaptor was 5'-GACGATGAGTCCTGAG-3', 3'-TACTCAGGACTCAT-5'.

2.6.2 Preselective amplification reaction

Preselective amplification of target sequence was performed with *Eco*RI and *Mse*I adaptor- homologous primers. Each possessing one additional nucleotide at the 3' primer end. Polymerase chain reactions were set up with 4 µl diluted restriction-ligation DNA, 2.5 pmol *Eco*RI +A Primer, 2.5 pmol *Mse*I + C Primer, 0.4 U *Taq* DNA polymerase (Qiagen GmbH, Hilden, Germany), 0.2 mM of each dNTP (Amersham- Pharmacia Biotech, Uppsala, Sweden) and 1x Qiagen PCR buffer in a volume of 20 µl. The PCR reaction was performed in a PE 9600 thermal cycle programmed for 20 cycles of 94 ° C (1 s), 56 ° C (30 s), 72° C (2 min).

To verify successful amplification,10 µl of the PCR mixture was electrophoresed on a 1.5% agarose gel in 1x TAE buffer stained with 0.5 µg / mL ethidium bromide: a smear of amplified target fragments was visible in the range 100 - 1500 bp. The remaining 10 µl were diluted 20-fold by adding 190 µl $TE_{0.1}$ buffer, and stored at - 20 ° C.

2.6.3 Selective amplification

Selective amplification was achieved with *Eco*RI + ANN and *Mse*I + CNN primers. Only *Eco*RI primers were labeled using either 5-carboxy-fluorescein (5-FAM), or 2',7'-dimethoxy-4',5'-dichloro-6carboxy-fluorescein (JOE), or N,N,N',N'-tetramethyl-6-carboxyrhodamin (TAMRA). Polymerase chain reactions were carried out using 3 µl diluted pre-amplified DNA,1 pmol labeled *Eco*RI + ANN primer, 5 pmol unlabeled *Mse*I + CNN primer, 0.4 U *Taq* DNA polymerase, 0.2 mM each of dNTP and 1 x Qiagen PCR buffer in a total vollume of 20 µl.

For amplification, the following cycle profile was used: one cycle of 30 s at 94° C,30 s at 65° C, 2 min at 72° C, followed by 8 cycles in which annealing temperature was subsequently lowered 1° C per cycle, and finally 23 cycles of 1 s at 94° C, 30 s at 56° C, 2 min at 72° C. Then, 0.5 µl of 5-FAM- labeled PCR products, 0.6 µl

of JOE – labeled PCR products and 0.9 µl of TAMRA-labeled PCR products were pooled. The mixture was made up with 0.15 µl of 6-carboxy-X-rhodamin (ROX)-labeled internal length standard GeneScan-500 ROX (PE-Applied Biosystems) and 0.85 µl formamide dye (98% formamide, 0.0055 dextran blue), denatured for 3 min at 90 ˚ C and chilled on ice.

Electrophoresis of 36 or 48 samples was carried out using 5% denaturing polyacrylamide gels (lang Ranger ™ , FMC Bioproducts, Rockland, Maine) in 1x TBE electrophoresis buffer (89 mM Trisbase, 89mM boric acid, 2.0 mM EDTA pH8.3) on an AABI Prism ™ 377 DNA sequencer (PE-Applied Biosystems) at 2500V for 4h. For raw data collection, the ABI PRISM ™ V.1.1 collection software was used. AFLP fragments were analysed using GENESCAN ™ V.2.0.2 analysis software (PE-Applied Biosystems) as described in the user's manuals.

For screening of AFLP markers 65 EcoRI + ANN-MseI + CNN primer combinations were applied to parents. 14 combinations which showed high level of polymorphism were used for F_3 families analysis. These combinations were:
E + ACA-M + CCC, E + ACA-M + CCG, E + ACA-M + CTA, E + ACG-M + CGT, E + ACG-M + CGG, E + ACT-M + CAT, E + ACT-M + CCA, E + ACT-M + CCC, E + ACT-M + CGA, E + ACT-M + CGG, E + AGA-M + CGG, E + AGT-M + CCC, E + AGT-M + CCG and E + AGT-M + CGG.

2.7 Microsatellite analysis

Wheat microsatellite (WMS) primer pairs were developed by Roeder et al. (1995) and Roeder et al. (1998). One primer of WMS primer pairs was labeled using either 5-carboxy-fluorescein (5-FAM), 4,7,2',7',-tetrachloro-6-carboxy-fluorescein (Tet) or 4,7',2',4',5',7'-hexachloro-6-carboxyrhodamin (Hex). Each PCR reaction contained 50 ng genomic DNA, 10 pmol of each labeled and unlabelled primer, 0.75 U *Taq* DNA polymerase (Qiagen), 2 µl. of 10 x PCR buffer containing 15 mM $MgCl_2$, 0.3 mM dNTPs in a total volume of 20 µl. The PCR reaction was carried out in a PE 9600 thermal cycler for 35 cycles at 95°C for 10 s, the annealing temperature was either 55°C or 60 °C according to the primer used for 10 s, and 72 °C for 30 s, with a final step at 72°C for 10 min. The PCR products amplified with Hex – labelled primer and 5- Fam- or Tet-labelled primer were diluted with water at 1:4 and 1:9, respectively.

The samples were mixed with 0.15 µl. GeneScan-500 TAMRA internal size standard (PE Biosystems) and 0.85 µl. formamide dye (98% formamide,0.01% dextran blue), denatured at 90°C for 3 min and chilled on ice.

Electrophoresis of 36 samples was carried out using 5% denaturing polyacrylamide gel (Long Ranger ^{TM} ,FMC Bioproducts) in 1 x TBE buffer (89mM T_{RIS} , 89mM boric acid, 2 mM EDTA, pH 8.3) on an ABI prism^{TM} 377 DNA Sequencer (PE Applied Biosystems) at 1200V for 1.5 h. ABI collection software version 1.1 was used for raw data collection . Microsatellite fragments were analysed using GENESCA^{TM} analysis software version 2.1 as described in the user's manuals.

2.8 Marker nomenclature

Microsatellite loci were designated Xgwm followed by a probe number, according to Roeder et al. (1998). AFLP marker designations were based on the primer combination used and the fragment sizes estimated accurately with reference to the internal lane standard Gene-Scan-500 ROX. The primer combinations are abbreviated according to the list which was provided by KeyGene and can be accessed in the Grain Genes database (http:///Wheat.PW.usda.gov). Accordingly, the abbreviations of the primers used were as follow:

Primer	Code
E + ACA	E35
E + ACG	E37
E + ACT	E38
E + AGA	E39
E + AGT	E42
M + CAT	M50
M + CCA	M51
M + CCC	M52
M+ CCG	M53
M + CGA	M55
M + CGG	M57
M + CGT	M58
M + CTA	M59

On the other hand, the same nomenclatures of RFLP of the original providers was used. Detected loci were marked with an 'X', the basic symbol for molecular marker loci of unknown function in wheat.

2.9 Statistical analysis

2.9.1 Normal distribution curve test

Normal distribution curve was calculated using the SPSS program ver. 10.0 (SPSS inc.) for FHB severity, plant height and heading date in all locations. Basically, the program tested the significance of the distribution using the Kolmogorov-Smirnov test. The formula used as follows:

$Z = \text{Max} (F_b - F_e) / n$

Where,

F_b = General mean of the population

F_e = Mean of the values under the normal curve.

n = Number of individuals in the population.

Data from the locations; Bergen, Obertraubling, Hadmersleben and Gruenbach was transformed into $\log_{10}$ data.

2.9.2 Correlation coefficients

Correlations were calculated between FHB severity and heading date, and FHB and plant height at Gruenbach location in 1998 and 1999. Also, correlations among different locations for FHB trait were calculated using SPSS ver. 10.0 . Formulas were as follows:

Correlation coefficients (r) = $Cov_{xy} / S_x S_y$

Where,

$Cov_{xy} = [\sum x_i y_i - (\sum x_i \sum y_i)/n]/ n-1$

$S_x^2 = [\sum x_i^2 - 1/n (\sum x_l)^2] / n-1$

$S_y^2 = [\sum y_i^2 - 1/n (\sum y_l)^2] / n-1$

$S_x S_y$ = (Standard deviation of x) (Standard deviation of y)

x_l = values of the first trait.

y_l = values of the second trait.

n = number of obsevations.

The statistical significance of each r was tested at 0.05 and 0.01.

2.9.3 Analysis of variance

Analysis of variance was performed using the program Costat (Cohort software, Minneapolis, MN). The ANOVA was estimated on two different basis; first ANOVA for the location Gruenbach in two different years for FHB severity and the second was for all locations including Gruenbach in the two different years for all traits. The calculation tables were as follows:

ANOVA for Gruenbach in two years for FHB severity

S.O.V	Df	MS	Expected MS
Block	r-1		
Genoypes (G)	G-1		$ry\sigma^2g + r\sigma^2gy + \sigma^2e$
Years (Y)	Y-1		
G X Y interaction	(G-1) (Y-1)		$r\sigma^2gy + \sigma^2e$
Error (e)	r (G-1) (Y-1)		σ^2e
Total	rGY –1		

ANOVA for different environments for all traits.

S.O.V	Df	MS	Expected MS
Environment (L)	L-1		
Genoypes (G)	G-1		$\sigma^2e + L\,\sigma^2g$
Error (e)	(L-1) (G-1)		σ^2e
Total	LG		

2.9.4 Heritability

For Gruenbach location, estimates of variance components σ^2g (genetic variation), σ^2y (years variation), σ^2gy (genotype x years interaction variance) and σ^2e (error variance) were calculated from ANOVA. Consequently, heritability (h^2) in broad-sense was calculated on an entry mean basis according to Hallauer and Miranda (1981).

Heritability was calculated for FHB severity trait in broad sense from the formula:

$$h^2 = \sigma^2g / (\sigma^2e/ry + \sigma^2gy/y + \sigma^2g)$$

Where,

$\sigma^2 g$ = the variance among the genotypes.

$\sigma^2 e$ = the error variance

$\sigma^2 gy$ = interaction between environment and genotypes

y = number of years

r = number of replications.

Genetic correlation for FHB severity at this location between the two years was calculated from the formula:

$$rG \text{ (Genetic correlation)} = V_G / V_{GY} + V_G$$

Accordingly, estimates of variance components $\sigma^2 g$ (genetic variation) and $\sigma^2 e$ (error variance) were calculated from ANOVA for all locations. Consequently, heritability (h^2) in broad-sense was calculated by substituting environments or years for replications according to Toojinda et al. (2000) as:

$h^2 = \sigma^2 g/(\sigma^2 g + \sigma^2 e/L)$

Where,

$\sigma^2 g$ = the variance among the genotypes

$\sigma^2 e$ = the error variance

L = number of environments or years

2.9.5 Predicted minimum numbers of the effective genes

The minimum numbers of effective genes controlling FHB resistance was estimate using Wright's method (1968). The modification for the level of inbreeding in the original formula is based on Cockerham (1983), the modified formula is:

$N = (GR)^2 / 4.27\ \sigma^2 g$

Where,

N = minimum number of effective genes

GR = the range between extreme genotypes

$\sigma^2 g$ = genetic variance of the F_3 familis.

GR was estimated by two methods. In method 1, GR was the range of the F_3 families means while in method 2, GR was the range of F_3 families means multiplied by heritability h^2 as calculated above. The assumptions of the formula were:

1. No linkage
2. No epistasis
3. No dominance
4. All loci have equal effects

2.9.6 X^2 distribution test for markers

Chi square distribution was calculated in the PLABQTL program for each marker in relation to a null hypothesis of the segregation ratio 1:2:1 co-dominant or 3:1 for the dominant markers. The formula used was:

$X^2 = \sum x$ [(observed – expected)^2 / expected]

df = number of classes – 1

The probability value for each marker data was obtained from table of chi square probability values for various degrees of freedom. The probability of chi square values were tested at 0.05 and 0.01 levels of significance.

2.9.7 Map construction

Linkage group analysis was performed using the MAPMAKER version 3.0 (Lander et al., 1987) using the Kosambi mapping function (Kosambi 1944) because of the independent cross-over events in different meiotic phases during the development from the F_1 to F_3. This analysis was carried out by evaluating the mapping populations as an F_2 using two-point analysis to identify linkage group at a LOD score of 3.0. Data was input as A, B and H to mark genotypes of parent A, parent B, and heterozygotes, respectively, for co-dominant marker. Other situations were coded by

C = not A; i. e. H or B (for dominant markers)

D = not B; i. e. H or A (for dominant markers)

"-" = missing data for the individual at a locus.

Two-point was used in order to determine the best order of marker loci within the linkage group. Marker loci and linkage group that were more than 40 cM apart were considered not significantly linked. The "ripple" command was conducted to

assign exact positions to markers. Marker not meeting that threshold, were placed in the interval using the Mapmaker "try" command

2.9.8 QTL analysis

The QTL analysis was performed as a composite interval analysis (CIM) to identify genomic regions associated with the traits evaluated using PLABQTL (Utz and Melchinger, 1996). The program performs a multiple regression on evenly distributed positions of the linkage map. It calculates the test statistic (LOD) based on the sum of squares of the regression in a model with a QTLs versus the sum of squares of the regression in a model without QTLs. The LOD threshold was set to 3.0 corresponding to an experiment – wise significance of 0.05 assuming chi square distribution test for the test statistics. Markers as cofactors for the final regressions were selected by the program using stepwise regression. Also, the proportion of phenotypic variance explained by each QTL marker was estimated using the coefficient of determination (R^2) which is based on the partial correlation of a putative QTL with the trait adjusted for cofactors in the multi-locus model. For each QTL, the regression coefficient from the multilocus-model was used to estimate the additive effect of the "Sgv.NB x MM.Sum3". or "Apollo" allele. Additive effect were negative if the allele of "Sgv.NB x MM.Sum3" decreased the FHB score or heading date measurement and positive if the "Apollo" increased the FHB score or heading date measurement. On the other hand, additive effect was negative if the allele of "Sgv.NB x MM.Sum3" decreased the plant height measurement and positive if the "Apollo" increased the plant height measurement. The QTL x environment interaction for traits were estimated by fitting a model to adjusted entry means of each environment which included all QTL detected in the analysis across environment by final ANOVA in PLABQTL.

In the PLABQTL program, the parameters for the QTL data were included in "*.qdt" data files. These parameters were marker data, linkage groups and the phenotypic observation values. Also, the commands for analysis of data were included in the controlling files "*.qin". These commands were:

FIRST-ANALYSIS = This provides a first analysis of the marker and phenotypic data, linkage map, segregation ratio with Chi-square test, frequencies of marker pairs, percentage of homozgosity and percentage of the genome inherited from

the first parent in the individuals assayed for markers, stepwise regression to pre-select cofactors and estimates of distribution parameters.

SCAN = This to perform interval scanning and to generate LOD score curve. Scanning for a putative QTL was carried out at regular increments spaced 1 or 2 cM units apart to get high resolution LOD score curve.

COV = This to use the cofactors in the form of marker numbers as covariates.

MODEL D = This to include the dominance effects in the analysis.

SMODEL = This to give the sequence of QTL positions.

ENVIRONMENTS E = This to include the number of environments and a QTL X environment analysis to be invoked.

STOP = This is a closing statement to terminate the progr

The output data for QTL identification as follows:

Chr.	Pos.	Flanking markers	Sup. Int.	LOD	R^2	add.

Where,

Chr. = The linkage group which included the putative QTLs.

Pos. = The position on the chromosome of the QTL in cM.

Supp. Interv. = Support interval with a LOD fall off of 1.0 expressed as position on the chromosome in cM.

Flanking markers = Names of the two markers linked to the putative QTL makers and located on the left and right sides of it.

LOD = Log10 of the likelihood odds ratios. The LOD score is calculated the F-value in the multiple regression

R2 = the percentage of the phenotypic variance, which is explained by a putative QTL

Add. = The estimated additive QTL effect at the location of the scanning. It is assumed that second parent carries the favorable alleles for the trait under study.

3 Results

3.1 Analysis of field experiments

In this study, FHB severity was recorded on the two parental lines "Apollo" and "Sgv.NB x MM.Sum3" and on 180 F_3 families in five different locations namely Bergen, Obertraubling, Herzogenaurach, Hadmersleben and Gruenbach. At the same time, two other traits namely; plant height and heading date were recorded to determine their correlation to FHB disease resistance.

3.1.1 FHB analysis

3.1.1.1 Phenotypic analysis of FHB severity

In this study, mean responses of the parents to scab were recorded as percent of infected spikelets in Gruenbach location for the years 1998 and 1999, while in the other four locations they were recorded for the year 1999 only. Mean responses of the parents at Gruenbach location in two years are given in (Table 1), where, significant differences were noticed between the parents' means, with the "Sgv.NB x MM.Sum3" parent found to be more resistant than "Apollo" parent.

Table 1: Mean performance of the parents for FHB severity, plant height and heading date at Gruenbach 1998/1999

	%FHB severity		Plant height		Heading date	
Parental lines	Gruenbach 1998	Gruenbach 1999	Gruenbach 1998	Gruenbach 1999	Gruenbach 1998	Gruenbach 1999
Apollo	50.6	55.1	92	85	230	227
Sgv.NB X MM.Sum3	4,4	2,8	87	76	224	223.5

The means for scab severity recorded for F_3 families and standard deviations for each location are given in (Table 2). The results indicated that, significant differences were noticed between the two years in Gruenbach. The means differed significantly among each others for all environments indicating the quantitative inheritance of all FHB resistance. Also, the highest average of disease severity

was at Herzogenaurach (55.8 %), while, the lowest was in Gruenbach (11.7 %) and the mean for all locations (34.3 %) was higher than the parental mean of all locations (29 %).

Frequency distributions for percent scab severity of the F_3 families for Gruenbach location in two years and for Obertraubling location, are shown in (Figure 2 and 4). The means of the distribution of Gruenbach environment were 20.1 and 12.0 for the years 1998 and 1999, respectively. The distributions of disease severity for the two years in Gruenbach were skewed to the left and deviated significantly from the normal distributions where the ranges were between 0 for the two years and 70% for the year 1998 and 40% for the year 1999. Therefore, the population means were shifted towards the more resistant parent "Sgv.NB x MM.Sum3" Also, in the Obertraubling environment, the distribution average was 31.4%, the range was between 5% and 70% and the standard deviation was 15.8. The distribution was the same like in Gruenbach environment and deviated to the "Sgv.NB x MM.Sum3". parent. While the distributions of the other three locations (Figures 2 and 3) fitted with normal distributions. The means of the normal distributions were 46.8%, 55.8% and 38.2; the ranges were (10% - 60%), (20% - 90%) and (2.5% - 54%); standard deviations were 9.7, 15.2, 15.5, 9.6, 10.3 and 18.7 for the environments Bergen, Obertraubling, Herzogenaurach, Hadmersleben, Gruenbach 99 and Gruenbach 98, respectively.

Table 2: Mean perecentage and standard deviation For Fusarium head blight (FHB) severity in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" in different environments

Environment	Mean	Standard Deviation
Bergen	46.8	9.7
Obertraubling	31.4	15.2
Herzogenaurach	55.8	15.5
Hadmersleben	38.2	9.6
Gruenbach99	12.0	10.3
Gruenbach98	20.1	16.2
Total mean	34.3	12.7
L.S.D:	**9.1 (5)%**	**14. 5 (1)%**

L.S.D: Least sgnificant difference at 0,05 and 0,01 probability level, respectively

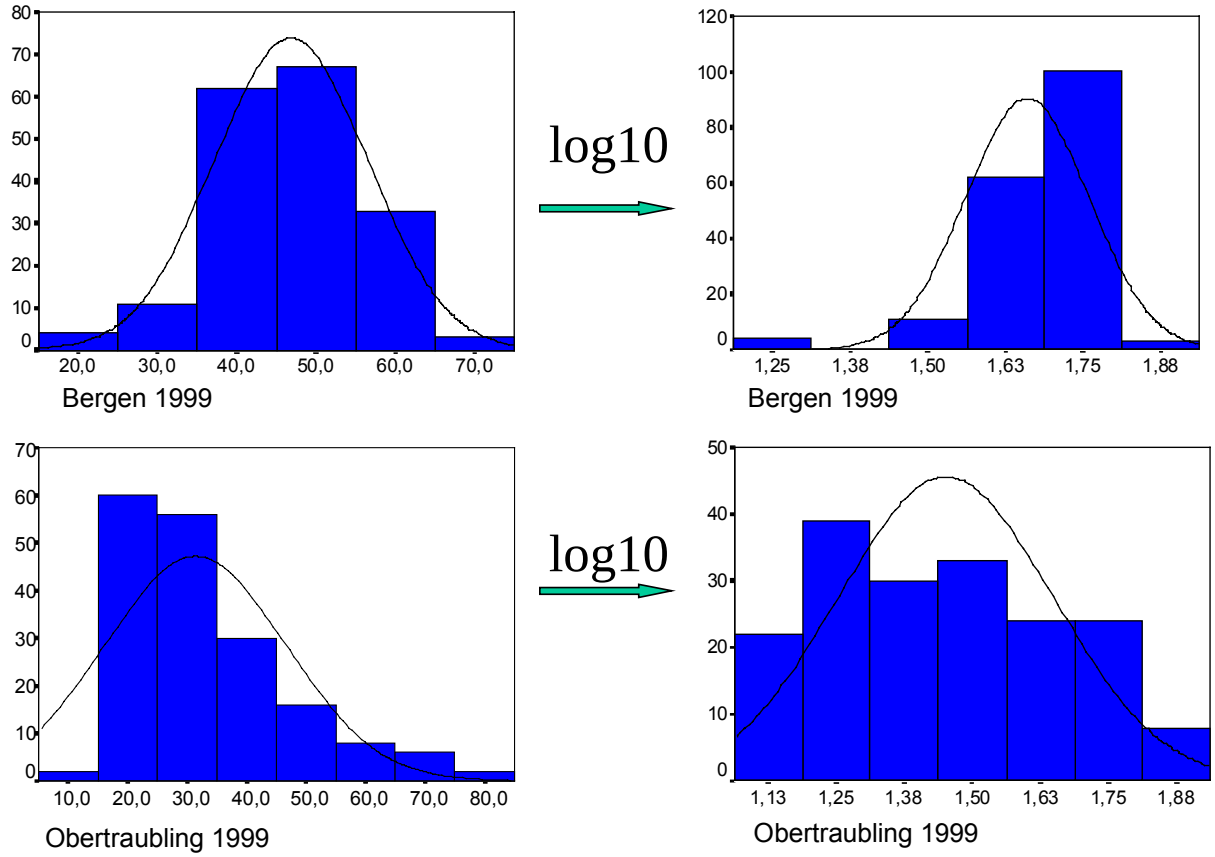


Fig. 2: Distribution of percentage Fusarium head bleight (FHB) severity in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for different environment.

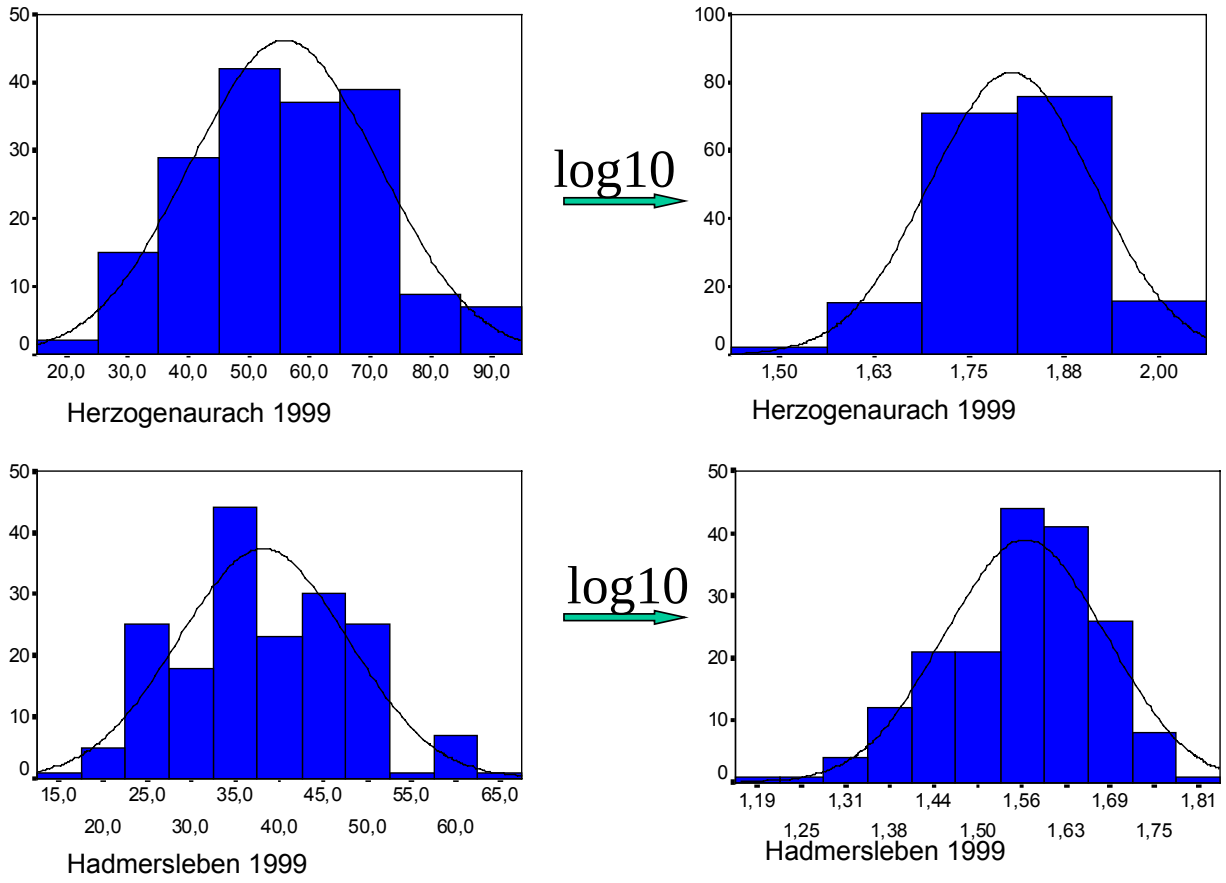


Fig. 3: Distribution of percentage Fusarium head bleight (FHB) severity in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for different environment.

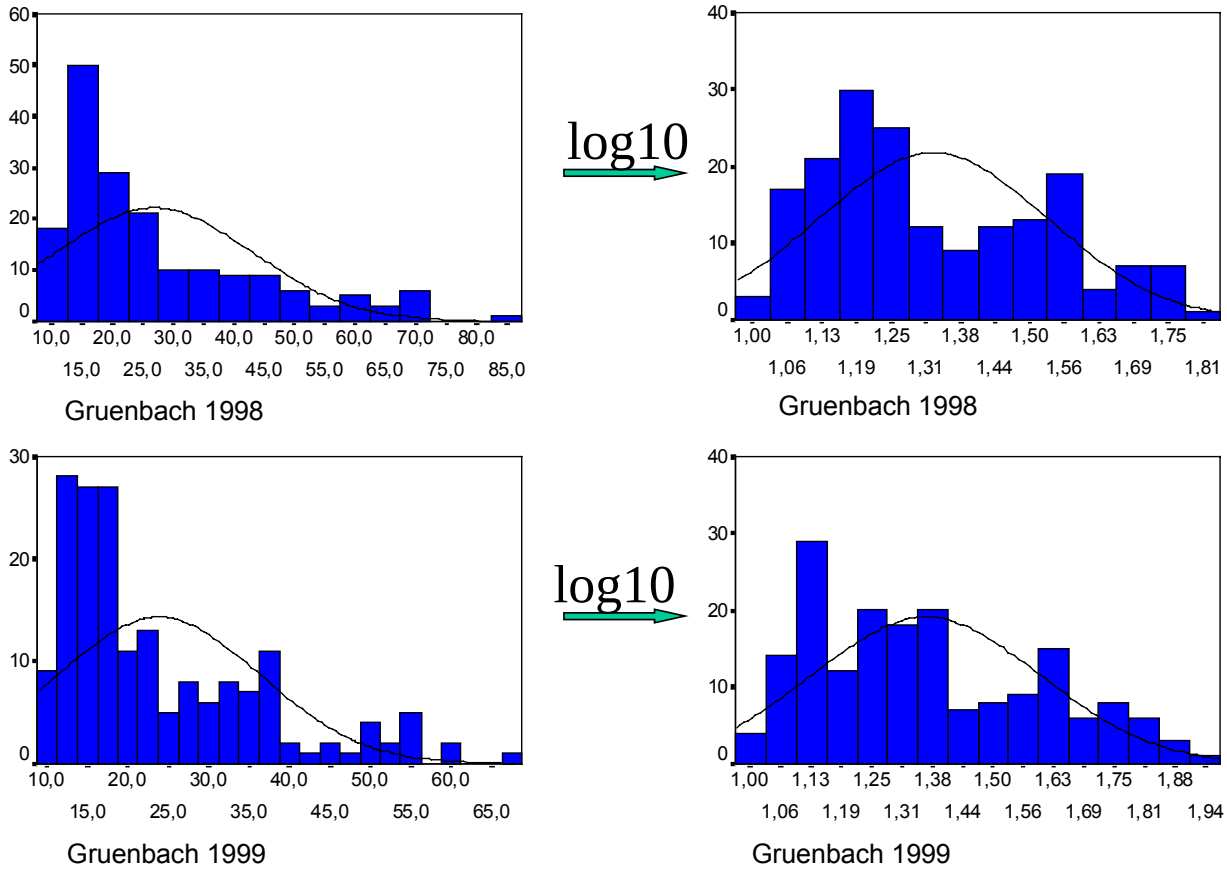


Fig. 4: Distribution of percentage Fusarium head bleight (FHB) severity in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for different environment.

3.1.1.2 ANOVA for FHB severity

Analysis of variance was estimated for all environments and for Gruenbach environment for two years. The analysis of variance for Gruenbach is presented in (Table 3), showing the mean squares for genotypes, years and the interactions between genotypes and years for scab severity. Mean squares of all source of variations were highly significant. In other words, there were significant differences among the 180 F_3 families for FHB severity. Also, significant differences were noticed between the two years, where genotypes differed significantly from one year to another at the same environment. Finally, interactions of genotypes with years were highly significant or the average interactions of each genotype at the same location from year to year differed significantly.

The mean squares for genotypes and the different environments are presented in (Table 4). Highly significant differences were indicated for all source of variations. Accordingly, there were significant differences among environments and among genotypes.

Table 3: Analysis of variance for Fusarium head blight (FHB) in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across two years

S.O.V	D.F	S.S	M.S
Block	2	33201.36	16600.68 **
Genotypes (G)	179	220900.16	1234.07 **
Years (Y)	1	19160.55	19160.55 **
G X Y interaction	179	27404.27	153.09 **
Error	718	36269.29	50.51
Total	1079	336935.66	

**** and * sgnificant at The 0,01 and 0,05 probability level, respectively.**
S.O.V= Source of variance, D.F=Degree of friedum, S.S=Sums of squares, M.S=Mean of square

Table 4: Analysis of variance for Fusarium head blight (FHB) in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for different environments

S.O.V	df	SS	MS	
Environment (L)	5	237707.23	47541.44	**
Genotypes (G)	179	89964.14	502.59	**
Error	895	93163.27	104.09	
Total	1079	420834.65		

**** and * sgnificant at The 0,01 and 0,05 probability level, respectively.**
S.O.V= Source of variance, D.F=Degree of friedum, S.S=Sums of squares, M.S=Mean of square

3.1.1.3 Heritability (h^2)

Estimates of variance components σ^2g (genetic variance), σ^2y (variance between years), σ^2gy (genotype x years interaction variance) and σ^2e (error variance) were calculated from ANOVA. Consequently, heritability (h^2) in broad-sense and genetic correlation were calculated. In Table 5 the value of heritability was 0.87 in Gruenbach and the genetic correlation between the two years was 0.68 for this trait. Also, the estimation of the heritability in broad-sense for F_3 families in different environments was 0.79 for FHB severity.

Table 5: Heritabilities and genetic correlation for Fusarium head blight (FHB), plant height and heading date in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3"

	Heritability h^2	Genetic correlation r_G
FHB	0.79 - 0.87	0.68
Plant height	0.85	0.74
Heading date	0.85	0.74

3.1.1.4 Determination of minimum number of effective genes

The minimum number of the effective genes was calculated according to the formula of Cockerham (1983). The calculated numbers using the method 1 were 6.3 in Gruenbach (average of two years) and 8.7 for across environments. While by using method 2 the values were; 5.5 in Gruenbach and 6.8 in across environments. In the second method, the correction tends to eliminate the environmental influence on the expression of this trait.

3.1.1.5 Phenotypic correlations among all environments

The values of the disease severity of the five environments were highly significant correlated to each other except in Herzogenaurach environment which was weakly correlated to all other environments (Table 6). The highest significant correlation value (0.81) was between Gruenbach environment in the two years. Also, Hatmersleben environment indicated significantly high correlations with other environments except the Herzogenaurach environment. Moderate type of correlations were demonstrated among each one of the two environments Bergen and Obertraubling to the rest environments. All correlatins were tested at the level of significance of 0.01 probability.

Table 6: Correlation cofficient for Fusarium head blight (FHB) severity among all different environments in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3"

	Bergen	Obertraubling	Herzogenaurach	Hadmersleben	Gruenbach 1998	Gruenbach 1999
Bergen	1.0					
Obertraubling	0.43**	1.0				
Herzogenaurach	0.22*	0.22*	1.0			
Hadmersleben	0.62**	0.67**	0.32**	1.0		
Gruenbach1998	0.26**	0.41**	0.22*	0.53**	1.0	
Gruenbach1999	0.28**	0.52**	0.26**	0.62**	0.81**	1.0

**** and * sgnificant at The 0,01 and 0,05 probability level, respectively.**

3.1.2 Phenotypic analysis of plant height and heading date

Plant height (HT) was measured at Gruenbach in 1998 and 1999 as the distance from the ground to the top of the spike in centimeter. Also, heading date (HD) was quantified as the number of days from planting to flowering of 50% of plants. The parents average of the two years are given in (Table 1) for the traits. Significant differences were noticed between the parents means for the two traits, where, the "Sgv.NB x MM.Sum3" parent was earlier in heading and shorter in length than "Apollo" parent.

Frequency distributions for the two traits of the F_3 families for Gruenbach location in two years are shown in (Figure 5). The mean for the families distribution was 227.5, the range was from 225 to 234 and the standard deviation was 1.54.

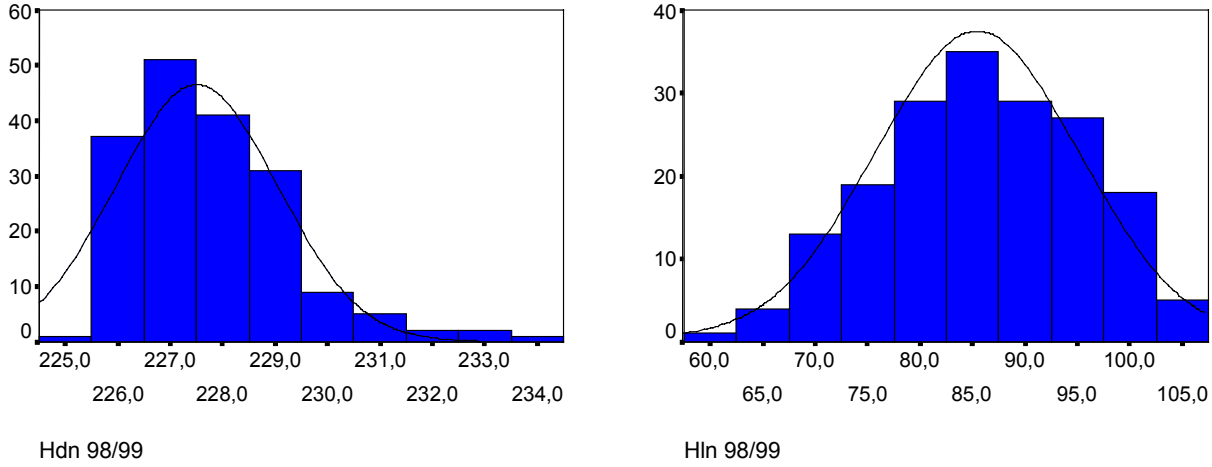


Fig. 5: Distribution of plant height and heading date in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for two years.

The distribution of heading date was skewed to the left and deviated significantly from the normal distributions. Therefore, the population means were shifted towards earlier heading date of parent "Sgv.NB x MM.Sum3" (226). While the mean of the families distribution for plant height was 87, ranged from 60 to 105 with a standard deviation of 9.7. The distribution fitted with the normal distribution.

Analysis of variances were estimated for Gruenbach environment for two years for the two traits which are presented in (Table 7 and 8), the mean squares for genotypes and years were calculated. Mean squares of all source of variations for both traits were highly significant. In other words, there were significant differences among the 180 F_3 families for the two traits. Also, significance differences were noticed between the two years for both traits, where, genotypes differed significantly from one year to another at the same environment.

Table 7: Analysis of variance for plant height in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across two years

S.O.V	df	SS	MS
Years (Y)	1	11787.7	11787.7 **
Genotypes (G)	179	34646.6	193.55 **
Error	179	5139.2	28.7
Total	359	51573.6	

**** and * sgnificant at The 0,01and 0,05 probability level, respectively.**
S.O.V= Source of variance, D.F=Degree of friedum, S.S=Sums of squares, M.S=Mean of square

Table 8: Analysis of variance for heading date in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across two years

S.O.V	df	SS	MS
Years (Y)	1	801.25	801.25**
Genotypes (G)	179	844.47	4.72**
Error	179	132.47	0.74
Total	359	1777.97	51573.6

**** and * sgnificant at The 0,01and 0,05 probability level, respectively.**
S.O.V= Source of variance, D.F=Degree of friedum, S.S=Sums of squares, M.S=Mean of square

Estimates of variance components σ^2g (genetic variance) and σ^2y (variance between years) and σ^2e (error variance) were calculated for both traits from ANOVA. Consequently, heritability (h^2) in broad-sense. In Table 5 the values of heritabilities were the same (0.85) for plant height and heading date.

3.1.3 Correlation of traits

Phenotypic correlation coefficients were calculated between FHB severity and the two traits heading date and plant height at Gruenbach location in 1998 and 1999 (Table 9) and tested at the 0.01 level . Generally, the results showed that the FHB severity is positively correlated with plant height and heading date. The correlation coffecients were highly significant which indicating that early heading and short plants are associated with FHB resistance. The correlations values were higher for the heading date trait than plant height with FHB severity in both seasons.

These correlations among resistance, shorter plants and early heading are consistent with the possibility that the genes that control these traits are linked or have pleiotropic effects. To determine the position and effects of genes controlling these traits, a linkage map was constructed with different marker systems that could be used to conduct a search of QTLs throughout the genome.

Table 9: Correlation cofficient among Fusarium head blight (FHB) ,plant height and heading date in F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" in two years

	FHB	
	1999	1998
Plant height	0.26 **	0.25 **
Heading date	0.52 **	0.56 **

**** and * sgnificant at The 0,01 and 0,05 probability level, respectively.**

3.2 Genetic mapping

3.2.1 Polymorphism rate

In this part of the study the genetic variation on the DNA level between the two parental lines "Apollo" and "Sgv.NB x MM.Sum3" was estimated using three molecularmarker techniques. These three analysis methods were; microsatellites, RFLPs and AFLPs offer great potential for generating large numbers of markers evenly distributed throughout the genome and have efficiently been used to give reliable and reproducible genetic markers.

3.2.1.1 Microsatellite (SSRs) and RFLPs

A total of 105 SSRs with known map positions were analysed with the DNA of the two parents in a polymerase chain reaction for polymorphism. Of the 105 SSR primer pairs tested, 63 showed polymorphims between the two parents (60%) (Table 10). With respect to reaction quality, 50 primer pairs were chosen to be analysed with the mapping popullation.

In the RFLP marker system, 60 probes were hybridized against restriction enzyme-digested DNA of the parents. 24 probes identified polymorphism (40%), of which 16 probes showed scorable RFLP patterns across the 180 F_3 samples.

Table 10: Rate of polymorphism detected by the microsatilite (SSR) and RFLP systems in "Apollo" and "Sgv.NB x MM.Sum3" genotypes.

Marker system	Total number of marker system tested	Number of polymorphic marker systems	Percentage of polymorohic marker systems	Number of marker analyzed in F3 families
SSRs	105	63	60	50
RFLP	60	24	40	16

3.2.1.2 AFLPs

The 65 AFLP marker combinations used in this study generated a total of 6700 fragments with an average of 103 fragments per primer pair, of which 447 were polymorphic (mean = 6.9 per primer pair). Thus, the rate of polymorphism was 6.7% in AFLP analysis (Table 11).

Table 11: Rate of polymorphism detected by the different primer combinations of AFLPs analysis in "Apollo" and "Sgv.NB x MM.Sum3" genotypes.

Category	Values
Number of primer combinations tested	65
Total number of fragments detected	6700
Average number of fragments detected per primer combinations	103
Number of polymorphic fragments	447
Rate of polymorphism	6.7%
Average number of polymorphic fragments per primer combinations	6.9

Out of the 65 primer combinations, 14 primer combinations with the highest levels of revealed polymrphisms were analysed across the progeny (Table 12). The total number of identified fragments was 161, the range among the chosen combinations was between 4 and 17, and the average was 11.5 per combination (Table 12).

Table 12: Number of Polymorphic AFLPs analyzed in F_3 families produced by 14 primer combinations.

Primer combination	Number of Polymorphic AFLPs
E35/M52	15
E35/M53	14
E35/M59	16
E37/M58	4
E37/M57	5
E38/M50	16
E38/M51	10
E38/M52	15
E38/M55	15
E38/M57	17
E39/M57	11
E42/M52	10
E42/M53	11
E42/M57	5
∑	161

The *Eco*RI + ANN - FAM , *Eco*RI + ANN - TAMRA and *Eco*RI + ANN - JOE primers indicated high, medium and low number of amplified fragments with all MseI-CNN primers, respectively. Therefore, the *EcoRI* + ANN - FAM and *Eco*RI + ANN – TAMRA were used in the F3 sample analysis.

3.2.2 Segregation of markers

A total of 150 mapped markers of F_2-derived F_3 families from the cross between "Apollo" and "Sgv.NB x MM.Sum3" were tested using a Chi square test for a null hypothesis of 1:2:1 for co-dominant markers or 3:1 for dominant markers (AFLP and some of the SSRs and RFLPs). (Table 13) showed the values of calculated Chi square and the statistical significance of each of them. The results indicated that 32 markers showed highly significant (at 0.01 and 0.001 levels) deviation form the single locus segregation ratio 3:1 or 1:2:1 while the rest fitted with these ratios. Out of the 32 markers deviated from the hypothetic ratio, 23 were dominant markers. These 32 markers were mainly on chromosme 1B (5), 2B (5), 6B (3), 7LG (3), 5A (3) and 2LG (2).

3.2.3 Linkage map construction

The map was constructed using the data of 227 molecuar markers (161 AFLPs, 16 RFLPs and 50 SSRs on the 180 F_3 families using the F_2 model in the Map-maker program. The results showed that out of the 227 markers, 150 (66.1%) markers were grouped to contruct the genetic linkage map. These identified markers were 104 AFLPs, 38 SSRs and 8 RFLPs.

The base map of 1656.7 cM is shown in (Figure 6). The total number of identified linkage groups was 27. 18 linkage groups were assigned to specific chromosome where SSRs and RFLPs served as anchor markers, while 7 linkage groups could not be allocated to specific chromosomes. The unidentified linkage groups included AFLP markers only. The two remaining groups were identified by low-copy RFLP anchor markers.

Consequently, 127 loci were mapped on genomes A, B and D of wheat (Figure 6). The number of mapped loci was the highest for B genome (77); moderate on A genome (29), and the least on B genome (12). In the B genome, the 77 loci were distributed across all 7 chromosomes. The two chromosome arms 7BS and 5BL formed one linkage group. Also, the two chromosomes 6B and 7BS/5BL included

the highest number of loci. The distribution of the loci was 12, 12, 7, 8,18, 20 on chromsomes 1B, 2B, 3B, 4B, 6B and 7BS/5BL respectively. In A genome, none of the loci was mapped to the chromosome 1A, while the others chromosomes included mapped loci. The chromosome 5A included the highest number of the genome A mapped loci. This number was 10 where 5 of them were SSRs. Chromosome 6A contained two linkage groups. In the D genome, which included the least number of mapped loci, the loci were mapped on 5 chromosomes where 4D and 5D were not tagged. Markers used for base map construction are shown on the left-hand side of each linkage group (Figure 6).

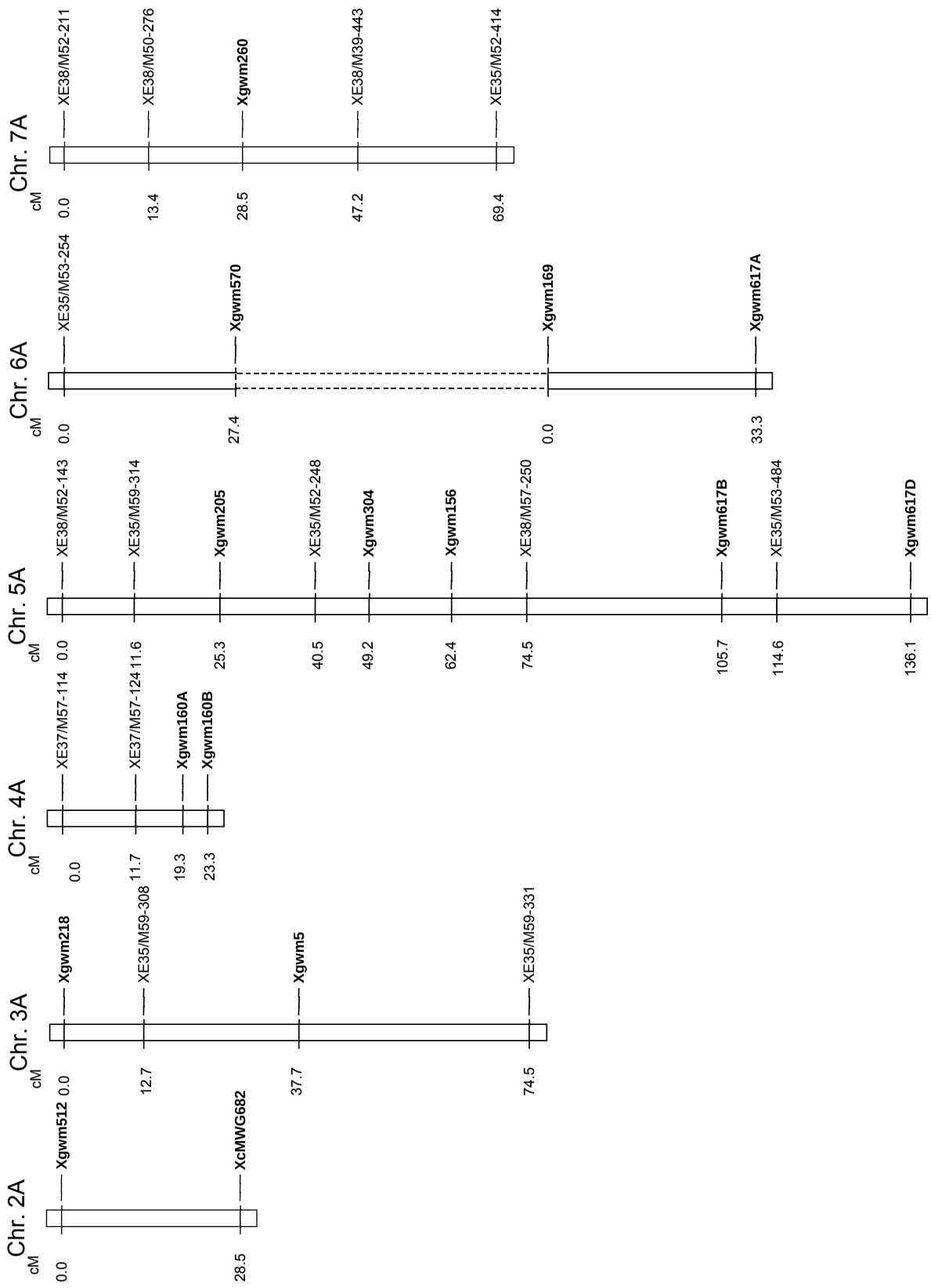
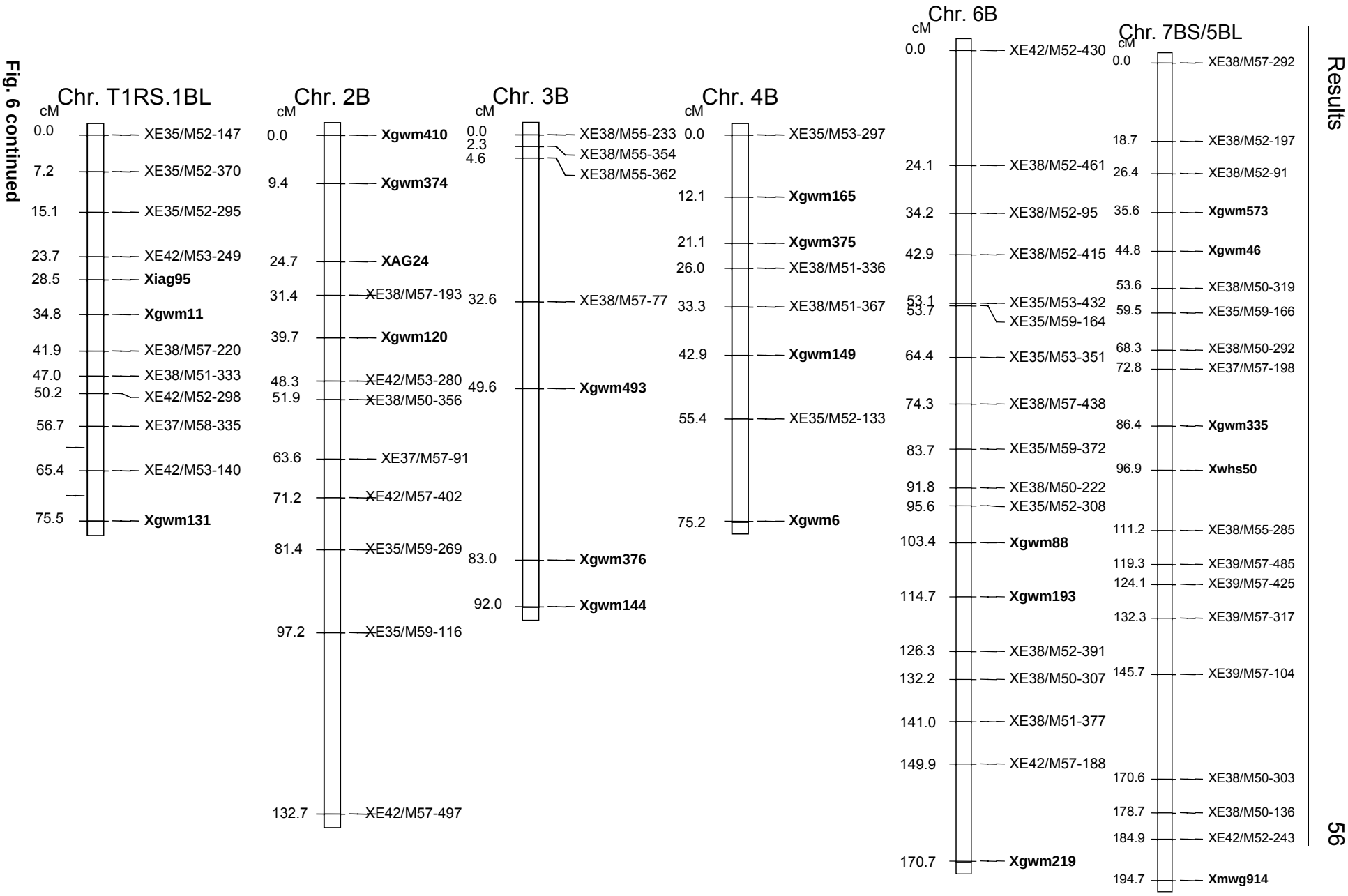


Fig. 6: Linkage map of the 180 F_3 families from the cross Apollo x Sgv.MM x NB. Sum3. Markers used for base map construction are shown on the left-hand side of each linke ge group and centiMorgans(cM) on the other side.

Fig. 6 continued



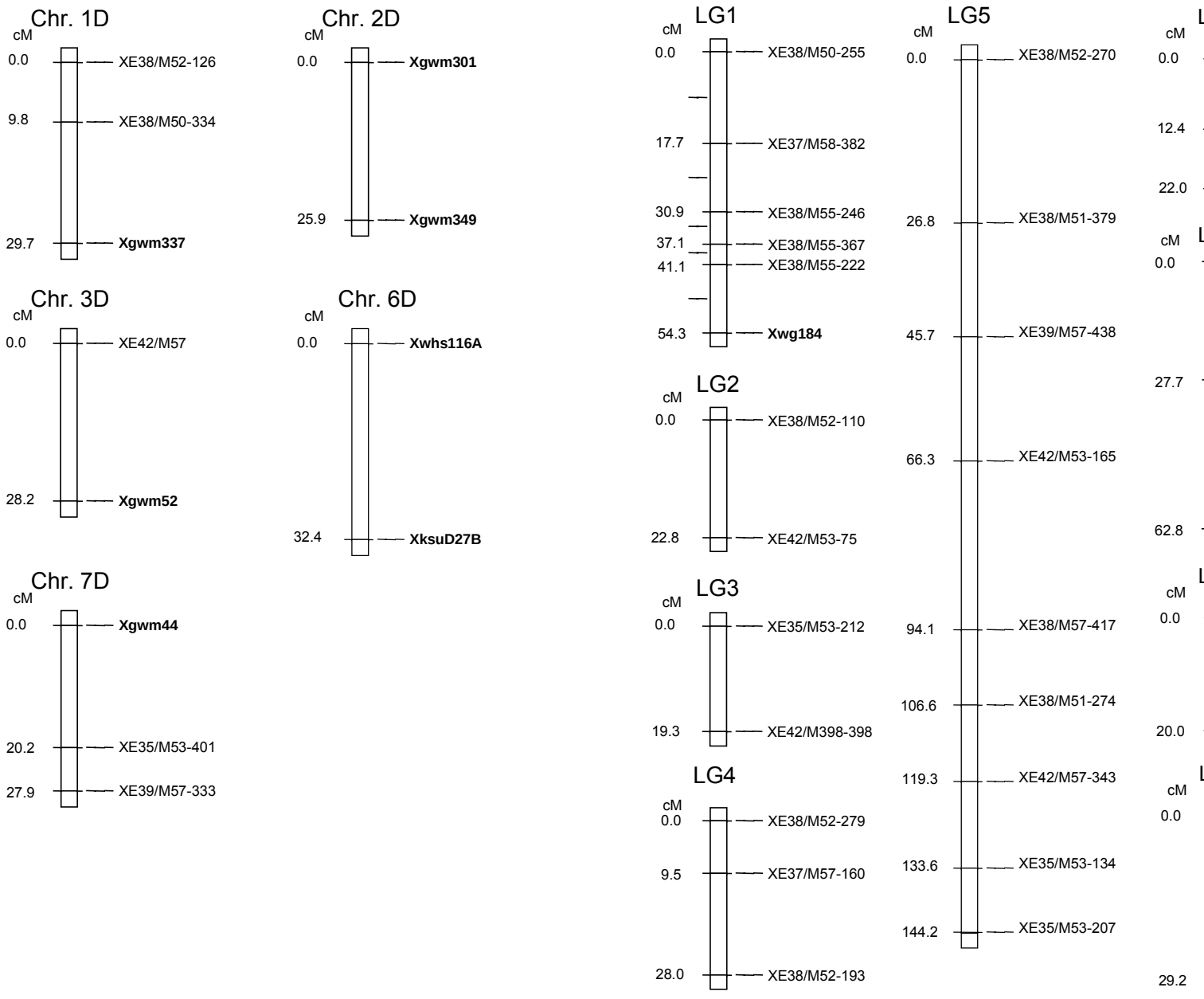


Fig. 6 continued

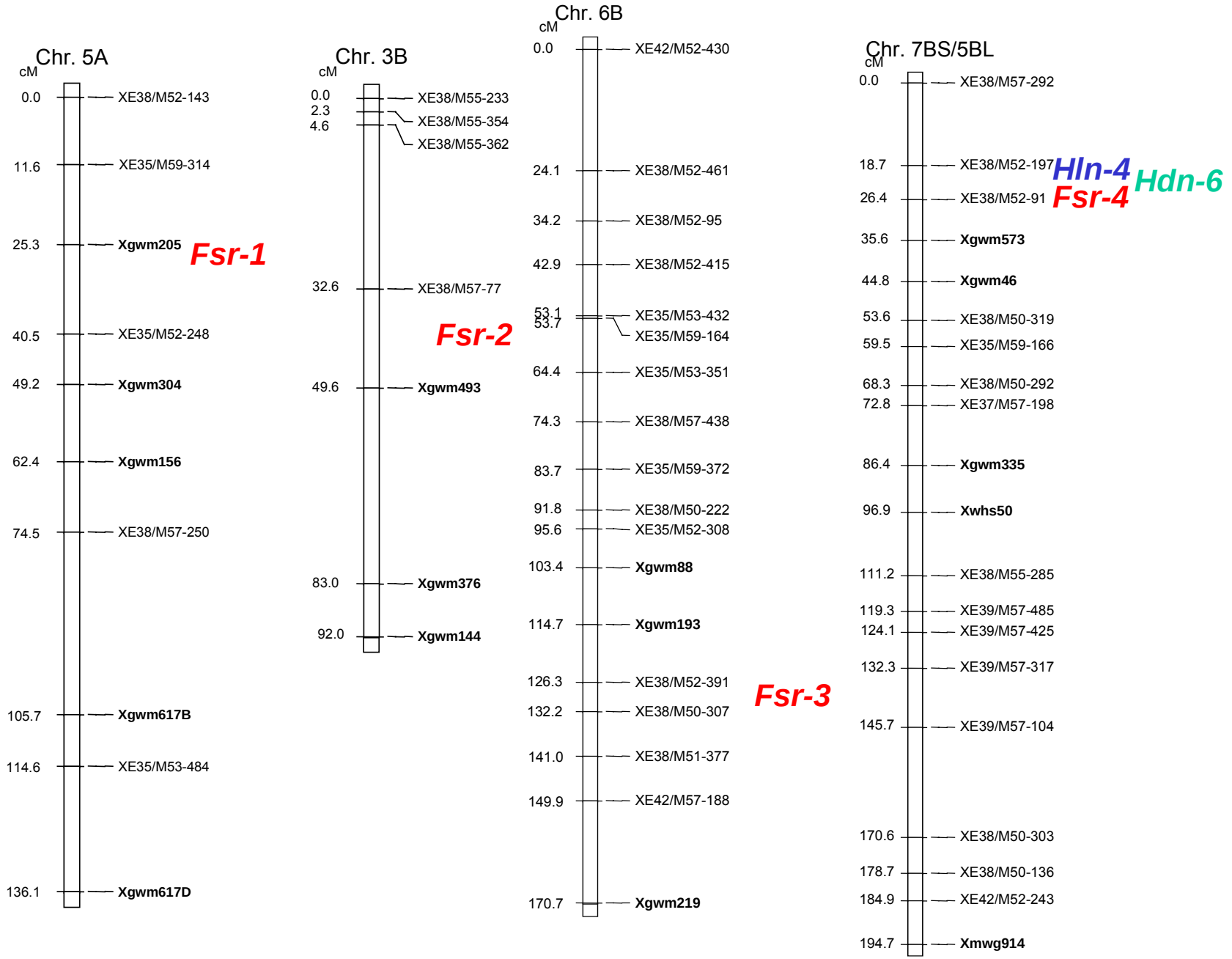


Fig. 7: Map positions of QTLs for resistance to *F.culmorum*

3.3 Detection and localization of QTLs

In this part, individual QTLs were detected for the traits FHB resistance, plant height and heading date. The FHB severity was recorded in five locations, therefore, the QTL analysis for FHB resistance was carried out for each location and over the five locations. While the traits plant height and heading date were recorded in one environment for two years, therefore they were analyzed in one location.

3.3.1 QTL analysis for FHB resistance in single environment

The individual QTLs for FHB resistance as identified by the CIM analysis using the PLAPQTL program are presented in (Table 14). The corresponding LOD scan over the chromosomes for FHB resistance are presented in (Figures chromosomes and linkage groups for LOD scan). Twelve QTL regions for FHB resistance were detected on chromosomes 5A, 3B, 6B, 7BS/5BL and the linkage groups 4LG, 5LG, 7LG and 9LG. Positive additive effect indicated that the FHB score was higher for the parental allele of "Apollo" i.e. susceptibility is inherited by the "Apollo" allele and resistance (smaller FHB score) by the "Sgv.NB x MM.Sum3" allele. The percentage of the phenotypic variance (R^2) explained by a single QTL ranged from 7.4% to 11.3% in the covariance analysis with cofactors. The total amount of phenotypic variance for FHB explained by all significant QTL, varied between 8.3% in Bergen and 45.5 % in Herzogenaurach.

Three QTLs were detected on chromosome 5A. The QTL at map position 26 cM was detected in both Herzogenaurach and Hadmersleben locations which explained 7.5% and 8.4% of the phenotypic variation, respectively. The additive effect values were –2.4 and –6.9 in the locations Herzogenaurach and Hadmersleben, respectively. This FHB resistance QTL was contributed by the more resistant wheat parent "Sgv.NB x MM.Sum3". The other two QTLs were identified at map positions 61 and 115 cM in Herzogenaurach and Hadmersleben, respectively. The QTL of 61 position explained 8.9% of the phenotypic variation and the additive effect score was –6.9 indicating the contribution from the more resistant parent “Sgv.NB x MM.Sum3“. While the QTL at position 115 cM explained 8.0% of the phenotypic variance with an additive effect of + 0.65, indicating that QTL was inherited from the "Apollo" parent.

On chromosome 3B, 2 QTLs were detected on map positions 4 and 50 cM. The first QTL was identified in Bergen location which explained 8.3% of the phenotypic variance and the second was identified in Hadmersleben and Gruenbach which explained 7.6% and 8.0% of the phenotypic variance, respectively. The additive effect of the first QTL was +1.2, inherited from the susceptible parent "Apollo", while the additive effect of the second was –0.23 and –0.34 and therefore was contributed from the parental line "Sgv.NB x MM.Sum3"

The chromosome 6B contained 2 putative QTLs on positions 85 and 36 cM, where they were discovered in Herzogenaurach and Obertraubling locations, respectively. The R^2 values were 11.3% and 8.2% and the additive effects were +43.9 and +1.4 of Herzogenaurach and Obertraubling locations, respectively, where both of them were donated by the parent "Apollo".

Chromosome 7BS/5BL and the linkage groups 4LG, 5LG, 7LG and 9LG were accommodated by a single QTL each. Their positions were 54 cM (R^2 = 7.6 , additive effect value = +0.27), 12 cM (8.0% , -0.16), 103 cM (7.4%, +17.5) 1 cM (9.0%, +0.63) and 15 cM(9.5%, +0.27), respectively. In Gruenbach, three QTLs of this group (4LG, 7LG and 7BS/5BL) were detected and the rest were identified in Herzogenaurach location. All of them were inherited by the same parent “Apollo“ except the QTL on 4LG.

Table 14: QTLs for FHB resistance in wheat F3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" for single environments

QTL Position			Hadmersleben			Gruenbach			Herzogenaurach			Obertaubling			Bergen		
Chr.	cM	Flanking marker	Lod	R2	Additive effect	Lod	R2	Additive effect	Lod	R2	Additive effect	Lod	R2	Additive effect	Lod	R2	Additive effect
5A	26	XE35/M59-314-Xgwm205	3.1	7.5	-2.4				3.4	8.4	-6.9						
	61	Xgwm304-Xgwm156							3.7	8.9	-6.9						
	115	XE35/M53-484-Xgwm617	3.2	8.0	+0.65												
3B	50	XE38/M57-77-Xgwm493	3.3	8.0	-0.23	3.1	7.6	-0.34									
	4	XE38/M55-354-XE38/M55-362													3.3	8.3	+1.2
6B	85	XE35/M59-372-XE38/M50-222										3.4	8.2	+1.4			
	36	XE38/M52-95-XE38/M52-415							4.7	11.3	+43.9						
7BS\5BL	54	XE38/M50-319-XE35/M59-193				3.1	7.6	+0.27									
4LG	12	XE37/M57-160-XE38/M52-193				3.7	8.0	-0.16									
5LG	103	XE38/M57-417- XE38/M51-274							3.1	7.4	+17.5						
7LG	1	XE39/M57-368-XE35/M59-317				3.6	9.0	+0.63									
9LG	15	XE42/M52-383-E42/M53-375							3.9	9.5	+3.3						
				23.5			32.2			45.5			8.2			8.3	

3.3.2 QTL analysis for FHB resistance across environments

In order to determine QTL that are important for the expression of the trait under different environmental conditions, QTL analysis was performed on the basis of the phenotypic values averaged over four different environments. Four QTLs were detected for FHB resistance on chromosomes 5AS (26 cM), 3BS (48 cM), 6BS (129 cM) and 7BS/5BL (28 cM) where they explained 37.0% of the phenotypic variance (Table 15).

Table 15: QTLs for *Fusarium* head blight (FHB) resistance in wheat F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across four different environments

QTL	Chr.	cM	Flanking marker	Lod	R2	Additive effect
fsr-1	5AS	26	XE35/M52-248 **Xgwm205**	3,5	8,5	-0,21
fsr-2	3BS	48	XE38/M57-77 **Xgwm493**	4,8	11,6	-0,25
fsr-3	6BS	129	XE38/M52-391 XE38/M50-307	3,3	8,1	-0,28**
fsr-4	7BS/5BL	28	XE38/M52-91 **Xgwm573**	3,6	8,8	-0,25**

****and* sgnificant at the 0,01 and 0,05 probability level for QTL x environment interaction.**

The range of R^2 values was between 11.6 % and 8.1%, therefore, the QTL which explained most of the phenotypic variance for FHB resistance was on 3BS on the interval 39 – 59 cM. The 4 QTLs for FHB resistance performed in additive manner and they were inherited by the parental resistant allele "Sgv.NB x MM.Sum3" The additive effect did not vary very much for the 4 QTLs and the values ranged from (–0.21 and –0.28) where the QTL which showed the greatest additive effect for FHB resistance was found on chromosome 6BS on the interval 126 – 133 cM. The two QTLs which identified on chromosomes 5AS and 3BS reached

significance in two environments (Hadmersleben and Herzogenaurach) and they were confirmed in the QTL analysis across environments. On the other hand, the other two QTLs did not reach significance in any environment but they were detected in the analysis of the averages over the four different environments. Only the QTL on chromosome 6BS showed significant QTL x environment interaction. The QTL showed markedly significant lower additive effect in two environments (-0.07 and -0.03) in Bergen and Obertraubling, respectively. The multiple analysis of the most significant genetic effects was performed by PLABQTL. The model for FHB resistant explained 8.6% of phenotypic variance and 18.2% of the genetic variance Table 15.

3.3.3 QTL analysis for plant height

Averaged over two years in Gruenbach location, 3 QTLs (LOD> 3.0) for plant height were detected (Table 16). These three QTLs were located on chromosomes 3BS (27 cM), 6BS (52 cM) and 7BS/5BL (19 cM), where individual QTLs explained 6.2 % ,10.1 % and 7.6 % of the phenotypic variance in composite interval mapping involving co-factors, respectively. The additive effect of the QTLs on chromosomes 3BS, 6BS and 7BS/5BL were –2.2, -5.5 and –16.3, respectively, while for, all for them the allele for shorter plant was inherited from the short parent "Sgv.NB x MM.Sum3". Two QTLs for plant height (3BS and7BS/5BL) were coincident with the QTLs for FHB resistance, where, the support interval of these QTLs were close to the support interval of two QTLs of FHB resistance. At these QTLs, the allele for shorter plants corresponded to the allele for more resistance for FHB.

Table 16: QTLs for plant height in wheat F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across two years

QTL	Chr.	cM	Flanking marker	Lod	R2	Additive effect
hln-1	3BS	27	XE38/M55-362 XE38/M57-77	2,4	6,2	-2,20
hln-2	6BS	52	XE38/M52-415 XE35/M53-432	4,3	10,1	-5,50
hln-3	7BS/5BL	19	XE38/M52-197 XE38/M52-91	3,1	7,6	-16,33

3.3.4 QTL analysis for heading date

Averaged over two years in Gruenbach location, 6 QTLs (LOD> 3.0) for heading were detected (Table 17). At five loci, the allele for early heading date at these loci was from the parent "Sgv.NB x MM.Sum3" (the additive effect scores were -0.39, -0.7, -0.57, -0.58 and –0.92), while for one QTL the allele was derived from the parent "Apollo" (additive effectscore was +0.16). A model fitting all QTLs explained 56.8 % of the phenotypic variance and the range was between 7.7 % and 12.3 %. They were identified on chromosomes 3A, 6AL, 2BL, 4BL, 6BL and 7BS/5BL at the map positions 8, 16, 123, 34, 150 and 24 cM, respectively. The QTL on chromosome 7BS/5BL was located at a close support interval to the QTLs for plant height and FHB resistance. At this QTL the allele for early heading date corresponded to the alleles for shorter plants and for higher FHB resistance.

Table 17: QTLs for heading date in wheat F_3 families of the cross "Apollo" x "Sgv.NB x MM.Sum3" across two years

QTL	Chr.	cM	Flanking marker	Lod	R2	Additive effect
hdn-1	3A	8	Xgwm218 XE35/M59-308	4,1	10,1	-0,39
hdn-2	6AL	16	Xgwm169 Xgwm617A	3,0	7,7	-0,70
hdn-3	2BL	123	XE35/M59-116 XE42/M57-497	3,1	7,8	+0,16
hdn-4	4BL	34	XE38/M51-367 Xgwm149	5,1	12,3	-0,57
hdn-5	6BL	150	XE42/M57-188 Xgwm219	4,6	11,2	-0,58
hdn-6	7BS/5BL	24	XE38/M52-91 Xgwm573	3,1	7,7	-0,92

4 Discussion

4.1 Genetic analysis of FHB resistance

4.1.1 Phenotypic frequency distributions

One of the objectives of this research was to elucidate the genetic basis of the quantitative resistance for FHB. Phenotypic frequency distributions (Figures 1, 2 and 3) of different environments support the quantitative inheritance of FHB resistance and observed transgressive resistance among F_3 families. Also, mean values of different environments showed significant differences among different environments with some exceptions. These differences in genotype responses confirmed the quantitative nature of the trait which were affected largely by the environmental conditions. Using the parental values as standards, there were more resistant transgressive segregants than susceptible transgressive segregants for FHB resistance at the environments Obertraubling and Gruenbach (1998 and 1999). However, the distribution of the Bergen environment indicated the opposite where susceptible transgressive segregants occured more frequently than resistant transgressive segregants. At the same time the other two environments Herzogenaurach and Hadmersleben allowed approximate equal distributions of the segregants. A similar phenomenon was reported in the progeny of two FHB resistant wheat genotypes (Van Ginkel et al., 1996). This phenomenon may be attributed to the fact that the phenotypic values of the resistance genotypes could not be distinguished as easily as those of the susceptible genotypes (Zhu et al., 1999). Also, it may be due to the level of environmental pressure which allow better expression of resistance in one environment than in the other. Finally the inoculation method used which may or may not allow the detection of resistant segregants (Van Ginkel et al., 1996).

ANOVA for F_3 families at different environments indicated that in Gruenbach significant differences were noticed among genotypes, years and interaction between genotypes and years for FHB severity. These observations demonstrated that the F_3 families under study were significantly different from each other in their FHB severity. Also, the families performances were fluctuated by environmental conditions. While significant mean square of seasons indicating that grand mean of seasons were differed form season to another.

On the other hand, in the different environmental analyses, significant mean squares for environment and genotypes were observed. These observations indicated that these environment differed significantly from each other. These different environments are important in studying quantitative traits like FHB resistance for many reasons. Since the FHB is a quantitative trait and affected by the environmental conditions, the responses of different genotypes would be different from one environment to another. Therefore, the gene expression of different QTLs for FHB resistance under different environments can be studied. Also, the obtained results confirmed that the F_3 families under study differed significantly form each other. Therefore, the differences among F_3 families genotypes allowed the study of FHB resistance segregation and consequently better understanding of the inheritance of the trait.

4.1.2 Heritability

In this study, the heritability in broad sense was 0.87 in Gruenbach (1998 and 1999 seasons) while it was 0.79 across different environments for FHB resistance. These measures showed that the proportion of the phenotypic variance among the F_3 families in the population under study resulted from genetic differences . These high values mean that the environmental factors influencing the FHB resistance were relatively uniform among the F_3 families (Falconer, 1989). Most of the previous studies agreed with the heritabiliy found in this study. Snijders (1990) estimated the heritability in different genotypes for FHB resistance to be 0.85 and 0.95 in groups of 32 and 54 genotypes, respectively. Also, Bai et al. (1989), Baird (1993) and Li and Yu (1988) estimated high values for heritability for FHB resistance trait. At the same time, Singh et al. (1995) estimated heritability in narrow sense and they found high values (0.66 – 0.92) in F_6 populations derived from different crosses. Finally, Bai et al. (2000) showed that the heritabilities were high ranging from 0.91, 0.80, 0.87 and 0.87 for the F_5, F_6, F_7 and F_{10} generations respectively. It can be concluded that since the heritability is a population parameter and it is not fixed for the trait, therefore, it depends on the genetic structure and the specific environment in which the population was analysed. Thus, the heritability estimated for one population and a specific environment cannot be applied to another population or even to the same population in a different environment.

Finally, the similarity in measurements for heritabilities does not mean that the populations are genetically similar for the trait (Falconer, 1989).

4.1.3 Determination of minimum number of effective genes

In this study, the estimated minimum number of the segregating genes govering FHB resistance in the F3 populations varied between 5 - 8. Although this number is little higher than other researchers, estimations, however, it confirms the oligogenic nature of this trait. This nature has been confirmed by others: Yu (1982); Liao and Yu (1985); Yu et al. (1986); Zhou et al. (1987); Bai et al. (1989); Yu (1991); Bai (1995); Singh et al. (1995); Van Ginkel et al. (1996) and Yao et al. (1997). For instance, Zhou et al. (1987) estimated two, Bai et al. (1989) three major resistance genes in the cultivar Sumai3 and Snijder ranged from 1 - 6 in different populations using quantitative genetic methods. Similarly, in Ning7840, a cultivar descended from Sumai3, two (Van Ginkel et al., 1996) and three (Bai, 1995) major resistance genes have been estimated. These differences among different researchers may be due to that these estimations of number of genes depend to certain extend on the nature of the population used, the type of resistance considered and the calculated range which depends on the extreme genotypes in the population.

However, the results of the quantitative methods used to calculate the number of genes should be viewed with caution. Besides the fact that Wright's and Cockerham's equations are not very accurate estimators, possibly also not all assumptions were met. The estimates of number of genes are based on independent assortment. If linkage occurs, the number of genes really present could be higher than the estimated number. Nevertheless, it is clear that FHB resistance governed by several minor genes (Snijders, 1990).

4.1.4 Phenotypic correlations among environments for FHB trait

Correlations for FHB severity between the two years for Gruenbach environment was high (0.81) suggesting a weak genotype X year interaction for FHB symptom expression. Also, these results indicated that the expression of genotypes for FHB resistance did not change markedly between the two years, which indicated the constancy of this population under these environmental conditions in

Gruenbach. Marina et al. (2000) did not agree with previous results where they found low values of correlation among years for FHB resistance in barley. This disagreement may be due to the difference between wheat and barley in their response to FHB disease and also, it may be due to highly changeable conditions of the environments studied by (Marina et al., 2000).

The correlations among different environments were highly significant with values ranged from 0.22 and 0.67. This range explained that, although there were significant correlations, the values were high among some environments and were low and moderate among others. These findings indicating that genotype X environment interaction was present. de la Pena et al. (1999) agreed, in part, with the findings of this research where they showed positive correlations among environments in two out of six cases according to their study on FHB resistance in barley.

4.2 Phenotypic correlations between FHB resistance and plant height and heading date

In this study, significant correlation between FHB resistance and plant height and heading date were noticed. This correlation was stronger between FHB resistance and heading date than between FHB resistance and plant height. Therefore, in the population under study at the environmental conditions of this study, the early heading date and shortness may be used as morphological markers for the presence of FHB resistance. This correlation was mentioned, before by Mesterhazy (1995) and Miedaner (1997) in wheat where they agreed that the inflorescence structure, maturity and plant height traits are associated with FHB resistance. However, Love and Seitz (1987) could not identify any morphological characters that correlate with susceptibility in USA wheat material. The same phenomenon was found in barley where Takeda (1990) and Steffenson (1998) indicated a correlation between heading date and plant height and FHB resistance. Also, de la Pena et al. (1999) confirmed these findings where they demonstrated negative correlation between the FHB resistance and these traits. However, Zhu et al. (1999) in barley could not find any correlation between these traits and FHB resistance. Therefore, it could be concluded from these discrepancies that the association between these traits and FHB resistance is not consistent (Zhou et al.,

1988; Lu et al., 1990; and Yu, 1990). Thus, morphological characters such as plant height and heading date cannot be the only markers to be facilitated in

breeding programs for FHB resistance and consequently in marker assisted selection. Therefore, the DNA-based markers may provide a powerful tool for improved selection in breeding for FHB resistance. The problems of inconsistent association with morphological markers can be eliminated by the use of molecular markers since the genomic DNA should remain constant under different environments and at all stages of growth.

4.3 Genetic mapping of gene loci for FHB resistance

4.3.1 Polymorphism detected by different marker systems

As it is mentioned before, the use of resistant cultivar is the best way to control the FHB disease (Bai and Shaner, 1994). Also, because FHB resistance is quantitatively inherited and evaluation of resistance is complicated and requires special equipment, breeding for FHB resistance with traditional methods requires substantial time and effort. Therefore, molecular identification of QTL has become the method of choice for FHB resistance. After a linkage between a QTL and molecular markers has been determined, the QTL can be transferred selectively into different genetic backgrounds by marker-assisted selection because these markers are not influenced by the environment which can be scored at all stages of plant growth. Therefore, it is important to start mapping protocol with group of markers which showed a high level of polymorphism in the organism under study. In this study, the large genetic distance between the two parental lines "Sgv.NB x MM.Sum3". and "Apollo" provided a high degree of polymorphism and a sufficient number of offspring differing in their FHB resistance.

Wheat is a segmental allopolyploid containing three distinct but genetically related (homoeologous) genomes, A, B and D. The haploid content of bread wheat genome is approximately 1.7 X 10^7 bp (Arumuganthan and Earle, 1991) with an average of 810 Mb per chromosome. Such a large genome of hexaploid wheat has resulted from polyploidy and extensive duplications, such that over 80% of the genome consists of repetitive DNA sequences (Gupta et al., 1999). For these reasons, there have been problems in the preparation of molecular maps and in

the development of markers for marker-aided selection in wheat. The main problem has been the failure of a variety of molecular markers to detect adequate and useful polymorphism. However, despite these problems, success has been achieved in recent years and molecular maps have become available for chromosomes of all homoeoelogous groups in wheat by using different types of molecular markers. In this research three types of molecular markers, namely RFLPs (hybridization-based DNA markers), AFLPs (PCR-based DNA markers) and SSRs (PCR-based DNA markers), were used to construct a linkage map to locate QTLs responsible for FHB resistance in hexaploid wheat. Each type of these markers has its own advantages in studying and demonstrating the genetic variations in wheat and consequently, among the parents and F_3 populations produced from the cross between the two parents used.

Among the various molecular markers used in this study, RFLPs have more limitations than others, since RFLP analysis is time-consuming and labour-intensive. In this study, 60 probes were screened and only 16 were useful for further studies. This result is in agreement with other researchers who indicated that RFLP analysis identified low level of polymorphism in wheat and because of low frequency of RFLPs, this approach has been relatively less useful in this crop. (Chao et al., 1989; Kam-Morgan et al., 1989 and Liu et al., 1990; and Cadalen et al., 1997). This low frequency may be attributed to the polyploid nature, high proportion of repetitive DNA, large genome size and recent origin of wheat. Despite of these difficulties, sufficient advantages have resulted in the application of RFLP technology in constructing the genetic map of the F_3 population. One of these advantages is that detailed RFLP linkage maps (Chao et al., 1989; Devos and Gale, 1993a; Nelson et al., 1995a,b and c; and Marino et al., 1996) have been published for all seven homologous groups, therefore, they become locus-specific in the genome of wheat. The 60 probes used in this study were specific for certain alleles of the wheat genome.

On the other hand, the AFLP is combining the features of RFLP and PCR while avoiding the disadvantages encountered in these markers. In this study, 65 markers combinations were screened and they showed a total of 6700 fragments. AFLPs amplified 103 fragments per primer combination and displayed polymorphisms between the two parents with almost every primer combination that ampli-

fied a clearly separated band profile. This large number of fragments was expected because AFLP is a powerful technique in generating large numbers of markers for the construction of high density genetic maps (Van Eck et al., 1995; Keim et al., 1997; Qi et al., 1998). The results obtained in this research showed that each primer combination in AFLP analysis detected 7 times more polymorphism than polymorphic probe in RFLP analysis. This result is in agreement with the results obtained by Ma and Lapitan (1998) who stated that; „In wheat, a single primer combination could detect up to eight times more polymorphism than a polymorphic RFLP probe". Also, other researchers (Barret and Kidwell, 1998; Burkhamer et al., 1998; Parker et al., 1998; Bai et al., 1999; Hartl et al., 1999) demonstrated that the AFLP markers in common wheat were useful in the evaluation of genetic diversity and identification of quantitative resistance genes because they produced high level of polymorphism. On the other hand, since the wheat genome is relatively large too many bands may be produced by some AFLP primer pairs. This may be a disadvantage of the application of AFLPs in wheat. When too many bands are produced with a primer combination, the bands are difficult to score. Thus, primer pairs with three selective nucleotides were used in this study, which recommended by (Bai et al., 1999).

Finally, microsatellite markers detected 2.6 times more polymorphim than RFLP markers. Although, the SSRs indicated less polymorphism than AFLPs, however, they were very informative, since they are chromosome-specific and evenly distributed along chromosmes (Roeder et al., 1998). In addition, Plaschke et al. (1995); Roeder et al. (1995); Ma et al. (1996); Bryan et al. (1997), agreed that SSRs showed a much higher polymorphism and informativeness in hexaploid wheat than any other marker systems. However, due to the large genome size, the development of microsatellite markers in wheat is extremely time-consuming and expensive (Roeder et al., 1998). The percentage of polymorphic SSRs used in this study was 60% of the total number of tested SSRs, although only 47% were useful. However, this number was higher than the numbers showed by Roeder et al. (1995) and Ma et al. (1996) who found that the percentage of polymorphic microsatellite were about 22% and 36% in common wheat, respectively. This discrepency may be due to that these two researchers used larger numbers of SSRs.

4.3.2 Linkage map

The population consisting of 180 F_3 families from the cross between the two parents "Sgv.NB x MM.Sum3" and "Apollo" proved to be suitable for constructing of a linkage map and in detecting QTL for FHB resistance. Since the number of loci controlling quantitative traits are large, the use of a large mapping population will give more chance in detecting these QTLs (Kicherer et al., 2000). Such large population sizes have been used by others such as Keller et al. (1999); Messmer et al. (2000) and Sourdille et al. (2000) who used progenies map of 226, 204 and 217 individuals, respectively, for constructing genetic maps. Although, other researchers used mapping populations with smaller numbers such as Bai et al. (1999); de la Pena et al. (1999); Mingeot and Jacquemin, (1999); Waldron et al. (1999) and Toojinda et al. (2000). These differences in the size of the mapping population may depend on the genome size of the organism, the generation of mapping population and the nature of the inheritance of the trait under study (Beavis, 1998).

In this study, 227 different polymorphic markers were used to construct the linkage map of 180 F_3 families. 77 were mapped on B genome, 29 on A genome and 12 on D genome and 32 were on unidentified linkage groups with a total of 150 molecular marker loci. The 150 polymorphic marker loci did not cover the whole genome. According to the extended linkage map data from integrated genetic maps of wheat (McGuire and Qualset, 1997) the average chromosome length varied between 150 and 200 cM which would result in a total genome length of 3200 to 4200 cM. The map of this study spans 1656.7 cM, therefore it might cover half of the wheat genome. The distribution of the marker loci across the 21 chromosomes indicated that there are more markers identified on B genome than on other genomes which covered approximately 2/3(66%) from markers in this study on the genome B according to the genetic map of wheat (McGuire and Qualset, 1997). This result is in harmony with genetic maps obtained by Keller et al. (1999) and Messmer et al. (1999, 2000) . The percentage of markers assigned to the respective genomes in this research is in good agreement with the numbers obtained from other researchers. Liu and Tsunewaki (1991) by using the RFLP markers, found only half as much polymorphism in the D genome as in the A and B genomes in mapping a progeny from a cross of Chinese Spring and *T. aestivum*

var. *spelta*. While Devos et al. (1992) observed approximately the same difference in polymorphism levels between 3A and 3B versus 3D in a study of several wheat varieties. Nelson et al. (1995 a) in a study of group 3 chromosomes found that only 25% of the surveyed RFLPs mapped to the D genome. This was also true in another study by Nelson et al. (1995 c) of groups 4, 5 and 7 chromosomes where only 21% of marker loci mapped to D genome. Finally, Marino et al. (1996) assigned 38% of 154 RFLP markers to 6A, 33% to 6B and 29% to 6D in the study of group 6 chromosomes. The same results were obtained by Roeder et al. (1998) who found that out of 279 microsatellite markers, 93 mapped to the A genome, 115 to B genome and 71 to D genome.

Thus it can be concluded, that the number of markers assigned to each genome is in part a reflection of the relative amount of genetic variation present among the different genomes of wheat. Therefore, in order to increase the number of A and D genome markers, they could be isolated from *T. monococcum* or *T. tauschii,* respectively. This idea of the potential of *Aegilops tauschii* the diploid progenitor of the D genome of wheat as a source of microsatellite markers for hexaploid bread wheat was investigated by Pestsova et al. (2000). Their obtained data confirmed the idea where all primer pairs that were functional in *Ae. tauschii* amplified well in hexaploid wheat which extended the existing wheat microsatellite map.

Also, using new developed systems of molecular markers like single nucleotide polymorphisms (SNPs, Gupta et al., 1999) may help in increasing the number of markers in different genomes including the A and D genomes. The use of SNPs system, which led to rapid advancement in developing human genetic map, would offer rapid and high automated genotyping of wheat genome (Gupta et al., 1999).

4.4 Detection of QTLs for FHB resistance

For the mapping of QTL for resistance to disease, consistent disease pressure is critical for accurate assessment of the resistance potential of plant genotypes and for determination of the magnitude of the genetic factors that contribute to resistance. This is especially important for FHB, since environment is one of the major determining factors for initiation and development of FHB. In this study, 180 F_3 families in five different environments were studied to minimize environmental variation. At the same time, 227 markers were used to construct the genetic map

yielding 150 loci that formed 28 linkage groups with 77 of the markers unlinked. Twelve QTLs for FHB resistance were detected to have influence on the expression of the FHB resistance. The number and location of significant QTL detected for FHB resistance varied among the five environments. Since single environment differed in the development of infection after inoculation and consequently in the infection pressure and most likely in the pathogen population, therefore, different genes might be relevant for resistance in different environments. Also, since the FHB resistance is controlled by group of QTLs which affected by environment, therefore, gene expression of these QTLs would differ in different environments (Bai et al., 1999; de la Pena et al., 1999; Keller et al., 1999; Messmer et al., 2000). This can be explained from the QTLs, which were identified in some environments at low LOD values less than 3.0 (insignificant) and their contributions to the phenotypic variance were (R^2) insignificant too. One of these QTLs, which was identified on 4LG in position 12 cM at LOD 3.7 in Gruenbach environment (1998 and 1999) was detected in the same support interval at approximately LOD 2.4 across environments (Figure. 8b in Appendix). Also, another QTL which was appointed in two different environments; at Hadmersleben and Herzogenaurach environments on chromosome 5A in the same support interval at LOD 3.2 and at LOD 2.7, respectively (Figure 8a in Appendix). These low values of LOD in one environment explained the low contribution of the QTL to the phenotypic variance in this environment. In other words, these QTLs are present at the specific loci and the different factors of environment pressure were not strong enough to let these QTLs to be expressed at the maximum level. Therefore, in the proper environments, the same QTLs showed high level of gene expression (LOD more than 3.0). However, in order to determine QTL that are important for the expression of the trait under different environmental conditions, QTL analysis was performed on the basis of the phenotypic values averaged over across environments and four QTLs were determined (Table 15).

Fusarium head blight resistant genes are reportedly cultivar dependent (Waldron et al. (1999) and are correlated to the parents of the mapping population. Using monosomic analysis, Yu (1982) located genes for resistance to FHB in cultivar Sumai3 on five chromosomes (2A, 5A, 1B, 6D and 7D), while Ban and Suenaga (1997), on the basis of the linkage with a suppressor gene for awned-

ness, located one of the resistance genes from Sumai3 on the long arm of chromosome 5A or 6B. Buerstmayr et al. (1997) positioned FHB resistance genes in one Sumai3 derivative on chromosmes 5A, 1B, 3B, 4B, 6B and 6D and in another derivative on chromosomes 3A, 3B, 6B and 4D. Therefore, results of this study partially agree with those of monosomic studies, where the chromosomes 5A, 3B, 6B were tagged. Those researchers used the Sumai3 as a parent for identifying the genes for resistance to FHB and at the time, the composed line "Sgv.NB x MM.Sum3". parent was used in this study as a parent for the mapping population. Therefore, this population is a derivative of Sumai3. Also, chromosomes 4A, 5A, 7A, 7B, 4D, 2A, 5B, 6B, 3D and 7D were involved in controlling FHB resistance in cultivars; Wangshiubai and Wenzhouhongheshang (Yu et al., 1986) and chromosomes 7A, 3B, 5B , 6B, 6D, 3A and 4D were found to control the FHb reistance in five other Chinese cultivars (Yu, 1991). In a study of five sets of substitution lines, Grausgruber et al. (1997) found six chromosomes 4B, 3A, 6B, 5B, 2A and 6D. Finally, Buerstmayr et al. (1999) used the parental winter wheat line U-136.1(dreived from"Sgv.NB x MM.Sum3") and the cultivar Hobbit-sib in back-cross reciprocal maonosomic analysis and found resistant genes on chromosomes 5A, 1B, 3B, 4B, 6B, 6D. Their results agreed with the findings of this study in identifying genes on chromosomes 5A, 3B and 6B.

From previous studies using the monosomic analysis, it can be concluded that, Fusarium head blight resistant genes are located throughout the genome of wheat (Bai and Shanner, 1994; and Buerstmayr et al., 1997).

In this study, the four QTLs determined for FHB resistance using different marker systems were contributed from the second parent "Sgv.NB x MM.Sum3". There is no complete agreement between different studies except where the same parents were used. The results obtained in this study agree with those of Waldron et al. (1999), who found QTLs on 3BS, 2AL, 4BL and 6BS with phenotypic variances (R^2) of 15%, 14%, 7.2% and 6%, respectively. Also, it agrees with those by Anderson et al. (1999) who used RFLP, microsatellite and AFLP marker systems and they found one QTL on chromosmes 3BS (R^2 16%) in two populations derived from crosses between Sumai3 x Stao and ND2603 x Puta. They mapped a QTL to the telomeric end of the chromosome 3BS. At the same time, in this research, a QTL was mapped on the telomeric end of the same chromosome on the

short arm, which explained approximately 12% of the phenotypic variance. Also, these resultes were confirmed by the findings of monosomic analysis by Yu et al. (1986) and Buerstmayr et al. (1997). Therefore, it is likely that this 3B QTL is the same QTL detected by Waldron et al. (1999) and by Anderson et al. (1999). Another explanation is that the QTL from this investigation is closely linked to their QTLs, which may indicate the presence of a cluster of resistance genes in this area of chromosome. Also, Waldron et al. (1999) have identified minor QTL on chromosome 6BS where the data of this research indicated a major QTL on the same chromosome arm. Thus, there is a partial agreement between their study and our study in this region of the chromosome arm.

The results of this research indicated the presence of four putative QTLs (4, 5, 7 and 9 LG) with no chromosomal of location which were found in single environment each. Similar results were obtained by Bai et al. (1997). The authors identified two QTLs for FHB resistance and several AFLP markers associated with these in recombinant inbred lines from a cross between the resistance Chinese wheat Ning 7850 and the susceptible cultivar Clark. Also, Moreno-Sevilla et al. (1997), found 12 genomic regions containing putative QTL associated with FHB resistance in a population derived from a cross of Sumai3 with Stoa. Finally, Bai et al. (1999) found that 11 AFLP marker loci showed significant association with FHB resistance and an individual marker explained up to 53% of the total variation R^2. Further studies on the same population may be needed to assign these linkage groups to their respective chromosomes. Although these markers are not assigned to certain chromosomes, they may be useful in marker-assisted breeding to improve resistance to FHB.

The findings of this research that two QTLs were identified on chromosomes 5A and 7B were confirmed by the monosomic studies (Yu et al., 1986 and Buerstmayr et al., 1999). Also, Bai et al (1999) suggested that the homoeologous group 7 of the wheat genome seems to play an important role in FHB resistance. These two QTLs were first identified using molecular marker analysis which confirmed that FHB resistance is a quantitative trait. Also, the values of R^2 (between 8.5% and 8.8% from the phenotypic variance) indicated that these QTLs are minor genes of the quantitative trait.

Although the monosomic studies showed some genes on D genome for FHB resistance, the findings of this study did not indicate any QTL on D genome, which was confirmed by Procunier et al. (1998) with microsatellite markers who suggested that the Sumai3 D genome lacks major FHB resistance genes. This discrepancy, may be due to several factors including genetic background effects from the susceptible parents that result in some QTL being masked while others are more pronounced or incomplete marker map coverage of genome D.

4.5 Sources of alleles resistance

Fusarium head blight resistant genes are located throughout the genome and are cultivar dependent (Buerstmayr et al., 1997). In this study, it was found that four putative QTLs were derived from "Sgv.NB x MM.Sum3" parent across environments and other 11 QTLs were found in studying every single environment. Some of the late QTLs were derived from susceptible parent "Apollo" and some others from the resistant parent "Sgv.NB x MM.Sum3". These findings further confirm that FHB resistance is a quantitative trait and that apparently susceptible parents may contain resistant alleles may not found in resistant cultivars. Other studies have indicated the presence of resistance genes in susceptible and moderately susceptible cultivars (Snijders, 1990; Singh et al., 1995; Keller et al., 1999; Lueberstedt et al., 1999; Xia et al., 1999; Toojinde et al., 2000). Therefore, it can be concluded that the moderately susceptible parent "Apollo" may contain some resistance alleles that when combined with alleles from resistant parent can result in increased level of resistance. This finding was confirmed by a previous study by (de la Pena et al., 1999; Zhu et al., 1999; Waldron et al., 1999).

4.6 QTL X environment interactions

Breeding for FHB resistance has been difficult due to the multiple components of resistance, the limited understanding of the genetic basis and the genotype X environment interaction (Wiersma et al., 1996; McMullen et al., 1997; Zhu et al., 1999). Therefore, the biological basis of G X E may not fully understood because of both, the underlying environmental and the genetic complexity (Hayward et al., 1993).

In this research, two QTLs, on 5A and 3BS, showed insignificant QTL X environment interactions. Although, the additive effects of these QTLs did not demonstrate significant differences among the different environments, they appeared only in two environments, at LOD score more than 3.0 (Table 14). Also, they were present with high significant LOD values in the across environments analysis. Therefore, they can be considered to show partial stability or adaptation.

However, the 6BS and 7BS/5BL QTLs were identified across environments. They indicated significant QTL X environment interactions where they showed highly significant differences among their additive effect values. They showed markedly low additive effects in every single environment, however they indicated high additive effect values in the across environments analysis. Because of the fact that the FHB resistance is a quantitative trait which is highly affected by the environments, the adaptation conditions and the interaction between the genotype x environment play a great role in the expression of these specific QTLs. In other words, these high effects of environmental conditions on these QTLs may be due to the quantitative nature of this trait and to the different factors of environmental pressure. These results suggest that the environmental effects should be considered during the process of marker-assisted selection for these two QTLs (Keller et al., 1999).

4.7 Localization of QTLs for plant height and heading date

In this study, three QTLs were detected for plant height on chromosomes 3BS, 6BS and 7BS/5BL and they were contributed from the short parent "Sgv.NB x MM.Sum3". As for heading date, six QTLs were identified in this research on 3A, 6AL, 2BL, 4BL, 6BL and 7BS/5BL chromosomes. There was a mapping report by Sourdille et al. (2000) who found three QTLS on 2BS, 5AL, 7BS chromosomes for heading date using. Furthermore, Keller et al. (1999) found 11 QTLs for plant height on chromosomes 1A, 2A, 4A, 5A, 6A, 1B, 4B, 5B and 7B while they detected 10 QTLs for heading date on 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 7A and 7B. In addition, Shah et al. (1999) appointed a QTL on 3AL chromosome for plant height and a QTL on 3A for heading date. Miura et al. (1999) identified two genes affecting earliness on chromosome 3A. Cadalen et al. (1998) using RFLP markers found QTLs on 4A, 4D, 7A and 7B for plant height. Kato et al. (1998) identified 3

QTLs on 5AL chromosome for plant height and indicated two QTLs for heading date on chromosome 5A. Also, Hyne et al. (1994) located QTLs for plant height on 6B and 7B and identified two QTLs on 7A and 7D chromosomes for heading date. Miura and Worland (1994) found a QTL on chromosome 3A for heading date. Roberts (1990) found another QTL on 5A for plant height. Hoogendoom (1985) detected genes involved in earliness on chromosomes 3A, 4A, 4D, 6B and 7B. Finally, Law et al. (1976) mapped the heading date on 5A and 5D and plant height on chromosome 2B. The results of this study agree with other reports for the location of QTLs for plant height and heading date except, the QTL on chromosome 3BS for plant height was not identified by others. In this study QTLs for heading date and plant height were found on 7BS/5BL in the same support interval. This finding agrees with the report of Keller et al. (1999) for plant height and heading date. This observation may indicate that these traits are linked to each other. Our results and those of other researchers indicate that QTLs for these two traits are spread over the genome. Also, genomes A and B play great role in controlling these traits and genome D has very little effect on these traits.

4.8 Coincidence of QTLs for plant height and heading date with FHB resistance

Significant phenotypic correlations among resistance to FHB, plant height and heading date were noticed in this study. These phenotypic correlations appear to be due in part, to coincidences of QTLs for these traits indicating the possibility that these QTL are either tightly linked or have pleiotropic effects. On chromosome 7BS/5BL, the QTL on position 28 cM spanning markers XE38/M52-91 to Xgmwm573 with support interval 20-38 is associated with a QTL for FHB resistance. While, a QTL for heading date and another one for plant height were assigned to chromosome 7BS/5BL between the same flanking markers at 24 and 19 cM regions with support intervals of 14 - 28 cM and 13 - 28 cM, respectively. These results indicated that the identified QTLs for the three different traits under study were located in the same marker interval on the genetic map. The position of these QTLs are very close to each other since their support interval was describing the most likely resistance of these QTL, overlapped. As for heading date and plant height, these QTLs most probably are at the same locus displaying,

these QTLs are either tightly linked or have pleiotropic effects. These results were in concordance with results from Keller et al. (1999) who identified heading date and plant height QTLs on chromosome 7BS in the same interval of their map. Since there was a correlation between early heading date and shorter plant height and FHB resistance, there are two possibilities: either the observed coincidence of these QTLs is due to close linkage between different loci or to pleiotropic effects of the same locus. Since, it seems likely that early heading and shorter plants have lower levels of FHB severity, then it is more likely that this situation is one locus situation, with pleiotropic effects. Early heading and short plants are desirable traits in wheat therefore, their association with FHB resistance is very useful in wheat improvement.

4.9 Perspectives for marker-assisted selection

Early generation selection for FHB resistance in the field is difficult and unpredictable, and genotype screening requires replications and is resource intensive. Consequently, marker-assisted selection could be used to facilitate the transfer of these genes for FHB resistance into well adapted genotypes. However, there are certain considerations need to be taken when the decision of which QTL should be emphasized in MAS strategy. It is necessary to be decided which region(s) has enough evidence for the presence of a QTL. This can be achieved by setting appropriate thresholds (LOD more than 3) for the identification of the QTLs and by detecting QTLs in the same region in different environments. In this study, two QTLs on chromosomes 3BS and 5AS fulfilled these criteria. They showed high values of LOD scores and they were detected in more than one environment. In addition, they were appointed in across environments analysis. Also, in the population under study, selection for FHB resistance genotypes can be done through MAS on The QTLs on The chromosomes 3BS, 5AL and 7BS/5BL. Beside MAS for FHB resistance, selection can be done by plant height and heading date to assure these traits correlated with FHB resistance for the plants which selected by MAS. Finally, The selected QTL for MAS should indicate high levels contribution to the phenotypic variance which measured by R^2. However, this consideration should be taken with caution because these values can be significantly influenced by population sizes and the number of markers used in the multi-locus model (Beavis, 1998). In simulation studies, QTL analyses using population sizes of 100 identified only subset of the total number of simulated QTLs and often over-estimated their effects (de la Pena et al., 1999).

It can be concluded that the QTLs on chromosomes 3BS and 5AS, which were constant under different environmental conditions, are promising candidates for MAS. Also, the QTLs on chromosome 7BS/5BL, which indicated that these QTLs are either tightly linked or have pleiotropic effects with other correlated phenotypic traits.

5 Summary

The FHB mapping population consisted of 180 F_3 families developed from a cross between the variety "Apollo" (susceptible) and the breeding line "Sgv.NB x MM.Sum3" (resistant). The FHB severity was evaluated in five locations. At the same time, two other traits namely, plant height and heading date were recorded to determine their correlation to FHB disease resistance.

The map was constructed using the data of 227 molecular markers (161 AFLPs, 16 RFLPs and 50 SSRs) on the 180 F_3 families using the F_2 model in the Map-maker program. The results showed that out of the 227 markers, 150 (66.1%) markers were grouped to construct the genetic linkage map. The base map spans 1656,7 cM with a total number of 27 linkage groups. 77 markers were mapped on B genome, 29 on A genome and 12 on D genome and 32 were on unidentified linkage groups with a total of 150 molecular marker loci. The five chromosomes 1B, 2B, 6B, 7BS/5BL, 5A and 5LG and one unknown chromosome 5LG included the highest number of loci.

The individual QTLs for FHB resistance, plant height and heading date were identified by the CIM analysis using the PLABQTL programe. In single environment analysis twelve QTL regions for FHB resistance were detected on chromosomes 5A, 3B, 6B, 7BS/5BL and the linkage groups 4LG, 5LG, 7LG, and 9 LG. The percentage of the phenotypic variance R^2 explained by a single QTL ranged from 7.4% to 11.3%. The total amount of R^2 explained by all QTL varied between 8.3% in Bergen and 45.5% in Herzogenaurach. Averaged over four different environments, four QTLs were detected for FHB resistance (derived from "Sgv.NB x MM.Sum3") on 5AS, 3BS, 6BS and 7BS/5BL where they explained 37.0% of the phenotypic variance.

In this study, three QTLs were detected for plant height on chromosomes 3BS, 6BS and 7BS/5BL and they were contributed from the short parent Sgv.NB x MM.Sum3". As for heading date, five QTLs were identified on 3A, 6AL, 4BL, 6BL and 7BS/5BL . At these loci were from the parent "Sgv.NB x MM.Sum3", while one QTL on chromosome 2BL was derived from the parent "Apollo". The total phenotypic variance R^2 was explained 23.9 and 46.8 for plant height and heading date, respectively.

The QTL on chromosome 7BS/5BL was located at a close support interval to the QTLs for shorter plant height, early heading date and higher FHB resistance, which indicated that these QTLs are either tightly linked or have pleiotropic effects with other correlated phenotypic traits in this study.

In the population under study, selection for FHB resistance genotypes can be done through marker-assisted selection (MAS) for the QTLs on the chromosomes 3BS, 5AL and 7BS/5BL. Beside MAS for FHB resistance, selection can be done by plant height and heading date to assure these traits correlated with FHB resistance for plants which selected by MAS.

6 References

Anderson AL (1948) The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:595-611.

Anderson AJ, Waldron BL, Moreno-Sevilla B, Stack RW, Frohberg RC (1998) Detection of *Fusarium* head blight resistance QTL in wheat using AFLPs and RFLPs. In Slinkard AE (eds) Proc 9th International Wheat Genetics Symposium. Saskatoon, Univ Extension Press, Univ of Sakatchewan, Saskatoon, Canada 3:135-137.

Anderson AJ, Waldron BL, Moreno-Sevilla B, Stack RW, Frohberg RC (1999) Update on DNA markers for *Fusarium* head blight resistance QTL in two wheat populations. In Jennifer et al. (eds) Proc 1999 National *Fusarium* Head Blight Forum. USA Univesity Printing, East Lansing, Minnesota:19-21.

Arumuganathan E, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208-218.

Arus P, Moreno-Gonzalez J (1993) Marker-assisted selelction. In Plant Breeding: Principles and Prospects. In Hayward MD, Bosemark MD, Romagosa I (eds) Publishing Champman and Hall, London:314-331.

Bai GH (1995) Scab of wheat: epidemiology, inheritance of resistance and molecular linked to cultivar resistance PhD thesis. Purdue Univ, W. Lafayette, Indiana, USA.

Bai GH, Dweikat I, Shaner GE (1995) Identification of QTLs for scab resistance in wheat by means of RAPD markers. Phytopathology 85:1201.

Bai GH, Kolb FL, Shaner GE, Domier LL (1997) Mapping QTL for wheat scab resistance using AFLPs. In Dill-Macky R , Jones RK (eds) Proc. National *Fusarium* Head Blight Forum. St Paul, MN, 10-12 Nov, 1997. Univ of Minnesota, St Paul:27.

Bai GH, Kolb FL, Shaner GE, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343-348.

Bai GH, Shaner GE, Ohm, H (1993) Inheritance of resistance to *Fusarium graminearum* in eight wheat cultivars. Phytopathology 83:1414.

Bai GH, Shaner GE (1994) Scab of wheat prospects and control. Plant Dis 78:760-766.

Bai GH, Shaner GE (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis 80:975-979.

Bai GH, Shaner GE, Ohm H (2000) Inheritance of resistance to *Fusarium graminearum* in wheat. Theor Appl Genet 100:1-8.

Bai GH, Xiao QP (1989) Genetic analysis on resistance to scab in six wheat cultivars. Acta Agric Shanghai 5:17-23.

Bai GH, Zhou CF, Ge YF, Qian C-M, Chen ZD, Yao GC (1989) A sudy on scab resistance in new wheat cultivars and advanced lines. Jiangsu Agric Sci 7:20-22.

Bai GH, Zhou CF, Qian C-M, Xia SS, Ge YF(1989) Analysis on combining ability of resistance to scab and other characters in eight wheat cultivars. Jiangsu Agric Sci 1:79-83.

Bai GH, Zhou CF, Qian C-M, Ge YF (1990) Genetic analysis on resistance to scab extension in wheat cultivars. In Zhu LH (eds) Advance in genetic researches on resistance to diseases in major crops. Zhu LH, ed Jiangsu Sci-Tech Publising House, Nanjing, China:171-177.

Baird RE (1993) Effects of foliar fungicides on wheat, 1992. Fungic Nematicide Tests 48:223.

Ban T, Suenaga K (1997) Inheritance of resistance to *Fusarium* head blight caused by *F. graminearum* in wheat. Cereal Res Commum 25:727-728.

Ban T (1997) Genetic analysis of *Fusarium* head blight resistance using wheat doubled haploids. in Dubin HL, Gilchrist L, Reeves J, McNab A (eds) *Fusarium* head scab. global status and future prospects CIMMYT, DF, Mexico:71-78.

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci 38:1261-1271.

Barrett BA, Kidwell KK, Fox PN (1998) Comparison of AFLP and pedigree-based genetic diversity assessment methods using wheat cultivars from the Pacific Northwest. Crop Sci 38:1271-1278.

Beavis WD (1998) QTL Analysis: power, precision and accuracy. In Paterson AH (eds) Molecular dissection of complex trait. CRC Press, Boca Raton, Fla:145-162.

Beckmann JS, Soller M (1986) Restriction fragment length polymorphisms in plant genetic improvement .Oxford Surv Plant Mol Cell Biol 3:197-250.

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27:835-845.

Bennett FT (1931) Gibberella saubinetii (Mont) Sacc. on British cereals. II Physiological and pathological studies. Ann Appl Biol 18:158-177.

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale D (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557-563.

Buerstmayr H, Lemmens M, Ruckenbauer P (1997) Chromosomal location of *Fusarium* head blight resistance genes in wheat. Cereal Res Commun 25:731-732.

Buerstmayr H, Lemmens M, Fedak G, Ruckenbauer P (1999) Back-cross reciprocal monsomic analysis of *Fusarium* head blight resistance in wheat. Theor Appl Genet 98:76-85.

Burkhamer RL, Lanning SP, Martens RJ, Martin JM, Talbert LE (1998) Predicting progeny variance from parental divergence in hard red spring wheat. Crop Sci 38:243-248.

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An in tervaietal molecular marker map in Triticum aestivum L. em. THell., and comparison with a map from a wide cross. Theor Appl Genet 94:367-377.

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leeroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled-haploid population. Theor Appl Genet 96:933-940.

Caetano-Anolles G, Basam J, Gresshoff PM (1991) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology 9:553-557.

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495-504.

Chen CH (1983) A study on the inheritance of scab-resistance in wheat. Acta Agric Univ Zhejiangensis 9:115-126.

Chen JL (1989) Research on the inheritance of resistance to scab in the famous scab-resistant cultivar Sumai3 and discussion on strategy of scab resistance breeding in wheat. Shannxi J Agric Sci 2:2-12.

Clarke B, Stancombe P, Money T, Foote T, Moore G (1992) Targeting deletion (homeologous chromosome pairing locus) or addition line single copy sequences from cereal genomes.Nucl Acids Res 20:1289-1292.

Clear RM, Abramson D (1986) Occurrence of *Fusarium* head blight and deoxynivalenol (vomitoxin) in two saples of Manitoba wheat in 1984. Can Plant Dis Surv 66(1):9-11.

Cockerham CC (1983) A study on the genetics of resistance to wheat scab. Sci Agric Sin 6:61-64.

de la Pena RC, Smith KP, Capettini F, Muehibauer GJ, Gallo-Meagher M, Dill-Macky R, Somers DA, Rasmusson DC (1999) Quantitative trait loci associated with resistance to *Fusarium* head blight and kernel discoloration in barley. Theor Appl Genet 99:561-569.

Devos KM, Atkinson MD, Chinoy CN, Gale MD (1992) RFLP-based genetic map of the homoelogous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931-939.

Devos KM, Bryan GJ, Collins AJ, Stephenson P, Gale (1995) Application Of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90:247-252.

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group 3 chromosomes of wheat , rye and barley. Theor Appl Genet 85:469-652.

diaz de Ackermann M, Kohli MM (1996) Research on *Fusarium* head blight of wheat in Uruguay. in Dubin HL, Gilchrist L, Reeves J, McNab A (eds) *Fusarium* head scab. global status and future prospects CIMMYT, DF, Mexico:13-18.

Eastwood RF, Lagudah ES, Appels R (1994) A direct search for DNA sequences tightly lined to cereal cyst nematode resistance genes in *Triticum tauschii*. Genome 37:311-319.

Falconer DS (1989) Introduction to Quantitative Genetics, 3th edn. Longman, England, Essex.

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragments to high specific activity. Anal Biochem 132:6-13.

Fischer SG, Lerman LS (1983) DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: Correspondence with melting theory. proc Natl Acad Sci USA 80:1579-1583.

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jai J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat .In Chen S (eds), Proc. 8th International Wheat Genetics Symposium. 29-40. China Agricultural Scientech Press, Beijing:29-40.

Galich MTV de (1996) Fusarium head blight in Argentina. in Dubin HL, Gilchrist L, Reeves J, McNab A (eds.) *Fusarium* head scab: global status and future prospects. CIMMYT, DF, Mexico:19-28.

Gilbert J, Leisle D, Procunier JD, Aung T, Fedak G, Tekauz A (1995) Status of breeding for resistance to *Fusarium* head blight in spring wheat in western Canda. International Seminar on *Fusarium*:Mycotoxins, Taxonomy and Pathogenicity:136.

Goodwin SB, Hu X, Shaner G (1998) An AFLP marker linked to a gene for resistance to *Septoria tritici* blotch in wheat. In Slinkard AE (eds) Proc 9th International Wheat Genetics Symposium. Univ Extension Press, Univ of Sakatchewan, Saskatoon 3:108-110

Gottlieb L D (1986) The genetic basis of plant form. Philos. Trans R Soc Lond 313: 197-208.

Grausgruber H, Lemmens M, Buerstmayr H, Ruckenbauer P (1997) Genetic analysis of *Fusarium* head blight resistance and toxin tolerance in wheat, using inter-varietal chromosome substituttion lines. Cereal Res Commun 25:743-744.

Gupta M, Chyi J, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet 89:998-1006.

Gupta M, Balyan HS, Sharma PC, Ramesh B (1998) Genetics and molecular biology of seed storage proteins in wheat. In Gupta PK (eds.) Genetics and Biotechnology in Crop Improvement. Rastogi Publications, Meerut:126-157.

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their application in wheat breeding. Plant Breeding 118:369-390.

Hallauer AH, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames/IA.

Hartl L, Mori S, Schweizer G (1998) Identification of a diabnostic molecular marker for the powdery mildew resistance gene *Pm4b* based on fluorescently labelled AFLPs. In Slinkard AE (eds) Proc 9th International Wheat Genetics Symposium. Univ Extension Press, Univ of Sakatchewan, Saskatoon 3:111-113.

Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999) Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1a* and *Pm4a* in common wheat. Genome 42:322-329.

Hayward MD, Bosemark NO, Romagosa I (1993) Plant Breeding Principles and Prospects. Published by, Great Britain at the university Press, Cambridge.

He S, Ohm H, Mackenzie S (1992) Detectionof DNA sequence polymorphisms among wheat varieties. Theor Appl Genet 84:573-578.

Helentjaris T, King G, Slocum, Sidenstrang C, Wegman S (1985) Restriction fragment length polymorphism as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109-118.

Hoogendoorn J (1985) A reciprocal F_1 monosomic analysis of the genetic control of time ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum L*.). Euphytica 34:545-558.

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101:407-414.

Hyne V, Kearsey MJ, Martinez O, Gang W, Snape JW (1994) A partial genome assy for quantitative trait loci in wheat (*Triticum aestivum*) using different analytical techniques. Theor Appl Genet 89:735-741.

Innis MA, Gelfand DH, Snisky JJ, White TJ (1990) PCR protocole. A guide to methods and application. San Diego, CA, USA, Academic Pres: 482.

Ittu M, Saulescu NN, Hagima I, Ittu G, Mustatea P (2000) Association of *Fusarium* head blight resistance with gliadin loci in a winter wheat cross. Crop Sci 40:62-67.

Kam-Morgen LNW, Gill BS, Muthukrishnan S (1989) DNA restriction frgment length polymorphisims: Astrategy for genetic mapping of genome of wheat. Genome 32:724-732.

Kato K, Miura H, Sawada S. (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor Appl Genet 98:472-477.

Keim P, Schupp Jm, Travis SE, Clayton K, Zhu T, Shi LA, Ferreira A, Webb DM (1997) A high-density soybean genetic map based upon AFLP markers. Crop Sci 37:357-543.

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat X spelt population. Theor Appl Genet 98:903-912.

Kicherer S, Backes G, Walther U, Jahoor A (2000) Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare L*). Theor Appl Genet 100:881-888.

Kochert G (1994) RFLP technology. In DNA-Based Markers in Plants, Phillipps RL and Vasil IK (Hrsg), Kluwer Academic Puplished by , Dortrecht, Boston, London:8-38.

Koebner RMD, Martin PK (1990) Association of eyespot resistance in wheat cv. Capa Desprez with endopeptidase profile. Plant Breeding 104:312-317.

Koebner RMD, Krisch F, Thorpe C, Prins R (1998) AFLPs as a source of STS markers in alien introgression. In Slinkard AE (eds) Proc 9th International Wheat Genetics Symposium. Univ Extension Press, Univ of Sakatchewan, Saskatoon 1:118-123.

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172-175.

Korzun V, Roeder MS, Worland AJ, Boerner (1997b) Intrachromosomal mapping of genes for dwarfing (*Rht12*) and vernalization response (*Vrn1*) in wheat by using RFLP and microsatellite markers. Plant Breeding 116:227-232.

Korzun V, Roeder MS, Ganal MW, Worland AJ, Law CN (1998) Genetic analysis of the dwarfing gene *Rht8* in wheat.Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum L*.). Theor Appl Genet 96:1104-1109.

Lander E, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constucting primary

genetic linkage maps of experimental and natural population. Genomics 1:174-181.

Law CN, Sutka J, Worland AJ (1978) A genetic study of day-lenght response in wheat. Heredity 41:185-191.

Li YF, Yu YJ (1988) Monosomic analysis for scab resistance index in wheat cultivar "WZHHS" J Huazhong. Agric Univ 4(2):6-14.33.

Liao YC, Yu YJ (1985) Genetic analysis on scab-resistance in local wheat cultivar Wangshuibia. J. Huazhong Agric Univ 4:6-14.

Liu DJ, Liu JY, Tao WJ, Chen PD (1998) Molecular markers and breeding wheat for powdery mildew resistance. In Slinkard AE (eds) Proc 9th International Wheat Genetics Symposium. Univ Extension Press, Univ of Sakatchewan, Saskatoon 3:128-131.

Liu YG, Mori N, Tsunewaki K (1990) Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn J Genet 65:367-380.

Liu YG, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617-633.

Liu ZZ, Wang ZY (1991) Improved scab resistance in China: Sources of resistance and problems. In: Saunders D A (eds) Wheat for the Nontraditional Warm Areas, Proc Int Conf CIMMYT, DF, Mexico:178-188.

Love GR, Seitz LM (1987) Effects of location and cultivar on FHB in wheat from Kansas in 1982-83. Cereal Chem 64:124-128.

Lu WZ, Liu FL, Lin YB, Wang CL (1990) Correlation analysis between resistance to scab and plant height in wheat. Advances in Researches on Inheritance to diseases in Major Crops. Zhu LH (eds) Jiangsu Sci-Tech. Publishing House, Nanjing, China:229-234.

Luebberstedt T, Xia XC, Tan G, Liu X, Melchinger AE (1999) QTL mapping of resistance to Sporisorium reiliana in maize. Theor Appl Genet 99:593-598.

Ma ZQ, Lapitan NLV (1998) A comparison of amplified and restriction fragment length polymorphism in wheat . Cereal Res Commun 26:7-13.

Ma ZQ, Roeder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri- and tetra- nucleotide microsatellites in wheat. Genome 39:123-130.

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum L*) Genome 39:359-366.

Market CL, Moller F (1959) Chemical and biochemical techniques for varietal identification. Seed Sci Technol 1:181-199.

Martin RA, Johnston HW (1982) Effects and control of *Fusarium* diseases of cereal grains in the Atlantic Provinces. Can J Plant Pathol 4:210-216.

McGuire PE, Qualset CO (1997) Progress in genome mapping of wheat and related species. Joint Proc 5th and 6th Public Wrkshps Int Triticeae Mapping Initiative, 1-3 Sept 1995, Norwich UK, and 30- 31 Aug 1996, Sydeny Australia. Report No 18. Univ of California Genetic Resources Conservation Program. Davis, California, USA.

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81:1340-1348.

Messmer MM, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of a wheat X spelt cross. Theor Appl Genet 98:1163-1170.

Messmer MM, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet C, Keller B (2000) Genetic analysis of durable leaf rust resistance in winter wheat. Theor Appl Genet 100:419-431.

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

Miedaner T (1997) Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116:201-220.

Mingeot D, Jacquemin JM (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98:1132-1137.

Miura H, Worland AJ (1994) Genetic control of vernalization, day-lenght response and earliness per se by homologous group-3 chromosomes in wheat. Plant Breeding 113:160-169.

Moreno-Sevilla B, Anderson JA, Waldron BL, Stack RW, Frohberg RC (1997) RFLP mappin of *Fusarium* head blight resistance genes in wheat. In Dill-Macky R , Jones RK (eds) Proc National *Fusarium* Head Blight Forum. St Paul, MN, 10-12 Nov. 1997. Univ of Minnesota, St Paul:18.

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant Journal 3: 175-182.

Mullius KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase chain reaction. Method Enzymol 155:335-350.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H (1986) Specific enzymatic amplification of DNA in vitro the polymerase chain reaction. Cold Spring Harbor Symp Quant Biol 51:263-273.

Myers RM, Maniatis T, Lerman LS (1987) Detection and localization of single base changes by denaturing-gradient gel electrophoresis. Methods in Enzmol 155:501-527.

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 94:597-602.

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995a) Molecular mapping of wheat homoeologous group 2. Genome 38:516-524.

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995b) Molecular mapping of wheat homoeologous group 3. Genome 38:525-533.

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Autrique E, Bernard M, Leroy P, Faris JD, Anderson JA (1995c) Molecular mapping of wheat. Major genes and rearrangements in homoeologous group 4,5, and 7. Genetics 141:721-731.

Old RW, Primose SB (1989) Principles of gene manipulation. An introduction to genetic engineering 4th ed. Blackwell Scientific Publication, Oxford.

O,Brien J (1993) Genetic Maps. Book 6. plant 6th ed. Cold Spning Harbour Laboratory Press, USA.

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of chloroplast variation. Am. Nature 103:516-529.

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downey midew resistance in lettuce. Theor Appl Genet 85:985-993.

Parker GD, Chalmers KJ, Rathjen AJ, Langridge P (1998) Mapping loci associated with flour in wheat (*Triticum aestivum L*.). Theor Appl Genet 97:238-245.

P$\bar{e}$rez de la Vega M (1993) Biochemical characterization of populations. in Plant Breeding: Principles and Prospects. Edited by Hayward MD, Bosemark NO, Romagos I. Publishing Champman and Hall, London.

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.

Peter JR (1990) Genetics. 2th edn. Published by Scott, Foresman and Compan, Glenview, Illinois, Boston, USA.

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

Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theor Appl Genet 99:341-345.

Procunier JD, Gilbert J, Aung T, Gray M, Prashar S (1998) Microsatellite identification of specific D chromosomes for *Fusarium* head blight resistance in

hexaploid wheat. In Slinkard AE (eds) Proc of the 9th International Wheat Genetics Symposium. Saskatoon, Univ Extension Press, Univ of Sakatchewan, Saskatoon 3:143-144.

Qi X, Stam P, Linndhout P (1998) Use of locus-specific AFLP markers to construct ahigh-density molecular map in baley. Theor Appl Genet 96:376-384.

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gel to nylon membranes. Nucl Acid Res 20:7207-7221.

Reis EM (1990) Integrated diseases management-The changing concepts of controlling head blight and spot blotch. Saunders D A (eds) Wheat for the Nontraditional Warm Areas. Proc Int Conf CIMMYT, DF, Mexico:165-177.

Roberts DWQ (1990) identification of loci on chromosome 5A involved in control of cold hardiness, vernalization, leaf length, growth habit and height of hardened plants. Genome 33:247-259.

Roeder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327-333.

Roeder MS, Plaschke J, Wendehake K, Plaschke J, Tixer MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023.

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance . Theor Appl Genet 99:336-340.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Nat1 Aca Sci USA 81:8014-8018.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of B-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350-1354.

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8:552-560.

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831-838.

Shah MM, Gill KS, Baenzger PS, Yen Y, Kaeppler SM, Ariyarathe HM. (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:172 8-1732.

Sheng CS, He JM (1989) Our experience with wheat breeding for scab resistance. Jiangsu Agric Sci Suppl 1:58-59.

Singh RP, Ma H, Rajaram S (1995) Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis 79:238-240.

Sinha RC, Savard ME, Lau R (1995) Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. J Agric Food Chem 43:1740-1744.

Snijders CHA (1990) *Fusarium* head blight and mycotoxin contamination of wheat , a review. Neth J Plant Pathol 96:187-198.

Snijders CHA (1990 a) Diallel analysis of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:1-9.

Snijders CHA (1990 b) The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:11-18.

Snijders CHA (1990 c) Response to selection in F_2 generations of winter for resistance to head blight caused by *Fusarium culmorum*. Euphytica 50:163-169.

Snijders CHA (1990 d) Genetic variation for resistance to *Fusarium* head blight in bread wheat. Euphytica 50:171-179.

Snijders CHA (1994) Breeding for resistance to *Fusarium* in wheat and maize. In Mycotoxins in Grains. Eagan Press, St Paul MN:37-58.

Snijders CHA, Van Eeuwijk FA (1991) Genotype by strain interaction for resistance to *Fusarium* head blight caused by *Fusarium culmorum* in winter wheat. Theor Appl Genet 81:239-244.

Sourdille P, Snape JW, Cadalen T, Charmet G, Nakata N, Bernard S (2000) Detection of QTLs for heading time and photoperiod response in wheat using a doubled- haploid population. Genome 43:487-494.

Stephenson P, Bryan G, Kirby J, Collins A, Devos KM, Busso C, Gale MD (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946-949.

Strangge RN, Smith H (1987) Specificity of choline and betaine as stimulants of *Fusarium grminearum*. Trans Br Mycol Soc 70:187-192.

Sutton JC (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol 4:195-209.

Takeda K (1990) Selection response and parent-offspring correlation of the resistance to *Fusarium* head blight in barley. Japan J Breed 40:91-101.

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of sequence-tagged-site-facilitated PCR products as molecular markers in wheat. Theor Appl Genet 87:789-794.

Tanksley S (1983) Molecular markers in plant breeding. Plant Mol Biol Rep 1:3-8.

Tanksley SD (1993) Mapping Polygenes. Annual Rev Genetic 27:205-233.

Tanksley SD, Miller J, Paterson A, Bernatzky R (1988) Molecular mapping of plant chromosomes.In: Chromosome structure and function, Gustafson JP, Appels R (Hrsg) Plenum Publishing Corp, Plenum Press, USA:157-173.

Teich AH, Hamilton JR (1985) Effect of culture practices, soil phosphorous, potassium, and pH on the incidence of *Fusarium* head blight and deoxynivalenol levels in wheat. Appl Environ Microbiol 49:1429-1431.

Toojinda T, Broers LH, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Ramsay L, Vivar H, Waugh R (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Genet 101:580-589.

Tuite J, Shaner GE, Everson RJ (1990) Wheat scab in soft red winter wheat in Indiana in 1986 and ist relation to some quality measurements. Plant Dis 74:959-962.

Utz HF, Melchinger AE(1996) PLABQTL: A program for composite interval maping of QTL. J Quant Trait Loci. http//probe.nalusda.gov:8000/otherdocs/jqt11996-01/utz.htm1.

Van Eck HJ, van der Voort JR, Draaistra J, van Zanvoort P, van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal location of AFLP markers in non-inberd potato offspring. Mol Breed 1:397-410.

Van Ginkel M, Van der Schaar W, Yang Z, Rajaram S (1996) In heritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863-867.

Vos P, Hogers R, Bleeker M, Reijans T, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. Nucl Acids Res 23: 4407-4414.

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP Mapping Of QTL for *Fuarium* head blight resistance in wheat. Crop Sci 39: 805-811.

Wang YZ, Yong XN, Xiao QP (1982) The impovement of identification technique of scab resistance of wheat and the development of resistant sources. Sci Agric sinica 5:67-77.

Wang YZ, Miller JD (1988) Screening techniques and sources of resistance to *Fusarium* head blight. In Klatt AR (eds.) Wheat production Constraints in Tropical Environments. CIMMYT, Mexico:239-250.

Wang YZ, Liu ZZ, Zhao WJ, Huang DZ, Huang XM (1989) Advance of scab resistance testing and improvement in wheat varieties. Jiangsu Agric Sci 1:64-68.

Weising K, Nybom H, Wolff K, Meyer W (1995) DNA Finger-printing in Plant and Fungi. CRC Press, Boca Raton.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucl Acids Res 19: 861-866.

Welz HG, Xia XC, Bassetti P, Melchinger AE, Luebberstedt T (1999) QTLs for resistance to Setosphaeria turcica in an early maturing Dent x Flint maize population. Theor Appl Genet 99:649-655.

Wiese MV (1987) Scab (head blight) In: Compendium of Wheat Diseases .2nd ed. American Phytopathological Socciety, St Paul, MN:16-18.

Wiersma JV, Peters EL, Hanson MA, Bouvette RJ, Bush RH (1996) *Fusarium* head blight in hard red spring wheat : cultivars responses to natural epidemics. Agron J 88:223-230.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18: 6531-6535.

Worland AJ, Korzun V, Roeder MS, Ganal MW, Law CN (1998) Genetic analysis of the dwarfing gene *Rht8* in wheat. Part 1I. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor Appl Genet 96:1110-1120.

Wright S (1968) Evolution and genetics of population. Vol. 1. Genetic and biometric foundations. University of Chicago Press, Chicago.

Wu ZS (1986) Development of a gene pool with improved resistance to scab in wheat by using the dominant male sterile gene Ta1. Proc. Int. Wheat Conf., 1986, Rabat, Morocco.

Wu ZS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241:225-235.

Wu KS, Jones R, Danneberger L, Scolnik PA (1994) Detection of microsatellite polymorphism without cloning. Nucleic Acids Res 22: 3257-3258.

Xia XC, Melchinger AE, Kuntze L, Luebberstedt T (1999) Quantitative Trait Loci Mapping of Resistance to Sugarcane Mosaic Virus in Maize. Phytopathology 89:660-667.

Xiao QP, Wu ZF, Pen LX (1989) Dynamic observation of interaction between *Fusarium graminearum* causing wheat scab and varieties with different types of resistance. Jiangsu Agric Sci 1:69-71.

Yao JB, Ge YF, Wang SW, Yao GC, Zhou CF, Qian CM (1997) Chromosomal location of genes for scab resistance in wheat cultivars Sumai3. Acta Agron Sinica 23:450-453.

Yu YJ (1982) Monosomic analysis for scab resistance and yield components in the wheat cultivar Sumai3. Cereal Res Commun 10(3-4):185-189.

Yu YJ (1990) Genetic analysis for scab resistance in four wheat cultivars,Phjzm, Hhdtb, Cyhm and Ygfz. In: Advances in Researches on inheritance to Diseases in Major Crops. Zhu LH (eds.) Jiangsu Sci-Tech. Publishing House, Nanjing, China:197-205.

Yu YJ (1991) Genetic analysis for scab resistance in wheat cultivars Pinghujianzi-mai, Honghudataibao, Chongyangmai, Yangangfangzhou and Wannian 2. Acta Agron Sinica China 17:248-254.

Yu YJ, Yu CL, You FL (1986) Monosomic analysis for scab resistance genes in wheat cultivars. Proc 1st Int Symp Chromosome Engineering in Plants. Xian, China:38-40.

Zabeau M, Vos P (1993) Keygene, Wageningen. In European Patent Application, Publication NR 0 534 858 A1.

Zhou Ch, Xia S, Qian C, Yao G, Shen J (1987) On the problem of wheat breeding for scab resistance. Sci Agric Sinica 20:19-25.

Zhou ML, Bai GH, Wang S, Zhao YH (1988) Comparison of scab resistance among the wheat cultivars and their isogenic lines with different plant heights. Acta Agric. Jiangsu 4(1):25-30.

Zhu H, Fan Y (1989) Wheat scab epidemics and efficacy of testing for resistance in field in northern Fujiang province. Jiangsu Agric Sci 1:75-78.

Zhu H, Gilchrist L, Hayes P, Kleinhofs A, Kudrna D, Liu Z, Prom L, Steffenson B, Toojinda T, Vivar H (1999) Does function follow form? Principal QTLs for *Fusarium* head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley. Theor Appl Genet 99:1221-1232.

Zhuang QS, Li ZS (1993) Present status of wheat breeding and relateed genetic study in China. Wheat Inf Serv 76:1-15.

7 Appendix

7.1 Chemicals

Substance	Specification	Supplier
Agarose seakem		FMC, Rockland
Agarose seaplaque		FMC, Rockland
Albumin fration V (BSA)		Roth, Karlsruhe
Ammoniumpersulfate	p.a.	Amresco, Solon, Ohio
Ampicillin	pure	Boehringer, Mannheim
ATP		New England Biolabs, Beverly
Bacto-agar		Difco, Detroit
Bacto-typton		Difco, Detroit
Blocking reagent		Boehringer, Mannheim
Boric acid	pure	Amresco, Solon, Ohio
Bromophenolblue		Riedel-de Haen, Seelze
Chloroform	p.a.	Roth, Karlsruhe
CTAB	p.a.	Sigma, St. Louis
Dextran blue		Fluka, Darmstadt
dATP		Boehringer, Mannheim
dGTP		Boehringer, Mannheim
dNTP		Pharmacia, Uppsala
EDTA	p.a.	Serva, Heidelberg
EtBr	pure	Serva, Heidelberg
Ethanol	p.a.	Roth, Karlsruhe
Formamide	deionisiert	Amresco, Solon, Ohio
Glycerin	99,5%	Roth, Karlsruhe
Urea	p.a.	Amresco, Solon, Ohio
HCl	p.a.	Baker, Phillipsburg
Isoamyl alcohol	p.a.	Baker, Phillipsburg
Isopropanol	p.a.	Roth, Karlsruhe
Lang ranger gel-solution		FMC, Rockland
ß-Mecaptoethanol	p.a.	Merck, Darmstadt

Substance	Specification	Supplier
$MgCl_2$	p.a.	Merck, Darmstadt
NaAc	p.a., cryst.	Merck, Darmstadt
NaCl	p.a.	Roth, Karlsruhe
NaOH	p.a.	Merck, Darmstadt
Sodiumcitrat	p.a.	Sigma, St. Louis
NH_4Ac	p.a.	Riedel-de Haen, Seelze
^{32}P-dCTP	3000 Ci/mmol	ICN, Costa Mesa
Rnase		Roth, Karlsruhe
SDS	pure	Serva, Heidelberg
SDS	2 x cryst.	Serva, Heidelberg
Sephadex G50	fine	Pharmacia, Uppsala
TEMED	p.a.	Amresco, Solon, Ohio
Tris	p.a.	Riedel-de Haen, Seelze

7.2 Chi square test for markers segregation

Table 13: Chi square test for markers segregation

		Obs. segr.no			
Chrom.	Marker	mm	Mm	MM	chi-sq
1B	XE35/M52-147	34	0	142	3.03
	XE35/M52370	35	0	141	2.45
	XE35/M52-295	37	0	139	1.48
	Xiag95	22	96	57	15.65*
	XE42/M53-249	28	0	151	8.36
	Xgwm11	28	0	152	8.56
	XE38/M57-220	116	0	64	10.70*
	XE38/M51333	122	0	55	3.48
	XE42/M52-298	116	0	64	10.70*
	XE37/M58-335	107	0	72	22.12*
	XE42/M53-140	124	0	55	3.13
	Xgwm131	27	98	55	10.13

Table 13 continued

1D	XE38/M52-126	43	0	132	0.02
	XE38/M50-334	41	0	139	0.47
	Xgwm337	43	94	40	0.79
1KG	XE38/m50-255	112	0	68	15.67*
	XE37/M58-382	121	0	58	5.23
	XE38/M55-246	121	0	55	3.67
	XE38/M55-367	130	0	46	0.12
	XE38/M55-222	131	0	45	0.03
	Xwg184	53	88	37	2.90
2A	Xgwm512	57	0	121	4.68
	Xcmwg68	38	92	47	1.19
2B	Xgwm410	30	94	54	7.03
	Xgwm374	22	103	54	15.51*
	XAG24	27	96	55	9.91
	XE38/M52-193	112	0	68	15.67*
	Xgwm120	29	90	61	11.38*
	XE42/M53-280	42	0	137	0.23
	XE38/M50-356	122	0	58	5.01
	XE37/M57-91	37	0	143	1.90
	XE42/M57-402	121	0	57	4.68
	XE35/M59-269	112	0	65	12.97*
	XE35/M59-116	89	0	89	59.33*
	XE35/M59-497	121	0	57	4.68
2D	Xgwm301	32	95	44	3.80
	Xgwm349	43	86	43	0.12
2KG	XE38/M52-110	67	0	108	16.47*
	XE42/M53-75	82	0	97	41.34*

Table 13 continued

3A	Xgwm218	48	75	55	4.96
	XE35/M59-308	129	0	48	0.42
	Xgwm5	58	87	33	7.11
	XE35/M59-331	56	0	121	4.16
3B	XE38/M55-233	46	0	130	0.12
	XE38/M55-354	44	0	132	
	XE38/M55-362	46	0	130	0.12
	XE38/M57-77	43	0	137	0.12
	Xgwm493	34	111	32	11.49*
	Xgwm376	39	101	31	6.37
	Xgwm144	41	88	44	0.16
3D	XE42/M57-462	110	0	70	18.52*
	Xgwm52	48	81	48	1.27
3KG	XE35/M53-212	119	0	60	6.93
	XE42/M52-398	60	0	120	6.67
4A	XE37/M57-114	51	0	129	1.07
	XE37/M57-124	48	0	132	0.27
	Xgwm160	54	82	42	2.72
	Xgwm160	139	0	39	0.91
4B	XE35/M53-297	54	0	125	2.55
	Xgwm165	50	90	35	2.71
	Xgwm375	55	90	33	5.46
	XE38/M57-336	55	0	122	3.48
	XD38/M51-367	59	0	120	6.05
	Xgwm149	51	88	36	2.58
	XE35/M52-133	47	0	129	0.27
	Xgwm6	47	93	39	0.99

Table 13 continued

4KG	XE38/M52-279	38	0	139	1.18
	XE37/M57-160	40	0	140	0.74
	XE38/M52-193	31	0	144	4.95
5A	XE38/M52-143	65	0	110	13.76
	XE35/M59-314	130	0	47	0.23
	Xgwm205	38	90	33	2.55
	XE35/M52-248	45	0	131	0.03
	Xgwm304	41	94	26	7.32
	Xgwm156	42	103	33	5.31
	XE38/M57-250	47	0	133	0.12
	Xgwm617	154	0	24	12.59*
	XE35/M53-484	77	0	102	30.99*
5KG	XE38/M52-270	135	0	44	0.02
	XE38/M51-379	153	0	24	12.36*
	XE39/M57-438	139	0	38	1.18
	XE42/M53-165	134	0	45	0.00
	XE38/M57-417	126	0	54	2.40
	XE38/M51-274	119	0	58	5.70
	XE42/M57-343	53	0	125	2.16
	XE35/M53-134	55	0	124	3.13
	XE35/M53-207	62	0	117	8.87
6A1	XE35/M53-254	55	0	124	3.13
	Xgwm570	50	90	40	1.11
6A2	Xgwm169	28	100	50	8.16
	Xgwm617	31	123	24	26.53*
6B	XE42/M52-430	105	0	75	26.67*
	XE38/M52-461	108	0	67	16.47*

Table 13 continued

	XE38/M52-95	105	0	70	21.00*
	XE38/M52-415	119	0	56	4.57
	XE35/M53-432	116	0	63	9.92
	XE35/M59-164	31	0	146	5.29
	XE35/M53-351	121	0	58	5.23
	XE38/M57-438	139	0	38	1.18
	XE35/M59-372	118	0	59	6.56
	XE38/M50-222	132	0	48	0.27
	XE35/M52-308	48	0	128	0.48
	Xgwm88	47	76	49	2.37
	Xgwm193	34	96	45	3.03
	XE38/M52-391	40	0	135	0.43
	XE38/M50-307	44	0	136	0.03
	XE38/M51-377	48	0	129	0.42
	XE42/M57-188	40	0	138	0.61
	Xgwm219	43	94	43	0.36
6D	Xwhs116	23	105	50	13.94*
	XksuD27	29	101	50	7.59
6KG	XE39/M57-472	124	0	53	2.31
	XE38/M55-450	139	0	37	1.48
	XksuD27	8	139	33	60.30*
7A	XE38/M52-211	47	0	128	0.32
	XE38/M50-276	46	0	134	0.03
	Xgwm260	39	98	40	2.05
	XE38/M39-443	122	0	54	3.03
	XE35/M52-414	113	0	63	10.94*
7BS/5BL	XE38/M57-292	122	0	58	5.01
	XE38/M52-197	53	0	122	2.61

Table 13 continued

	XE38/M52-91	49	0	126	0.84
	Xgwm573	45	95	39	1.08
	Xgwm46	44	92	41	0.38
	XE38/M50-319	48	0	132	0.27
	XE35/M59-166	127	0	50	1.00
	XE38/M50-292	41	0	139	0.47
	XE37/M57-198	128	0	52	1.45
	Xgwm335	43	100	33	4.41
	Xwhs50	38	0	138	1.09
	XE38/M55-285	54	0	122	3.03
	XE39/M57-485	56	0	121	4.16
	XE39/M57-425	52	0	125	1.81
	XE39/M57-317	51	0	126	1.37
	XE39/M57-104	40	0	137	0.54
	XE38/M50-303	50	0	130	0.74
	XE38/M50-136	55	0	125	2.96
	XE42/M52-243	63	0	117	9.60
	Xmwg914	59	0	115	7.36
7D	Xgwm44	31	86	48	3.80
	XE35/M53-401	56	0	123	3.77
	XE39/M57-333	132	0	45	0.02
7KG	XE39/M57-368	77	0	100	32.32
	XE35/M59-317	114	0	63	146.59*
	XE42/M53-177	108	0	71	119.20*
8KG	XE35/M53-72	56	0	123	3.77
	XE42/M53-435	50	0	129	0.82
9KG	XE42/M52-383	91	0	89	62.70*
	XE42/M53-375	55	0	124	3.13

7.3 LOD scans over the chromosommes

QTLs for FHB resistance were identified by the CIM analysis using the PLAPQTL program . The LOD scans are shown in chromosomes 5A, 3B, 6B, 7BS/5BL, LG4, LG7 and LG9 (Figure 8). LOD values are shown on the Y-axis while map distances in cM are on the X-axis and horizontal lines indicate the maximum significance threshold (LOD>3).

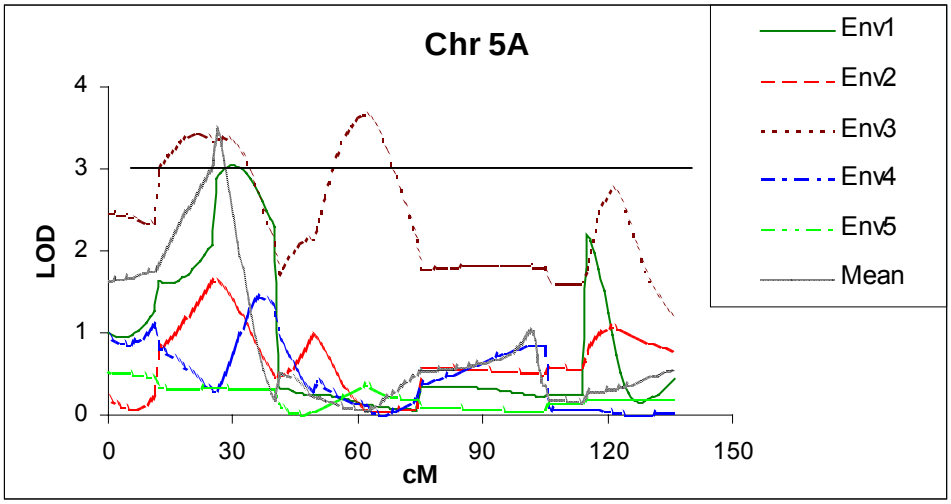


Fig.8a

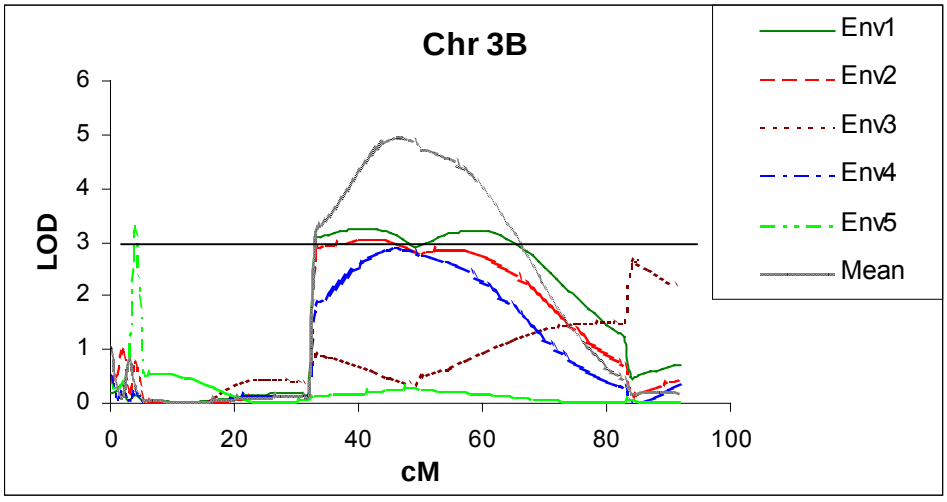


Fig.8b

Env1	Hadmersleben	Env2	Gruenbach
Env3	Herzogenaurach	Env4	Obertraubling
Env5	Bergen	Mean	across environments

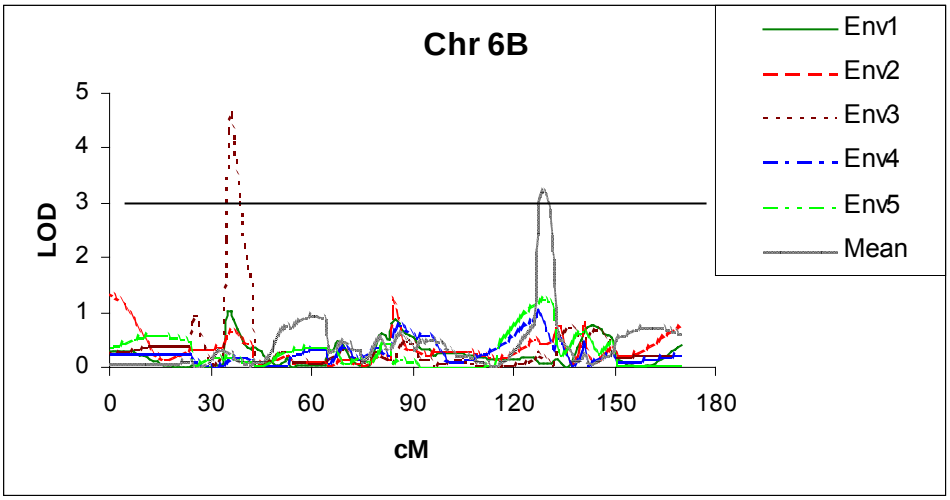


Fig.8c

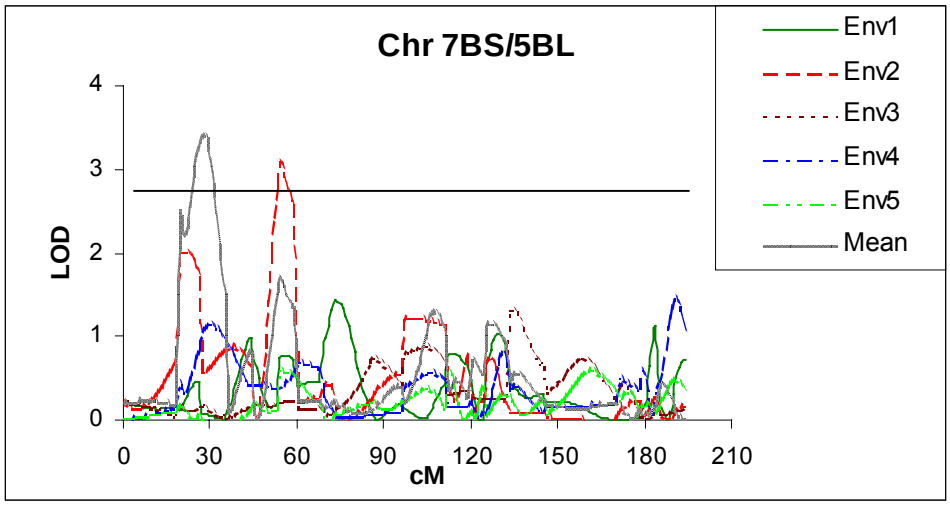


Fig.8d

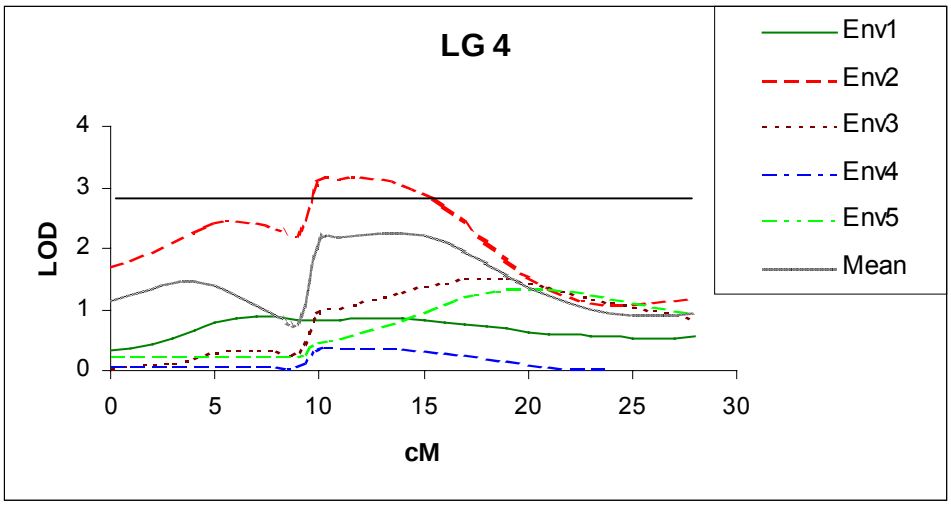


Fig.8e

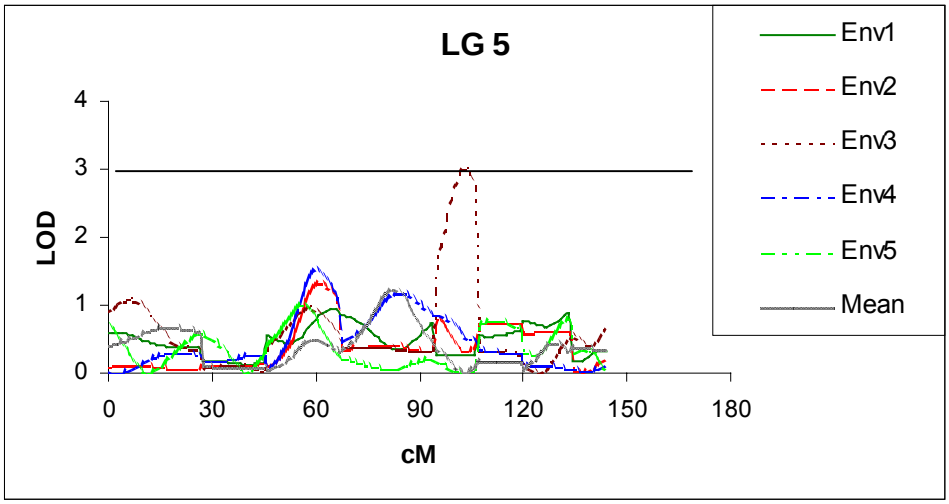


Fig.8f

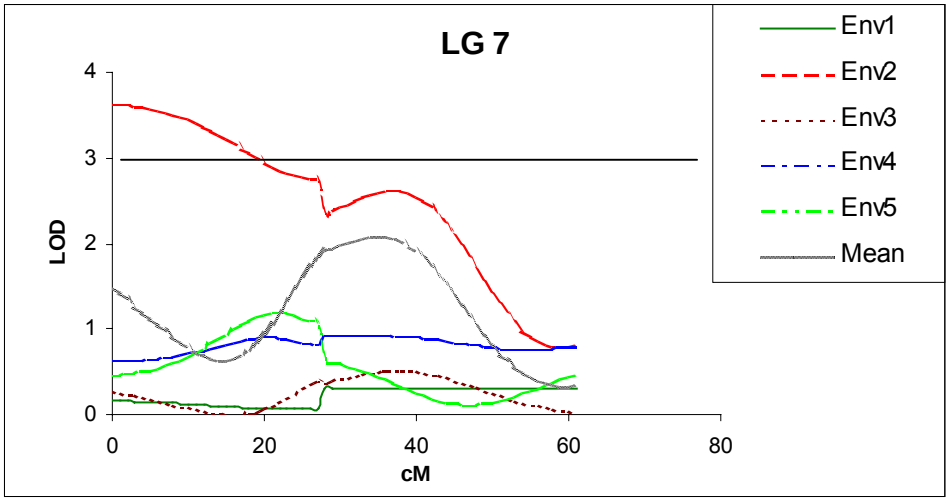


Fig.8g

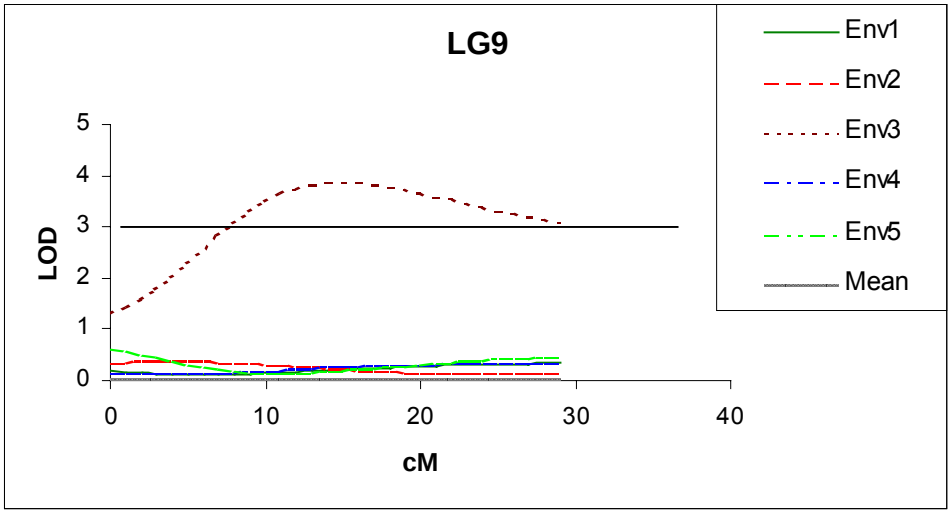


Fig.8h

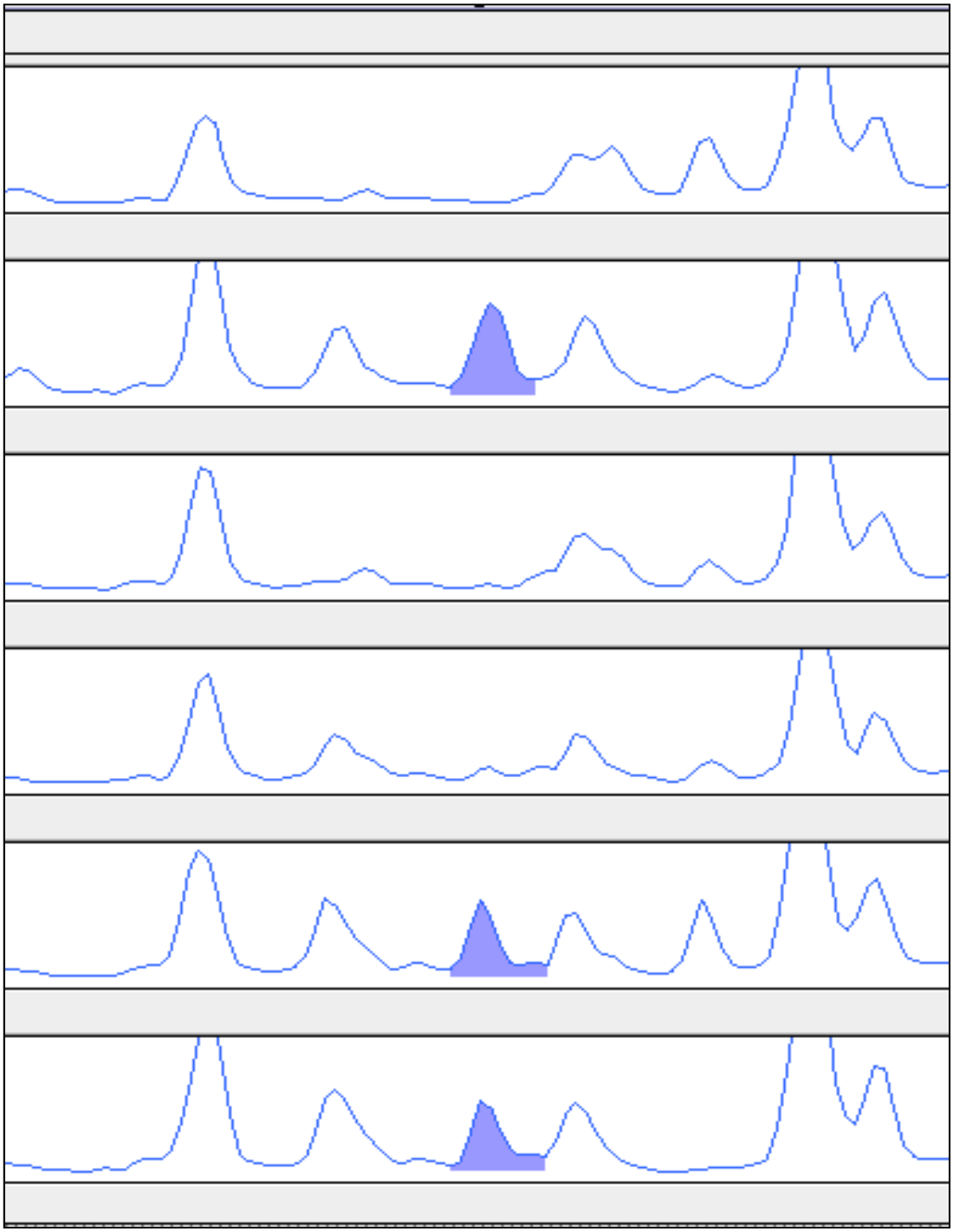


Fig. 9a: Electropherograms, analysed using GENESCAN™ analysis software version 2.0.2, showing polymorphic AFLP marker (shaded) of 91 bp in F_3 families and two different parental lines (Apollo and Sgv. NB x MM. sum 3), amplified with the primer combination E38/M52-91. The fragment was assigned to chromosome 7BS/5BL and was linked with one QTL of FHB.

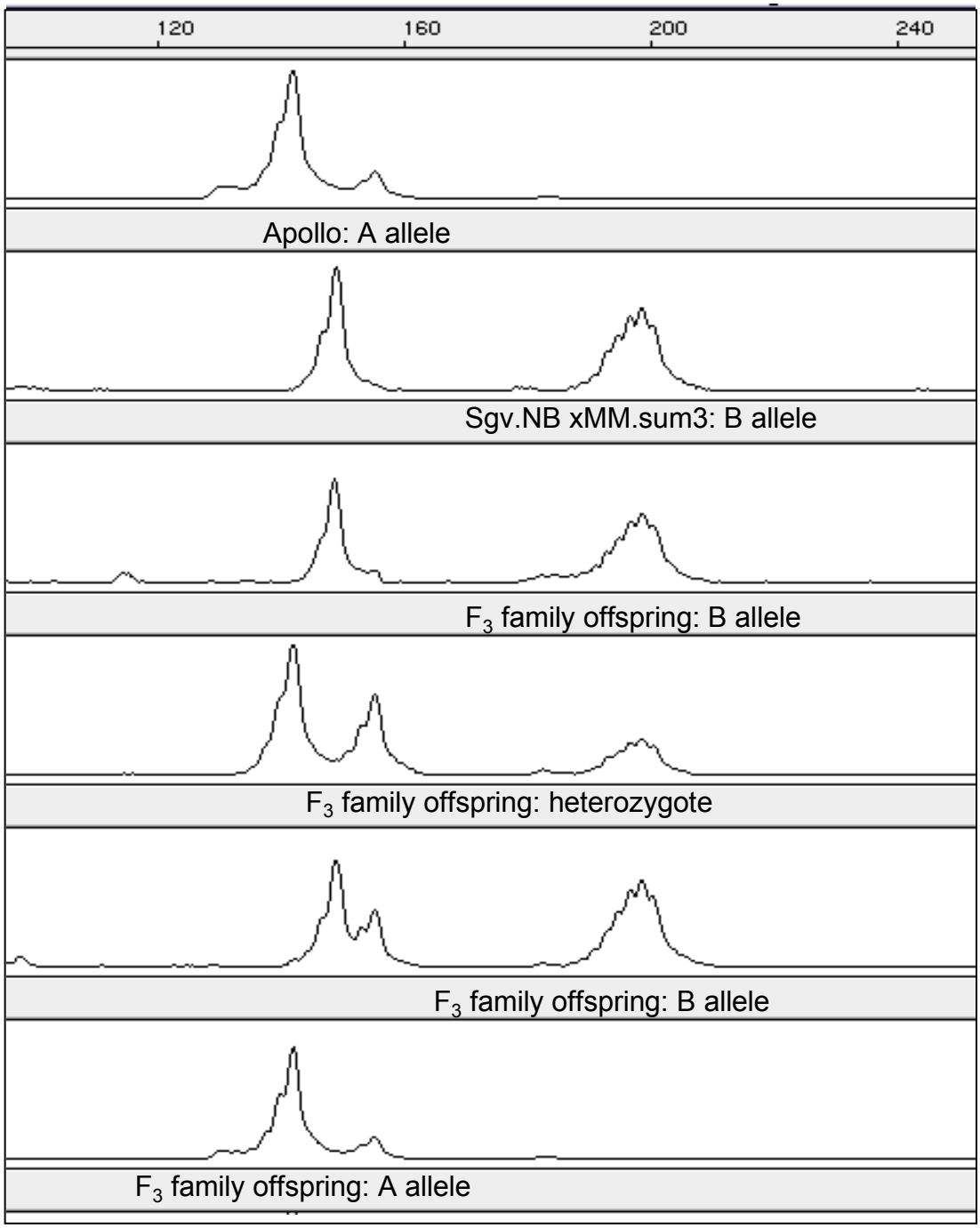


Fig. 9b: Electropherograms, analysed using GENESCAN™ analysis software version 2.0.2, of polymorphic microsatellite markers in F_3 families and different two parental lines (Apollo and Sgv. NB x MM. sum 3), amplified with the SSR marker gwm 493. The fragments were assigned to the short arm of chromosome 3BS. This marker was linked with one QTL for FHB resistence.

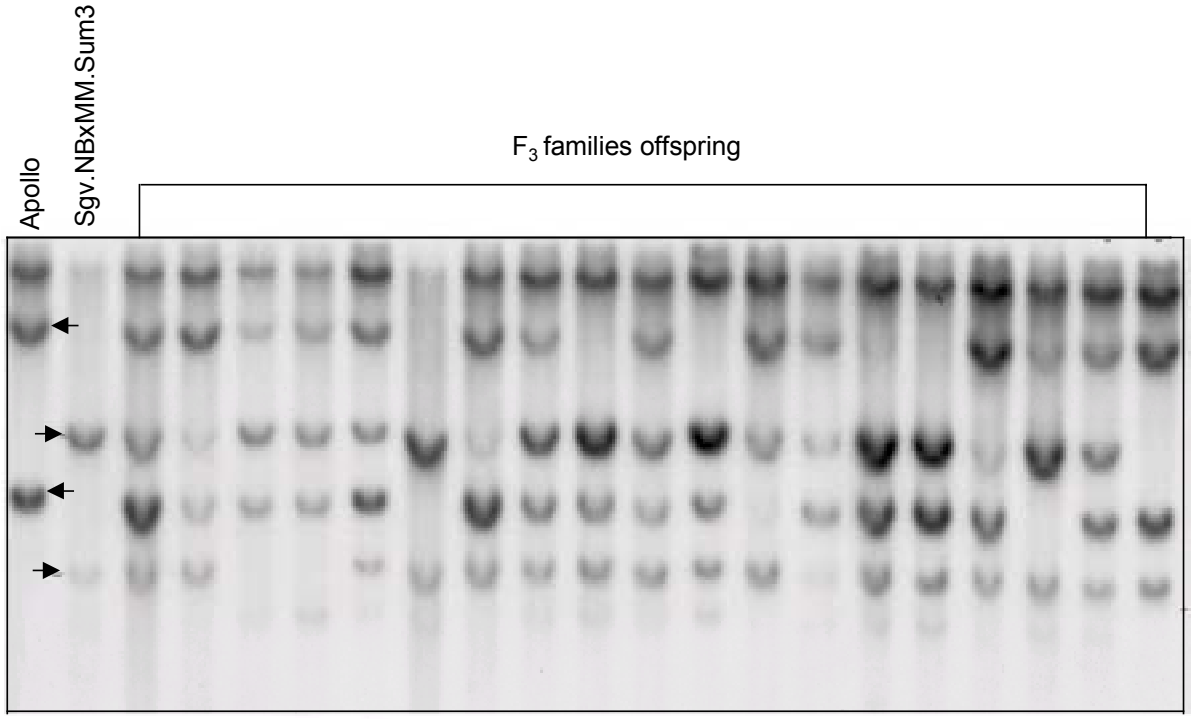


Fig.9c: Southern blot hybridization with the wheat probe KSUD27 of *Eco*RI-digested genomic DAN. The data obtained from F_3 families and different two parental lines (Apollo and Sgv. NB x MM. sum 3) are shown. Polymorphic bands are seen in the two parents. whereas each F_3 families offspring display the polymorphic bands, depending upon whether there is homozygosity or heterozygosity the locus. The fragments were assigned to chromosome 6D and LG6.

7.4 Abbreviations

A	adenine
AFLP	amplified fragment length polymorphisms
ANOVA	analysis of variance
APS	ammonium per sulfate
bp	base pair
BSA	bovine serum albumine
C	cytosin
CDNA	copy or complementary DNA
Chr	chromosome
CIM	composite intervall mapping
cM	centimorgan
CTAB	cetyltrimethylammonium bromide
DAF	DNA amplification fingerprinting
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DGGE	denaturing gradient gel electrophoresis
dGTP	deoxyaguanosine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
DON	deoynivalenol toxins
EDTA	ethylenediaminetetraacetic acid
Et al	et aliter
ET Br	ethidium bromide
FHB	fusarium head blight
F_n	nte filialgeneration

G	guanine
Kb	kilobase
Krist	kristallin (crystalline)
LOD	logarithm of odds
MAS	marker assisted selection
ML	maximum likelihood
p.a.	pro analysis
PCR	polymerase chain reaction
p32-dctp	p32-deoxcitidin triphosphate
QTL	quantitative trait locus
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphisms
RNA	ribonucleic acid
RNase	ribonuclease
SCAR	sequence characterized amplified regions
sCIM	simple composite intervall mapping
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphisms
SSR	simple sequence repeat
STS	sequence tagged site
Tris	2-amino-2-(hydroxymethyl)-propane-1,3-diol
v/v	volume/volume
w/v	weight/volume

Acknowledgments

I would like to express my deep gratitute and sincere appreciation to my suppervisor Prof. Dr. G. Wenzel for his permanent support, kind encourgement and the scientific advises throuhout my study in Germany, as well as his carefully reading and preperation of my thesis.

I am very much indebted to Dr. V. Mohler for his untiring help, understanding and his correct guidance, as well as for his freedom in discussions and constructive criticisim in editing of the manuscript.

Moreover, I thank all my colleagues in the Departement of Crop sciences and Plant Breeding at the Technical University Munich in Freising-Weihenstephan. Deep thanks are also given to Dr. G. Schwarz and Dr. M. Herz for their kind cooperation and valuable comments, as well as many thanks to Ms. C. Moeller for excellent technical assistance.

Special appreciation is expressed to Dr. H. A. Jahoor and Dr. G. Backes for their suport and valuable helps in statistical analysis and I must express my appreciation for their kind host at Risø National Laboratory in Roskilde, Denmark.

I am extremely thanksfull to Dr. Walter for evaluating symptoms of FHB severity in the field trials.

Special thanks to Prof. Dr. A. Elhossary and Dr. A. Fahmi for their help in preparation in my Ph. D. thesis.

Also, I would like to express my appreciation to Egyptian government for their financial support.

Moreover, my thanks for my family who provides the proper conditions to finish this study.

CURRICULUM VITAE

OF

Mahmoud EL-Zaabalawy Mahmoud EL-Badawy

Full Name	Mahmoud EL-Zaabalawy Mahmoud EL-Badawy
Date of Birth	02.01.1969
Place of Birth	Moshtohor, Qalyoubia, Egypt
Nationality	Egyption
Marital Status	Married and have three Children
Home address	Moshtohor, Toukh, Qalyoubia, Egypt P.O.Box.13736
Work address	Egypt, Faculty of Agriculture at Moshtohor, P.O.Box.13736
Professional	Assistant Lecturer in Agronomy Department , Zagazig University

Education

1974 -1980, Primary school

1980 -1983, Prep school

1983 -1986, Secondary school

University

B.Sc. in Agronomy Department, Faculty of Agriculture at Moshtohor, Zagazig University Banha Branch 1990

M.Sc.in Plant Breeding, Faculty of Agriculture at Moshtohor, Zagazig,University Banha Branch 1994

1997-2001, Ph.D. student at Technical University Munich in Freising-Weihenstephan, Departement of Crop Sciences and Plant Breeding. Scholarship financed by Egyptian government.

Lebenslauf

Name	Mahmoud El -Zaabalawy Mahmoud EL-Badawy
Geburtsdatum	02.01.1969
Geburtsort	Moshtohor
Nationalität	Ägypter
Familienstand	Verheiratet, drei Kinder
Heimatsanschrift	Ägypten, Qalyoubia, Toukh, Moshtohor, 13736
Dienstanschrift	Ägypten, Fakltät für Landwirtschaft in Moshtohor, 13736
Beruf	Wissenschaftlicher Assistent an der Fakultät für Landwirtschaft der Universität Zagazig in Moshtohor
Schulausbildung	
1974 –1980	Grundschule in Moshtohor
1980 –1983	Mittlere Reife in Moshtohor
1983 – 1986	Gymnasium in Moshtohor
1986	Abitur
1986 – 1990	Studium der Agrarwissenschaft an der Fakultät für Landwirtschaft der Universität Zagazig in Moshtohor
1990 – 1991	Militärdienst
1991 –1994	Demonstrator an der Abteilung für Pflanzenbau der Fakultät für Landwirtschaft der Universität Zagazig in Moshtohor
1994	Erlangung des Grades eines Masters (M.Sc.) im Fachgebiet Pflanzenzüchtung an der Fakultät für Landwirtschaft der
1997	Universität Zagazig in Moshtohor Bewilligung eines Promotionsstipendiums durch die Studienmission der Ägyptischen Regierung
1997 - 2001	Ph.D. Student im Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am Lehrstuhl für Pflanzenbau und Pflanzenzüchtung