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Association Mapping of QTLs for Fusarium Head Blight Tolerance in a Structured Barley Population

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Zusammenfassung

Ährenfusariosen (FHB) verursachen eine der am schwierigsten zu bekämpfenden Pflanzenkrankheiten. Es reduziert das Korngewicht, führt zu erheblich Ertragsausfällen und Qualitätseinbußen die auftreten, wenn Mykotoxine und insbesondere Deoxynivalenol (DON) im infizierten Samen produziert werden. DON Kontaminationen geben auch in der Lebensmittelsicherheit Anlass zur Besorgnis. Darüber hinaus könnte Gersten- oder Weizenstroh, das als Viehfutter dient signifikante Mengen von DON im Spreu enthalten. Association mapping eines Merkmals dient zur Identifizierung einer chromosomalen Region, deren Gene die untersuchte Information enthalten. Die Entdeckung von immer mehr polymorphen Markern über das gesamte Genom gibt uns die Möglichkeit mit deren Hilfe Regionen zu lokalisieren, die nah an den interessanten Genen liegen.
Ein association mapping in Gerste wurde zur Identifizierung von vielversprechenden Toleranz QTLs gegen FHB durchgeführt. Dazu diente eine Gerstenpopulation bestehend aus 103 Wild forms (*H. vulgare* ssp. *spontaneum*) als "Core Collection" und 21 Sommergerstensorten (*H. vulgare* ssp. *vulgare*). Die Experimente wurden in den Jahren 2009, 2010 und 2011 im Gewächshaus und unter Feldbedingungen im Jahr 2011durchgeführt. In fünf Wiederholungen wurden die Experimente in einem vollständig zufälligen Design angeordnet und anschließend mit zwei verschiedenen Pilz-Isolaten infiziert. Die Auswertung der Infektionsstärke wurde mit leaves disease scoring (LDS) und spikes disease scoring (SDS) durchgeführt. Im Jahr 2011 wurde auch der Tag des Ährenschiebens (HD) als verwandtes Merkmal für FHB Infektion mit in die Untersuchungen aufgenommen. Die Erfassung der genetischen Informationen wurden mit 895 Diversity Array Technology (DArT) Markern vorgenommen. Eine Strukturanalyse wurde für alle Genotypen mittels Hauptkomponentenanalyse (PCA) durchgeführt und konnte diese in 3 Cluster aufteilen. Der relative Verwandtschaftskoeffizient (K-Matrix) unter allen Genotypen wurde mit der "SPAGeDi-1.3d" Software berechnet. Die Assoziationsstudie wurde in einem mixed linear model (MLM) unter Berücksichtignug der PCA-Werte und der K-Matrix durchgeführt. Alle untersuchten Merkmale LDS, SDS und HD zeigten hochsignifikant Unterschiede in den drei Jahren. Wenn man die Mittelwerte aus zwei Jahren und zwei Isolaten kombiniert kann gezeigt werden, dass 18 Genotypen einen Befall von weniger als 20% für LDS aufwiesen. Nach der Datenerfassung mit dem SDS konnten 48 Genotypen mit einen Befall von weniger als 20% und 9 mit einem Befall von weniger als 10% bewertet werden. Positive und signifikante phänotypische Korrelationen konnten zwischen LDS und SDS berechnet werden. Eine negative Korrelationen zeigte sich zwischen SDS und HD im Jahr 2011. Dreiundsiebzig Marker korrelierten signifikant mit allen

untersuchten Merkmalen und waren über das gesamte Genom der untersuchten Population verteilt. Verschiedene QTLs konnten für LDS, leaves disease scoring isolates in 2011 (LDSI), SDS, spikes disease scoring isolates in 2011 (SDSI), visual leaves disease scoring (VS), HD, marker isolate interaction within leaves disease scoring (MILDS) und marker isolate interaction within spikes disease scoring (MISDS) identifiziert werden. Einige QTLs wurden in zwei Merkmalen identifiziert. Marker bpb-0522 wurde mit SDSI und MISDS und der Marker bPb-6466 durch LDS und MILDS nachgewiesen. Einige dieser QTLs wurden für zwei Merkmale auf der selben, beziehungsweise nahezu der gleichen Position auf dem Chromosom identifiziert. Auf der Position 122,08cM auf Chromosom 6H wurden zwei Marker (bPb-4379 und bPb-3375) die mit SDS und HD assoziieren entdeckt. Auch im Chromosom 2H auf 70,8 cM wurden zwei Marker für LDS und SDS erkannt. In der Studie wurde ein mixed model für die epistatische Interaktion zwischen allen DArT Markern und den untersuchten Merkmalen getestet. Wechselwirkungen zwischen 11 Paaren von QTLs für FHB Resistenz, beziehungsweise Toleranz und eine Interaktion zu dem Beginn des Ährenschiebens, an dem 24 QTLs auf sechs Chromosomen beteiligt sind wurden erkannt. Die Ergebnisse zeigten, dass Gerste FHB Resistenz und Toleranz eint kompliziertes Merkmal ist und durch eine kompliziertes Gen Netzwerk gesteuert wird.

ABSTRACT

Fusarium head blight (FHB) disease is one of the most challenging crop diseases; it reduces kernel weight, cause significant yield losses and quality reductions that may occur if fungal mycotoxins, especially deoxynivalenol (DON) are produced in infected seed. DON contamination has raised serious food safety concerns. Furthermore, straw of barley or wheat may be consumed by livestock could contain significant amounts of DON in chaff. Association mapping of a trait is to identify chromosomal regions that contain genes affecting the trait. The discovery of dense polymorphic markers covering the entire genome provides us an opportunity to localize these regions by trying to find the markers closest to the genes of interest. Association mapping was conducted in a structured barley population consists of (103 accessions of wild barley (*H. vulgare ssp. spontaneum*) core collection and 21 spring barley cultivars (*H. vulgare ssp. vulgare*)) in this study for identification of promising tolerance QTLs in barley against FHB. These experiments were carried out in the green-house during years 2009, 2010 and 2011 and under field-potted conditions in 2011, arranged in a completely randomized design with five replications, by using two different ways of scoring for disease infection leaves disease scoring (LDS) and spikes disease scoring (SDS) and two different fungus isolates. Heading date (HD) also had been recorded as a related trait for FHB in 2011. The accessions were genotyped by using 895 Diversity Arrays Technology **(**DArT) markers. Structure analysis was carried out for the all accessions using principal component analysis (PCA) the accessions were divided into 3 clusters and the relative kinship coefficients (K matrix) among all pairs of accessions were calculated by "SPAGeDi-1.3d" Software. The association analysis was performed in mixed linear model (MLM) including PCA values and K matrix. All studied traits LDS, SDS and HD were exhibited highly significantly differences in three years. Results from combined means in two years and two isolates showed that; 18 accessions had lower than 20 infection percentage for LDS and 48 accessions had lower than 20 infection percentage, 9 accessions from this group were lower than 10 infection percentage for SDS. Positive and significant phenotypic correlation has been recorded between LDS and SDS and negative correlation also have been recorded between SDS and HD in 2011. Seventy three markers were correlated significantly with all studied traits and covered the whole genome of the studied population. Different QTLs have been identified for LDS, leaves disease scoring isolates in 2011 (LDSI), SDS, spikes disease scoring isolates in 2011 (SDSI), visual leaves disease scoring (VS), HD, marker isolate interaction within leaves disease scoring (MILDS) and marker isolate interaction within spikes disease scoring (MISDS). Some of these QTLs were identified in two traits; marker

bPb-0522 detected to SDSI and MISDS and also marker bPb-6466 detected to LDS and MILDS. Few of this QTLs were identified for two traits in the same/nearly position on the chromosome; in the position 122.08 cM on chromosome 6H two markers (bPb-4379 and bPb-3375) were identified to be associated with SDS and HD. Also in chromosome 2H on 70.8 cM two markers detected to LDS and SDS. In the study a mixed model was tested for the epistatic interaction between all DArT markers and studied traits. Interactions were detected between 11 pairs of QTLs for FHB resistance/tolerance and one interaction for heading date, which involved 24 QTLs on 6 chromosomes. The results indicated that barley FHB resistance/tolerance is a complicated trait and may be controlled by a complicated gene network.

Index of content

1. Introduction, objectives and Literature review

Association mapping seeks to identify specific functional variants (i.e.,loci, alleles) linked to phenotypic differences in a trait, to facilitate detection of trait causing DNA sequence polymorphisms and/or selection of genotypes that closely resemble the phenotype.

1.1 Introduction

Association genetics is a relatively new approach to dissect complex traits that is based on the establishment of causal relationships between genotypes and phenotypes in natural or breeding populations. The use of new high-throughput techniques, which allow in a single assay genotyping of thousands of single nucleotide polymorphisms (SNPs), has strengthened the application of this approach both in animal and plant research. Originating in human genetics, association genetics is now widely used in plant breeding, in particular in undomesticated organisms such as forest trees. The main advantage of association mapping in comparison with other common approaches (e.g. QTL mapping) is that the multiple generations of recombination that have taken place in natural populations result in a tight linkage of causal polymorphisms with nearby genomic regions, avoiding the large blocks of linkage that are often obtained from two- or three-generation pedigrees and facilitating the identification of polymorphisms that are associated with quantitative traits. As a complement to traditional linkage studies, association mapping or linkage disequilibrium (LD) mapping offers a powerful alternative approach for fine-scale mapping of flowring time in maize (Thornsberry et al. 2001), yield traits in barley (Kraakman et al. 2004), Iron deficiency in soybean (Wang et al. 2008), and disease resistance in rice (Garris et al. 2003), potato (Gebhardt et al. 2004; Simko et al. 2004) , corn (Szalma et al. 2005) and fusarium head blight resistance in barley (Massman et al. 2011).

Fusarium Head Blight (FHB), or head scab, caused by *Fusarium graminearum* Schwabe (telomorph = *Gibberella zeae* (Schw.)). is a historically devastating disease of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and other cereal crops across the world (Boutigny et al. 2011). The People's Republic of China, Canada, parts of southern Africa, Eastern Europe, South America, and the United States all have recorded FHB outbreaks and each country continues to struggle with this destructive disease. The head blight develops in warm, humid weather during the formation and ripening of the kernels. Infection begins in the flowers and frequently spreads to other parts of the head. The diseased area turns light brown. A pink, moldy growth often develops around the base of the infected

flower, and black fruiting bodies may be found on the glumes, kernels of diseased heads are grayish brown.

FHB is a preharvest disease, it reduces kernel weight, cause significant yield losses and quality reductions (Goswami and Kistler 2004; Liddell 2003). Yield losses in all crops occur from floret sterility; additional yield and quality losses can occur when shriveled, light test-weight kernels are produced as a result of infection. The germination rate and seedling vigour are also reduced when the seeds are infected. *Fusarium* species digest proteins and starch and the use of infected kernels generates technical problems for bread production (Betchel et al. 1985; Boutigny et al. 2011). FHB can reduction the seeds quality that may occur if fungal toxins (mycotoxins) are produced in infected seed. The toxins are unacceptable for certain end uses, so toxin-containing grain is downgraded at the market. Furthermore, straw of barley or wheat may be consumed by livestock could contain significant amounts of DON in chaff, and DON can be minimized if straw is sourced from low-symptom crops (Cowger and Arellano 2012).

1.2 Objectives of this study

1. The main goal of this research was to apply association mapping approaches to identify DArT markers associated with fusarium head blight disease tolerance in a structured barley population.
2. Investigation of new sources for barley genotypes those have tolerant against *Fusaruim.*
3. Evaluation of leaves disease scoring efficiency as a disease assessment method.
4. Identification of promising tolerance QTLs in barley against fusarium head blight.

1.3 Literature review

Breeding resistant cultivars could be an effective strategy to manage FHB in barley, but unfortunately this strategy faces significant challenges. All barley genotypes investigated so far express only partial resistance to FHB. Further, several genetic mapping studies have shown that resistance to FHB and to the accumulation of the mycotoxin deoxynivalenol (DON) that is produced by the pathogen are conditioned by many genes distributed throughout the genome (Kolb et al. 2001). In addition, quantitative trait loci (QTL) associated with resistance are often inconsistently detected among environments and are usually associated with agronomic and morphological traits such as late heading, tall plant height, lax spike, and two-rowed spike (Steffenson 2002). Breeding for FHB resistance has been difficult, because resistance to FHB is conditioned by many genes distributed throughout the

genome (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Kolb et al. 2001; Mesfin et al. 2003; Dahleen et al. 2012). This has led several researchers to conclude that most QTLs for FHB resistance result from the pleiotropic effect of morphological or developmental genes, and consequently that the "function" of FHB resistance is primarily related to plant morphology (Zhu et al. 1999; Ma et al. 2000).

Progress in exploiting genetic resistance to FHB and DON accumulation has been slow due to the technical difficulties and expense in disease screening, complex nature of resistance, the preponderance of genotype × environment interaction, and coincidence of QTLs for FHB with other QTLs associated with plant development and spike morphology.

1.3.1 Barley crop

1.3.1.1 World barley production and utilization

Barley is a cereal grain, early maturing grain with a high yield potential and widely adaptable crop (Harlan 1976). Barley (*Hordeum vulgare* ssp. *vulgare* L.) ranks fourth in world cereal crop production and is used for, in order of importance, animal feed, brewing malts and human food. World barley production in 2010 was approximately 123.5 million metric tons (MMT) produced on 47.59 million hectares (MH). It is grown for animal feed, human food, and malt. However, in developing countries, most barley is grown in marginal environments, often on the fringes of deserts and steppes or at high elevations in the tropics, receiving modest or no inputs. This partly explains why yields there are nearly half of those in developed countries. Although barley is considered to be one of oldest cultivated cereal grains and was used extensively as a food in the past, only a small amount of barley is used for human consumption. In recent years there has been a growing research interest for the utilization of barley in a wide range of food applications (Bhatty, 1999; Bilgi and Çelik, 2004 and Köksel et al. 1999).

1.3.1.2 Taxonomic position and origin of barley

Linnaeus was the first to provide a botanical description of barley in his species Plantarium in 1753. Barley is a grass belonging to the family Poaceae, the tribe *Triticeae* and the genus *Hordeum*. There are 32 species, for a total of 45 taxa in the genus Hordeum that are separated into four sections (Bothmer 1992). The four sections proposed by Bothmer are as follows: *Hordeum*, *Anisolepis*, *Critesion*, and *Stenostachys*. The division of the genus into sections puts plants into groups that have similar morphological characteristics, similarities in ecology,

life forms and geographical area of origin. The basic chromosome number of x = 7 is represented across the 45 taxa as diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42). Six species are listed in the section *Hordeum; H. bulbosum, H. murinum* ssp. *glaucum, H. murinum* ssp. *leporinum, H. murinum* ssp. *murinum L., H. vulgare* ssp. *vulgare,* and *H. vulgare* ssp. *spontaneum*. The genomes of *H. vulgare* ssp. *vulgare* (cultivated barley) and *H. vulgare* ssp. *spontaneum* (wild barley) are identical and interfertile.

Barley is a short season crop, early maturing grain found in widely varying environments around the world. Barley provides an very good system for genome mapping and genetic studies, due to (1) its diploid nature, (2) low chromosome number (2n=14), (3) relatively large chromosomes (6-8 μm), (4) high degree of self fertility, and (5) ease of hybridization (Sreenivasulu et al. 2008, Hussain 2006). Barley is a self-pollinated crop. Consequently, its variation is structured in true breeding lines. Hundreds of modern varieties and thousands of landraces are known.

1.3.2 Differences between wild barley and cultivated barley

Barley (*Hordeum vulgare* L.) is one of the founder crops of Old World agriculture and was one of the first domesticated cereals. *H. vulgare* is divided into two subspecies: *Hordeum vulgare* ssp. *vulgare,* and *H. vulgare* ssp. *spontaneum* (C. Koch.) Thell. *H. vulgare* ssp. *vulgare* is cultivated barley. The wild species *H. vulgare* ssp. *spontaneum* has centres of diversity in central and south western Asia, western North America, southern South America, and in the Mediterranean (Bothmer 1992). Most of the wild perennial species grow in moist environments whereas the annual species are mostly restricted to open habitats and disturbed areas. Many species have adapted to extreme environments and many have tolerance to cold and saline conditions (Bothmer 1992). It is particularly common in the Near East Fertile Crescent (Zohary 1969). Generally, wild barley is not tolerant to extreme low temperatures.

Wild barley has a quite similar morphology to cultivated 2-rowed barley. The most marked differences are wild barley's brittle rachis and its hulled grain. Six-rowed barley has evolved during domestication, the trait being controlled by a single gene on chromosome 2 (Komatsuda et al. 1999, Tanno et al. 2002). Wild barley is the only wild *Hordeum* species that can produce fully fertile hybrids (with normal chromosome pairing and segregation in meiosis) when crossed with cultivated barley. Hybrids can also be formed in nature when these two occur at the same location (Asfaw & Von Bothmer 1990). Studies with wild and cultivated barley have reported that there is more variation within the wild than in the

cultivated barley (Saghai Maroof et al. 1995), although in some cases the opposite has been reported for some isozymes and mitochondrial DNA (Nevo, 1992). The larger genetic variation within wild barley gives good opportunity to use this variation for breeding purposes like drought, salinity and diseases resistance.

1.3.3 Wild barley (*H. vulgare* ssp. *spontaneum)*

Wild barley represents an important genetic resource for cultivated barley, which has a narrowed gene pool due to intensive breeding. Therefore, it is inevitably to study the genetics of different traits in wild barley, if it can use for cultivars improvement. *Hordeum vulgar* ssp. *spontaneum* (wild barley) is the ancestor of cultivated barley. It belongs to the Poaceae-family of grasses and within it to the Triticeae-tribe. Triticeae is a temperate plant group mainly concentrated around central and South-eastern Asia, although the species belonging to it are distributed around the world. Triticeae includes many economically important cultivated cereals and forages but also about 350 wild species. The wild species are of great interest as potential gene donors for commercial breeding (Vanhala 2004).

Wild ancestry: The wild ancestor of the cultivated barley is well known. The crop shows close affinities to a group of wild and weedy barley forms which are traditionally grouped in *Hordeum spontaneous* C. Koch, but which are in fact, the wild race or subspecies of the cultivated crop. The correct name for this wild is therefore *H. vulgare* L. ssp. *spontaneum* (C. Koch). These are annual, brittle, two-rowed, diploid (2n = 14), predominantly self-pollinated barley forms and the only wild *Hordeum* stock that is cross compatible and fully interceptive with the cultivated barley, *spontaneum* X *vulgare* hybrids show normal chromosome pairing in meiosis (Bothmer 1992). Morphologically, the similarity between wild spontaneous and cultivated two-rowed distichal varieties is rather striking. They differ mainly in their modes of seed dispersal. Wild barley ears are brittle and maturity disarticulates into individual arrow-like triplets. These are highly specialized organs, which ensure the survival of the plant under wild conditions. Under cultivation this specialization broke down and non-brittle mutants were automatically selected for in the man-made system of sowing, reaping and threshing (Harlan and Zohary 1966, Zohary 1969).

The development of new barley cultivars tolerant of abiotic and biotic stress is an essential part of the continued improvement of the crop. The domestication of barley, as in many crops, resulted in a marked truncation of the genetic variation present in wild populations. This process is significant to agronomists and scientists because a lack of allelic variation will prevent the development of adapted cultivars and hinder the investigation of the genetic

mechanisms underlying performance. Ellis et al. (2000) reported that wild barley would be a useful source of new genetic variation for abiotic stress tolerance if surveys identify appropriate genetic variation and the development of marker-assisted selection allows efficient manipulation in cultivar development.

The close genetic links between the cultivated crop and wild *spontaneum* barleys are indicated also by spontaneous hybridizations that occur sporadically when wild and cultivated forms grow side by side. Some of such hybridization products, combining brittle ears and fertile lateral spikelets, were in the past erroneously regarded as genuinely wild types and even given a specific rank (*H. agriocrithon* Åberg). Seed storage proteins, extensive isozyme and DNA tests have already been carried out in barley (Nevo 1992). The results confirm the close relationships between the wild and cultivated entities grouped in the *H. vulgare* complex.

1.3.4 Causal organism, inoculums sources

Approximately 19 *Fusarium* species can cause FHB or Scab (Liddell 2003), Among major causal species, including *F. culmorum, F. graminearum, Microdochium nivale, M. majus, F. avenaceum,* and *F. poae* as (Xu and Nicholson, 2009), *F. graminearum* Schwabe (telomorph = *Gibberella zeae* (Schw.)) is the pathogen that has caused most of the outbreaks of FHB in most countries of the world (Stack, 1999). Within *F. graminearum*, isolates may differ in virulence. For example, Chinese isolates may be more virulent than the isolates from U.S.A. (Bai et al. 2001; Lu et al. 2001). However, consistent specificity of genotype resistance and pathogen virulence was not observed and proof evidence for race differentiation has not been found (Lu et al. 2001; Bai et al. 1999). Thus use of a mixture of different *F. graminearum* isolates as inoculums to screen FHB resistance is a common practice for inoculation (Bai et al. 1999, Zhou et al, 2000).

Fusarium can survive in crop residues between host crop cycles. Ascospores, macroconidia, chlamydospores, and hyphal fragments can be all used as initial inoculums for infection (Bai and Shaner 2004, Dill-Macky 2003) with ascospores as the primary inocula during natural infection. However, *F. graminearum* conidia are often used as inoculums for experimental inoculation due to its easiness for production (Dill-Macky, 2003). Naturally, *F. graminearum* forms perithecia to produce ascospores (*Gibberrella zeae* (Schw.) Petch). Very thick wall of perithecia can keep the fungus viable throughout the winter, which provides the pathogen a potential epidemiological advantage to overwinter (Xu and Nicholson, 2009). In late spring, matured perithecia forcibly discharge their ascospores into air when high moisture is available

to initiate initial infection in wheat during wheat heading (Webster and Weber, 2007). Thus, crop residuals from previous crop seasons are major sources of inoculum, and increased tillage may lower residue retention and the amount of overwintering inocula.

The optimal stage for infection is flowering, as demonstrated by (Schroeder and Christensen 1963). They provided data where wheat heads were very resistant to infection before flowering and mostly susceptible at anthesis. After flowering, infection can occur until the soft dough stage. The first visible lesions can be seen after 3-4 days of infection. The lesions may be brown, purplish-brown or brown with a bleached center. Water soaked lesions may appear on the florets at the onset of the disease. Salmon-colored light pink spores may become evident on the florets of infected heads under heavy disease pressure.

Mesterhazy et al. (1999) reported no host specificity among isolates. In two experiments the researchers tested twenty and twenty-five genotypes with different degrees of resistance with seven and eight isolates, respectively, of *Fusarium graminearum* and *Fusarium culmorum* of diverse origin. The results indicated that a race-specific pattern in these two species is not significant, although some data reveal some isolates have a very small preference for some genotypes. They also emphasized that the resistance background to *F. graminearum* and *F. culmorum* is the same and no specific resistance to these two species exists.

1.3.5 Resistance types and disease assessment

Five mechanisms for resistance to FHB described by Mesterhazy (1995). These included type I: resistance to initial infection, type II: resistance to spread within the spike, type III: resistance to kernel infection, type IV: yield tolerance and type V: resistance to mycotoxin accumulation. Some morphological traits are also associated with increased disease. Generally, awned genotypes with a short peduncle and a compact spike have faster disease spread than genotypes that are awnless, have a lax spike, and a long peduncle (Mesterhazy, 1995). Also, short genotypes are more severely infected than tall genotypes.

Schroeder and Christensen (1963) proposed two types of resistance in wheat: Type I and type II resistance are the most commonly used, and several methodologies have been developed to assess these types of resistance by plant breeders. Type II resistance is primarily measured by carrying out experiments in the greenhouse, through the use of point inoculations with a syringe or needle. Resistance to spread of FHB symptoms within a spike has been considered to be a major component of FHB resistance (Bai et al. 1999). Different procedures to prepare

the spore suspension and produce the infection exist; typically a single central floret is inoculated at anthesis with 2 µl to 10 µl of a macroconidial spore suspension. Approximately 21 days after inoculation the number of infected spikelets is counted and the percentage of infection is obtained divided by the total number of spikelets.

Type I resistance is usually measured by inoculating plants in one of the two ways: (1) spraying plants at the time of anthesis with a conidial suspension and then counting infected spikes 20-22 days after inoculation, or (2) by grain spawn inoculation. This method is mostly used for the evaluation of large amounts of material in field nurseries. The protocols developed for this method are similar to those developed utilizing spray inoculation, with the exception that the inoculum comes from colonized grain (corn or wheat) that has been spread throughout the field. The disease assessment is done around 21-25 days after flowering and this method probably comes closest to simulating natural epidemics (Rudd et al. 2001).

Type III resistance, or resistance to kernel infection is generally measured by threshing infected spikes and observing the damage to the kernels. Kernel number reduction, kernel weight, test weight, or visual estimates of *Fusarium* damaged kernels (tombstones) are common measurements to assess Type III resistance.

Screening genotypes for FHB resistance is not a simple, quick or cheap task. Other abiotic or biotic factors such as freeze damage or glume blotch can mask classic disease symptoms making the disease evaluations more difficult. Another point to consider is the environmental conditions that are necessary for FHB to develop. High humidity and a mean temperature of 25 C are required for the infection and spread of the disease. Humidity chambers are commonly used in greenhouse experiments and in field experiments sprinkler irrigation systems are utilized as well as bagging techniques. There are few estimates in the literature of the costs of scoring FHB phenotype. Cost estimates provided by researchers reach six US dollars per data point (Van Sanford et al. 2001).

1.3.5.1 Type I and type II resistance screening methods

Screening for FHB resistance is carried out in many different ways and by many national and international breeding programs. Research is most often focused on type I and type II resistance which are: resistance to initial infection and resistance to spread throughout the spike, respectively. These two types of resistance can be screened for using distinct inoculation procedures. Grain spawn inoculum (infested corn *Zea mays* L.) for example) or a macroconidial spray is most often used when screening for type I resistance. Type II resistance is measured through the use of a point inoculation technique that involves injecting

a spore suspension directly into the barley spikelet. Disease Incidence is usually recorded as the percentage of diseased spikes over a total number of spikes.

Singh et al. (2008) rated for FHB incidence (Type I resistance) and severity (Type II resistance) and on a 1-9 disease rating scale. Griffey et al. (2009) reacted of wheat cultivar 5205 and four check cultivars for to (powdery mildew, leaf rust, stripe rust, leaf blotch, glume blotch, Wheat spindle streak mosaic virus, Soilborne mosaic virus, Barley yellow dwarf virus, FHB, stem rust, and Hessian fly) by a disease scale from 0-9 (0 = immunity to 9 = very susceptible). Osborne and Jin (2002) rated severity of FHB for each spike on a 0-9 scale roughly based on percent of the spike visually blighted (0 to 90+ %). Incidence rate was calculated by: number of infected spikes divided by total spikes counted per replicate. Severity was calculated for infected spikes by: (sum of spike severity ratings) divided by the number of infected spikes per replicate.

1.3.6 Barley breeding for tolerance /resistance to Fusarium head blight

Barley is a major cereal grain. Important uses include use as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and stews, and in barley bread of various cultures. Barley grains are commonly made into malt in a traditional and ancient method of preparation. The main breeding objectives are high yield, and resistance to biotic and abiotic stresses. Furthermore, malting cultivars need to have high malting quality, which includes plump kernels, rapid and uniform germination, and optimal values for protein content and enzymatic activity (Kraakman 2005).

Many breeders have attempted to find resistant sources to FHB. Although completely FHB immune cultivars have not been found (Fang et al. 1997). In the United States, extensive FHB screening programs were established in barley in the 1990s to evaluate barley germplasm for FHB resistance and low DON content (Steffenson 1998). Few sources of FHB resistance have been found in barley and the level of their resistance is modest. Although FHB in barley usually does not spread from spikelet to spikelet within a spike, barley seems to be very susceptible to initial infection. Severe disease usually results from multiple initial infections in the spike (Bai and Shaner 2004).

Takeda and Heta (1989), Zhou et al. (1991) have been screened more than 10,000 barley accessions from different countries for FHB resistance, but only several dozen accessions had a low level of FHB. Prom et al. (1996) and Urrea et al. (2002) in multiple screenings of barley

germplasm for FHB resistance, the Chinese accession CIho 4196 was identified as having one of the highest levels of Type I FHB resistance and lowest levels of DON accumulation.The results from the United States and Europe, two-rowed lines were more resistant than were six-rowed lines, and hulled types were more resistant than were hull-less types. Despite, these resistant accessions had less severe FHB, they were still infected by *Fusarium* (<20% infection) under favorable weather conditions and accumulated DON at concentrations greater than 0.5 ppm (Legge 1999).

The resistance in wheat and barley to FHB is considered to be a quantitative trait; therefore it is likely controlled by several genes (Buerstmayr et al. 1999; Bai&Shaner, 2004). In Buerstmayr et al. (1999) study six eastern European resistant lines and one susceptible line were intercrossed, heterosis for resistance was common, indicating that the parental genotypes possess different resistance genes. Bai and Shaner (1994) reported that because the genes for resistance in different cultivars appear to be on different chromosomes, crosses between these cultivars may yield transgressive segregates with greater resistance than any of the parents.

Estimates of the number and location of FHB resistance genes in wheat and barley vary with resistant lines studied, research methods, and experimental conditions (Bai & Shaner 2004). Kolb et al. (2001) referred to several possible reasons for inconsistent results from different investigations including polygenic control of FHB resistance in wheat, effect of different genetic backgrounds, genotype and environment interactions, different types of resistance evaluated, heterogeneous sources of a resistant parent, or inoculation techniques used in different studies. Molecular marker technology may be able to provide more precise information on the number and location of QTL for FHB resistance.

1.3.7 QTLs detected to FHB in barley and related traits

Quantitative trait locus (QTL) mapping is a tool for investigating traits showing complex inheritance, such as FHB. QTLs determining both qualitative and quantitative disease resistance have been mapped in barley (Chen et al. 1994, de la Pena et al. (1999), Dahleen et al. (2003, 2012), Yu et al. (2010), Massman et al. (2011)). Marker-QTL associations are useful for introgression and the pyramiding of resistance QTL alleles (Tanksley and Nelson 1996; Toojinda et al. 1998). Information regarding the number, genome location, and effects of genes determining the multiple components of resistance to FHB, and the relationships with loci determining morphological and developmental traits, should expedite the development of FHB-resistant varieties Zhu et al. (1999).

De la Pena et al. (1999) identified 10, 11, and 4 QTLs for FHB severity, kernel discoloration score, and low DON content, respectively, in a molecular mapping of a population of RILs derived from the cross between the six-rowed barley cultivars Chevron and M69. These QTL are distributed over all seven barley chromosomes. QTLs explaining 10% or more of the variation in FHB severity were found in chromosomes 2H and 7H, while QTLs explaining 10% or more of the variation in DON accumulation were found in chromosomes 1H, 2H, and 7H. In Ma et al. (2000) study, Chevron did not show any QTL with major effect on FHB resistance, but markers linked to QTL on chromosomes 1, 2, and 4 were proposed for marker-assisted selection.

Dahleen et al. (2003) identified nine QTLs for Type I resistance to FHB in an F1–derived DH population from the cross 'Zhedar 2'/ND9712//Foster. Two of the nine QTL identified for FHB resistance were located in chromosome 2H, and they were detected in all environments. Three additional QTL were identified in chromosome 1H; however, each of these QTL was detected in only one environment. In a fine mapping study two QTL for heading date and Type I FHB resistance, respectively, 47 cM from each other in chromosome 2H, suggesting FHB and heading date are controlled by linked loci and not pleiotropic effects of a single locus Nduulu et al. (2003).

Mesfin et al. (2003) created a genetic map of 143 molcular markers (SSR) from a population derived from the parents Fredrickson (two-rowed, moderately resistant) and Stander (six-rowed, susceptible) and developed an F4:6. They detected three QTLs distinct regions in chromosome 2H associated with FHB resistance; two of these regions also were associated with resistance to DON accumulation. One Type I FHB resistance QTL was also associated with heading date. A Type II FHB resistance QTL was detected only in the greenhouse but was coincident with a QTL for resistance to DON accumulation in the field.
Horsley et al. (2006) by using a recombinant inbred line mapping population from the cross Foster × CIho 4196, found the centromeric region of chromosome 2H flanked by the markers ABG461C and MWG882A contained two QTL controlling Type I FHB resistance and plant height, and one QTL each for DON accumulation, days to heading, and rachis node number. The resistance QTL in the bin 8 region was provisionally designated Qrgz-2H-8, and the one in the bin 10 region was provisionally designated Qrgz-2H-10.

Sato et al. (2008) evaluated five recombinant inbred (RI) populations for FHB resistance. The RI populations consisted of top-cross progeny derived from a diallel set of crosses. Each of

five two-row barley lines differing in response to FHB were crossed with 'Harbin 2-row'. Thirty two QTLs were detected using all data sets (individual populations and years). Thirteen QTLs were detected using averages across years; 10 of these were consistent across the individual year and average data sets. These QTLs clustered at 14 regions, with clusters on all chromosomes. At 11 of these clusters, Harbin 2-row contributed FHB resistance alleles. No QTLs were detected near the row type (vrs1) locus in any of the five RI populations, suggesting that the FHB resistance QTL in this region reported in two-row 9 six-row crosses may be pleiotropic effect of vrs1. QTL were coincident with the flowering type locus (cly1/Cly2) on chromosome 2H in every population. Some QTL 9 QTL interactions were significant, but these were smaller than QTL main effects.

A common result in all mapping papers mentioned is the identification of one or more QTL for Type I FHB resistance in the bin 8–10 region of chromosome 2H. On the basis of mapping data alone, no conclusion can be made whether these QTL may or may not be the same Lamb et al. (2009). Massman et al. (2011) summarized FHB QTL locations from seven resistance sources. Most of the resistant cultivars showed two FHB QTL on the long arm of chromosome 2H, the first with a coincident QTL for HD and the second associated with *vrs1*, the gene for spike morphology.

Dahleen et al. (2012) improved the mapping of FHB resistance and DON accumulation QTL in 'Zhedar 2'. An enzyme-linked immunosorbent assay (ELISA) using an antibody specific to *Fusarium* was proposed as an alternative for measuring FHB instead of counting infected kernels per spike. QTL analysis located 24 significant regions for the six traits (FHB, DON, heading date, height and spike density and/or the gene for cleistogamy). Also confirmed the locations of multiple QTL for FHB, DON, heading date and height and identified four regions associated with spike density. Only two ELISA QTL were found, but the FHB and DON QTL regions showed large allelic effects for ELISA, supporting ELISA as an alternative to time-consuming measurements of FHB severity in the field.

1.3.8 Detached leaf assay

A detached leaf assay was used by Diamond & Cooke (1999), Browne & Cooke (2004) and Browne *et al.* (2005) to study the partial disease resistance (PDR) components in commercial cultivars and germplasm of winter and spring wheat having a range of FHB resistance. Several PDR components were found to be significantly correlated with whole plant reactions. They concluded that this method of assay can be used as a pre field-screening tool,

as it offers the advantages that conditions that can be controlled, requires relatively little space, can be more readily repeated compared with whole plant tests, and individual measurement of a number of PDR components can be taken rather than just disease incidence or severity alone. Brown and Cooke (2005) reported that some detached leaf assay-derived PDR components were not effective in identifying wheat lines with high levels of whole-plant FHB resistance. Murakami & Ban (2005) reported that an oval lesion resulted when a spore suspension of *F. graminearum* was inoculated onto wounded portions of wheat leaves, and that lesion size increased significantly when leaf tissue was inoculated with both a spore suspension and purified deoxynivalenol (DON) toxin. Using wounded wheat leaves, their bioassay system was able to detect differences in disease reaction between resistant and susceptible cultivars.

A detached leaf assay also used by Kumar et al. (2011) to measure fusarium head blight resistance components in barley. Results showed genotypes previously identified or known to have a level of field resistance to FHB, exhibited resistance in the detached leaf assay based on measurement of latent period, lesion size or sporulation at room temperature, suggesting that measuring these PDR components to identify genotypes may have potential to complement assessments of FHB reaction in the field. The relationship among barley PDR components was inconsistent and several were poorly correlated based on the detached leaf assay.

1.3.9 Heading Date (HD) and FHB Resistance

Because the fungus infects flowers, flowering time is of key concern when rating disease on germplasm or segregating populations. Several studies have observed coincident QTLs for HD and FHB resistance (de la Pena et al. (1999); Ma et al. (2000); Mesfin et al. (2003); Zhu et al (1999)). The first observation of differences in FHB susceptibility between cultivars was made by Arthur (1891), who noted that early-maturing wheat cultivars tended to be more resistant than cultivars which matured later. Late heading is usually associated with low FHB severity in barley (Steffenson et al. 1996). In their results, the level of FHB severity in early heading near-isogenic lines was nearly six times greater than the late heading near-isogenic lines. Another mapping studies also indicated that there are coincidental QTL for FHB severity and heading date (de la Pena et al. 1999; Ma et al. 2000).

QTLs associated with HD and plant height were mapped on all barley chromosomes and have been reviewed by Hayes et al. (2000), and Börner et al. (2002). Mesfin et al. (2003) found

One FHB resistance QTL was associated with heading date in a Two-Rowed by Six-Rowed Population. Canci et al. (2004) detected a QTL for FHB on chromosome 2H was confirmed and was also associated with kernel discoloration and heading date. Previously showed in this study the correlation between FHB and related traits including HD.

1.3.10 Diversity Arrays Technology (DArT) Technology

The early maps were based primarily on RFLP markers, with more recent maps also including AFLPs and SSRs and very recently, Diversity Arrays Technology (DArT) is a microarray hybridization based technique that permits simultaneous screening thousands of polymorphic loci without any prior sequence information (Jaccoud et al. 2001). DArT is one of the recently developed molecular techniques and it has been used in barley (Wenzl et al. 2004, Mohamed 2009, Suárez et al. 2012 and Sayed et al. 2012), sorghum (Mace et al. 2008), lolium (Kopecký et al. 2009), rye (Brągoszewska et al. 2009) and apple (Schouten et al. 2012).

The DArT methodology offers a high multiplexing level, being able to simultaneously type several thousand loci per assay, while being independent of sequence information. DArT assays generate whole genome fingerprints by scoring the presence versus absence of DNA fragments in genomic representations generated from genomic DNA samples through the process of complexity reduction (Mace et al. 2008). DArT a microarray, hybridization-based platform - has a capacity to deliver several thousand of sequence-specific markers without relying on sequence information (Jaccoud et al. 2001). Polymorphic clones (DArT markers) show variable hybridization signal intensities for different individuals. These clones are subsequently assembled into a "genotyping array" for routine genotyping. The inventors promote it as an open source (nonexclusive) technology with a great potential for genetic diversity and mapping studies in a number of 'orphan' crops relevant in Third World countries (Semagn et al. 2006) (www.cambia.org or http://www.diversityarrays.com for information).

1.3.10.1 The advantages of DArT technique:

I. It does not need prior sequence information for the species to be studied; this makes the method applicable to all species regardless of how much DNA sequence information is available for that species.

II. It is a high throughput, quick, and highly reproducible method.

III. It is cost effective, with an estimated cost per data point tenfold lower than SSR markers (Xia et al. 2005).

IV. The genetic scope of analysis is defined by the user and easily expandable.

V. It is not covered by exclusive patent rights, but on the contrary open-source (i.e. it is designed for open use and shared improvement). (Semagn et al. 2006)

1.3.10.2 This technique has also its own limitations:

I. DArT is a microarray-based technique that involves several steps, including preparation of genomic representation for the target species, cloning, and data management and analysis. The latter requires dedicated software's such as DArTsoft and DArTdb. The establishment of DArT system, therefore, is highly likely to demand an extensive investment both in laboratory facility and skilled manpower.

II. DArT assays for the presence of a specific DNA fragment in a representation. Hence, DArT markers are primarily dominant (present or absent) or differences in intensity, which limits its value in some applications.

For quantitative trait analysis, DArT has many potential applications. Till now, DArT marker patterns have been principally applied to the assessment of genetic variability in a group of organisms, such the development of wild barley (*Hordeum chilense*) by Suárez et al. (2012). Wenzl et al. (2004, 2006) gives an example of such a map, showing how the standard techniques of map construction using linkage disequilibrium can be applied using DArT markers. DArT is especially appropriated to QTL mapping (Wittenburg et al. 2005), and can be used to construct medium-density linkage maps relatively quickly. As these studies clarified, the most accurate diversity analysis requires proportional amounts of clones from all individuals tested to be present on the array. If alleles from a genotype are under-represented on an array, then DArT will indicate potentially greater differences from the population average.

DArT markers can be used to track phenotypic traits in breeding like other molecular markers, and the high throughput and low cost nature of the technology makes DArT more affordable for marker assisted selection. Multiple loci can be involved in the selection process, but using an array means all loci is dealt with simultaneously. Such markers can then be tracked though an introgression or crossing program, and used to supplement phenotyping to reduce potential miss-identification of a trait due to environmental effects (Lande & Thompson 1990), as per any other marker-aided selection tool. Even though DArT can be applied in the absence of sequence information, individual DArT markers are sequence-ready and can be used in the development of probe-based markers for further research (Kilian, 2004). One shortcoming of DArT is the number of positions on a DArT array that are consistently non-polymorphic, i.e. non-marker clones. This has been recognised since the inception of this technology (Jaccoud

et al. 2001), and recent studies detail how polymorphic markers can be identified in an initial discovery array process, then re-arrayed for genotypic applications as polymorphism-enriched arrays (Wenzl et al. 2004, Xia et al. 2005).

Benson et al. (2012) used DArT markers for characterize the population structure and linkage disequilibrium in a population of North American winter wheat selected from programs developing cultivars for FHB-prone regions by using a collection of 251 winter wheat lines.

1.3.11 Linkage Map

The aim of genetic mapping studies is to identify quantitative trait loci (QTLs) that are responsible for phenotypic variation. Although often viewed as mainly different, linkage and association mapping share a common strategy that exploits recombination's ability to break up the genome into fragments that can be correlated with phenotypic variation.
Linkage mapping indicate to the specification of a particular chromosomal segment or segments within the genome that carry a causal DNA variant or mutation leading to a biological phenotype of interest. The appropriate chromosomal segment is determined through the use of anonymous polymorphic DNA markers as tags in different individuals who share the phenotype. Statistical analysis of data is usually critical in the determination. For whole-genome linkage analysis, the most commonly used polymorphic markers are short tandem repeats, known as microsatellites or STRs. The experimental use of these markers has many subtleties and pitfalls, which are reviewed. Successful linkage mapping for a phenotypic trait is followed by the process of positional cloning, whereby the true underlying genetic variant is discovered. The final step from anonymous chromosomal segment to sequence variant detection can be relatively straightforward or highly demanding, depending on the complexity of the phenotype, the severity of the mutation in affecting gene function, and the extent to which carriers of the mutation are predisposed to the phenotype.

One of the first well developed classical genetic maps for barley included isozymes and morphological markers (Sogaard and von-Wettstein-Knowles 1987). Genetic mapping of barley accelerated with the application of molecular markers to doubled haploid (DH) populations (Chen and Hayes, 1989). Subsequently, molecular markers were added, beginning with RFLP and PCR markers (Shin et al. 1990), and these maps became more dense (Graner et al. 1991, and Kleinhofs et al. 1993) enabling the mapping of many important agronomic qualitative and quantitative traits. New molecular markers were developed, improving the barley genetic map with AFLP markers (Waugh et al. 1997, Qi et al. 1998, Yin et al. 1999 and Hori et al. 2003).

1.3.12 Association mapping (AM), methods and applications

In the past decade, association mapping has emerged as a tool for studying the genetics of natural variation and economically important traits in plants (Atwell et al. 2010). Flowering date, disease resistance, chemical composition and many other economically and evolutionarily important traits have been studied in crop species (Zhu et al. 2008). Apart from agriculturally relevant crops, the model plant Arabidopsis (*Arabidopsis thaliana*) is of great value for understanding complex traits using AM.

The presence of recombination events that have accumulated in plants over thousands of generations is both an advantage as well as a potential pitfall of AM, because functional QTLs that are correlated with population structure can result in many false positives (Yu and Buckler 2006). Several statistical methods have been developed that use neutral genotypic information to account for confounding effects of population structure in AM studies (Price et al. 2006; Zhao et al. 2007; Feng et al. 2009).

Yu et al. (2006) developed new methodology, a mixed linear model (MLM) that combines both population structure information (Q-matrix) and level of pairwise relatedness coefficients—"kinship" (K-matrix) in the analysis, where the mixed linear model (MLM) approach found to be effective in removing the confounding effects of the population in association.

Overall approach of population-based association mapping in plants varies based on the methodology chosen, assuming structured population samples, the performance of association mapping includes the following steps as described by Abdurakhmonov and Abdukarimov (2008).

(1) Selection of a group of individuals from a natural population or germplasm collection with wide coverage of genetic diversity. (2) Recording or measuring the phenotypic characteristics (yield, quality, tolerance, or resistance) of selected population groups. (3) Genotyping a mapping population individuals with available molecular markers. (4) Assessment of the population structure (the level of genetic differentiation among groups within sampled population individuals) and kinship (coefficient of relatedness between pairs of each individual within a sample). And (5) based on information gained through population structure, correlation of phenotypic and genotypic/haplotypic data with the application of an appropriate statistical approach that reveals, consequently a specific gene(s) controlling a

QTL of interest can be cloned using the marker tags and annotated for an exact biological function. Association mapping offers three main advantages: increased mapping resolution, reduced research time, and greater allele numbers (Reich et al. 2001).

Association mapping, also known as linkage disequilibrium mapping, is a relatively new and promising genetic method for complex trait dissection. Association mapping has the promise of higher mapping resolution through exploitation of historical recombination events at the population level that may enable gene level mapping on non-model organisms where linkage based approaches would not be feasible (Varshney and Tuberosa 2007). Differences between linkage mapping and association mapping methods and the aim represented in table (1).

Table: (1) Comparison of family-based (QTL) and population-based (association mapping) methods that aim to unravel the genetic basis of complex traits in plants[a] (Kloth et al. 2012).

	QTL-mapping[b]	Candidate gene association mapping	Genome-wide association mapping
Main advantages	- No population structure effects - Identification of rare alleles - Few genetic markers required	- Allows fine mapping - Relatively low costs	- Allows untargeted fine mapping (blind approach) - Detection of common alleles
Main disadvantages	- Limited genetic diversity - Not always possible to create crosses - Cannot distinguish between pleiotropic and physically close genes	- Detailed functional knowledge of trait is required - No novel traits will be found	- Confounding effects due to population structure -Will miss rare and weak effect alleles
General requirements	- Small 'original population size', low number of genetic markers, many replicates needed - Generated mapping material, e.g. F2 population, (AI-)RILs, MAGIC lines, NILs, HIFs, etc.	- Large population size, small number of genetic markers, the bigger the population size, the less replicates needed - Prior genetic and biochemical knowledge on trait of interest - Prior knowledge on LD, nucleotide polymorphism, breeding system and population structure	- Large population size, many genetic markers, the bigger the population size, the fewer replicates needed - Prior knowledge on LD, nucleotide polymorphism, breeding system and population structure
Recent case study in *Arabidopsis*	- QTL mapping with AI-RILs on flowering time (Balasubramanian et al. 2009) two AI-RIL populations (approximately 280 individuals each) 181 and 224 markers 12 to 70 replicates	- Candidate gene approach on flowering time (Ehrenreich et al. 2009) 251 accessions 51 SNPs 10 replicates per accession	- Whole-genome approach on multiple phenotypic traits (Atwell et al. 2010) 199 accessions in total 216 150 SNPs Four replicates in general

[a] Combinations of these three approaches can allow the identification of false positives and negatives, but is much more laborious: a recent dual QTL mapping–GWA study (Bergelson and Roux 2010) involved phenotyping nearly 20 000 individual plants, including 184 worldwide natural accessions genotyped for 216 509 SNPs and 4366 RILs derived from 13 independent crosses. See (Zhao et al. 2007) for an overview of different linkage mapping populations mentioned in this table.

[b] Abbreviations: AI-RIL, advanced intercross-recombinant inbred line; HIFs, heterogeneous inbred family; LD, linkage disequilibrium; MAGIC, multiparent advanced generation intercross; NIL, near-isogenic line; QTL, quantitative trait locus; RIL, recombinant inbred line; SNPs, single nucleotide polymorphisms. (Kloth et al. 2012).

The most difficulties and problems with association mapping is that population structure can lead to spurious association between a candidate marker and a phenotype. One common solution has been to abandon case-control studies in favour of family-based test of association, but this comes at a considerable cost in the need collect DNA from close relative of affected individuals (Pritchared et al. 2000).

Breseghello and Sorrells (2006) studied AM in wheat for identification of genetic markers associated with kernel morphology and milling quality. They used in their study a population of 149 cultivars of soft winter wheat (*Triticum aestivum* L.), were genotyping with 93 SSR markers, Association between markers and traits was tested using a linear mixed-effect model, where the marker being tested was considered as a fixed effects factor and subpopulation was considered as a random-effects factor. Significant markers were detected in the three chromosomes tested; kernel width was associated with the locus Xwmc111-2D in both Ohio (OH) and New York (NY) and with Xgwm30-2D in NY only. A tow-marker model including both loci was significantly (P = 0.0002) more informative for KW in NY than either marker separately. The locus Xgwm539-2D was associated with kernel length in NY, although in this location it did not achieve the corrected threshold. Six loci in the LD block near the centromere of 5A were associated with kernel area, length, and weight, but not with kernel width.

Yu et al. (2006) observed six gene expression phenotypes as phenotypic traits in mapping expression quantitative trait loci (eQTL). For the sample containing complex familial relationships and population structure, and they studied three quantitative traits measured on 277 diverse maize inbred lines, representing the diversity present in public breeding programs around the world. The population differentiation (Fst) among the major subgroups in our sample ranged from 0.047 (SSR) to 0.073 (SNP)., Although 80% of the pair-wise kinship estimates were close to 0, the remaining estimates were distributed from 0.05 to 1.0, as expected from complex familial relationship and population structure. Furthermore they found 37.6% of SNPs were associated with flowering time at $P < 0.05$ by the simple model, compared with 14.1% by the Q model, 6.1% by the K model and only 6.0% by the Q+K model. For flowering time and ear height, the Q+k model had the highest power. For ear diameter, the k model yielded a slightly higher power than the Q+k model did. The most benefit of the Q+K model is able to systematically account for multiple levels of relatedness among individuals.

Kantartzi and Stewart (2008) analysed of genetic distance and population structure provided evidence of significant population structure in the *Gossypium arboreum* accessions and identified the highest likelihood at k=6 . A total of 30 marker-trait association were identified with 19 SSR markers located on 11 chromosomes, the association analysis identified marker-trait associations (P=0.05) for all traits evaluated. Lint%, lint colour, elongation, micronaire and perimeter were associated with four markers each, length with three markers, and strength and maturity with tow and five markers respectively, Furthermore the LD (R2 values) between markers ranged from 10% to 20%. Of the 30 marker-trait associations, four identified 15% or more of the total variation for lint% (BNL0256 and BNL1122), lint colour (BNL0542) and length (BNL1122).

Benson et al. (2012) selected a collection of 251 winter wheat lines out of collaborative FHB screening nurseries this collection were genotyped with simple sequence repeat (SSR), sequence tagged site (STS), and Diversity Array Technology (DArT) markers to assess LD, genetic diversity, and population structure, for understanding population structure, linkage disequilibrium, and genetic diversity in soft winter wheat enriched for Fusarium head blight resistance. Cluster analysis was done using Nei's genetic distance (Nei, 1972). Principal component analysis (PCA) was performed in SAS 9.2 (SAS Institute, 2010). The first 10 eigenvectors of PCA were calculated from the correlation matrix derived from marker genotypes and eigenvectors were graphed to visualize relatedness of lines. Model-based analysis of PS was done with the program STRUCTURE (Pritchard, 2000) with the conditions of admixture and non correlated allele frequencies. They reported that the genome wide average of LD decay to $r^2 < 0.2$ was 9.9 cM and moderate levels of LD ($r^2 > 0.2$) were generally constrained to markers less than 5 cM apart. Although the lines evaluated were targeted to distinct production zones of the eastern winter wheat region, cluster and principal component analyses did not detect separation of lines into subpopulations.

1.3.13 Linkage disequilibrium (LD)

Linkage disequilibrium (LD) is the non-random association of alleles in a sample population and forms the basis for the construction of genetic maps and the localization of genetic loci for a variety of traits. The principles leading to LD apply to both biparental mapping populations (F_2, RILs, etc) and natural populations. Because of its inherent advantages, LD mapping approaches are increasingly being applied for plant species, in particular maize. Due to the out-breeding character of this species, LD extends only over a few kb and thus leads to

a high genetic resolution, up to the level of individual candidate genes that can be associated with a given trait (Rafalski and Morgante 2004, Gupta et al. 2005).
A European germplasm collection of 146 two-rowed spring barley cultivars was used to carry out LD mapping of yield traits using 236 AFLP markers (Kraakman et al. 2004). Associated markers were identified that are located in similar regions where QTLs for yield had been found in barley (Romagosa et al. 1999 and Li et al. 2006).

A survey of 953 cultivated barley accessions representing a broad spectrum of the genetic diversity in barley genetic resources revealed that LD extends up to 50 cM but is highly dependent on population structure (Kraakman et al. 2004 and Malysheva-Otto et al. 2006). On the one hand, the high level of LD in barley is due to the inbreeding mating type of this species; on the other hand, the selection of germplasm plays an important role analysis of a germplasm collection of European cultivars, land races and wild barley accession from the Fertile Crescent region provided hints that the level of LD increases from cultivars to landraces to wild barley (Caldwell et al. 2006). Similarly, Morell et al. (2005) reported low levels of LD in wild barley by examining LD within and between 18 genes from 25 accessions.

The genotyping database for 953 cultivated barley accessions profiled with 48 SSR markers was established. The principal coordinate analysis revealed structuring of the barley population with regard to (i) geographical regions and (ii) agronomic traits. Geographic origin contributed most to the observed molecular diversity. Genome-wide linkage disequilibrium (LD) was estimated as squared correlation of allele frequencies (r^2). The values of LD for barley were comparable to other plant species (conifers, poplar and maize). The pattern of intrachromosomal LD with distances between the genomic loci ranging from 1 to 150 cM revealed that in barley LD extended up to distances as long as 50 cM with $r^2 > 0.05$, or up to 10 cM with $r^2 > 0.2$. Few loci mapping to different chromosomes showed significant LD with $r^2 > 0.05$. The number of loci in significant LD as well as the pattern of LD was clearly dependent on the population structure. The LD in homogenous group of 207 European 2-rowed spring barleys compared to the highly structured worldwide barley population was increased in the number of loci pairs with $r^2 > 0.05$ and had higher values of r^2, although the percentage of intrachromosomal loci pairs in significant LD based on $P < 0.001$ was 100% in the whole set of varieties, but only 45% in the subgroup of European 2-rowed spring barley. The value of LD also varied depending on the polymorphism of the loci selected for genotyping (Malysheva-Otto et al. 2006).

Steffenson et al. (2007) used 318 Wild Barley accessions to perform association mapping studies using DArT markers to identify rust resistance genes. In addition LD analysis has been performed based on haplotypes derived from 131 accessions by covering 83 SNPs within 132 kb around the gene HveIF4E, which confers resistance to barley yellow mosaic virus.

To conduct robust association mapping (AM) studies of economically important traits in US barley breeding germplasm, population structure and LD decay were examined in a complete panel of US barley breeding germplasm (3840 lines) genotyped with 3072 single nucleotide polymorphisms (SNPs). Nine subpopulations (sp1–sp9) were identified by the program STRUCTURE and subsequently confirmed by principle component analysis (PCA). LD was found to decay across a range from 4.0 to 19.8 cM. This result indicates that the germplasm genotyped with 3072 SNPs would be robust for mapping and possibly identifying the causal polymorphisms contributing to disease resistance and perhaps other traits (Zhou et al. 2012).

2. Materials and Methods

2.1 Plant material

One hundred fourty barley accessions were used in this study. They were distributed as follows:

a) 108 accessions of wild barley (*H. vulgare ssp. spontaneum*) and 7 landraces (*H. vulgare ssp. vulgare*) from the ICBB core collection (gene banks in Gatersleben and Braunschweig).

b) 21 spring barley cultivars representative for the breeding pool of spring barley (*H. vulgare ssp. vulgare*) in the North Rhine Westphalia (NRW), Germany, (Reetz and Léon 2004) and 4 common cultivars (Scarlett, Lerche, Barke (1996 breeder Josef Breun) and Thuringia). The seeds of these cultivars were provided by the Institute of Crop Science and Resource Conservation (INRES), chair of plant breeding.

2.2 Experiments Design

2.2.1 Green-house Experiments

Experiments were carried out in glass green-house during 2009, 2010 and 2011 at INRES, chair of Plant Pathology, Faculty of Agriculture, Rheinische Friedrich-Wilhelms-University of Bonn. Green-house conditions were, with a 16-h light photoperiod at 24 ± 2 ºC. The intensity of light was 300-µmol m^{-2} s^{-1} in plant height. These conditions were the same during three years.

2.2.1.1 Year 2009

This experiment was conducted in autumn season (August- November) using 30 Barley genotypes consists of 21 spring barley cultivars, 7 Landraces and 2 common German cultivar Scarlett and Barke. The experiment was arranged in a completely randomized design using 5 replications. Each replicate was sown with one seed in plastic pots of 15-cm width and 25-cm depth. Pots contained a 7:3:1 mixture of organic potting substrate, field soil (soil horizon C) and sand, respectively. No fertilizers added to pots till harvest.

2.2.1.2 Years (2010 and 2011)

These experiments were conducted in winter/spring season using 140 barley accessions. The experiment was arranged in a completely randomized design using 5 replications, seeds of all accessions have been germinated in Petri dishes onto wetted tissue paper in refrigerator at 4 ^{0}C for 7 days, after that 5 seeds from each genotype (as replications) were sown separately in

plastic pots of 15-cm width and 25-cm depth. Pots were filled with same previous soil. Also in these two years no fertilizer added.

2.2.2 Field-potted Experiment 2011

This experiment was carried out using 32 Barley genotypes consisting of 21 spring barley cultivars, 7 Landraces and 4 common German cultivars (''Scarlett'', ''Lerche'', ''Barke'' and '' Thuringia''), in field-potted conditions during spring/summer season 2011 at INRES, Chair, of Plant Pathology. The experiment was arranged in a randomized block design with 5 replications, 12 seeds in two rows were sown in plastic pots of dimensions 22 x 22 x 25 cm, with 4 holes pierced at the bottom for drainage. This experiment was done without fertilizer. These pots had the previous mentioned soil. The experiment were done in normal conditions and covered from rain.

2.3 Pathogen

Two different isolates from *Fusarium graminearum* Schwabe (teleomorph : *Gibberella zeae* (Schw.) Petch) were used in green-house and field-potted experiments; first isolate was used in 2009, 2010 and 2011 and second isolate was used only in 2011 experiments. First isolate was FG 5.1 and second isolate was FG 5.3. Both isolates were from the Institute of Crop Science and Resource Conservation, Chair of Plant Pathology, University of Bonn, Germany.

2.4 Inoculum production

Fusarium graminearum isolates were cultured in potato-dextrose broth (24 gl^{-1}) in 1000 mL Erlenmeyer flasks for five days in darkness on a shaker (200 rpm) at 22 ºC. After 3 days of incubation, 500-1000 microliter suspension were spread over the surface of petri-dishes containing LSPDA, water agar (agar-agar 20 g/l^{-1}), the cultures were dried under a laminar flow cabinet for 20 to 30 min.

The plates were incubated under near ultra violet light at 22 ºC for 3 days. Conidia were harvested by adding sterile distilled water including some droplets of Tween 20 and slightly scraping with a spatula. The suspension was passed through double layered cheesecloth. The number of conidia per mL was determined using a haemocytometer.

2.5 Evaluations of disease scoring

2.5.1 Leaves evaluation

At seedling growth (5 leaves unfolded), an experiment was carried out for leaves evaluation by cutting third leaf from each plant and dividing it into 5 pieces (2 cm). These pieces were washed in Ethyl alcohol 75% for 3 seconds, then in distilled water three times, and cultured it in petri dichs included kinetin agar media (6g Agar-Agar/l + 10mg Kinetin/l) (Browne et al. 2004). Then, each leaf piece was inoculated with 10^4 conidia mL^{-1}/piece of *Fusarium graminearum.* Finally, the petri dichs were incubated in growth chamber at 12/12 light/darkness and temperature 24°C.

2.5.2 Spikes evaluations

2.5.2.1 Spikes evaluations in green-house

At anthesis, 5 central florets from the main spike of each plant with were inoculated with ca. 10^3 conidia/floret (Zhou et al. 2010), then covered with paper bag for each spike (for 24 h). At the plant maturity time, were harvested by hand and the spike was placed into the paper envelopes in order to avoid the loss of grains.

2.5.2.2 Spikes evaluations in field-potted

At anthesis, each pot was inoculated with 100 mL spore suspension (10^5 conidia mL^{-1}). The plants were covered with big plastic bag for each pot (for 24 h), to ensure high relative humidity for optimum infection conditions.

2.6 Experimental design

The experiments tested in green-house 2009, 2010 and 2011 were conducted in a completely randomized design with 5 replicates; each replicate was a plant per pot. For the first scoring test (leaves disease scoring), 25 readings were taken from each accession (third leaf from 5 plants, each leaf cut onto 5 pieces). In the second scoring test (spikes disease scoring), 5 readings/accession were taken. All these scores were conducted in 2009 and 2010 for one *Fusarium* isolate (5.1).

In 2011, a second Isolate (5.3) was used. The experiment was conducted in two parts: first the Isolate 5.1 was used with 3 replicates and 15 readings were taken from each accession for leaves disease scoring (third leaf from 3 plants, each leaf cut onto 5 pieces). Second part, the Isolate 5.3 was used with 2 replicates and 10 readings were taken from each accession for leaves disease scoring (third leaf from 2 plants, each leaf cut onto 5 pieces).

In second scoring (spikes disease scoring), 5 readings were taken for each accession; 3 readings from 5.1 isolate and 2 readings from 5.3 isolate.

Field-potted experiment 2011 was conducted in a completely randomized block design with 4 blocks (2 blocks/isolate), each block consisted of 32 pots; 12 plants were sown in each pot in two rows. For first scoring test (leaves disease scoring), 4 random leaves (third leaf) were taken from four plants and each leaf was cut down into 5 pieces (20 scoring reads/pot). Second scoring (spikes disease scoring) was done after harvesting inoculated spikes (one read/pot).

2.7 Phenotypic data measurements

2.7.1 Disease scoring

2.7.1.1 Leaves Disease Scoring (LDS)

Seven days after inoculation, the disease symptoms were scored using scale from 0 to 9; (0 = immunity to 9 = very susceptible) (Osborne and Jin 2002), for each part from five parts of third cut leaf. In 2009, visual scoring was carried out without monitoring leaves, while in 2010 and 2011 all inoculated leaf parts were monitored using electronic microscope, for assessment disease infection or scoring photos, image analysis software for plant disease quantification (APS Assess) was used for evaluation of the images (this program calculates the whole area of the leaf and the infected area gives % infection percentage).

2.7.1.2 Spikes Disease Scoring (SDS)

The symptoms were visible to assess, disease severity was assessed as the percentages of bleached spikes, using a nine-class rating scale from 0 to 9 (0 = no bleached spikes and 9 = completely bleached). Diseased spikelet inoculated spike with considering if the seeds were shrivelled or not. The disease scoring scale 0-9 (0 = immunity to 9 = very susceptible) was used (Osborne and Jin 2002).

2.7.2 Heading Date (HD)

Heading date (HD) was scored in 2011 only, and was calculated in green-house as days from planting (10th February) to anthesis (de la Pena et al. 1999). In field-potted calculated from date of sowing (23rd May) to 50% of spikes were fully emerged from the boot in each pot (Lamb et al. 2009).

2.8 DNA extraction for DArT markers

DNA has been extracted from freeze dried leaves by using "Qiagen®Kit" procedure according to DNeasy Plant Handbook 07/2006 as follows:

1. Place sample material (10 mg lyophilized tissue) into each tube in 2 collection microtube racks. Add one tungsten carbide bead to each collection microtube. Seal the microtubes with the caps provided. Cool the racks of collection microtubes in liquid nitrogen. Ensure that the microtubes remain tightly closed.
2. Place a clear cover (saved from step 1) over each rack of collection microtubes, and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the microtubes. Ensure that no liquid nitrogen remains, but do not allow the leaf material to thaw. Remove the clear cover.
3. Sandwich each rack of collection microtubes between adapter plates and fix into TissueLyser clamps as described in the TissueLyser User Manual. Work quickly so that the plant material does not thaw. Grind the samples for 1 min at 20 Hz.
4. Remove and disassemble the plate sandwiches, noting the orientation of the racks of collection microtubes during the first round of disruption. Ensure that the collection microtubes are tightly closed.
5. Cool the racks of collection microtubes again in liquid nitrogen. Place a clear cover over each rack of collection microtubes and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the microtubes. Ensure that no liquid nitrogen remains, but do not allow the leaf material to thaw. Remove the clear cover.
6. Ensure that the collection microtubes are tightly closed. Reassemble the plate sandwiches so that the collection microtubes nearest the. Reinsert the plate sandwiches into the TissueLyser. Work quickly so that the plant material does not thaw.
7. Grind the samples for another 1 min at 20 Hz. Remove the plate sandwiches from the TissueLyser and remove the adapter plates from each rack of collection microtubes. Knock the racks against the bench 5 times to ensure that no tissue powder remains in the caps. Keep the samples frozen until working lysis solution is added.
8. Combine Buffer AP1, RNase A, and Reagent DX according to the table below to make a working lysis solution. Carefully remove the caps from the collection microtubes. Immediately pipet 400 µl working lysis solution into each collection microtube.
9. Seal the microtubes with new caps (provided); ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection

microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

10. Remove and discard caps. Add 130 µl Buffer AP2 to each collection microtube. Close the microtubes carefully with new caps (provided); ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
11. Incubate the racks of collection microtubes for 10 min at –20°C. Centrifuge the racks of collection microtubes for 5 min at 6000 rpm. Remove and discard the caps. Carefully transfer 400 µl of each supernatant to new racks of collection microtubes (provided), ensuring that the new tubes are in the correct orientation. Add 1.5 volumes (typically 600 µl) of Buffer AP3/E to each sample.
12. Close the collection microtubes with new caps (provided); ensure that the tubes are properly sealed to prevent leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
13. Place two DNeasy 96 plates on top of S-Blocks (provided). Mark the DNeasy 96 plates for later sample identification. Remove and discard the caps from the collection microtubes. Carefully transfer 1 ml of each sample to each well of the DNeasy 96 plates. Seal each DNeasy 96 plate with an AirPore Tape Sheet (provided). Centrifuge for 4 min at 6000 rpm.
14. Remove the tape. Carefully add 800 µl Buffer AW to each sample. Centrifuge for 15 min at 6000 rpm to dry the DNeasy membranes. To elute the DNA, place each DNeasy 96 plate in the correct orientation on a new rack of Elution Microtubes RS (provided), add 100 µl Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided). Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 6000 rpm. Repeat step 26 with another 100 µl Buffer AE.

2.9 DArT Markers analysis

The extracted DNA of the accessions was sent to Diversity Arrays Technology P/L -

Triticarte P/L,1 (http://www.triticarte.com.au/default.html) Wilf Crane Crescent, Yarralumla ACT 600, AUSTRALIA (Wenzl et al. 2004) for genotyping. For DArT marker analysis, the genotyping with 1081 DArT markers had been done by hybridization based markers. In this set of 1081 two sorts of marker were included polymorphic and monomorphic markers, after filtering the monomorphics it is became 895 markers. The chromosomal positions of the DArT markers are according to Wenzl et al. (2006). Their technology involves reducing the complexity of the sample by cutting with restriction enzymes and annealing adaptors. Then fragments are amplified from the adaptors. The fragments are labelled and hybridized to a microarray of variable fragments representing the diversity within the species. DArT markers are biallelic dominant markers, so the data was received as 0/1 (absent/present). The linkage map has been drawn by using MapChart ver.2.2 (Voorrips 2002).

2.10 Statistical analysis

The statistical analysis was conducted in five parts as follows

2.10.1 Phenotypic data

The phenotypic data were analysed each season separately as a one way ANOVA using Proc GLM procedure, by SAS version 9.2 (SAS Institute 2008). Pearson correlation coefficients (r) between traits under disease infection stress conditions were calculated by the SAS Procedure too.

The analysis for each year was carried out using a mixed linear model

$$Y_{ijk} = \mu + T_i + A_j + T_i*A_j + \varepsilon_{ijk}$$

Where μ is the general mean, T_i is the fixed effect of *ith* disease isolates, A_j is the random effect of *jth* accession, T_i*A_j is the random interaction effect of the *ith* disease isolates with *jth* accession, and ε_{ijk} is the residual effect.

2.10.2 Structure analysis and relatedness relationships

Population structure analysis was carried out using "STRUCTURE" software version 2.2. Software package (Pritchard et al. 2000), to estimate the number of subpopulations within the mapping set by using filtered DArT data, the admixture setting with correlated allele frequencies, and a burn in and run time of 100,000 with K = 1–22 with two iterations and the number of replications (MCMC) was 200,000.

The number of subpopulations (K) present in the dataset was estimated by plotting the probability of data ln Pr (X/K) for each for value of K. The variation of ln Pr (X/K) among independent simulation runs with the same value of K and the rate of ln Pr (X/K) change from K-1to K-22 was used to select the optimal number of subpopulations in the population under study.

The recognized subgroups from 124 genotypes are shown in table (2): Based on the suggestions of Pritchard and Wen (2007),

All analyses were done for 124 genotypes only (from total 140). However DArT markers data for 16 genotypes were not available.

The relative kinship coefficients (K-matrix) among all pairs of accessions were calculated using 895 DArT marker data by "SPAGeDi-1.3d" Software (Hardy and Vekemans 2002).

2.10.3 Principal Component Analysis (PCA)

This analysis was carried out by using SAS 9.2 program PROC PRINCOMP, according to (price et al. 2006) for study of the population structure. The significance for PCA was evaluated using Franklin et al. (1995) method. The significant determined how many Principal Components PCs can be used in the statistical model.

Table: (2) List of 140 accessions of the studied barley population.

No.	Accession	Type	Origin	Subp.	No.	Accession	Origin	Type	Subp.	No.	Accession	Type	Origin	Subp.
1	ICB180001	*Hsp*	SYR	6	48	ICB180882	AFG	*Hsp*	12	95	ICB181492	*Hsp*	TJK	4
2	ICB180006	*Hsp*	SYR	6	49	ICB180887	IRN	*Hsp*	7	96	ICB181498	*Hsp*	SYR	4
3	ICB180007	*Hsp*	PAL	2	50	ICB180902	TUR	*Hsp*	3	97	ICB181500	*Hsp*	UZB	4
4	ICB180013	*Hsp*	PAL	9	51	ICB180923	PAL	*Hsp*	9	98	ICB191338	*Hsp*	PAL	4
5	ICB180014	*Hsp*	PAL	2	52	ICB180927	JOR	*Hsp*	7	99	IG119451	*Hsp*	PAL	10
6	ICB180018	*Hsp*	PAL	2	53	ICB180973	JOR	*Hsp*	9	100	IG121857	*Hsp*	PAL	2
7	ICB180044	*Hsp*	PAL	4	54	ICB180982	JOR	*Hsp*	3	101	IG123991	*Hsp*	SYR	4
8	ICB180046	*Hsp*	PAL	9	55	ICB180994	JOR	*Hsp*	12	102	IG12400	*Hsp*	UZB	4
9	ICB180051	*Hsp*	PAL	4	56	ICB181150	JOR	*Hsp*	12	103	IG124017	*Hsp*	UZB	4
10	ICB180052	*Hsp*	SYR	14	57	ICB181154	SYR	*Hsp*	5	104	Ingrid	*Hv*	GER	14
11	ICB180063	*Hsp*	IRN	8	58	ICB181156	SYR	*Hsp*	5	105	Emir	*Hv*	GER	14
12	ICB180068	*Hsp*	IRN	13	59	ICB181158	SYR	*Hsp*	5	106	Contra	*Hv*	GER	14
13	ICB180069	*Hsp*	SYR	8	60	ICB181160	SYR	*Hsp*	4	107	Carina	*Hv*	GER	14
14	ICB180070	*Hsp*	IRN	13	61	ICB181162	SYR	*Hsp*	11	108	Aramir	*Hv*	GER	14
15	ICB180072	*Hsp*	SYR	7	62	ICB181164	SYR	*Hsp*	11	109	Kym	*Hv*	GER	14
16	ICB180079	*Hsp*	IRN	9	63	ICB181168	SYR	*Hsp*	11	110	Candice	*Hv*	GER	14
17	ICB180092	*Hsp*	IRN	9	64	ICB181170	SYR	*Hsp*	11	111	Camelot	*Hv*	GER	14
18	ICB180102	*Hsp*	IRN	12	65	ICB181172	PAL	*Hsp*	11	112	Cheri	*Hv*	GER	14
19	ICB180109	*Hsp*	IRN	12	66	ICB181176	PAL	*Hsp*	11	113	Otis	*Hv*	GER	14
20	ICB180117	*Hsp*	IRN	4	67	ICB181180	PAL	*Hsp*	15	114	Peragis	*Hv*	GER	14
21	ICB180148	*Hsp*	IRN	13	68	ICB181182	PAL	*Hsp*	15	115	Schwarze G.V. Strube	*Hv*	GER	14
22	ICB180199	*Hsp*	IRN	12	69	ICB181184	PAL	*Hsp*	13	116	Alpine Pfauengerste	*Hv*	GER	14
23	ICB180211	*Hsp*	SYR	4	70	ICB181186	IRN	*Hsp*	5	117	Dummersdorf	*Hv*	GER	14
24	ICB180215	*Hsp*	TKM	9	71	ICB181216	TKM	*Hsp*	2	118	Jassener Land	*Hv*	GER	14
25	ICB180217	*Hsp*	UZB	4	72	ICB181228	IRN	*Hsp*	16	119	Neuhaus Landgerste	*Hv*	GER	14
26	ICB180231	*Hsp*	JOR	9	73	ICB181230	IRN	*Hsp*	16	120	Oberpfälzer	*Hv*	GER	14
27	ICB180260	*Hsp*	TUR	12	74	ICB181238	IRQ	*Hsp*	4	121	Danubia	*Hv*	GER	14
28	I*CB180303*	*Hsp*	TUR	12	75	ICB181243	SYR	*Hsp*	4	122	Voldagsen	*Hv*	GER	14
29	ICB180329	*Hsp*	SYR	9	76	ICB181268	TKM	*Hsp*	9	123	Reisgersten Linie II	*Hv*	GER	14
30	ICB180389	*Hsp*	JOR	8	77	ICB181323	TUR	*Hsp*	6	124	Heidesandgerste	*Hv*	GER	14
31	ICB180410	*Hsp*	SYR	12	78	ICB181324	UNK	*Hsp*	9	125	Ackermanns Bavaria	*Hv*	GER	--
32	ICB180430	*Hsp*	LBY	12	79	ICB181330	SYR	*Hsp*	9	126	Ackermanns Danubia	*Hv*	GER	--
33	ICB180452	*Hsp*	LBY	8	80	ICB181331	SYR	*Hsp*	12	127	Barke	*Hv*	GER	--
34	ICB180508	*Hsp*	RUS	12	81	ICB181381	TKM	*Hsp*	9	128	Criewenes 403	*Hv*	GER	--
35	ICB180533	*Hsp*	JOR	9	82	ICB181399	PAL	*Hsp*	9	129	Heils Franken	*Hv*	GER	--
36	ICB180554	*Hsp*	JOR	9	83	ICB181405	PAL	*Hsp*	9	130	Heines Hanna	*Hv*	GER	--
37	ICB180573	*Hsp*	JOR	8	84	ICB181412	PAL	*Hsp*	9	131	Lerche	*Hv*	GER	--
38	ICB180631	*Hsp*	JOR	8	85	ICB181418	PAL	*Hsp*	2	132	Pflugs Intensiv	*Hv*	GER	--
39	ICB180687	*Hsp*	JOR	6	86	ICB181424	PAL	*Hsp*	2	133	Ragusa	*Hv*	CRO	--
40	ICB180743	*Hsp*	JOR	9	87	ICB181430	PAL	*Hsp*	9	134	Scarlett	*Hv*	GER	--
41	ICB180802	*Hsp*	JOR	3	88	ICB181436	PAL	*Hsp*	9	135	Thuringia	*Hv*	GER	--
42	ICB180827	*Hsp*	JOR	6	89	ICB181442	PAL	*Hsp*	2	136	ICB180024	*Hsp*		--
43	ICB180857	*Hsp*	JOR	7	90	ICB181448	PAL	*Hsp*	1	137	ICB180847	*Hsp*		--
44	ICB180862	*Hsp*	UNK	3	91	ICB181454	PAL	*Hsp*	1	138	ICB181174	*Hsp*		--
45	ICB180867	*Hsp*	AFG	3	92	ICB181466	PAL	*Hsp*	15	139	ICB181178	*Hsp*		--
46	ICB180872	*Hsp*	IRQ	7	93	ICB181475	IRN	*Hsp*	2	140	ICB181267	*Hsp*		--
47	ICB180877	*Hsp*	AFG	3	94	ICB181488	PAK	*Hsp*	9					

Hsp =Hordeum spontaneum, Hv = Hordeum vulgare, SYR= Syria, JOR= Jordan, AFG= Afghanistan, IRQ= Iraq, IRN= Iran, TUR= Turkey, PAL= Palestine, TKM= Turkmenistan, PAK= Pakistan, LBY= Libya, RUS= Russia, UZB= Uzbekistan, TJK= Tajikistan, UNK= Unknown, GER= Germany, CRO= Croatia. Subp. = subpopulation numbers which resulted from STRUCTURE software analysis. -- = Accessions without subpopulation Number.

2.10.4 Marker-Trait association analysis

A major problem with association mapping is the presence of a population structure, which can lead to false positives and failure to detect genuine associations (i.e., false negatives), particularly in highly selfing species (Iwata et al. 2007).

The association analysis was performed with a mixed linear model (MLM) including Q and K matrix using SAS Software version 9.2. This analysis was achieved to identify DArT markers which are associated with the *Fusarium graminearum* stress tolerance traits and heading date trait considering the population based on population structure and the relatedness relationships.
Two statistical models used as follows:
First one with year's effect

$$Y_{ijkmnf} = \mu + Y_i + M_j + Y_i*M_j + \Sigma PCA_k + A_m(M_j)K_n + Y_i*A_m(M_j)K_n + \varepsilon_{f(ijkmn)}$$

where μ is the general mean, Y_i is the fixed effect of the *i*-th Year, M_j is the fixed effect of j-th marker, Y_i*M_j is the fixed interaction of *i*-th year with *j*-th marker, PCA_k is the fixed effect of *k*-th subgroup of the population structure (PC values), $A_m(M_j)K_n$ is the random effect of *m-th* accession nested in the j-th marker associated with *n*-th kinship coefficient, $Y_i*A_m(M_j)K_n$ is the random interaction effect of j-th year with *m*-th accession nested in the *j*-th marker associated with nth kinship coefficient, $\varepsilon_{f(ijkmn)}$ is the error.

Second one with the fungus isolates effect

$$Y_{ijkmnf} = \mu + T_i + M_j + T_i*M_j + \Sigma PCA_k + A_m(M_i)K_n + T_i*A_m(M_j)K_n + \varepsilon_{f(ijkmn)}$$

where μ is the general mean, T_i is the fixed effect of the *i*-th disease isolates, M_j is the fixed effect of *j-th* marker, T_i*M_j is the fixed interaction effect of *j*-th disease isolates with *l*-th marker, PCA_k is the fixed effect of *k*-th subgroup of the population structure (PC values), M_j is the fixed effect of *l*-th marker, $A_m(M_l)K_n$ is the random effect of *mth* accession nested in the *l*-th marker associated with *n*-th kinship coefficient , $T_i*A_m(M_j)K_n$ is the random interaction effect of *i-th* disease isolates with *m*-th accession nested in the *j*-th marker associated with *n-th* kinship coefficient, $\varepsilon_{f(ijkmn)}$ is the error.

2.10.5 Epistatic effects

According to Sayed et al. (2012) method, the epistatic interactions between all DArT markers pairs were tested with SAS procedure MIXED (SAS ver. 9.2, SAS Institute, 2008) using the following two mixed hierarchical model:

First one with year's effect

$$X_{ijkmo} = \mu + M1_i + M2_j + M1_i*M2_j + L_k(M1_i*M2_j) + Y_{m+} L_j*Y_m + \varepsilon_{o(ijkm)}$$

Second one with fungus isolates effects

$$X_{ijkmo} = \mu + M1_i + M2_j + M1_i*M2_j + L_k(M1_i*M2_j) + T_m + L_j*T_k + \varepsilon_{o(ijkm)}$$

Here μ is the general mean, $M1_i$ and $M2_j$ are the fixed effects of the *i*-th marker (*M1*) and *j*-th marker (*M2*). $M1_i*M2_j$ is the fixed interaction effect of the *i*-th *M1* genotype with *j*-th *M2* genotype, $L_k(M1_i*M2_j)$ is the random effect of the *k*-th genotypes nested in the *i*-th *M1* and *j*-th *M2* marker genotype interaction. Epistatic effects were accepted based on probability of false discovery rate (PFDR < 0.05) and has been calculated by PROC MULTTEST procedure in SAS.

2.11 Validation test of spike disease scoring

2.11.1 DNA extraction from infected seeds

DNA has been extracted from 30 mg milled seeds according to (QIAGEN) Kit (Mini Potocol).

Procedure:

1. Add 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml) to a maximum of 100 mg (wet weight) or 20 mg (dried) disrupted plant or fungal tissue and vortex vigorously.
2. Incubate the mixture for 10 min at 65°C. Mix 2 or 3 times during incubation by inverting tube.
3. Add 130 µl Buffer AP2 to the lysate, mix, and incubate for 5 min on ice.
4. Recommended: Centrifuge the lysate for 5 min at 20,000 x g (14,000 rpm).
5. Pipet the lysate into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at 20,000 x g (14,000 rpm).
6. Transfer the flow-through fraction from step 11 into a new tube (not supplied) without disturbing the cell-debris pellet.
7. Add 1.5 volumes of Buffer AP3/E to the cleared lysate, and mix by pipetting.
8. Pipet 650 µl of the mixture from step 13, including any precipitate that may have formed, into the DNeasy Mini spin column placed in a 2 ml collection tube (supplied). Centrifuge for 1 min at _6000 x g (corresponds to _8000 rpm for most microcentrifuges), and discard the flow-through.* Reuse the collection tube in step 9.

9. Repeat step 8 with remaining sample. Discard flow-through and collection tube.
10. Place the DNeasy Mini spin column into a new 2 ml collection tube (supplied), add 500 µl Buffer AW, and centrifuge for 1 min at ≥6000 x g (≥8000 rpm). Discard the flow-through and reuse the collection tube in step 11.
11. Add 500 µl Buffer AW to the DNeasy Mini spin column, and centrifuge for 2 min at 20,000 x g (14,000 rpm) to dry the membrane.
12. Transfer the DNeasy Mini spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 100 µl Buffer AE directly onto the DNeasy membrane.
    Incubate for 5 min at room temperature (15–25°C), and then centrifuge for 1 min at ≥6000 x g (≥8000 rpm) to elute.

2.11.2 Real time PCR (qPCR)

For validation of spikes disease scoring, real time polymerase chain reaction (qPCR) was conducted for 9 random samples from the population with different SDS using StepOnePlus Real-Time PCR System machine and StepOne Software, Version 2.2.2.

The following reaction mixture was used:

1- DNA sample

2- TaqMan ® Universal PCR Master Mix

- -MgCl2
- Puffer-Komponenten
- Deoxynucleosid-Triphosphate (dNTPs)
- AmpliTaqGold DNA-Polymerase

3- Target probe *F. graminearum* probe AGATATGTCTCTTCAAGTCT

4-Forward and reverse primer target (Waalwijk et al. 2004)

F. graminearum gram MGB-F GGCGCTTCTCGTGAACACA

F. graminearum gram MGB-R TGGCTAAACAGCACGAATGC

5- Internal PCR Control (IPC) forward and reverse primers

6- IPC probe

7- IPC DNA

8- Distilled water

The quantity of *F. graminearum* in each sample was calculated using the following equation:

(Mean quantity/2/initial weight) X 100 = pg DNA/mg dry weight.

3. Results:

The barley population of wild and cultivated forms was evaluated under Fusarium head blight disease stress under greenhouse and field-potted conditions for three successive seasons (2009, 2010 and 2011). In parallel, the population was genotyped with 895 DArT Markers to identify DArT markers associated disease infection tolerance. Structure analysis was conducted using Structure software 2.2 and Principal Component Analysis (PCA), Kinship coefficients matrix calculated by SPAGeDi 1.3 software and then the association analysis was achieved including population structure (PC values) and relatedness relationship coefficients (K matrix) to avoid the superiors association to detect the marker genotype which associated with studied traits by SAS 9.2 software. The following part presents the phenotypic variation, phenotypic correlation among traits, and the markers which associated with each trait.

3.1 Phenotypic measurements

In this study 140 accession were evaluated for quantitative traits (Leaves Disease Scoring; visual and Image analysis software for plant disease quantification (APS Assess) scoring (VS and LDS), Spikes Disease scoring (SDS) and Heading date (HD)) for two different isolates from *Fusarium graminarum* (5.1 and 5.3) the phenotypic differences between genotypes in 2009, 2010 and 2011 seasons are shown in table (3). All traits showed highly significantly genotype differences for both seasons and for the combined analysis. Table (4) presents a summary statistics of the studied traits under disease infection across three years.

Fig.1,2,3,4,5,6,7 and 8 shows the normal distribution of LDS, SDS,VS as traits under disease infection stress conditions and HD in 2009, 2010 and 2011 years in the green-house and field-potted experiments. Where the **Fig. 1** refers to the distribution analysis of VS in green-house 2009 which ranged from 4.00 up to 52.00. **Fig. 2** represents the genotypes frequency in green-house 2010 for (a) LDS which ranged from 0.00 up to 82.00 and (b) VS which ranged from 0.30 up to 86.04. **Fig. 3** shows the genotypes frequency in green-house 2010 for SDS which ranged from 0.00 to 70.00. **Fig. 4** refers to the distribution of the genotypes frequency in 2011 for (a) LDS 5.1 isolate ranged from 1.05 to 76.45 and (b) LDS 5.3 isolate ranged from 1.38 to 72.28. **Fig. 5** shows the genotypes frequency in 2011 for (a) SDS 5.1 isolate ranged from 0.00 to 70.00 and (b) SDS 5.3 isolate ranged from 0.00 to 60.00. **Fig. 6** represents the frequency of genotypes in 2011 for HD (a) in green-house which ranged from 58.00 to 93.00 and (b) in field-potted which ranged from 48.00 to 65.00. **Fig. 7** shows the genotypes frequency in field-potted 2011 for (a) LDS 5.1 isolate ranged from 1.56 to 41.73 and (b) LDS 5.3 isolate ranged from 2.13 to 30.56.

Table: (3) Analysis of variance of studied traits under *Fusarium graminarum* stress conditions in 2009, 2010 and 2011 years for structured barley population.

Year 2009 Green-house		
Trait	VS 5.1 isolate	
SOV	DF	MS
Geno.	29	479.38 ***
Rep.	4	35.37 **NS**
Error	116	24.35

Year 2010 Green-house						
Trait	VS 5.1 isolate		LDS 5.1 isolate		SDS 5.1 isolate	
SOV	DF	MS	DF	MS	DF	MS
Geno.	138	711.43 ***	138	780.38 ***	139	715.39 ***
Rep.	4	86.48 **NS**	4	78.56 **NS**	4	63.02 **NS**
Error	530	216.88	530	126.21	534	25.82

Year 2011 Green-house										
Trait	LDS 5.1 isolate		LDS 5.3 isolate		SDS 5.1 isolate		SDS 5.3 isolate		HD	
SOV	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Geno.	137	550.72***	137	281.79***	137	502.75***	137	314.46***	137	182.26***
Rep.	2	103.05 **NS**	1	24.56 **NS**	2	33.57 **NS**	1	36.23 **NS**	4	1.62 **NS**
Error	274	67.73	137	61.09	274	20.43	137	15.79	548	2.08

Year 2011 Field-potted										
Trait	LDS 5.1 isolate		LDS 5.3 isolate		SDS 5.1 isolate		SDS 5.3 isolate		HD	
SOV	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Geno.	31	542.27***	31	271.56***	31	417.20***	31	216.88***	31	54.40***
Rep.	1	0.11 **NS**	1	2.15 **NS**	2	9.375 **NS**	1	76.56 **NS**	4	5.99 **NS**
Error	220	7.93	220	3.64	62	26.57	31	18.49	124	1.487

***, is significant at 0.001 level and NS not significant. MS is the mean square of the studied trait. VS = Visual leaves disease scoring, LDS = Leaves disease scoring, SDS = Spikes disease scoring and HD = Heading date.

Table: (4) Summary statistics of evaluated traits under disease infection stress conditions in 2009, 2010 and 2011 years for structured barley population.

Trait	Isolate	Mean	Min.	Max.	CV	SD	SE	Cosign.
VS 2009 GH	5.1	28.43	4.00	52.00	17.36	4.93	24.35	
VS 2010 GH	5.1	36.19	0.00	82.00	31.04	11.23	126.2	
LDS 2010 GH	5.1	34.21	0.30	86.04	43.04	14.72	216.8	
SDS 2010 GH	5.1	26.25	0.00	70.00	19.36	5.08	25.83	
LDS 2011 GH	5.1	31.29	1.05	76.45	26.29	8.23	67.74	***
	5.3	25.05	1.38	72.28	31.21	7.81	61.09	
SDS 2011 GH	5.1	24.88	0.00	70.00	18.17	4.52	20.43	NS
	5.3	23.77	0.00	60.00	16.72	3.97	15.79	
HD 2011 GH	--	78.15	58.00	93.00	1.85	1.44	2.08	
LDS 2011 FP	5.1	19.54	1.56	41.73	14.42	2.81	7.94	***
	5.3	13.50	2.13	30.56	14.14	1.91	3.65	
SDS 2011 FP	5.1	22.50	0.00	60.00	22.91	5.15	26.58	NS
	5.3	21.41	0.00	50.00	20.09	4.3	18.49	
HD 2011 FP	--	56.98	48.00	65.00	2.14	1.21	1.48	

***, is significant at 0.001 level and NS not significant. VS = Visual leaves disease scoring, LDS = Leaves disease scoring, SDS = Spikes disease scoring, HD = Heading date, GH =Green-house experiment and FP = Field-potted experiment. Cosign.= combined significant between two isolate

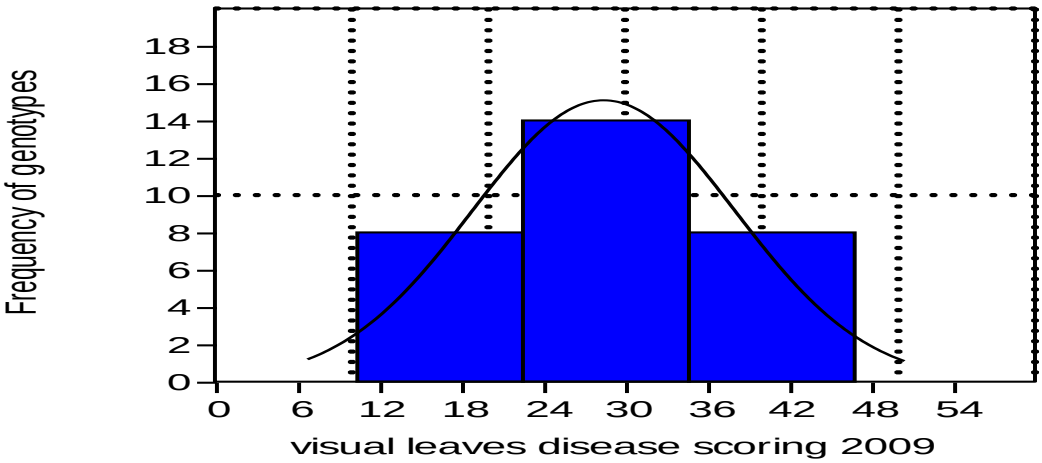
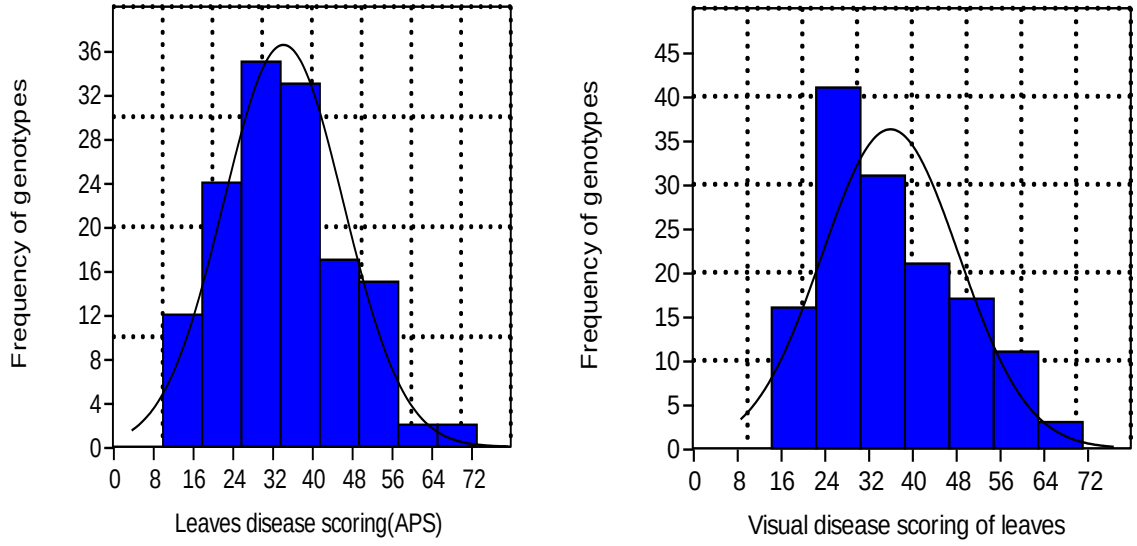


Fig. (1) Frequency of means of genotypes for visual leaves disease scoring 2009.



(a) (b)

Fig. (2) Frequency of means of genotypes for: (a) APS and (b) visual leaves disease scoring 2010.

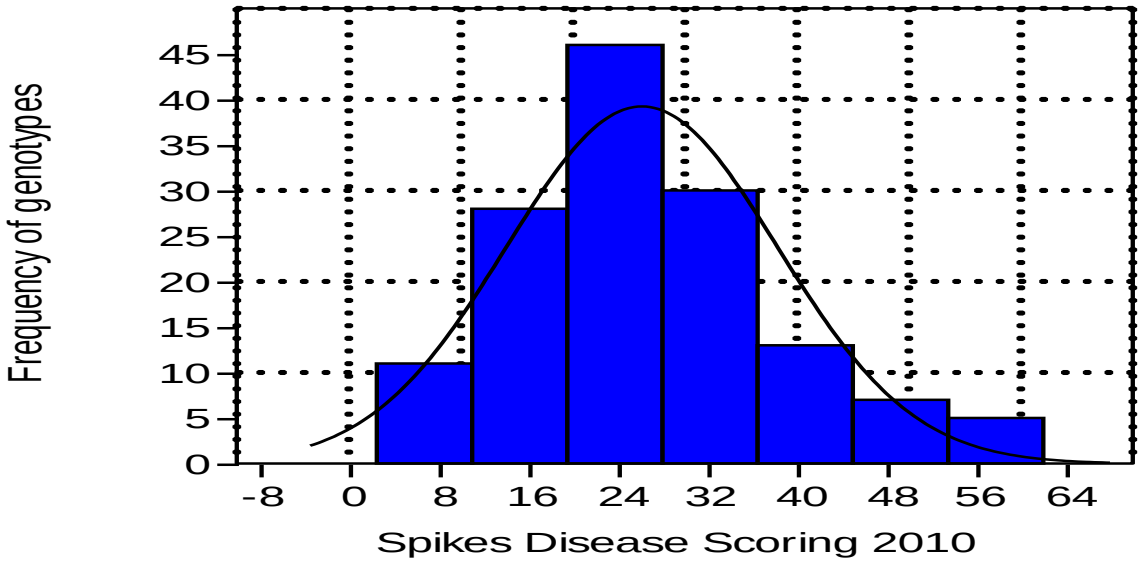
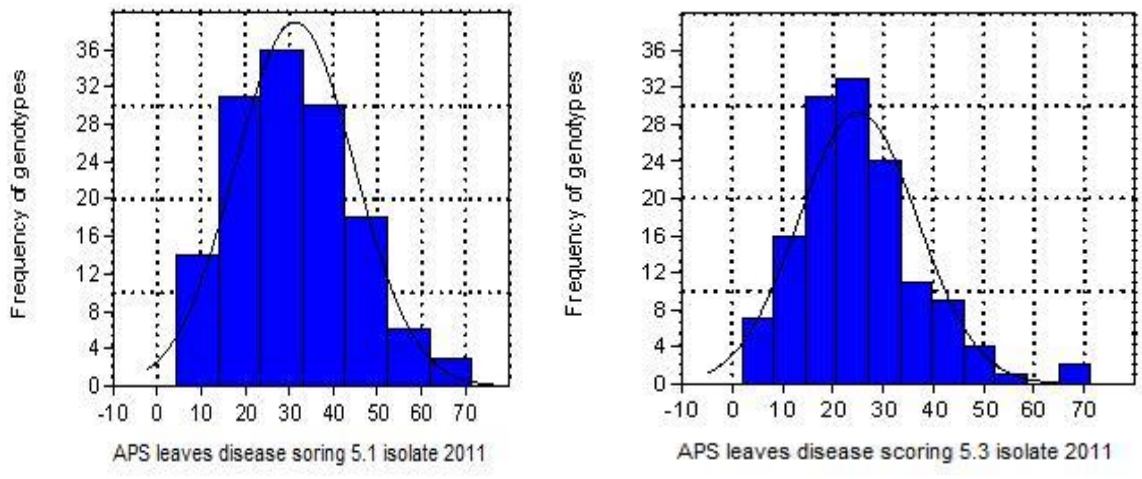
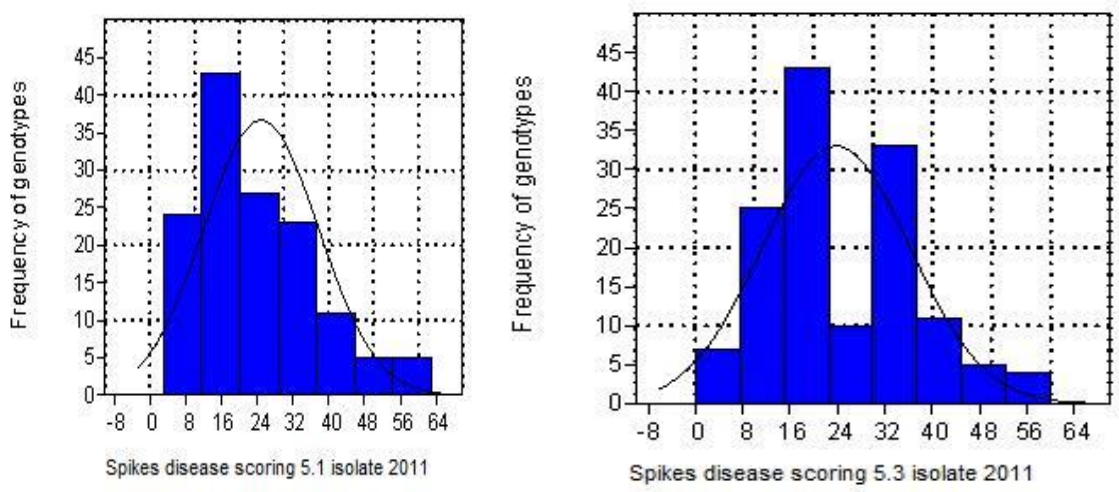


Fig. (3) Frequency of means of genotypes for spikes disease scoring 2010.



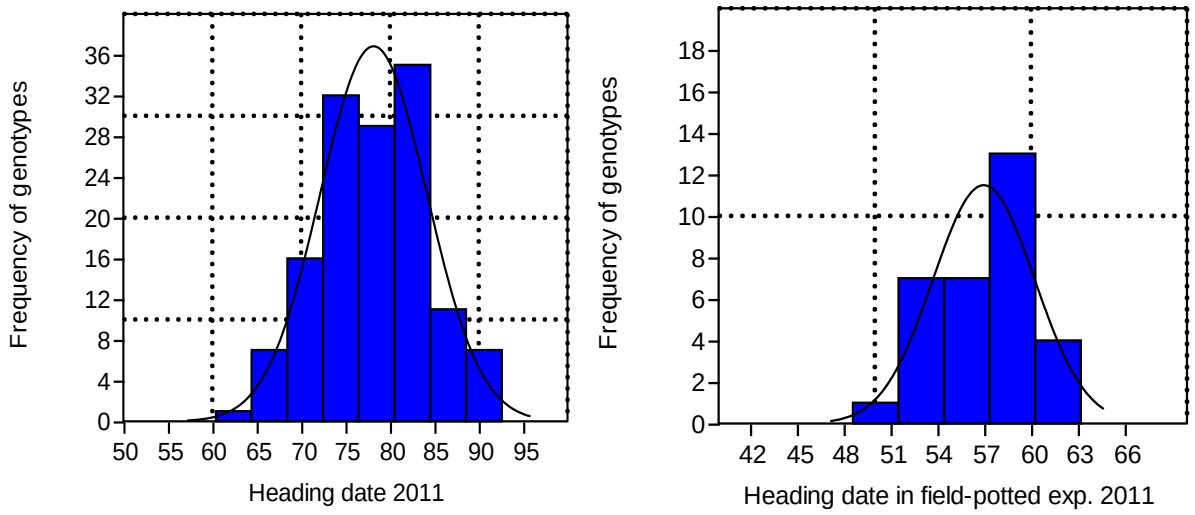
(a) (b)

Fig. (4) Frequency of means of genotypes for APS leaves disease scoring (a) 5.1 isolate and (b) 5.3 isolate 2011.



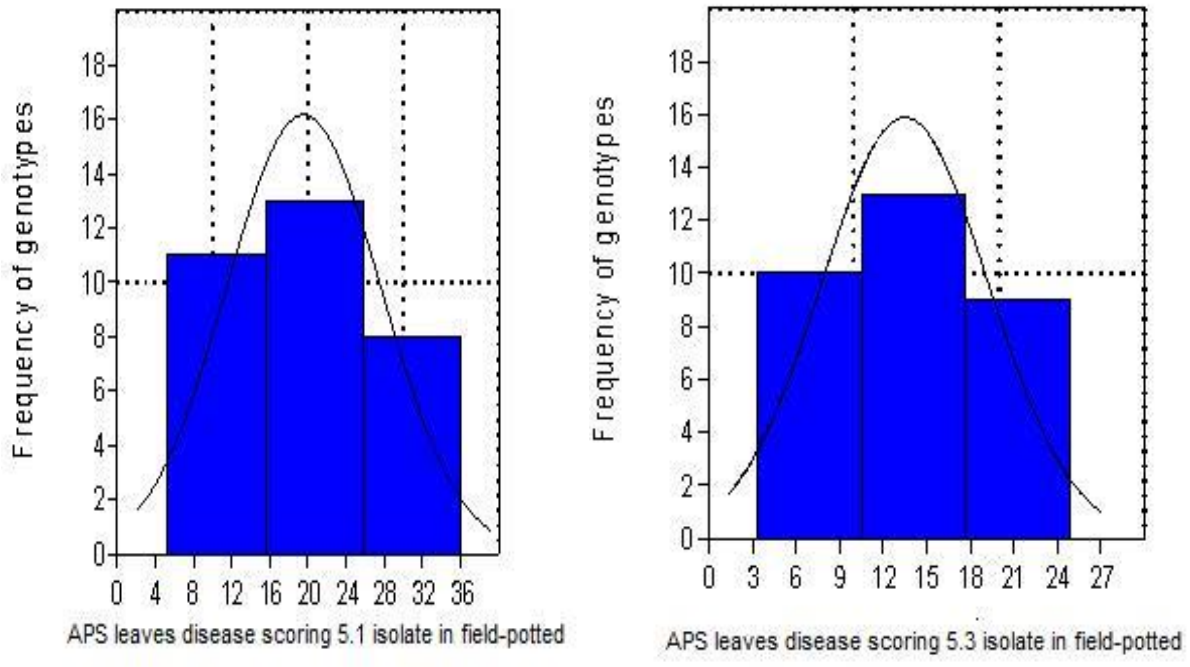
(a) (b)

Fig. (5) Frequency of means of genotypes for spikes disease scoring (a) 5.1 isolate and (b) 5.3 isolate 2011.



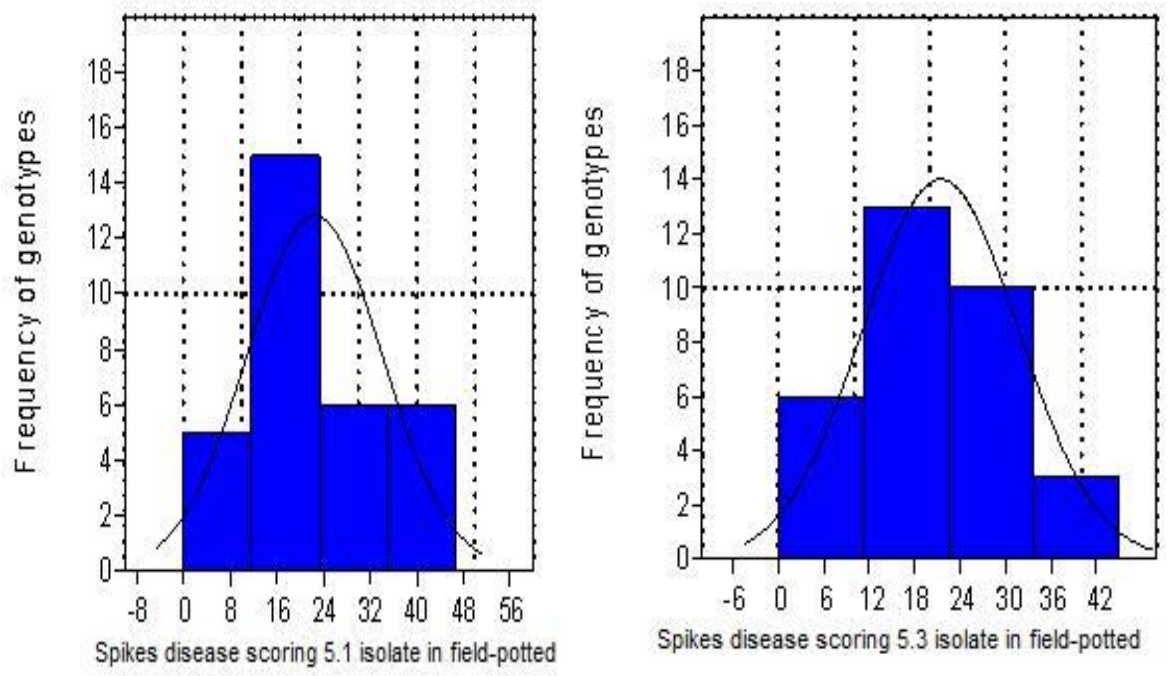
(a) (b)

Fig. (6) Frequency of means of genotypes for heading date 2011(a) green-house days after 10th Feb. and (b) field-potted days after 23rd May.



(a) (b)

Fig. (7) Frequency of means of genotypes for APS leaves disease scoring (a) 5.1 isolate (b) 5.3 isolate field-potted experiment 2011.



(a) (b)

Fig. (8) Frequency of means of genotypes for spikes disease scoring (a) 5.1 isolate (b) 5.3 isolate field-potted experiment 2011.

Fig. 8 showed the genotypes frequency in field-potted 2011 for (a) SDS 5.1 isolate ranged from 0.00 to 60.00 and (b) SDS 5.3 isolate ranged from 0.00 to 50.00.

3.1.1 Fusarium head blight disease assessment under green-house conditions

Table 5. represented the means for two years 2010 and 2011 in green-house experiments for two different *Fusarium graminarum* isolates 5.1 and 5.3 and two different disease scoring leaves and spikes. Accessions mean squares were highly significant for all experiments with highly variation in means of infection.

3.1.1.1 Leaves disease scoring (LDS) 2010, 5.1 isolate

Means of infection percentage was follow normal distribution with highly significant different within the population, ranged from 10.05 – 73.16%. Within the group of cultivated and landraces the lowest disease symptoms scored for 4 accessions Heidesandgerste, Alpine Pfauengerste, Barke and Dummersdorf with 11.91, 17.24, 18.53 and 19.44%, respectively. In wild types, 13 accessions recorded disease symptoms lower than 20%. The lowest one was ICB180211 with 10.05%.

3.1.1.2 Visual leaves disease scoring (VS) 2010, 5.1 isolate

This kind of score was done visually for the same leaves in 2010. Means of infection percentage also followed normal distribution with few exceptions with highly significant differences between the accessions, varied from 14.4 – 71.20%. One landraces and one cultivar had a score lower than 20%; Raguse and Barke with 17.60, 18.40, respectively. Twelve wilds from wild group had a score lower than 20%, the best one was ICB181164 scored 14.4%.

3.1.1.3 Spikes disease scoring (SDS) 2010, 5.1 isolate

Spikes disease scoring is the main disease scoring for FHB assessment in barley and wheat, because the yield loses always happened when the fungus attacks spikes of the plants. The means of this score was also followed the normal distribution in the studied population. Analysis of variance for this score showed highly significant different between the accessions with highly varied ranges of means 2.5 – 62%. Among cultivated types 4 accessions were having infection % lower 10% one landrace Pflugs Intensiv and 3 varieties Danubia, Oberpfälzer, Voldagsen with same score 8%. The lowest score in this year was 2.5% for wild

Table: (5) Means of two years for all studied traits under green-house conditions and combined means over twoyears and isolates

No.	Year		2010			2011				Combined means in two years			
	Accession	Type	LDS	VS	SDS	LDS	LDS	SDS	SDS	LDS	SDS	LDS	SDS
		Isolate	5.1	5.1	5.1	5.1	5.3	5.1	5.3	5.1	5.1	5.1 and 5.3	
1	Ingrid	Cultivated	31.99	33.20	14.00	15.74	9.22	10	10	23.87	12	18.98	11.33
2	Emir	Cultivated	40.41	34.80	18.00	12.37	17.75	20	10	26.39	19	23.51	16.00
3	Contra	Cultivated	52.69	57.20	26.00	22.67	18.12	23	20	37.68	24.5	31.16	23.00
4	Carina	Cultivated	36.21	36.80	34.00	26.01	24.37	33	20	31.11	33.5	28.86	29.00
5	Aramir	Cultivated	39.98	39.60	22.00	17.87	12.26	20	20	28.93	21	23.37	20.67
6	Kym	Cultivated	33.39	42.80	14.00	33.11	19.70	10	10	33.25	12	28.73	11.33
7	Candice	Cultivated	34.72	32.40	18.00	4.48	2.01	13	35	19.60	15.5	13.74	22.00
8	Camelot	Cultivated	27.92	26.80	24.00	8.09	5.36	20	10	18.01	22	13.79	18.00
9	Cheri	Cultivated	34.37	32.00	22.50	14.83	4.93	23	30	24.60	22.75	18.04	25.17
10	Otis	Cultivated	31.95	40.80	34.00	15.00	8.92	33	40	23.48	33.5	18.62	35.67
11	Peragis	Cultivated	33.93	54.80	18.00	17.98	22.80	17	10	25.96	17.5	24.90	15.00
12	Schwarze G.V. Strube	Cultivated	39.28	60.80	20.00	28.65	33.85	17	30	33.97	18.5	33.93	22.33
13	Alpine Pfauengerste	Cultivated	17.24	31.60	36.00	17.85	17.48	37	30	17.55	36.5	17.52	34.33
14	Dummersdorf	Cultivated	19.44	29.20	18.00	19.77	24.41	13	20	19.61	15.5	21.21	17.00
15	Jassener Land	Cultivated	33.73	40.40	16.00	22.59	18.13	13	15	28.16	14.5	24.82	14.67
16	Neuhaus Landgerste	Cultivated	--	--	12.00	11.59	28.20	10	15	11.59	11	19.90	12.33
17	Oberpfälzer	Cultivated	30.50	26.00	8.00	17.95	18.35	7	10	24.23	7.5	22.27	8.33
18	Danubia	Cultivated	21.04	21.20	8.00	10.30	19.30	10	5	15.67	9	16.88	7.67
19	Voldagsen	Cultivated	27.07	41.60	8.00	16.98	13.42	7	10	22.03	7.5	19.16	8.33
20	Reisgersten Linie II	Cultivated	23.23	26.80	26.00	28.14	22.48	20	10	25.69	23	24.62	18.67
21	Heidesandgerste	Cultivated	11.91	24.50	30.00	28.86	18.01	27	20	20.39	28.5	19.59	25.67
22	Ragusa	Landrace	23.38	17.60	20.00	27.22	25.52	23	15	25.30	21.5	25.37	19.33
23	Barke	Cultivated	18.53	18.40	16.00	20.37	27.31	13	10	19.45	14.5	22.07	13.00
24	Heils Franken	Landrace	37.74	26.80	22.00	24.71	19.87	23	15	31.23	22.5	27.44	20.00
25	Heines Hanna	Landrace	29.22	29.20	14.00	9.69	8.31	10	10	19.46	12	15.74	11.33
26	Criewenes 403	Landrace	27.80	26.00	28.00	19.64	28.58	30	20	23.72	29	25.34	26.00
27	Pflugs Intensiv	Landrace	25.57	32.00	8.00	18.07	18.53	7	15	21.82	7.5	20.72	10.00
28	Ackermanns Danubia	Landrace	51.04	36.00	18.00	9.28	8.39	20	20	30.16	19	22.90	19.33
29	Ackermanns Bavaria	Landrace	32.86	28.00	13.34	13.80	9.82	10	5	23.33	11.67	18.83	9.45
30	Scarlett	Cultivated	25.41	32.50	14.00	21.25	13.67	10	5	23.33	12	20.11	9.67
31	Lerche	Cultivated	25.94	36.40	28.00	42.14	8.59	23	30	34.04	25.5	25.56	27.00
32	Thurigina	Cultivated	31.99	33.20	38.00	11.02	6.66	33	20	21.51	35.5	16.56	30.33
33	ICB180001	Wild	22.27	36.80	22.50	28.81	35.26	20	25	25.54	21.25	28.78	22.50
34	ICB180006	Wild	18.01	18.00	30.00	25.85	33.03	30	50	21.93	30	25.63	36.67
35	ICB180007	Wild	27.76	26.80	14.00	21.34	34.90	10	10	24.55	12	28.00	11.33
36	ICB180013	Wild	18.47	33.20	42.00	36.73	44.32	43	15	27.60	42.5	33.17	33.33
37	ICB180014	Wild	41.97	40.40	26.00	23.98	21.40	27	35	32.98	26.5	29.12	29.33
38	ICB180018	Wild	53.52	60.80	22.00	42.83	35.12	20	35	48.18	21	43.82	25.67
39	ICB180024	Wild	48.13	52.80	42.00	50.23	47.95	40	45	49.18	41	48.77	42.33
40	ICB180044	Wild	21.26	40.00	38.00	45.99	44.46	37	50	33.63	37.5	37.24	41.67
41	ICB180046	Wild	35.51	44.00	22.00	15.85	21.05	17	25	25.68	19.5	24.14	21.33
42	ICB180051	Wild	31.72	30.00	14.00	37.99	23.29	10	5	34.86	12	31.00	9.67
43	ICB180052	Wild	34.86	47.20	46.00	27.22	16.74	47	35	31.04	46.5	26.27	42.67
44	ICB180063	Wild	34.49	48.80	50.00	36.66	20.92	53	55	35.58	51.5	30.69	52.67
45	ICB180068	Wild	36.09	50.80	36.00	31.40	27.56	33	25	33.75	34.5	31.68	31.33
46	ICB180069	Wild	38.52	46.40	34.00	38.93	20.62	30	5	38.73	32	32.69	23.00
47	ICB180070	Wild	25.91	32.80	28.00	44.23	32.71	27	30	35.07	27.5	34.28	28.33
48	ICB180072	Wild	34.69	55.20	46.00	28.19	19.47	43	20	31.44	44.5	27.45	36.33
49	ICB180079	Wild	44.37	47.60	36.00	37.62	29.18	33	30	41.00	34.5	37.06	33.00
50	ICB180092	Wild	24.07	28.80	34.00	46.86	33.66	33	55	35.47	33.5	34.86	40.67
51	ICB180102	Wild	16.27	24.40	20.00	29.96	18.33	23	20	23.12	21.5	21.52	21.00
52	ICB180109	Wild	20.22	18.00	44.00	21.55	23.50	47	40	20.89	45.5	21.76	43.67
53	ICB180117	Wild	27.96	25.20	20.00	24.24	17.99	17	20	26.10	18.5	23.40	19.00
54	ICB180148	Wild	68.45	71.20	22.00	24.51	23.92	20	30	46.48	21	38.96	24.00
55	ICB180199	Wild	33.97	32.40	28.00	69.60	71.19	27	30	51.79	27.5	58.25	28.33
56	ICB180211	Wild	10.05	17.60	14.00	29.49	27.81	10	25	19.77	12	22.45	16.33
57	ICB180215	Wild	16.53	20.80	12.00	11.98	42.30	3	15	14.26	7.5	23.60	10.00
58	ICB180217	Wild	16.25	18.40	44.00	13.35	25.50	43	30	14.80	43.5	18.37	39.00

Table: (5) continued

No.	Year		2010			2011				Combined means in two years			
	Accession	Type	LDS	VS	SDS	LDS	LDS	SDS	SDS	LDS	SDS	LDS	SDS
		Isolate	5.1	5.1	5.1	5.1	5.3	5.1	5.3	5.1	5.1	5.1 and 5.3	
59	ICB180231	Wild	25.00	34.00	56.00	13.58	16.93	57	60	19.29	56.5	18.50	57.67
60	ICB180260	Wild	24.85	25.60	36.00	27.44	29.45	37	40	26.15	36.5	27.25	37.67
61	ICB180303	Wild	17.36	18.00	6.00	30.96	35.69	10	10	24.16	8	28.00	8.67
62	ICB180329	Wild	43.11	38.00	16.00	21.13	29.30	13	35	32.12	14.5	31.18	21.33
63	ICB180389	Wild	64.25	38.00	26.00	47.28	14.19	27	30	55.77	26.5	41.91	27.67
64	ICB180410	Wild	42.25	46.40	46.00	71.01	45.28	43	20	56.63	44.5	52.85	36.33
65	ICB180430	Wild	47.89	47.20	48.00	35.03	26.80	47	35	41.46	47.5	36.57	43.33
66	ICB180452	Wild	49.35	60.40	24.00	44.56	29.02	20	10	46.96	22	40.98	18.00
67	ICB180508	Wild	55.63	64.00	14.00	41.52	54.56	13	15	48.58	13.5	50.57	14.00
68	ICB180533	Wild	54.56	62.40	30.00	59.19	67.50	33	25	56.88	31.5	60.42	29.33
69	ICB180554	Wild	42.02	48.80	22.00	47.96	40.56	20	40	44.99	21	43.51	27.33
70	ICB180573	Wild	56.70	58.40	28.00	40.86	23.68	30	20	48.78	29	40.41	26.00
71	ICB180631	Wild	46.75	61.20	26.00	51.62	30.65	23	30	49.19	24.5	43.01	26.33
72	ICB180687	Wild	47.76	45.20	24.00	41.32	24.96	20	30	44.54	22	38.01	24.67
73	ICB180743	Wild	34.81	39.20	24.00	48.22	23.12	20	20	41.52	22	35.38	21.33
74	ICB180802	Wild	51.20	49.00	28.00	27.97	33.05	30	10	39.59	29	37.41	22.67
75	ICB180827	Wild	42.10	51.60	35.00	46.79	18.15	33	10	44.45	34	35.68	26.00
76	ICB180847	Wild	40.84	50.00	10.00	50.14	21.13	10	15	45.49	10	37.37	11.67
77	ICB180857	Wild	32.21	47.20	2.50	39.19	23.63	7	30	35.70	4.75	31.68	13.17
78	ICB180862	Wild	57.98	57.60	12.00	28.57	29.75	10	10	43.28	11	38.77	10.67
79	ICB180867	Wild	51.65	49.50	40.00	53.36	22.09	37	20	52.51	38.5	42.37	32.33
80	ICB180872	Wild	43.65	24.00	45.00	49.31	51.84	43	40	46.48	44	48.27	42.67
81	ICB180877	Wild	50.46	25.20	17.50	39.10	13.60	13	15	44.78	15.25	34.39	15.17
82	ICB180882	Wild	31.40	26.00	42.00	40.08	26.42	43	30	35.74	42.5	32.63	38.33
83	ICB180887	Wild	38.84	30.80	26.00	27.58	18.72	23	20	33.21	24.5	28.38	23.00
84	ICB180902	Wild	36.22	40.40	24.00	37.81	19.06	23	20	37.02	23.5	31.03	22.33
85	ICB180923	Wild	51.55	52.80	28.00	33.76	24.67	27	30	42.66	27.5	36.66	28.33
86	ICB180927	Wild	40.91	28.80	22.00	48.32	27.74	20	20	44.62	21	38.99	20.67
87	ICB180973	Wild	36.52	27.60	44.00	47.24	48.89	47	50	41.88	45.5	44.22	47.00
88	ICB180982	Wild	73.16	70.40	28.00	35.22	6.89	27	10	54.19	27.5	38.42	21.67
89	ICB180994	Wild	42.30	50.40	60.00	71.36	36.52	63	40	56.83	61.5	50.06	54.33
90	ICB181150	Wild	43.62	32.00	46.00	38.89	45.35	43	40	41.26	44.5	42.62	43.00
91	ICB181154	Wild	45.93	47.60	18.00	39.64	18.08	17	20	42.79	17.5	34.55	18.33
92	ICB181156	Wild	51.31	29.60	44.00	40.14	7.23	43	10	45.73	43.5	32.89	32.33
93	ICB181158	Wild	40.90	24.00	62.00	46.18	14.68	60	25	43.54	61	33.92	49.00
94	ICB181160	Wild	38.34	19.60	26.00	36.87	31.53	23	30	37.61	24.5	35.58	26.33
95	ICB181162	Wild	34.70	29.60	24.00	30.16	24.00	20	35	32.43	22	29.62	26.33
96	ICB181164	Wild	29.03	14.40	20.00	33.39	19.96	17	35	31.21	18.5	27.46	24.00
97	ICB181168	Wild	17.07	25.20	16.00	23.40	18.48	17	15	20.24	16.5	19.65	16.00
98	ICB181170	Wild	31.41	30.00	56.00	13.55	17.80	60	20	22.48	58	20.92	45.33
99	ICB181172	Wild	29.54	29.50	28.00	23.79	14.43	30	30	26.67	29	22.59	29.33
100	ICB181174	Wild	19.75	28.80	22.50	23.97	7.18	27	20	21.86	24.75	16.97	23.17
101	ICB181176	Wild	29.56	38.80	10.00	20.38	12.77	10	20	24.97	10	20.90	13.33
102	ICB181178	Wild	42.25	43.20	20.00	32.60	33.34	23	35	37.43	21.5	36.06	26.00
103	ICB181180	Wild	34.16	33.20	36.00	57.06	18.11	33	30	45.61	34.5	36.44	33.00
104	ICB181182	Wild	56.22	43.20	22.00	36.91	28.38	20	30	46.57	21	40.50	24.00
105	ICB181184	Wild	54.10	46.00	22.00	56.07	25.51	23	20	55.09	22.5	45.23	21.67
106	ICB181186	Wild	47.97	30.00	18.00	54.33	22.60	17	0	51.15	17.5	41.63	11.67
107	ICB181216	Wild	41.18	40.40	22.00	54.68	39.45	20	15	47.93	21	45.10	19.00
108	ICB181228	Wild	35.58	43.20	34.00	--	--	--	--	35.58	34	35.58	34.00
109	ICB181230	Wild	54.08	58.80	28.00	--	--	--	--	54.08	28	54.08	28.00
110	ICB181238	Wild	30.87	30.40	22.00	41.76	31.79	20	30	36.32	21	34.81	24.00
111	ICB181243	Wild	51.15	55.20	28.00	31.41	30.37	27	35	41.28	27.5	37.64	30.00
112	ICB181267	Wild	28.46	34.80	26.00	44.92	28.98	23	20	36.69	24.5	34.12	23.00
113	ICB181268	Wild	24.77	16.50	28.00	29.44	34.15	27	60	27.11	27.5	29.45	38.33
114	ICB181323	Wild	20.64	30.00	22.50	32.02	29.64	20	20	26.33	21.25	27.43	20.83
115	ICB181324	Wild	35.60	37.60	60.00	22.37	16.83	63	35	28.99	61.5	24.93	52.67
116	ICB181330	Wild	31.62	40.80	22.00	36.58	21.20	20	30	34.10	21	29.80	24.00

Table: (5) continued

No.	Year		2010			2011				Combined means in two years			
	Accession	Type	LDS	VS	SDS	LDS	LDS	SDS	SDS	LDS	SDS	LDS	SDS
		Isolate	5.1	5.1	5.1	5.1	5.3	5.1	5.3	5.1	5.1	5.1 and 5.3	
117	ICB181331	Wild	28.54	32.40	24.00	38.67	35.20	23	45	33.61	23.5	34.14	30.67
118	ICB181381	Wild	35.60	47.00	18.00	22.77	20.10	17	20	29.19	17.5	26.16	18.33
119	ICB181399	Wild	30.82	37.20	20.00	38.36	42.59	20	20	34.59	20	37.26	20.00
120	ICB181405	Wild	26.21	28.50	36.00	33.46	38.60	37	40	29.84	36.5	32.76	37.67
121	ICB181412	Wild	30.22	35.20	37.50	31.01	8.66	37	10	30.62	37.25	23.30	28.17
122	ICB181418	Wild	25.20	29.60	24.00	33.43	21.84	23	20	29.32	23.5	26.82	22.33
123	ICB181424	Wild	34.33	34.50	20.00	16.22	24.93	17	10	25.28	18.5	25.16	15.67
124	ICB181430	Wild	23.20	28.40	40.00	26.27	17.62	43	20	24.74	41.5	22.36	34.33
125	ICB181436	Wild	16.82	23.60	22.00	24.40	13.33	17	10	20.61	19.5	18.18	16.33
126	ICB181442	Wild	26.86	26.00	22.00	19.48	21.47	20	40	23.17	21	22.60	27.33
127	ICB181448	Wild	16.74	18.00	22.00	29.29	20.17	17	25	23.02	19.5	22.07	21.33
128	ICB181454	Wild	32.87	32.80	12.00	10.40	16.64	10	10	21.64	11	19.97	10.67
129	ICB181466	Wild	23.34	16.80	20.00	22.28	25.95	20	25	22.81	20	23.86	21.67
130	ICB181475	Wild	38.57	29.60	32.00	19.92	23.59	20	25	29.25	26	27.36	25.67
131	ICB181488	Wild	28.72	36.80	8.00	45.19	49.46	10	40	36.96	9	41.12	19.33
132	ICB181492	Wild	24.60	28.40	40.00	37.30	42.43	40	40	30.95	40	34.78	40.00
133	ICB181498	Wild	24.57	23.60	26.00	29.86	40.09	23	10	27.22	24.5	31.51	19.67
134	ICB181500	Wild	28.22	26.00	16.00	22.11	23.64	13	5	25.17	14.5	24.66	11.33
135	ICB191338	Wild	17.79	18.00	26.00	32.29	28.43	27	25	25.04	26.5	26.17	26.00
136	IGI19451	Wild	24.66	32.80	12.00	23.52	24.64	10	30	24.09	11	24.27	17.33
137	IGI21857	Wild	35.71	16.80	10.00	22.30	22.21	10	10	29.01	10	26.74	10.00
138	IGI23991	Wild	16.33	37.60	8.00	29.96	28.64	10	15	23.15	9	24.98	11.00
139	IGI24000	Wild	28.10	30.40	36.00	19.79	14.17	37	10	23.95	36.5	20.69	27.67
140	IGI24017	Wild	31.80	41.20	28.00	39.54	19.51	30	20	35.67	29	30.28	26.00

--, Missing values

accession ICB180857, 3 wilds also having lower score with 6, 8 and 8% for ICB180303, ICB181488 and IG123991, respectively.

For both scoring, 23 accessions were having nearly same score in LDS and SDS, 7 from the group of cultivated and landraces (Dummersdorf and Barke were scored lower than 20%). Sixteen wild types ICB180102, ICB180211, ICB180215 and ICB181168 were scored lower than 20%) .

3.1.1.4 Leaves disease scoring (LDS) 2011, 5.1 isolate

Means of infection percentage were followed the normal distribution with highly significant differences between the accessions, means ranged from 4.48 to 71.36%. Four accessions; 2 varieties and 2 landraces were having disease symptoms lower than 10%, they were Candice, Camelot, Ackermanns Danubia and Heines Hanna with score 4.48, 8.09, 9.28 and 9.69%, respectively. Ten wild forms had infection % lower than 20% the best 3 of this tens were ICB180215, ICB180217 and ICB181170 with 11.98, 13.35 and 13.55%, respectively.

3.1.1.5 Leaves disease scoring (LDS) 2011, 5.3 isolate

In year 2011 another isolate from the fungus was added to the work, to study if the response for the infection will changed or not. Highly significant differences were recorded between these two isolates, this means each isolate had different disease severity with the accessions under study. Also, highly significant different found within the population for this scores. Means were not completely followed the normal distribution, ranged from 2.01- 71.19%. Ten out of 32 of varieties and landraces had a disease symptoms lower than 10%, the best one of these tens was Candice with 2.01 infection percentage. Four wilds had infection % lower than 10%, they were ICB180892, ICB181174, ICB181156 and ICB181412 with 6.89, 7.18, 7.23 and 8.66%.

3.1.1.6 Spikes disease scoring (SDS) 2011, 5.1 isolate

This trait was not completely followed the normal distribution, there were highly significant differences between the accessions, means ranged 3- 63%. Three accessions Voldagsen, Oberpfälzer and Pflugs Intensiv had lower and same score 7% from the group of cultivate and landraces. Two wild accessions were having scored 3 and 7 %, ICB180215 and ICB180857, respectively.

3.1.1.7 Spikes disease scoring (SDS) 2011, 5.3 isolate

There are highly significant differences between the accessions for this trait. Means were not followed the normal distribution with range 0.0 – 60%. Ackermanns Bavaria, Scarlett and Danubia were having lower score with 5%. The lowest recorded score in the population was 0.0 for the accession ICB181186 also 3 wild accessions had 5% infection %, they were ICB180051, ICB181500 and ICB180069. Among both isolate for SDS no significant differences in infection percentage for the studied population.

In year 2011, the results for two different scores and isolates showed that, 12 accessions were having very close results to LDS and SDS. Three from the cultivated and landraces group, (Heines Hanna had infection % lower than 10, Ingrid scored 10% in SDS and 9.22-15.74% for LDS and third one ‘Carina’ had score ranged from 18.12 for LDS isolate 5.3 to 23 for SDS isolate 5.1). The nine wilds scored up and around 20% for both methods of scoring (ICB180117 (17% SDS 5.1 isolate – 24.24% LDS 5.1 isolate) and ICB181381 (17% SDS5.1 isolate – 22.77% LDS 5.1 isolate)).

Combined means for two years 2010 and 2011 showed that; 10 accessions were having lower than 20 infection percentage in LDS and SDS. They were 7 cultivated and 3 wild types (Ingrid,

Camelot, Neuhaus Landgerste, Danubia, Voldagsen, Heines Hanna, Ackermanns Bavaria, ICB181168, ICB181436 and ICB181454) Over two years and two different isolates from fungus for LDS; 18 different accessions had lower than 20% infection; Candice, Camelot, Heines Hanna, Thurigina, Danubia, ICB181174, Alpine Pfauengerste, Cheri, ICB181436, ICB180217, ICB180231, Otis, Ackermanns Bavaria, Ingrid, Voldagsen, Heidesandgerste, ICB181168 and ICB181454, with infection percentages; 13.74, 13.79, 15.74, 16.56, 16.88, 16.97, 17.52, 18.04, 18.18, 18.37, 18.50, 18.62, 18.83, 18.98, 19.16, 19.59, 19.65 and 19.97 %respectively.

Over two years and two different isolates from fungus for SDS showed that; 48 accessions had lower than 20% infection percentage, 9 from this group were lower than 10% infection percentage; Danubia, Ackermanns Bavaria, Scarlett, ICB180051, Oberpfälzer, Voldagsen, ICB181186, ICB180303 and ICB181500 with infection percentages; 7, 8.34, 8.5, 8.5, 8.75, 8.75, 8.75, 9 and 9.75% respectively.

Generally, over two year's green-house experimental work, 2 accessions had good results against two isolates from *Fusarium graminarium* fungus and these results were low also over two different ways of disease scoring. These two were Danubia and Ackermanns Bavaria with LDS 16.88, 18.83% and with SDS 7, 8.34% respectively

3.1.2 Fusarium head blight disease assessment under field-potted conditions

Table 6. Showed the means of field-potted experiment in 2011 for two different *Fusarium graminarum* isolates 5.1 and 5.3 and two different disease scoring leaves and spikes. Among 32 accessions (cultivated and landraces types) mean squares were highly significant for both disease scoring types, this highly significant different also found within the same group under green-house conditions in 2009. Means of studied traits were followed the normal distribution, except the means of SDS for isolate 5.1 and HD both traits were not completely followed the normal distribution curve.

3.1.2.1 Leaves disease scoring (LDS) 5.1 isolate

The range of means of infection percentage was 5.27 - 35.13%. Results showed that 2 accessions were having lower disease symptoms (Otis and Ragusa with means 5.27 and 8.45%). Eighteen cultivars and landraces were having means under 20 %.

Table: (6) Means of studied traits for 32 barley accessions under field-potted conditions 2011 and visual leaves disease scoring for year 2009 under green-house conditions.

Accessions	Type	LDS 5.1I.	LDS 5.3I.	SDS 5.1I.	SDS 5.3I.	VS 2009
Ingrid	Cultivated	12.83	8.08	10	0.0	10.4
Emir	Cultivated	11.65	7.71	10	20	24
Contra	Cultivated	24.63	13.82	23.33	20	18.4
Carina	Cultivated	11.24	8.51	13.33	5	27.6
Aramir	Cultivated	17.79	14.08	20	15	29.2
Kym	Cultivated	17.37	13.11	26.67	20	24.4
Candice	Cultivated	16.64	7.12	23.33	15	34.4
Camelot	Cultivated	16.29	13.48	16.67	10	20.8
Cheri	Cultivated	15.78	9.25	16.67	45	25.2
Otis	Cultivated	5.27	5.76	30	30	32
Peragis	Cultivated	19.87	17.33	13.33	10	31.2
Schwarze G.V. Strube	Cultivated	31.77	19.48	40	30	37.6
Alpine Pfauengerste	Cultivated	11.74	3.41	46.67	30	24
Dummersdorf	Cultivated	13.96	20.22	20	25	14.8
Jassener Land	Cultivated	28.95	18.98	36.67	25	36.8
Neuhaus Landgerste	Cultivated	29.81	13.61	33.33	15	42.4
Oberpfälzer	Cultivated	11.75	12.51	20	20	26
Danubia	Cultivated	21.02	13.36	16.67	15	34.4
Voldagsen	Cultivated	25.10	17.72	20	30	37.6
Reisgersten Linie II	Cultivated	36.05	24.82	26.67	35	46.8
Heidesandgerste	Cultivated	25.40	13.76	43.33	30	44.8
Ragusa	Cultivated	8.45	5.69	40	30	12.8
Barke	Cultivated	27.99	13.44	26.67	30	32.4
Heils Franken	Landrace	35.13	23.26	36.67	10	41.6
Heines Hanna	Landrace	13.98	7.50	23.33	20	21.6
Criewenes 403	Landrace	19.03	20.07	3.33	20	14
Pflugs Intensiv	Landrace	17.57	20.66	0.0	15	27.6
Ackermanns Danubia	Landrace	28.31	12.40	13.33	15	37.2
Ackermanns Bavaria	Landrace	19.41	12.51	13.33	25	26
Scarlett	Cultivated	10.16	4.25	6.66	10	16.8
Lerche	Cultivated	10.64	12.75	16.66	20	--
Thurigina	Cultivated	29.61	23.28	33.33	45	---

3.1.2.2 Leaves disease scoring (LDS) 5.3 isolate

Means of infection percentage were varied from 3.41 – 24.82%. Ten accessions were having infection % lower than 10, the best 4 were Alpine Pfauengerste, Scarlett, Ragusa and Otis with 3.41, 4.25, 5.69, 5.76%, respectively. Some accessions recorded nearly the same score for both isolates, cultivar 'Otis' was the nearly the same for both isolates in LDS 5.27 and 5.76, also for Aramir, Kym, Oberpfälzer, Lerche, and Ragusa. Highly significant differences were recorded between these two isolates for this score.

3.1.2.3 Spikes disease scoring (SDS) 5.1 isolate

Highly means range 0.0- 46.6% recorded for this trait. The best 3 lower means scored for disease symptoms were 0.0, 3.33, 6.66% for Pflugs Intensiv, Criewenes 403 and Scarlett, respectively.

3.1.2.4 Spikes disease scoring (SDS) 5.3 isolate

Means of infection percentage varied from 0.0 to 45 %. Two cultivars were having so lower infection percentage 0.0 and 5.0% for Ingrid and Carina. The analysis of variance showed no significant different between the isolates in SDS. Nine accessions were having same or nearly the same score for both isolates.

3.1.2.3 Visual leaves disease scoring (VS) 5.1 isolate under green-house conditions 2009

Among 30 barley accessions assessed in 2009 only, the means range was 10.4- 46.8%. Six accessions were having disease infection percentage lower than 20%. The lowest two were Ingrid and Ragusa were having 10.4 and 12.8 % infection percentage. Over this 2 different experiments (field-potted 2011 and green-house 2009); two cultivars showed nearly means under both isolates and scores, they were Ingrid and Scarlett.

3.2 The phenotypic correlation between studied traits

3.2.1 The phenotypic correlation between studied traits under green-house conditions

Table (7) shows the Pearson correlation coefficients (r) between 7 pairs of studied traits under disease infection stress at green-house in 2010 and 2011 years (Leaves Disease Scoring 5.1 Isolate (LDS5.1I. 2010), Visual leaves disease Scoring 2010 (VS 2010), Leaves Disease Scoring 5.1 Isolate 2011(LDS5.1I. 2011), Leaves Disease scoring 5.3 Isolate (LDS5.3I. 2011), Spikes Disease Scoring 5.1 Isolate 2010 (SDS5.1I. 2010), Spikes disease Scoring 5.1 Isolate 2011 (SDS5.1I 2011), Spikes Disease Scoring 5.3 Isolate 2011 (SDS5.3I. 2011) and Heading Date 2011 (HD 2011)). Highly significant positive correlation between the VS and LDS in 2010 that also shown in **Fig. 9 (**refers to the correlation between visual and APS scoring in leaves disease for 2010**)**, and it was the same in LDS 2011. LDS 5.1 isolate 2011 had a highly significant correlation, positively with LDS 5.3 isolate 2011 and SDS 2010, SDS 5.1 and 5.3 isolate 2011 and negatively, with HD. This means the disease infection resistance/tolerance are correlated with late heading. SDS 2010 had a highly significant correlation, positively with SDS first and second isolate 2011 ($r = 0.97$, $r = 0.46$) and LDS 5.1 isolate 2011 ($r = 0.47$) and negatively, with HD. SDS 5.1 and 5.3 isolate 2011 were highly significant correlated, positively with each other

and negatively with HD. HD was negatively correlated with all studied traits. The correlation coefficients were very low and not significant between studied traits in 2010 showed that, LDS5.1I. 2010 and LDS 5.3I. 2011, SDS 5.1I. 2010, SDS 5.1I. 2011, SDS 5.3I. 2011, HD 2011 with values varied from -0.07 to 0.10. Also the same between VS5.1I. and LDS 5.3I. 2011, SDS 5.1I. 2010, SDS 5.1I. 2011, SDS 5.1I. 2011, HD 2011with values varied from 0.017 to 0.11. This values indicated to the correlation between SDS and LDS was not strong. However, in 2011 the r values between both scoring method were highly significant with values 0.28, 0.28 and 0.22 this correlation values indicated that there are a correlation between LDS and SDS.

Table: (7) Pearson correlation coefficient (r) calculated between 7 pairs of traits under disease infection stress and heading date in a structured barley population over two years in the green-house

Trait	LDS 5.1I. 2010	VS 5.1I. 2010	LDS 5.1I. 2011	LDS5.3I. 2011	SDS 5.1I. 2010	SDS 5.1I. 2011	SDS 5.3I. 2011
VS 2010	0.72***						
LDS 5.1I. 2011	0.36***	0.32**					
LDS 5.3I. 2011	0.05	0.11	0.54***				
SDS 5.1I. 2010	0.10	0.08	0.28**	0.09			
SDS 5.1I. 2011	0.09	0.076	0.28**	0.11	0.97***		
SDS 5.3I. 2011	-0.01	0.017	0.22**	0.31***	0.46***	0.47***	
HD 2011	-0.07	-0.079	-0.21*	-0.106	-0.28**	-0.27**	-0.25**

*, ** and *** are significant effects at 0.05, 0.01 and 0.001 levels, respectively.

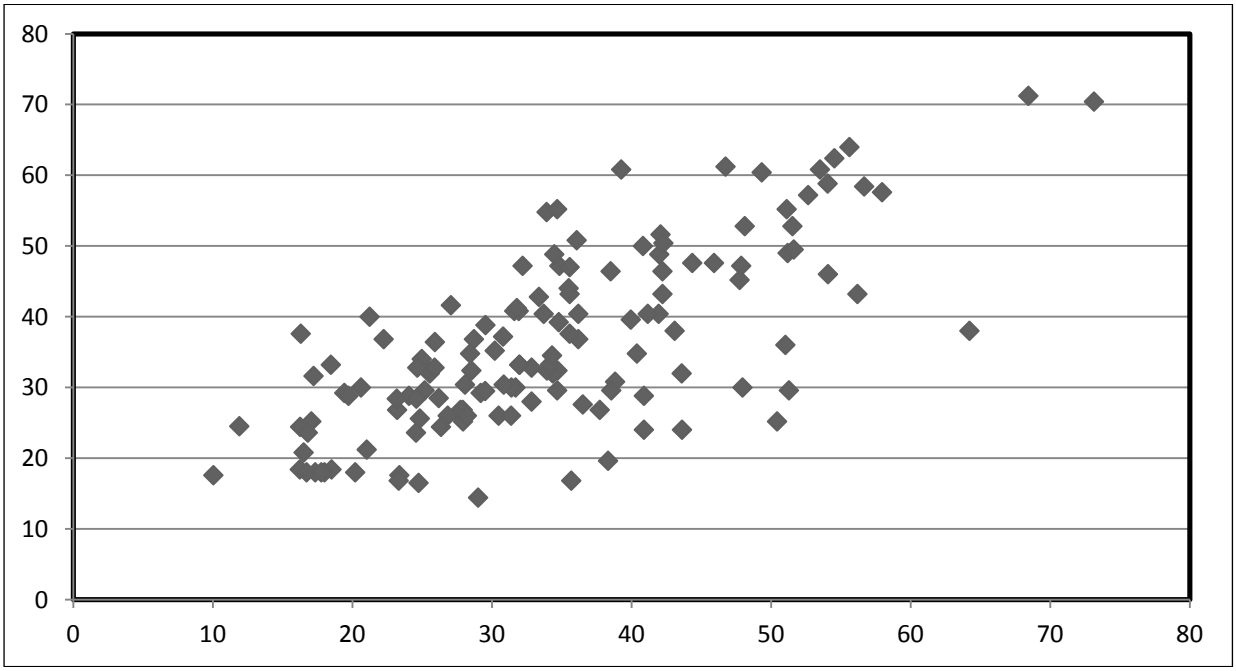


Fig. (9) Correlation between visual and APS scoring in leaves disease for 2010 (r = 0.725***).

3.2.2 The phenotypic correlation between studied traits under field-potted conditions

Table (8) shows the Pearson correlation coefficients (r) between 5 pairs (Leaves Disease Scoring 5.1 Isolate (LDS 5.1I.), Leaves Disease Scoring 5.3 Isolate (LDS 5.3I.), Spikes Disease Scoring 5.1 Isolate (SDS 5.1I.), Spikes Disease Scoring 5.3 Isolate (SDS 5.3I.) , Visual leaves disease Scoring 2009(VS2009) and Heading Date 2011 (HD2011)) of studied traits under disease infection stress at field-potted in 2011, and visual disease scoring in the green-house 2009. LDS first isolate had a highly positive significant correlation with LDS 5.3 isolate and VS 2009. SDS first isolate was highly positive significant correlation with SDS second isolate and VS 2009. HD had a negative correlation with LDS 5.1 isolate, SDS 5.1 isolate and VLDS 2009, and positive correlation with LDS and SDS 5.3 isolate.

Correlation coefficient between LDS5.1I. and SDS5.1I., SDS5.3I. the value were not significant and were 0.33 and 0.21. This values refers to low correlation between SDS and LDS in the studied population as expected from using these two different scores. Almost no correlation found between LDS and SDS. However, correlation between VS 2009 (under green-house conditions) and LDS5.1I. with r = 0.71, LDS5.3I. with r = 0.42 and SDS5.1I. with r = 0.41 (under field-potted conditions), these correlation values showed that the response of infection for the accessions used in both experiments was nearly the same.

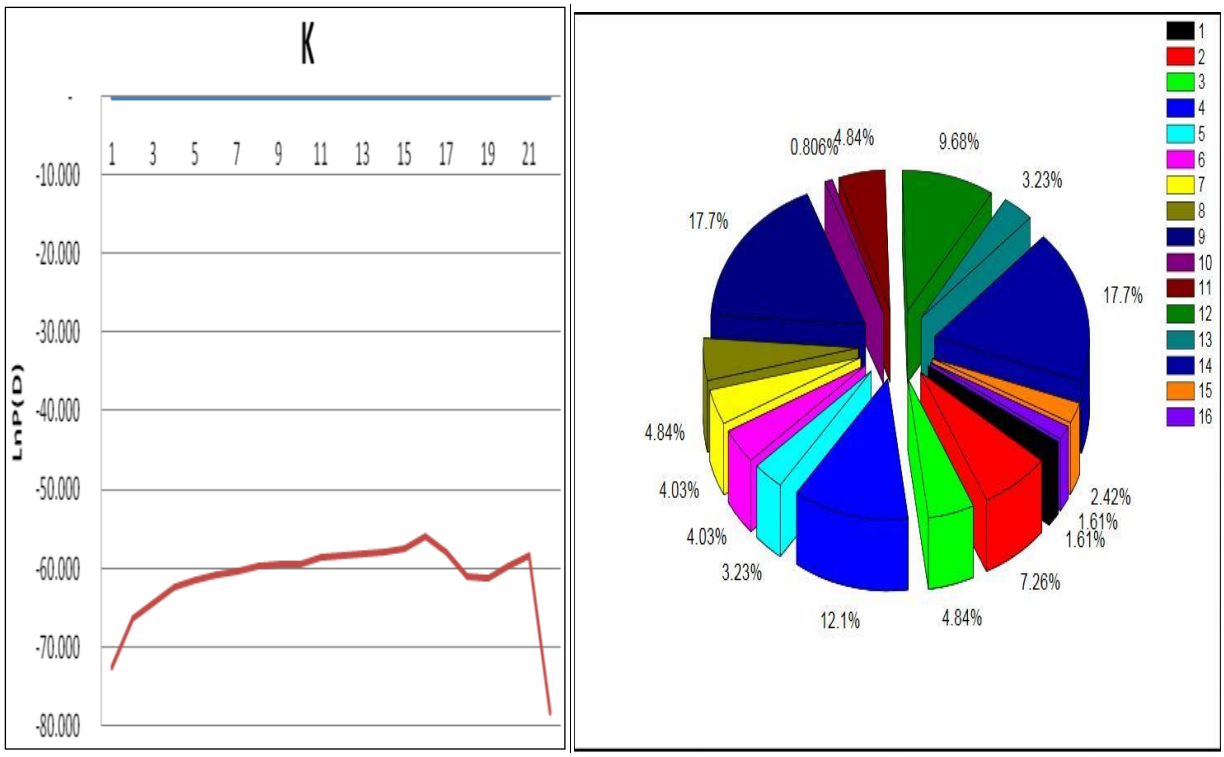
Table: (8) Pearson correlation coefficient (r) calculated between 5 pairs of traits under disease infection stress and heading date in the field-potted experiment 2011 and visual disease scoring in the green-house 2009

Trait	LDS5.1I.	LDS5.3I.	SDS5.1I.	SDS5.3I.	VS2009
LDS5.3I.	0.73***				
SDS5.1I.	0.33	0.02			
SDS5.3I.	0.21	0.18	0.46**		
VS2009	0.71***	0.42*	0.41*	0.21	
HD 2011	-0.03	0.10	-0.16	0.11	-0.17

* and *** are significant effects at 0.05 and 0.001 levels, respectively.

3.3 Population structure and Kinship coefficients

The Population structure analysis was conducted using genotypic data of 895 DArT markers by using Structure Software 2.2 (Pritchard et al 2000), and the accessions subdivided into 16 subpopulations, base on the suggestion of Pritchard and Wen (2007), by using the burn-in time 100 000 and the number of replications (MCMC) was 200 000, the individuals placed into k clusters, we set k (the number of subpopulations) from 1 to 22. To reach the appropriate K value, the estimated normal logharithm of the probability of fit (averaged for the two runs), the population structure matrix (Q) was defined by running structure at K = 16, where the highest likelihood has been obtained (**Fig.10a**).



(a) **(b)**

Fig. (10) (a) presents the number of clusters, which have the highest maximum likelihood, and (b) presents the percentage of the accessions in each cluster.

Table (2) in page number 31, presents the accessions, the country of origin, and the cluster number for each genotype, the cluster 1 included 2 accessions (1.61%), from Palestine. Cluster 2 included 9 accessions (7.26%) 7 out of them are Palestine, one Turkmenistan and one Iranian. Cluster 3 contained 6 accessions (4.84%), 2 from Jordan, 2 from Afghanistan, one from Turkey and one unknown origin country. Cluster 4 consist of 15 accessions (12.1%) 5 Syria, 4 Uzbekistan, 3 Palestine, one from Iraq, one Tajikistan and one Iranian. Cluster 5 included 4

accessions (3.23%) 3 Syrian and one Iranian. Cluster 6 included 5 accessions (4.03%) 2 Syrian, 2 from Jordan and one from Turkey. Cluster 7 contained 5 accessions (4.03%) 2 from Jordan and 3 from Syria, Iraq and Iran. Cluster 8 included 6 accessions (4.84%) 3 from Jordan and 3 from Syria, Iran and Libya. Cluster 9 contained 22 accessions (17.7%) 8 from Palestine, 5 from Jordan, 3 Turkmenistan, 2 Syrian, 2 Iranian, one from Pakistan and unknown origin country. Cluster 10 consists of 1 accessions (0.806%) from Palestine. 6 accession (4.84%) sited in cluster 11 four from Syria and 2 Palestinian. Cluster 12 included 12 accessions (9.68%) 3 of them from Iran, 2 Syrian, 2 from Jordan, 2 Turkey and 3 from Afghanistan, Russia and Libya. Cluster 13 contained 4 (3.23%) 3 Iranian and one Palestinian. Twenty two genotypes were in cluster 14 (17.7%) 21 German varieties and one accession from Syria. Three Accessions (2.42%) were from Palestine in cluster 15. Cluster 16 included 2 accessions (1.61%) from Iran (**Fig. 10 b**).

Fig. 11 represents that all genotypes were distributed within the 16 groups according to the relatively genetic distances using structure and cluster analysis, in the colored part above the diagram each individual is represented by a single vertical line broken into k colored segments, with lengths proportional to each of the k inferred clusters or subgroups. Whereas the part below of the diagram represents the cluster analysis based on the DICE dissimilarity index and the unweighted neighbour-jointing method was performed on the 895 DArT markers for 124 Accessions, 16 main clusters were identified which correspond well with genetic distances and origin of the genotypes.

3.4 Population structure by using Principal component analysis

Principal component analysis (PCA) also was used to analyze population structure by using genotypic data of 895 DArT markers. Results of the PCA analysis are shown in **Fig.12**. The PCA analysis was conducted by using SAS Software 9.2. The first dimension (PC1) was accounts for 11.68% of total variance. The second dimension (PC2) summarizes 4.57% of the variation. According to this analysis, the population was structured (Price et al. 2006), with three major clusters. According to Franklin et al. (1995) method, the first four PCs were significant, the first three used in the association mapping analysis as Q matrix.

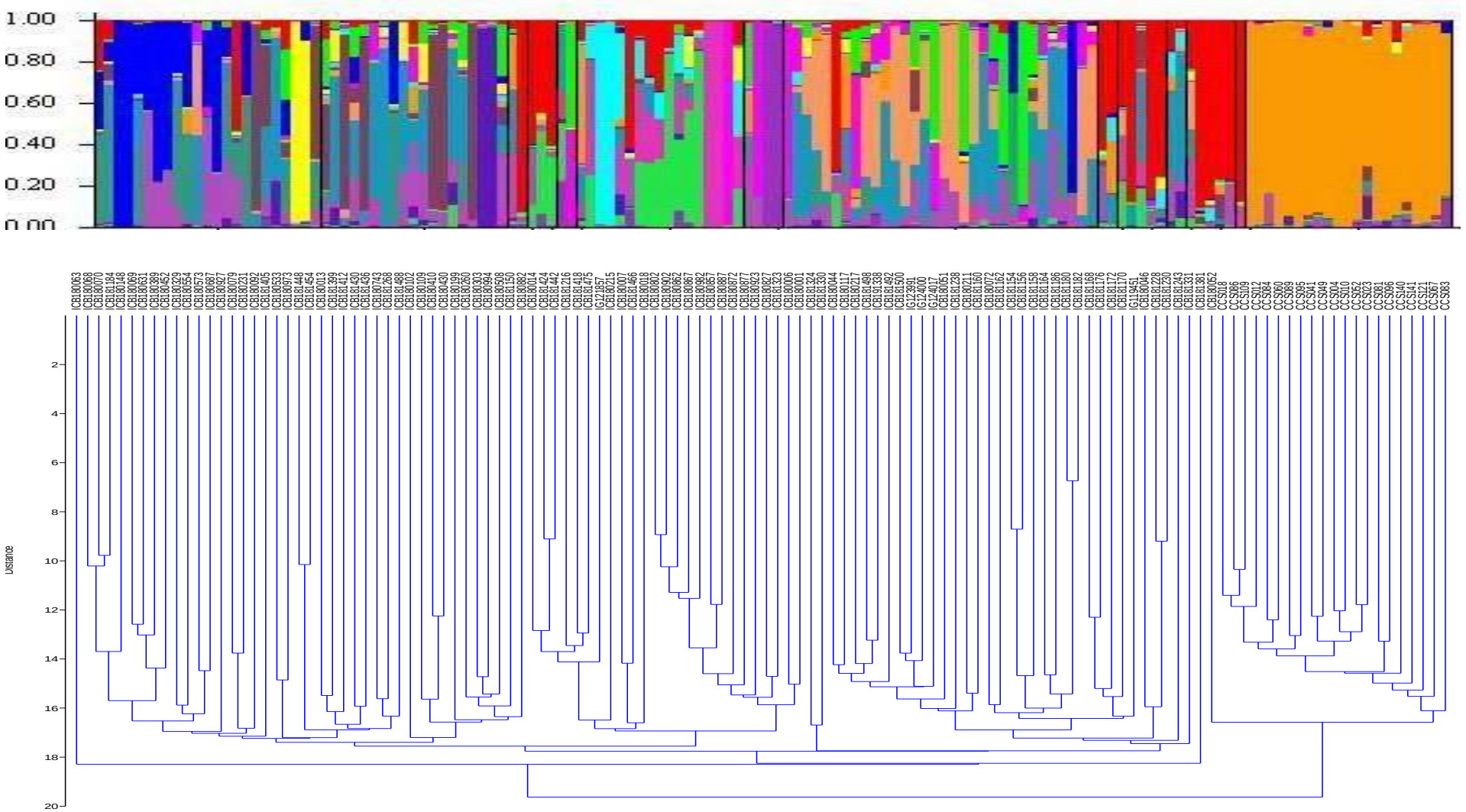


Fig.(11) The hierarchical clustering (UPGMA) of the accessions based on their genetic distances and the subdivision into 16 subpopuations according to the structure analysis and geographical distribution

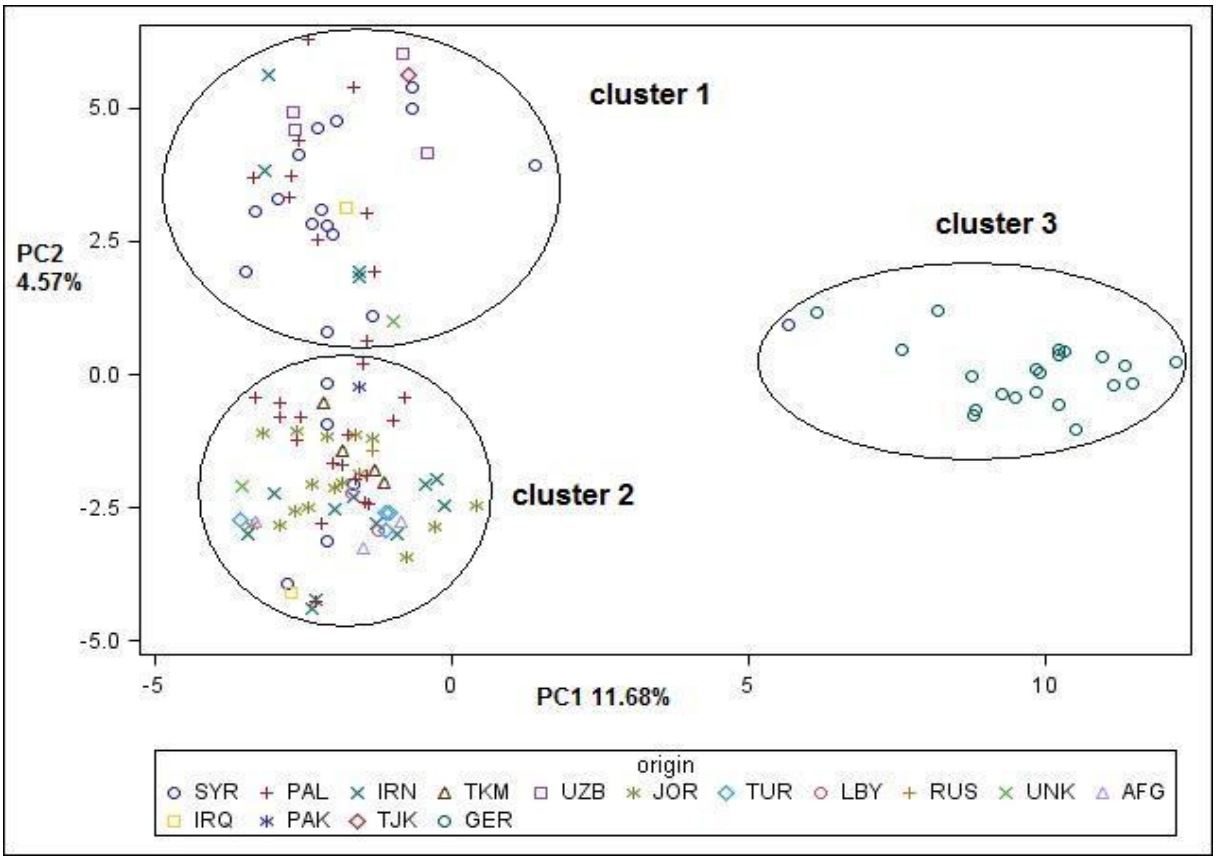


Fig. (12) Principal component analysis of the population of 124 barley accessions based on 895 DArT markers.

3.5 Structure of linkage disequilibrium among DArT markers

Estimation of composite linkage disequilibrium (LD) in order to determine population structure effects on LD in the studied population, after removed 368 DArT markers without positions, the DArT markers number became 527. The squared allele-frequency correlations, r^2, representing linkage disequilibrium (LD) were assessed for 4691, 5042, 3916, 562, 2556, 3081, 3403 and 23,251 combinations of DArT markers for chromosomes 1H, 2H, 3H, 4H, 5H, 6H, 7H and the whole genome, respectively. In the structured population all intrachromosomal loci pairs were in LD with $P < 0.01$, considering all 124 genotypes, the r^2 values for intrachromosomal pairs of loci ranged from 0.0 to 1.0. **Fig. 13** represent the pattern of LD decay plot of chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H separately and the whole genome, a loose curve that fitted the r^2 estimates did not reach the level of 0.1. This figure represent low or no LD decay in the structured population the decay was non line regression and the curve like a straight line. **Fig. 14** represents the heat map of pairwise linkage disequilibria (LD) for 527 DArT marker in the studied population. Both figures showed that very low LD (< 1 cM) observed in this population. This means high resolution in the population for association mapping.

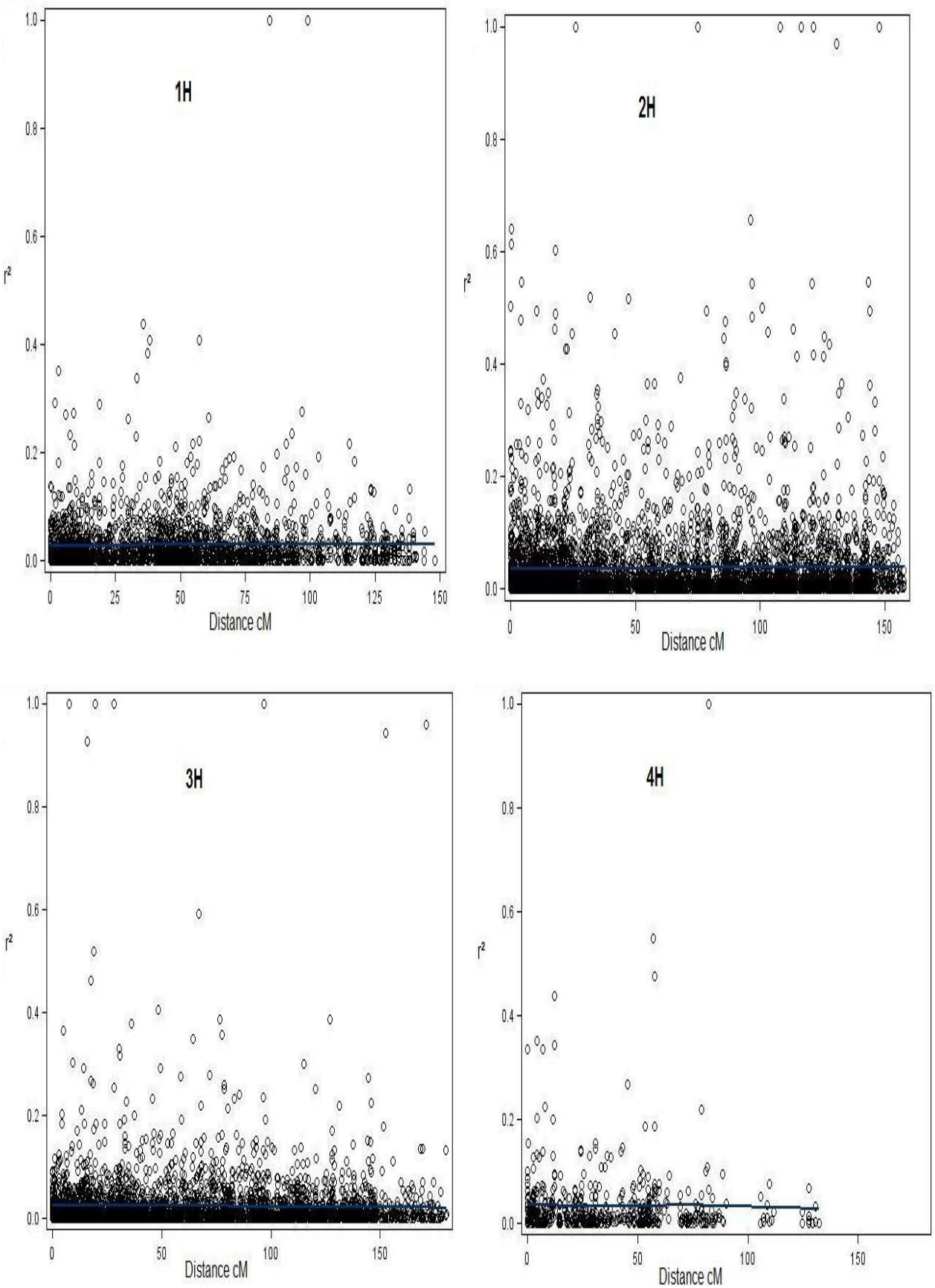


Fig. (13) The pattern of LD for 527 DArT loci in dependence on the population structure. Plots of LD represented by r² against genetic distance (in centiMorgan) in the population of 124 accessions in the seven chromosomes 1H, 2H, 3H, 4H, 5H, 6H, 7H and the whole genome.

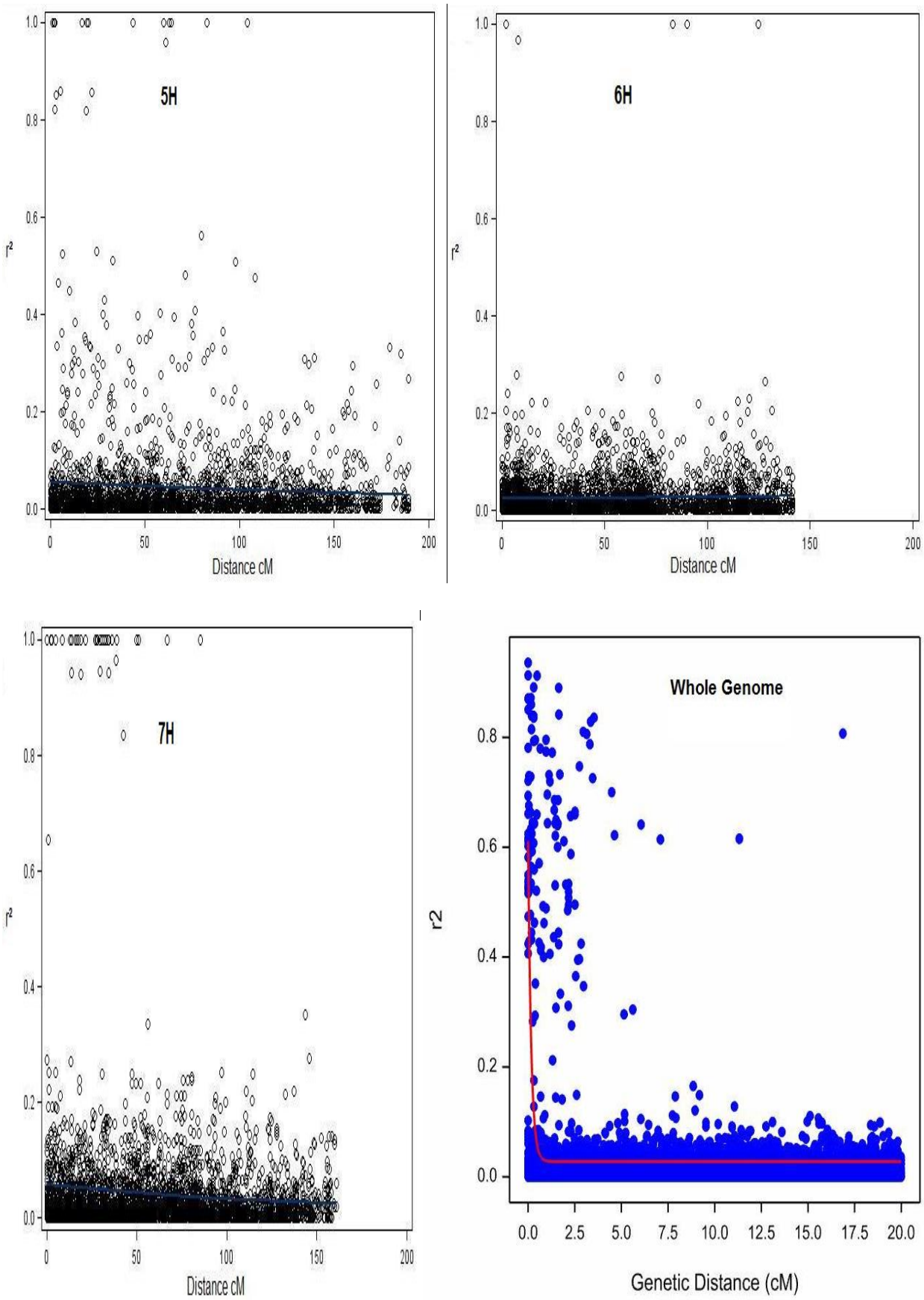


Fig. (13) The pattern of LD for 527 DArT loci in dependence on the population structure. Plots of LD represented by r² against genetic distance (in centiMorgan) in the population of 124 accessions in the seven chromosomes 1H, 2H, 3H, 4H, 5H, 6H, 7H and the whole genome.

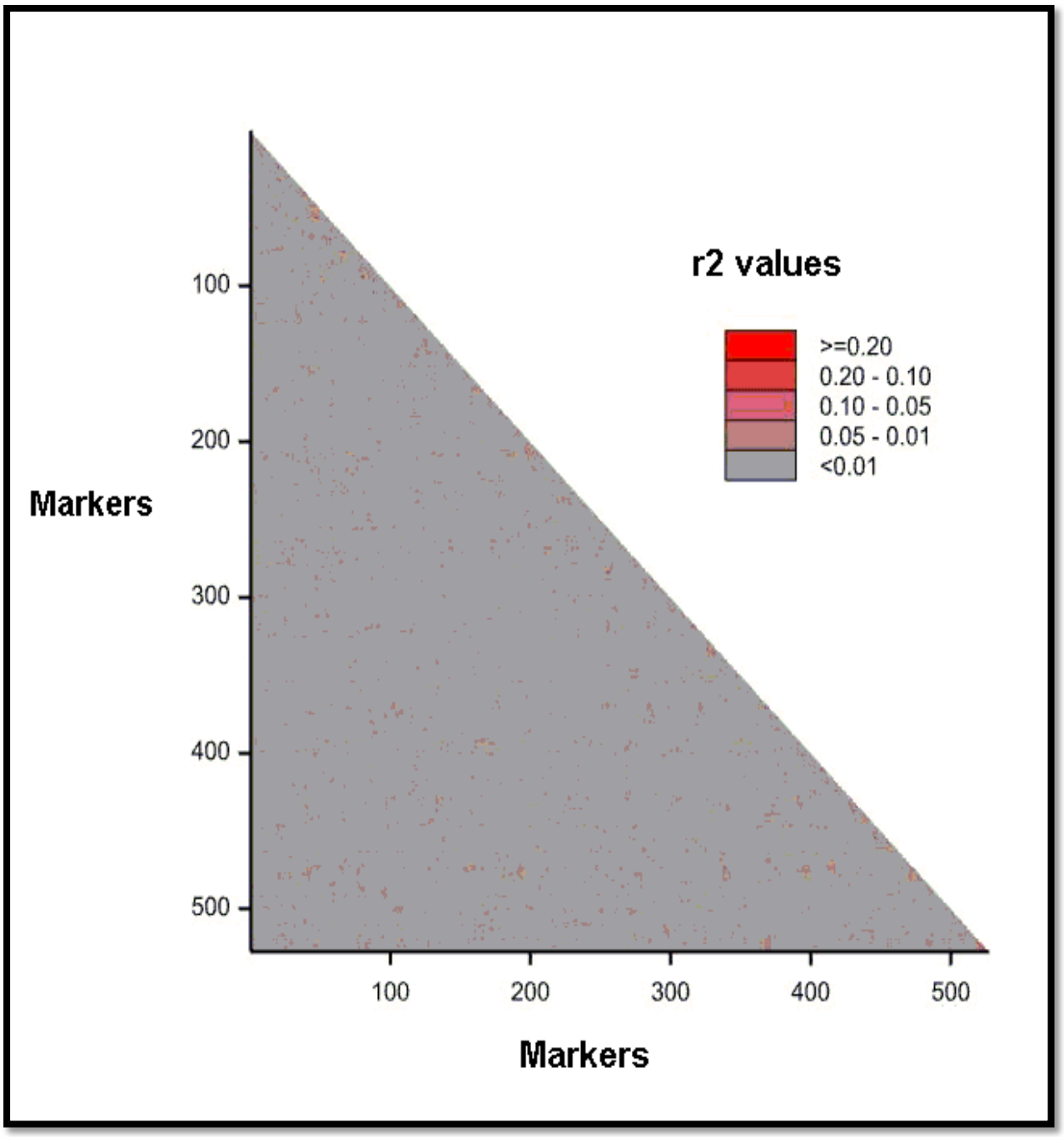


Fig. (14) Heat map of pairwise linkage disequilibria (LD) for 527 DArT markers in dependence on the population structure. Each pixel represents pairwise linkage disequilibrium (LD), which is measured by the squared allelic correlation coefficient r^2 between two DArT markers. As indicated by the color key, higher r^2 values are represented by red and lower by grey.

The Marker density was calculated for each chromosome separately the density values were 2.01, 1.42, 1.83, 3.5, 2.03, 1.45 and 1.64 cM for 1H, 2H, 3H, 4H, 5H, 6H and 7H, respectively.

3.6 Marker-trait associations

A Mixed linear Model (MLM) implemented which is comparable to the GRAMMAR estimation method. This technique was used with PCA and Kinship for full experimental design; like years, isolates,.. etc. This analysis was done using SAS Software Version 9.2 to conduct the association analysis and to identify the DArT markers associated with the disease scoring and heading date in the structured barley population based on population structure (Q matrix) and relatedness relationship (K matrix). A multiple QTL model was employed in this study iteratively extended and reduced by forward selection and backward elimination, respectively using the PROC MIXED procedure in SAS (Sayed et al. 2012). This QTL model bears the ability to utilize individual observations of each trait value simultaneously across year, blocks and therefore, trait values were not averaged across years for marker trait analysis. The association of DArT markers with the studied traits is described in table (9).

3.7 Detection of QTLs

Table (9) shows detected QTLs in this study at 0.05 significance onto different chromosomes of barley genome for all traits. However, Fig 15 , 16, 17, 18, 19 and 20 shows the manhatten plots for significant QTLs for each trait separately under study.

1) Leaves Disaese Scoring (LDS)

These detected QTLs for leaves disease scoring for two years 2010 and 2011. Ten markers were associated significantly with LDS, 3 out of these tens without positions the rest located on the chromosomes 2H, 5H, 6H and 7H. Eight markers affected by decreasing of the means of the trait, while the others affected increasing the means. The lowest and the highest differences values (-7.66 and 10.74) were observed for marker 4 and 6 on 6H (38.04 cM) and 7H (94.41 cM) respectively.

2) Leaves Disease Scoring Isolates (LDSI)

Leaves disease scoring isolates (LDSI) means that, leaves disease scoring when the isolates (two isolates) factor in 2011 included in the marker trait analysis. Fourten markers were associated significantly with LDSI and distributed on the whole genome of barley except chromosomes 2H and 7H did not contain any marker associated with this trait, 4 markers out of them without positions, 11 had range from (-6.7 to -8.18) the presence of the allele M1 led to decline that trait. Only 3 markers had another effect by increasing the infection percentage with 5.73, 7.7 and 11.14.

Table: (9) The detected QTLs of the significant markers at 0.05 for all studied traits

Trait	No.	Marker Name	Chr.	Pos.	Fvalue	PropF	Prob.	Effect		Diff.
								M0	M1	
LDS	1	bPb-6466	2H	7.59	8.476	0.00466	**	40.13	32.87	-7.29
	2	bPb-3574	2H	49.03	7.895	0.0064	**	36.51	45.77	9.26
	3	bPb-6881	2H	70.81	7.905	0.00655	**	38.96	31.52	-7.44
	4	bPb-0710	5H	115.64	7.242	0.00875	**	38.92	31.46	-7.46
	5	bPb-2058	6H	38.04	12.254	0.00074	***	42.57	34.91	-7.66
	6	bPb-9912	7H	94.41	15.546	0.00015	***	32.17	42.91	10.74
	7	bPb-0182	7H	123.08	7.115	0.00947	**	40.92	34.94	-5.98
	8	bPb-5727	-	----	8.770	0.00408	**	40.19	32.87	-7.32
	9	221193	-	----	8.998	0.00360	**	40.29	33.11	-7.18
	10	104868	-	----	11.852	0.00094	***	40.98	33.55	-7.43
LDSI	1	bPb-2813	1H	59.67	8.475	0.0048116	**	31.22	25.27	-5.95
	2	bPb-9957	1H	63.32	7.28	0.0089281	**	31.8	25.88	-5.92
	3	bPb-7989	3H	50.43	23.835	0.0000055	***	32.34	24.16	-8.18
	4	bPb-6576	4H	90.22	10.102	0.0021582	**	27.43	38.57	11.14
	5	bPb-0503	5H	56.76	9.782	0.0025579	**	34.51	27.35	-7.16
	6	bPb-0709	5H	76.76	9.848	0.0025136	**	34.43	27.04	-7.39
	7	bPb-8553	5H	120.44	9.033	0.0036828	**	34.4	27.33	-7.07
	8	bPb-7277	5H	139.48	8.20	0.0054875	**	34.16	27.46	-6.7
	9	bPb-3746	6H	60.92	10.602	0.0017648	**	34.42	26.95	-7.47
	10	bPb-1695	6H	143.8	9.43	0.0031145	**	34.17	27.17	-7.00
	11	223262	6H	-	7.401	0.0084366	**	31.85	26.03	-5.55
	12	223028	-	-	9.330	0.0031943	**	34.21	27.25	-6.96
	13	222475	-	-	8.031	0.0060712	**	26.01	31.74	5.73
	14	222651	-	-	9.803	0.0024983	**	25.56	32.96	7.4
VS	1	bPb-4645	3H	66.55	17.912	0.0000572	***	43.83	30.92	-12.91
	2	bPb-3623	3H	148.34	11.684	0.001011	***	42.26	32.65	-9.61
SDS	1	bPb-3056	2H	70.81	7.253	0.009179	**	29.15	23.55	-5.6
	2	bPb-6124	5H	190.97	13.825	0.0003714	***	21.95	29.86	7.91
	3	bPb-9051	6H	70.57	10.237	0.002006	**	33.15	24.06	-9.09
	4	bPb-4379	6H	122.08	7.936	0.0061106	**	30.97	24.04	-6.93
	5	bPb-8823	7H	133.4	19.442	0.000030	***	21.11	30.77	9.66
	6	bPb-1336	7H	141.81	10.907	0.0015016	**	30.09	23.03	-7.06
	7	bPb-5935	-	-	7.371	0.0083596	**	21.89	28.68	6.79
	8	bPb-5057	-	-	7.163	0.0093899	**	21.77	28.73	6.96
SDSI	1	bPb-9881	1H	7.21	11.22	0.001723	**	31.37	22.4	-8.97
	2	bPb-6568	5H	21.54	6.805	0.013045	*	22.37	29.8	4.43
	3	bPb-0730	6H	68.22	6.037	0.018963	*	25.87	18.73	-7.14
	4	bPb-9285	6H	134.62	9.207	0.003852	**	25.83	18.1	-7.73
	5	bPb-0522	6H	142.51	7.963	0.006839	**	25.44	19.01	-6.43
	6	bPb-8823	7H	133.4	12.289	0.000881	***	20.6	26.84	6.24
	7	224171	5H	-	8.287	0.00604	**	30.21	22.62	-7.59
	8	221054	-	-	6.791	0.012545	*	24.56	17.52	-7.04
HD	1	bPb-3375	6H	122.08	29.384	0.00000044	***	80.53	75.69	-4.84
	2	222472	-	-	11.489	0.000846	***	77.33	80.87	3.54
	3	223950	-	-	11.876	0.001041	***	76.34	79.87	3.23

*, ** and *** are the significances differences at 0.05, 0.01 and 0.001 levels. -, is missing position and chromosome.
M_0 and M_1 refer to the effect of the first allele (absence) and the second allele (presence) of the marker, respectively.

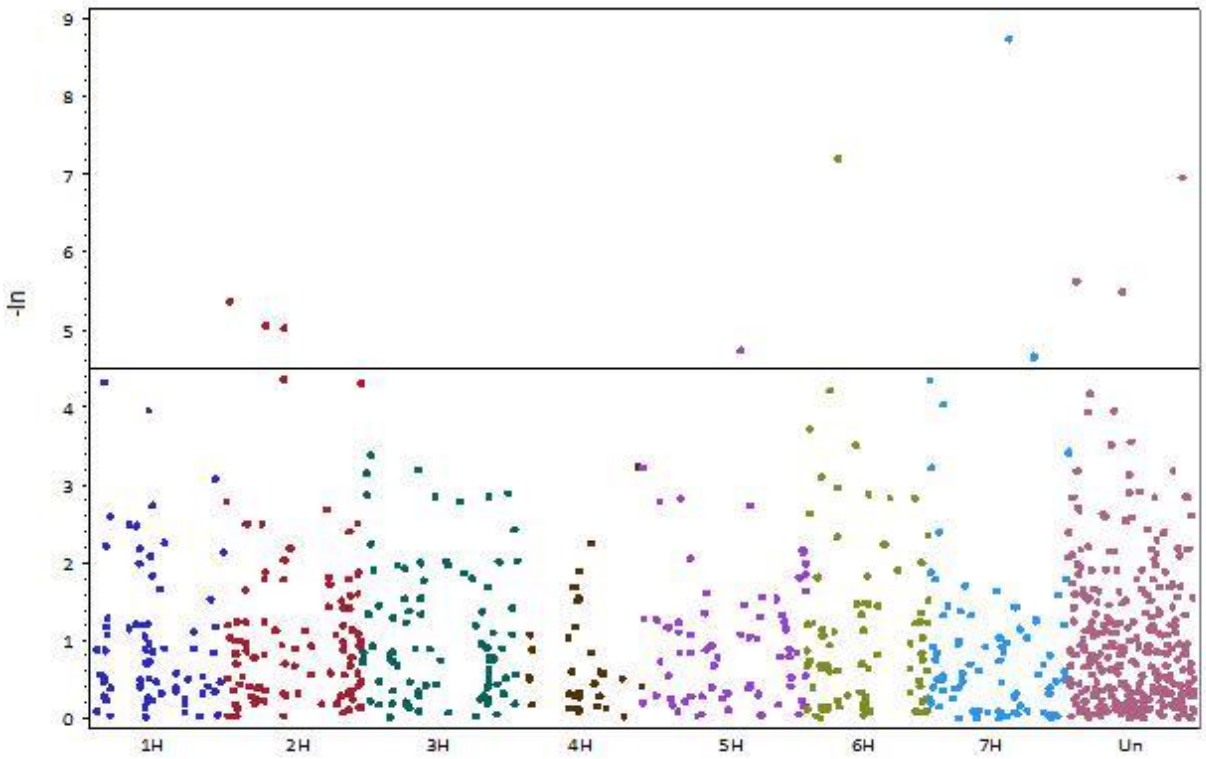


Fig. (15) The pattern of manhattan plot for detected QTLs of leaves disease scoring in 2010 and 2011.

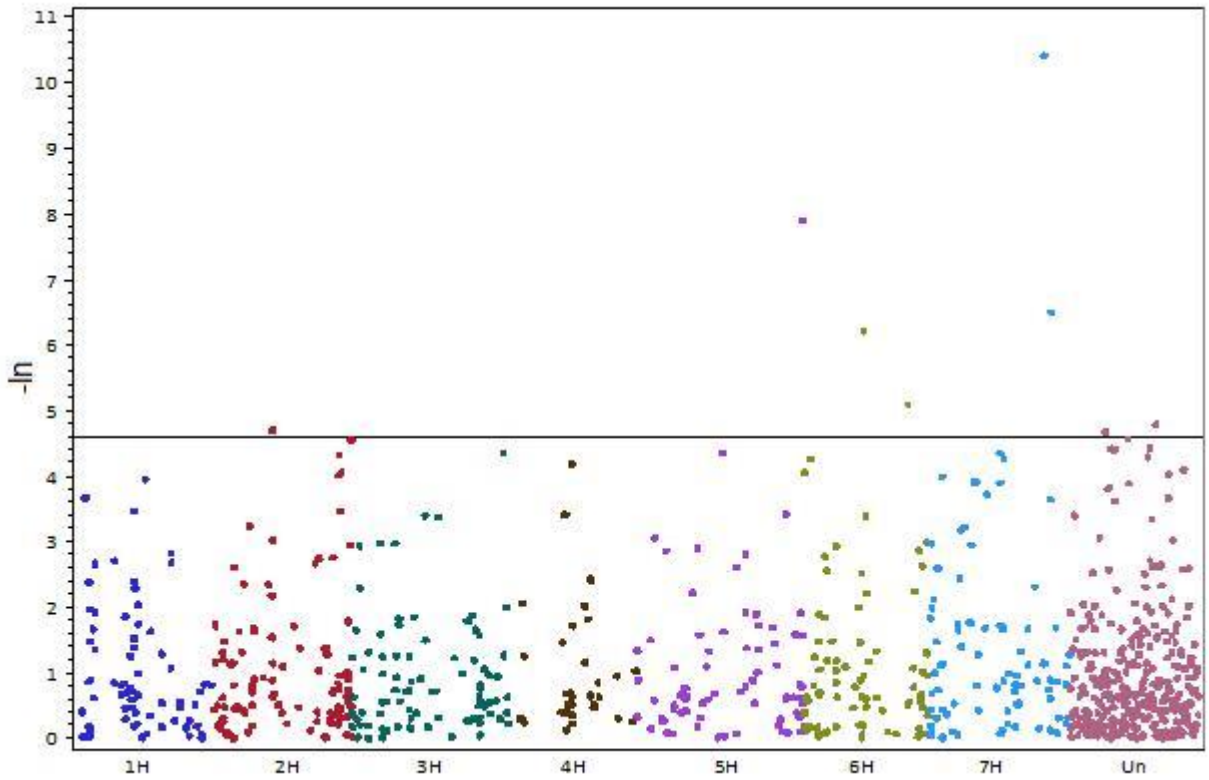


Fig. (16) The pattern of manhattan plot for detected QTLs of spikes disease scoring in 2010 and 2011.

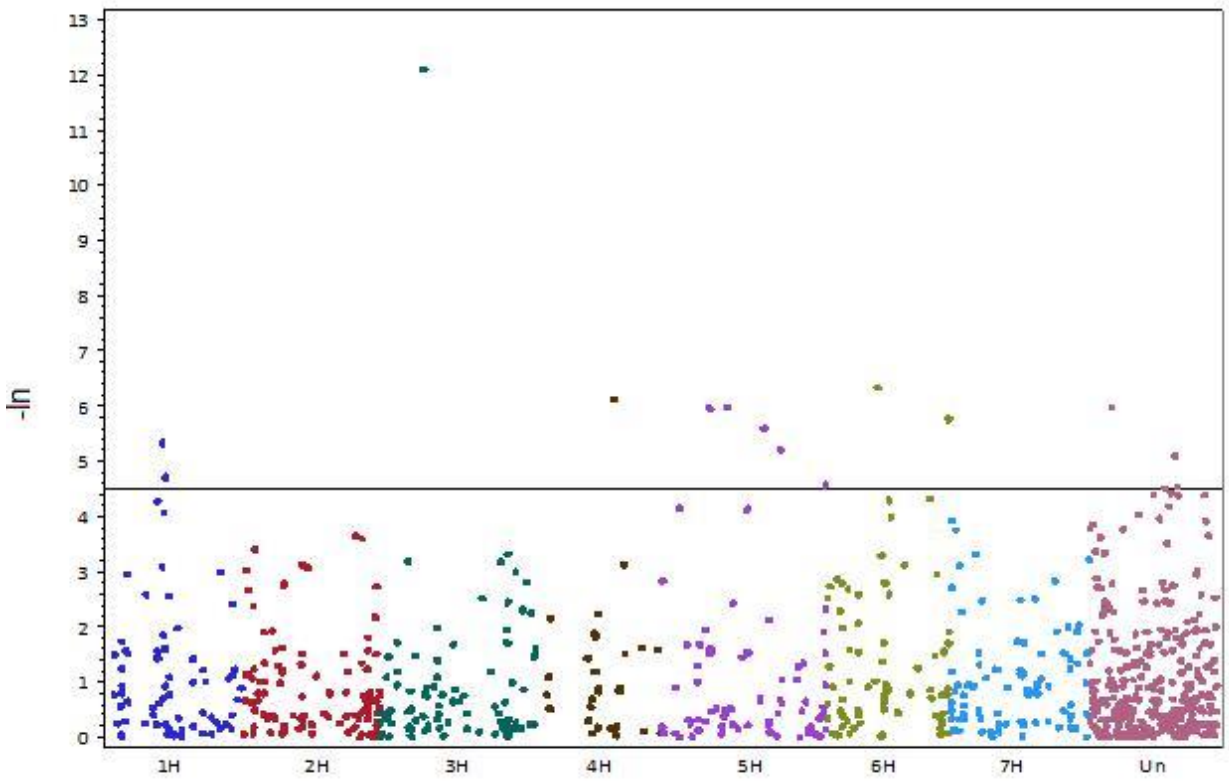


Fig. (17) The pattern of manhattan plot for detected QTLs of leaves disease scoring isolate in 2011.

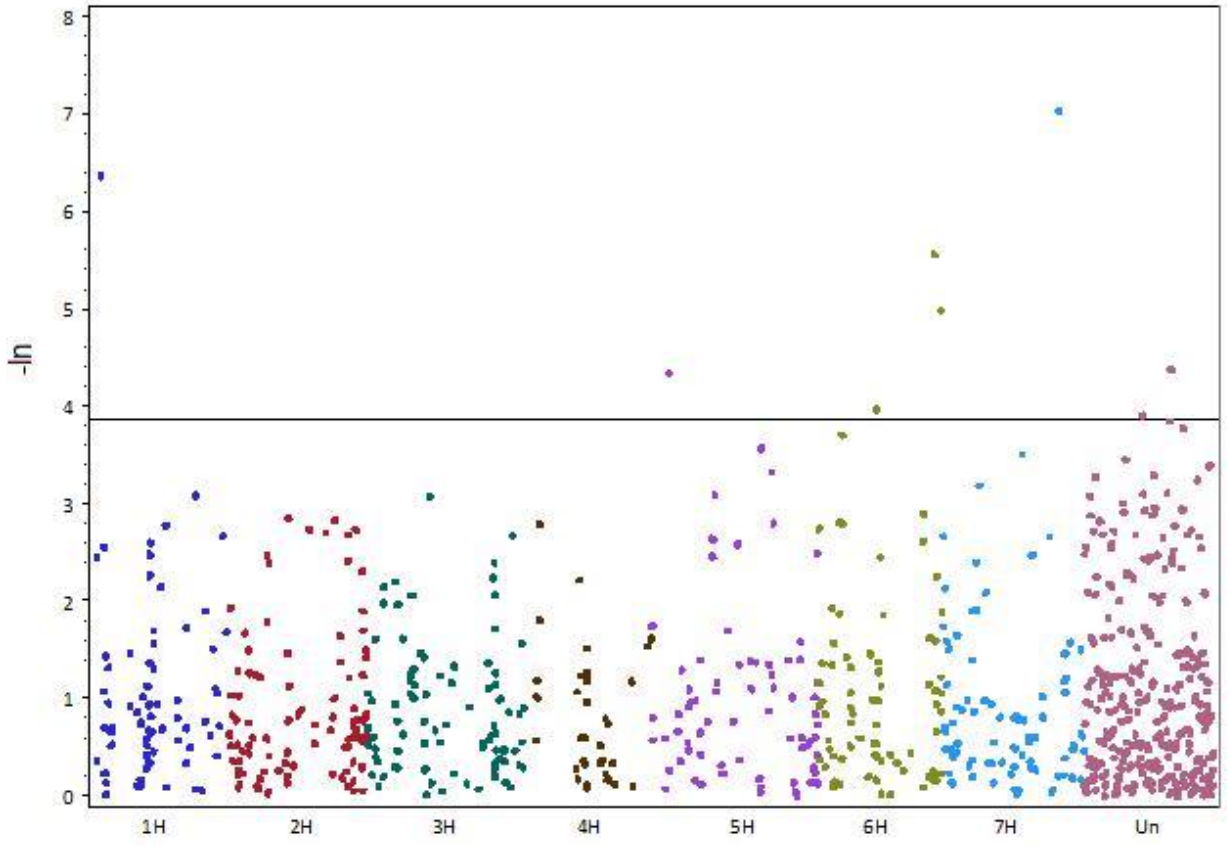


Fig. (18) The pattern of manhattan plot for detected QTLs of spikes disease scoring isolate in 2011.

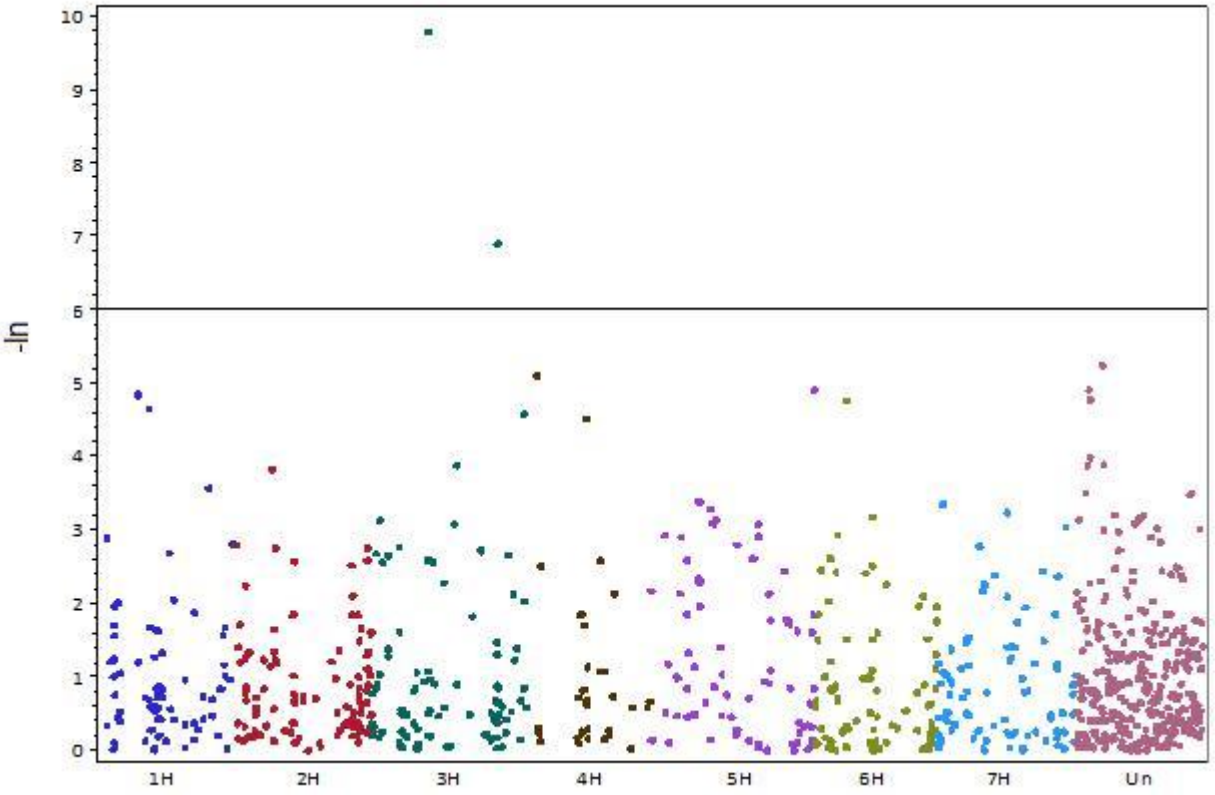


Fig. (19) The pattern of manhattan plot for detected QTLs of visual scoring 2010.

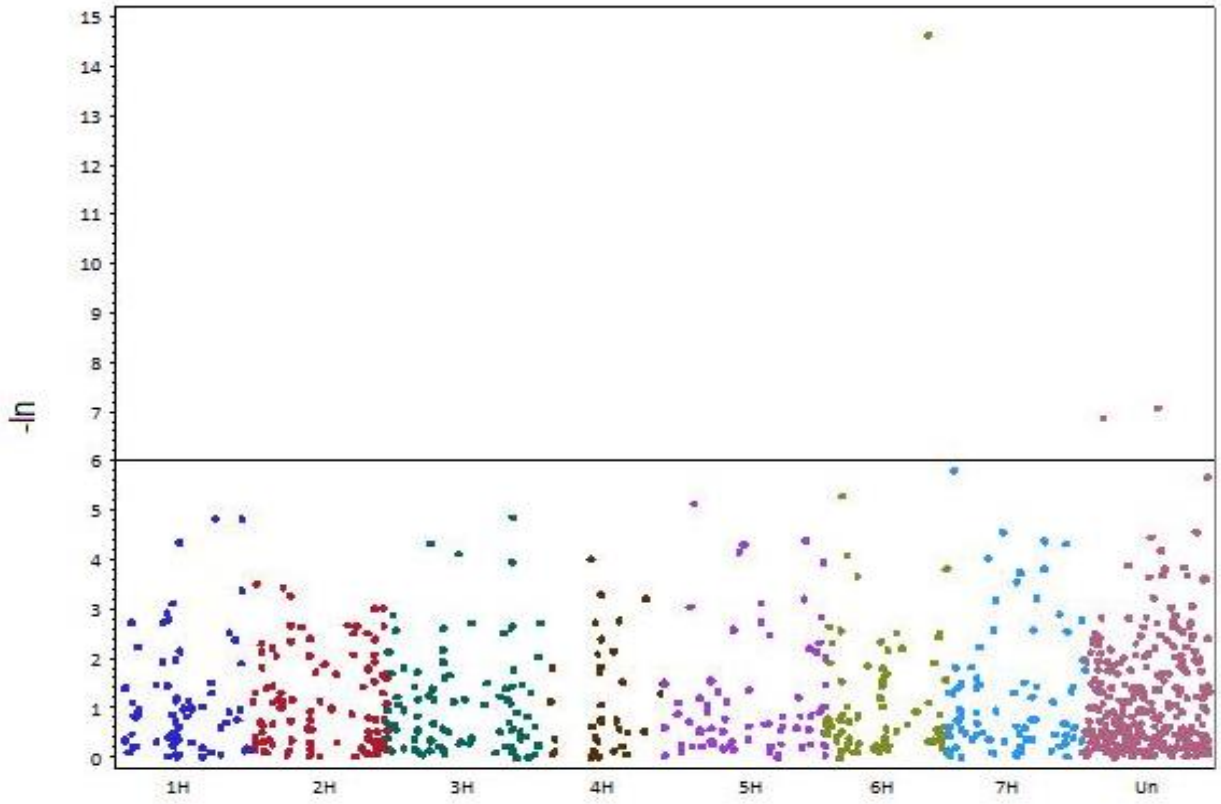


Fig. (20) The pattern of manhattan plot for detected QTLs of heading date 2011.

3) Visual Scoring (VS)

Two markers were associated with VS on chromosome 3H both had good effect on the trait with range from (-9.61 to -12.91).

4) Spikes Disease Scoring (SDS)

These detected QTLs for spikes disease scoring for two years 2010 and 2011. Eight Markers were associated significantly with SDS, distributed on chromosomes 2H, 5H, 6H and 7H, 2 out of this group without positions, 4 had affected negatively the best one was No. 3 (bPb-9051) on 6H with (-9.09). 4 had affected positively with the trait the higher one was No. 6 (bPb-8823) on 7H with 9.66. On the position of 70.81 cM on chromosome 2H two markers were identified to be associated with LDS and SDS they were markers bPb-6881 and bPb-3056.

5) Spikes Disease Scoring Isolates (SDSI)

Spikes disease scoring isolates (SDSI) means that, spikes disease scoring when the isolates (two isolates) factor in 2011 included in the marker trait analysis. Eight Markers were associated significantly with SDSI, distributed on chromosomes 1H, 5H, 6H and 7H, 2 from this group without positions, 2 had affected positively they on 5H and 7H. Six had negatively affected with the trait on 1H and 6H. The marker bPb-8823 on 7H on 133.4 cM. was detected also for SDS. Marker bPb-8823 on 7H on 133.4 cM were detected for pervious trait SDS.

6) Heading Date (HD)

Heading date recorded only for one year 2011. HD was associated significantly with 3 markers, 2 out of them without position and both affected with increasing heading date, thus mean this two associated to late heading. The third QTL (bPb-3375) on chromosome 6H (122.08 cM) was associated to early heading, another marker in same position (bPb-4379, 6H (122.08 cM)) was also detected to SDS trait.

3.8 Detection of interactions between QTLs and fungus isolates for two different scoring

Table (10) shows detected QTLs in this study at 0.05 significance onto different chromosomes of barley genome for the interaction between the treatments (isolates) and markers. In this table, 2 different types of interactions in both scoring (LDS and SDS); MILDS this interactions means marker*isolate interaction within leaves disease scoring and MISDS this means marker*isolate

interaction within spikes disease scoring. Each of these interaction have two means; when the interaction between the isolate and M0 (marker absence) and M1 (marker presence) as follows:

Table: (10) The detected QTLs of the significant markers at 0.05 for isolates*marker interaction.

Trait	No.	Marker Name	Chr.	Pos.	Fvalue	PropF	Prob.	Interaction effect (T*M)			
								Isolate 5.1 M0	Isolate 5.1 M1	Isolate 5.3 M0	Isolate 5.3 M1
MILDS	1	bPb-9957	1H	63.32	11.784	0.0010	**	42.68	34.86	34.95	25.88
	2	bPb-5339	1H	76.78	5.1184	0.0258	*	40.20	34.82	31.36	25.64
	3	bPb-6466	2H	7.59	6.8087	0.0104	*	40.58	34.77	33.38	25.13
	4	bPb-6881	2H	70.81	8.9986	0.0034	**	41.81	33.43	34.19	23.80
	5	bPb-7989	3H	50.43	5.9673	0.0163	*	41.44	33.03	33.18	23.92
	6	bPb-3843	3H	147.95	6.9572	0.0097	**	39.08	32.31	30.24	23.16
	7	bPb-0710	5H	115.64	7.3326	0.0079	**	41.51	33.73	34.18	23.76
	8	221908	-	-	7.2473	0.0083	**	39.04	32.32	30.29	23.85
MISDS	1	bPb-6661	6H	28.84	5.4304	0.024629	*	24.91	16.79	23.47	9.84
	2	bPb-9285	6H	134.62	9.2079	0.003852	**	26.69	20.04	25.12	16.22
	3	bPb-0522	6H	142.51	7.9640	0.006839	**	26.24	20.11	24.70	17.82
	4	bPb-3091	-	-	5.6109	0.022955	*	25.40	15.54	22.63	18.52
	5	bPb-1084	-	-	4.7213	0.033707	*	25.00	15.62	23.11	16.56
	6	bPb-5669	-	-	4.1009	0.049568	*	26.51	16.95	23.31	21.35
	7	bPb-7084	-	-	4.6164	0.037845	*	25.18	20.23	23.42	16.93
	8	221054	-	-	6.7913	0.012545	*	24.78	18.85	24.33	16.19

-, means unkown chromosomes and positions. MILDS = Marker isolate interaction within leaves disease scoring, MISDS = Marker isolate interaction within spikes disease scoring. Isolate 5.1 M0, Isolate 5.1 M1 refers to the interaction effect between First allele (absence) and the second allele (presence) of the marker and isolate 5.1, respectively. The same situation in 5.3 isolate

1) Detected QTLs for MILDS

Eight QTLs were detected to the interaction between the isolates and markers, one out of this group without chromsomal position the rest were distributed on IH, 2H, 3H and 5H. All of these interactions QTLs were nearly having same mean values with both isolates and allels. Five from these markers were detected in this study for LDS and LDSI traits on chromsomes 1H (bPb-9957), 2H (bPb-6881, bPb-6881), 3H (bPb-7989) and 5H (bPb-07010). Marker bPb-3843 on (147.95 cM, 3H) was so close to marker bPb-3623 148.34 cM which detected to VS trait in this study.

2) Detected QTLs for MISDS

Also 8 QTLs were found associated with the interaction between the two isolate and marker, 5 without chromosome positions. The other three were located on 28.34, 134.62, 142.51 cM chromosome 6H. The best interaction was with marker (bpb-6661) for both isolate 5.1 and 5.3. Two markers out of three on chromsome 6H were detected for SDSI trait.

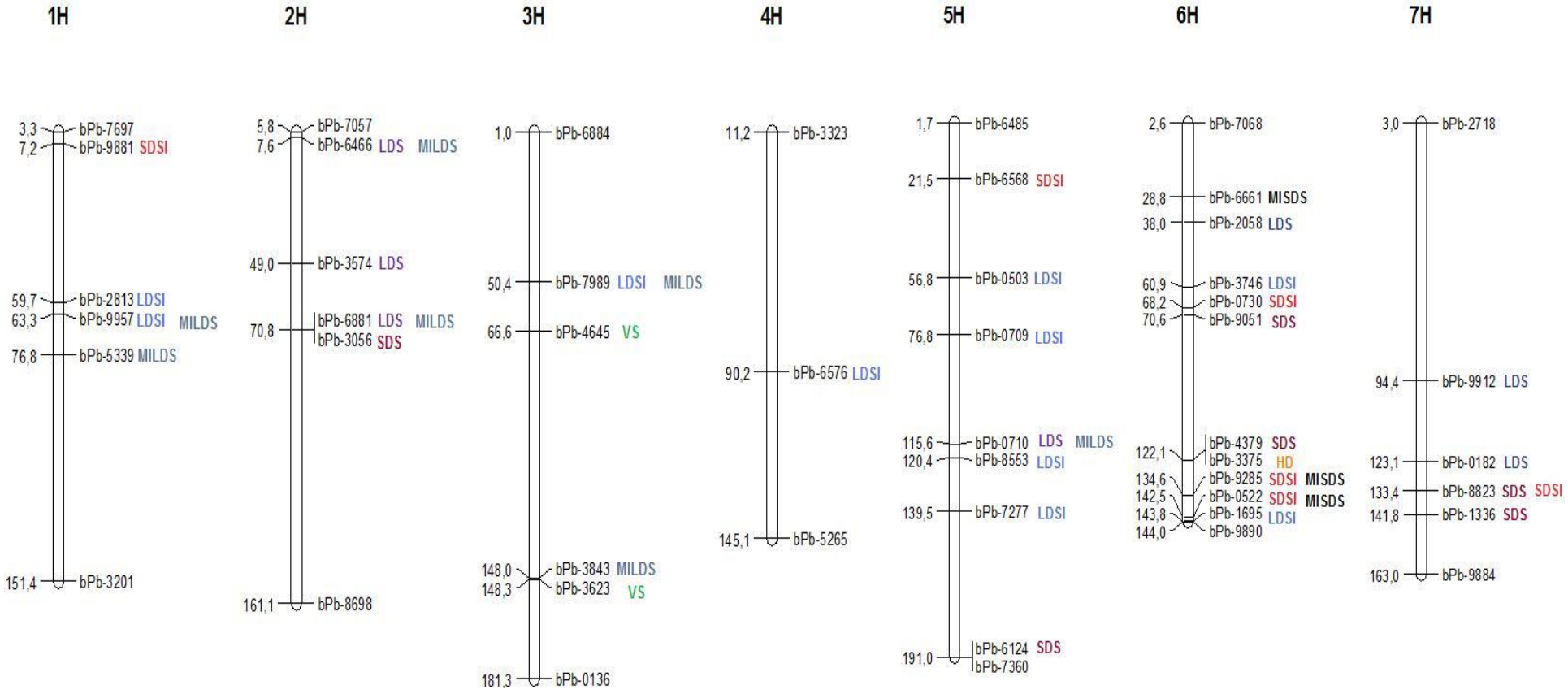


Fig. (21) DArT markers map for structured barley population, presents the detected QTLs associated with studied traits (see tables 9 and 10). Traits are written on the right hand side and their positions (cM) are written on the left hand side.

LDS = Leaves disease scoring, LDSI = leaves disease scoring isolate, SDS = spikes disease scoring, SDSI = spikes disease scoring isolate, VS = visual disease scoring, MILDS = Marker isolate interaction within leaves disease scoring, MISDS = Marker isolate interaction within spikes disease scoring and HD = heading date.

3.9 Epistatic interactions

Epistatic effects are statistically defined as interactions between effects of alleles from two or more genetic loci (Fisher 1918). Epistatic interaction analysis was carried out between markers with significant main effects and all other markers (Table11) also in Fig. 21, 22, 23 and 24. This study presents 4 different types for epistatic interaction effects between two different markers (Interactions between M_0 marker1 and M_0 marker2, M_1 marker1 and M_0 marker 2, M_0 marker1 and M_1 marker2 and M_1 marker1 and M_1 marker2). Whereas, M_0 means marker absence and M_1 means marker presence. All detected epistatic effect interactions were highly significant with highly FDR probability.

1) Leaves disease scoring (LDS)

Four highly significant epistatic interactions were detected to leaves disease scoring. Unfortunately, three out of these four interactions shared with unknown markers position. Only one epistatic effect (bPb-6408*bPb-4285) on 1H on 40.53 cM and on 2H on 3.54 cM. The best mean was (28.07) for this interaction when the allele 1 is presences in the two markers. However, the other three epistatic interactions gives means varied from 29.54 to 56.39. These four did not give the lower means against the infection by the fungus.

2) Spikes disease scoring (SDS)

Four epistatic interactions were detected for this trait. First interaction was in the same chromosome 2H in different positions 49.03 and 149.9 cM. The best means for this digenetic interaction was $M1_{M0}/M2_{M0}$ with 20.56. Second interaction effect was (bPb-6347* bPb-1420) on 3H and 5H. The interaction mean $M1_{M0}/M2_{M1}$ 18.44 recorded the lower mean within the four means and in also lower among this four epistatic effects. The other two interactions effects were detected with 2 unknowns markers position and 2 known's markers on chromosomes 5H and 7H. Third epistatic interaction (bPb-7067* bPb-8823) the marker bpb-8823 had a main effect for SDS (Table 9).

3) Leaves disease scoring isolate (LDSI)

Three epistatic interactions were detected for LDSI. First interaction (bPb-5755* bPb-6069) was having lower infection percentage mean (16.67) when the interaction the allele 1 is presences in both markers. However, the second one (bPb-6361* bPb-6048) have interaction values higher than the first and the third ranged from 24.74 - 48.93 for interactions $M1_{M0}/M2_{M1}$ and $M1_{M0}/M2_{M0}$, respectively. The third (bPb-6727*222522) have lower interaction means with 18.91 for the interaction

$M1_{M0}/M2_{M1}$. So near to marker bPb-6069 on chromosome 6H on 26.51 cM another marker (bPb-6661 on 28.8), was detected as a main effect for MISDS trait. Also, very close to marker bPb-6727 (6H on 134.08 cM) another marker (bPb-9285 on 134.6 cM) was associated to SDSI and MISDS traits as a main effect.

4) Heading date (HD)

Only one epistatic interaction was detected between marker bPb-2976 on chromosome 1H on 54.01 cM and marker bPb-3598 on 2H on 7.59 cM. The means of this interaction varied from 74.45 to 82.11 when the interactions $M1_{M1}/M2_{M0}$ and $M1_{M1}/M2_{M1}$, respectively. This means the effect when allele 1 are presences in both markers the interaction tends to the late heading.

There are no epistatic effects detected for SDSI and VS.

Table 12 and 13 represents information about the relationship between the favorable QTLs detected (main effects) in this study and the lowest accessions in the infection percentage for LDS and SDS.

Table 12 represents 14 accessions having infection percentage lower than 20% for LDS and 25 favorable QTLs detected to LDS, LDSI, MILDS (main effects). The total number of favorable QTLs was varied from 9 to 16 for accessions Voldagsen (19.16) and Otis (18.62), respectively. The most relevant QTLs also calculated in this table (The relevant ones were chosen by the effect of each on the trait under study in this case almost were have deacresing effect on the means of the trait ranged from (-8.18 to -7.00)). The number of relevant QTLs ranged from 2 -7 for Voldagsen (19.16) and Heidesandgerste (19.59), respectively.

The accession Candice, the lowest one in the infection percentage mean (13.74), had 13 favorable QTLs and 5 from this 13 were relevant QTLs from the total number 25.

Table 13 shows 7 accessions having infection percentage lower than 10% for SDS and 22 favorable QTLs detected to SDS, LDSI, MISDS (main effects). Low variation in the total number of favorable QTLs for SDS between these 7 accessions. The mean range of represented means of infection percentage for SDS was 7.00- 9.75.

The total number of favorable QTLs ranged from 9 – 13 for ICB181186 and Oberpfälzer (8.75). The relevant QTLs also represented in **Table 13** (The relevant ones were chosen by the effect of each on the trait under study in this case almost were have deacresing effect on the means of the trait ranged from (-9.09 to -7.04)). The number of relevant QTLs ranged from 4-9 for ICB181186 (8.75) and Oberpfälzer (8.75), respectively.

Table (11) Estimated of Lsmeans of 12 pairs of digenic interactions and epistatic effects for LDS, SDS, LDSI and HD traits.

Trait	No.	Effect	Marker 1			Marker 2			Fvalue	Sign	P_{FDR}	Lsmeans of digenitic interaction			
			Marker	Chr.	Pos.	Marker	Chr.	Pos.				$M1_{M0}/M2_{M0}$	$M1_{M1}/M2_{M0}$	$M1_{M0}/M2_{M1}$	$M1_{M1}/M2_{M1}$
LDS	1	bPb-6408* bPb-4285	bPb-6408	1H	40.53	bPb-4285	2H	3.54	6.834	***	< 0.001	34.19	40.31	38.94	28.07
	2	104846* bPb-3722	104846	--	--	bPb-3722	6H	68.53	55.82	***	< 0.001	32.15	30.99	56.39	34.55
	3	bPb-9486* bPb-9912	bPb-9486	--	--	bPb-9912	7H	94.41	121.5	***	< 0.001	42.80	29.72	29.54	41.54
	4	bPb-5245* bPb-3302	bPb-5245	--	--	bPb-3302	--	--	9.34	***	< 0.001	44.37	29.58	31.42	30.59
SDS	5	bPb-3574* bPb-8530	bPb-3574	2H	49.03	bPb-8530	2H	149.09	163.1	***	< 0.001	20.65	25.37	34.56	25.03
	6	bPb-6347* bPb-1420	bPb-6347	3H	55.63	bPb-1420	5H	138.99	9.94	***	< 0.001	34.27	26.12	18.44	24.56
	7	bPb-7067* bPb-8823	bPb-7067	--	--	bPb-8823	7H	133.4	50.96	***	< 0.001	28.71	29.16	30.29	18.68
	8	bPb-4199* bPb-6363	bPb-4199	--	--	bPb-6363	5H	36.1	56.51	***	< 0.001	23.21	26.89	22.69	46.09
LDSI	9	bPb-5755* bPb-6069	bPb-5755	2H	133.29	bPb-6069	6H	26.51	97.33	***	< 0.001	31.72	35.49	34.27	16.67
	10	bPb-6361* bPb-6048	bPb-6361	--	--	bPb-6048	2H	161.12	58.01	***	< 0.001	48.93	33.61	24.74	35.27
	11	bPb-6727*222522	222522	--	--	bPb-6727	6H	134.08	43.50	***	< 0.001	29.82	30.57	18.91	34.57
HD	12	bPb-2976* bPb-3598	bPb-2976	1H	54.01	bPb-3598	2H	7.59	32.75	***	< 0.001	75.62	74.45	75.52	82.11

--, missing chromosome numbers and positions. ***, significant at < 0.001. P_{FDR} probability of FDR values; FDR refers to the False Descovery Rate (Benjamini and Yekutieli 2005). M1and M2 refers to marker 1 and marker 2, respectively. M_0 and M_1 refer to the effect of the first allele (absence) and the second allele (presence) of the marker, respectively.

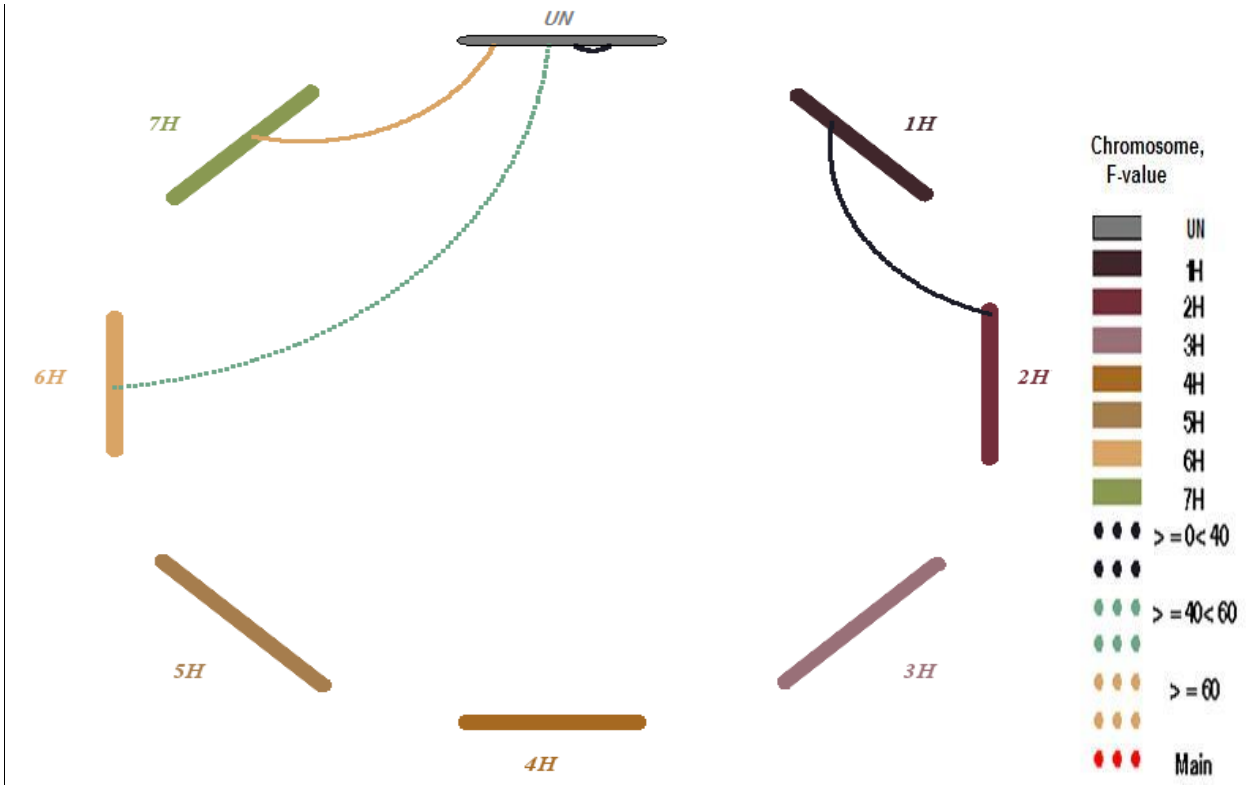


Fig. (22) Epistatic interaction effects between markers on different positions for leaves disease scoring trait in 2010 and 2011.

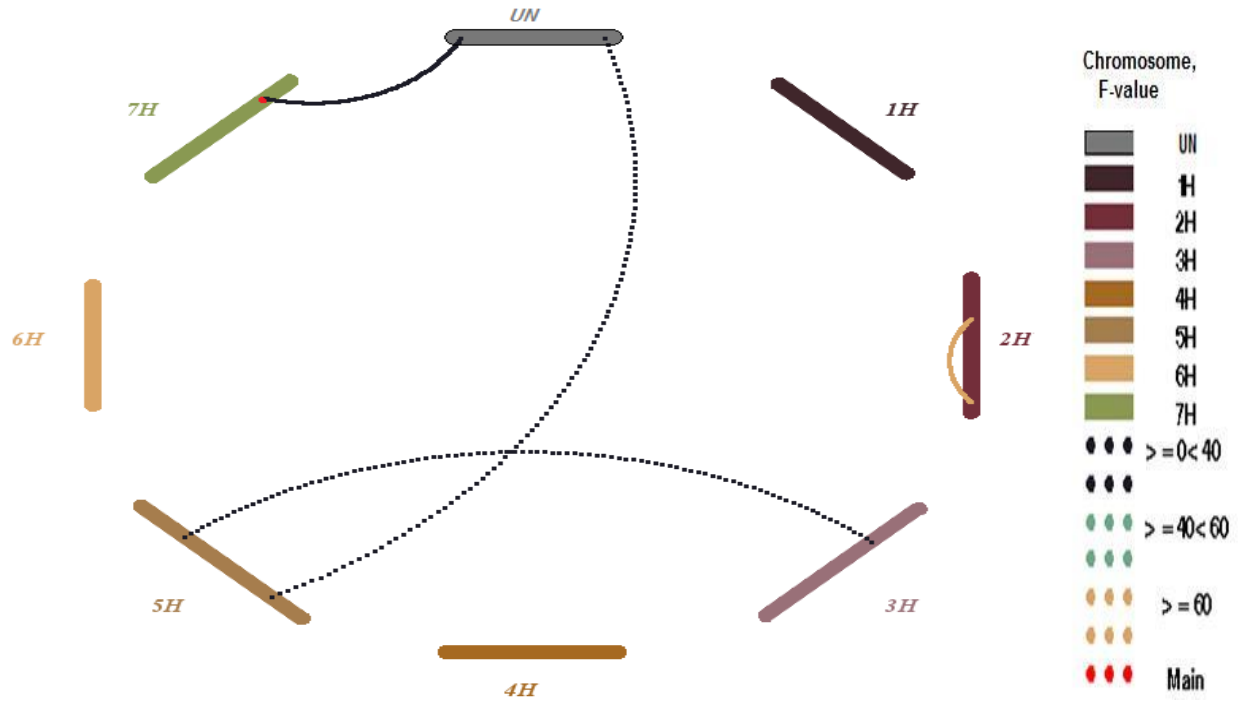


Fig. (23) Epistatic interaction effects between markers on different positions for spikes disease scoring trait in 2010 and 2011.

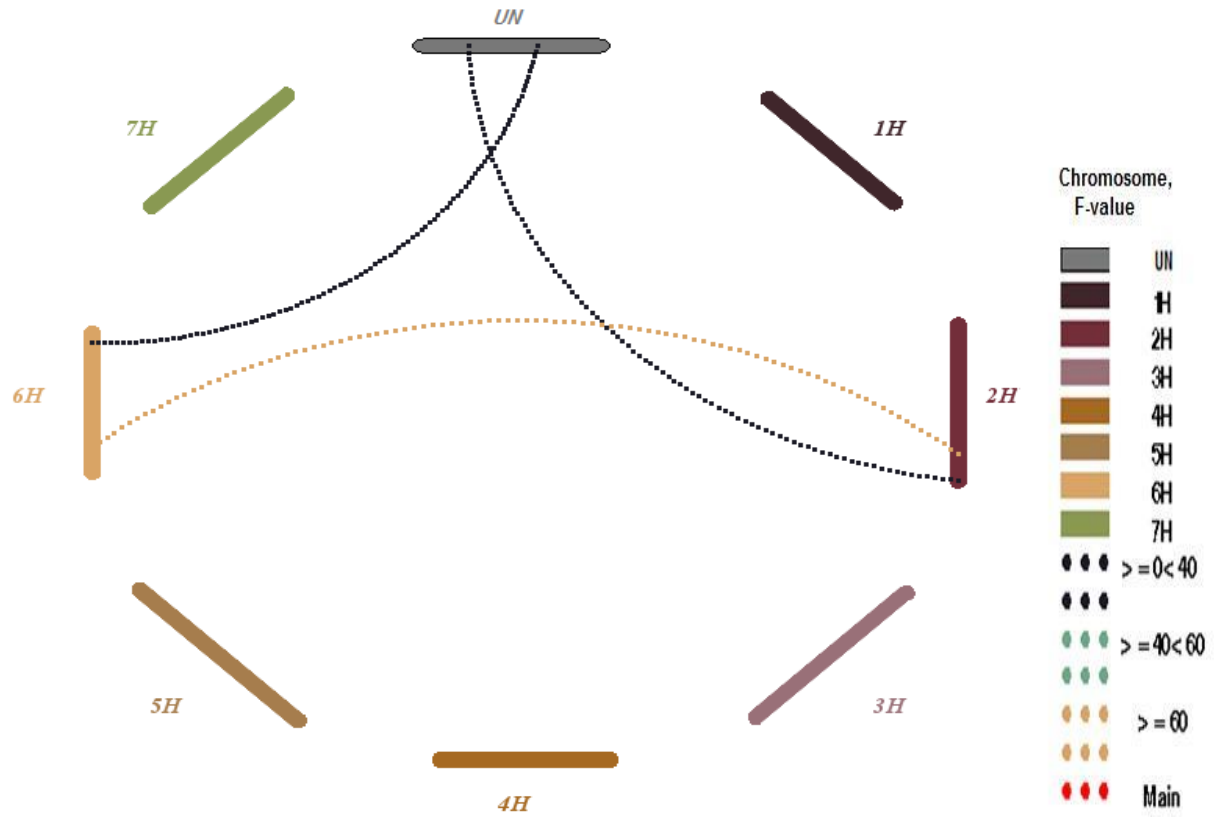


Fig. (24) Epistatic interaction effects between markers on different positions for leaves disease scoring isolate trait in 2011.

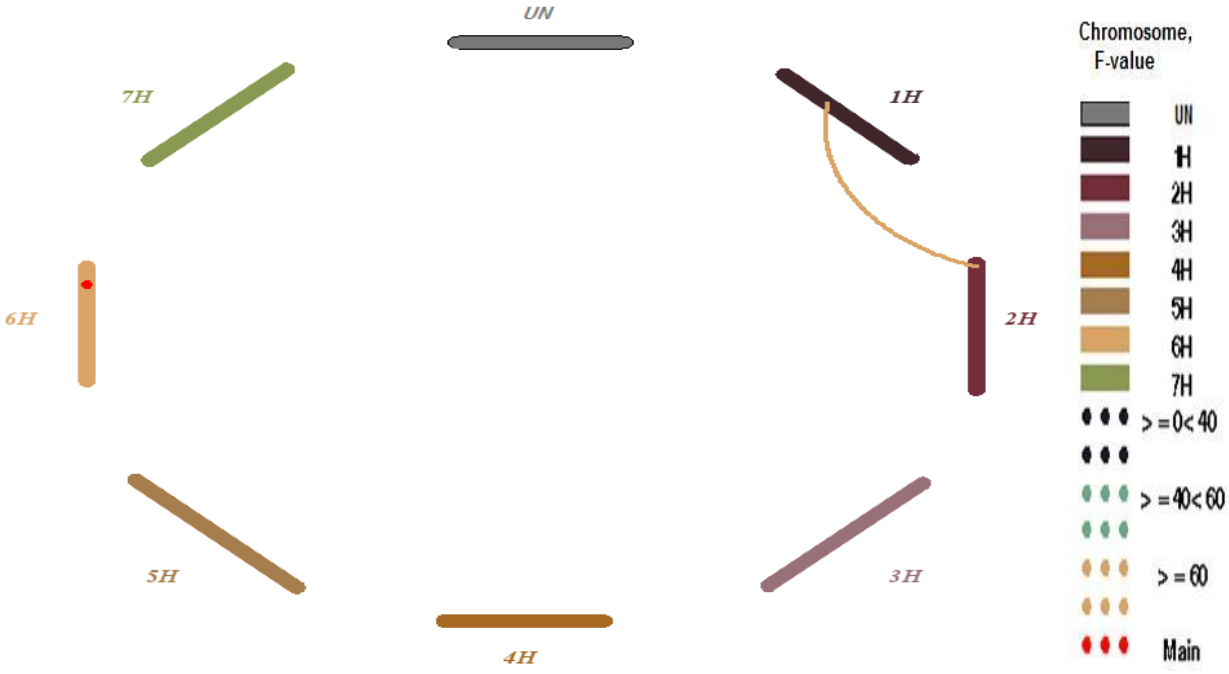


Fig. (25) Epistatic interaction effects between markers on different positions for heading date trait 2011.

Table (12) Relationship between promising accessions with lower infection percentage over two years and favorable QTLs detected for LDS.

Favorable QTL			Accessions													
No.	Marker name	Chr.	Candice	Camelot	Danubia	Alpine Pfauengerste	Cheri	ICB181436	ICB180217	ICB180231	Otis	Ingrid	Voldagsen	Heidesandgerste	ICB181168	ICB181454
Means of infection %			13.74	13.79	16.88	17.52	18.04	18.18	18.37	18.5	18.62	18.98	19.16	19.59	19.65	19.97
1	bPb-2813	1H	X	X	1	1	X	0	0	0	0	1	0	X	1	X
2	bPb-9957	1H	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	bPb-5339	1H	0	0	0	0	0	0	1	1	0	0	0	0	1	0
4	bPb-6466*	2H	0	1	0	0	0	1	0	0	0	0	0	1	1	0
5	bPb-6881*	2H	1	1	0	1	0	0	0	0	X	0	0	1	0	0
6	bPb-7989*	3H	1	1	0	1	1	0	0	0	1	1	0	1	0	0
7	bPb-3843	3H	1	1	0	1	1	0	0	1	1	0	1	1	0	0
8	bPb-6576	4H	1	1	1	1	1	0	1	1	1	1	1	1	1	1
9	bPb-0503*	5H	0	1	1	1	0	1	0	0	1	1	1	X	0	0
10	bPb-0709*	5H	0	0	0	0	0	1	0	0	0	0	X	X	0	0
11	bPb-0710*	5H	1	0	0	0	0	1	1	1	1	1	0	1	1	1
12	bPb-8553*	5H	0	0	0	0	0	0	0	1	0	0	0	1	0	X
13	bPb-7277	5H	0	0	0	1	1	1	0	0	1	0	0	0	0	0
14	bPb-2058*	6H	0	0	0	0	0	X	0	1	0	0	0	0	0	1
15	bPb-3746*	6H	0	0	1	0	1	0	0	0	1	0	0	1	1	X
16	bPb-1695*	6H	0	0	X	1	1	0	1	1	0	1	0	X	0	0
17	bPb-0182	7H	1	1	1	1	0	0	0	0	1	1	X	1	0	0
18	bPb-5727*	-	1	1	X	1	1	1	1	X	1	1	1	0	0	0
19	221193*	-	1	1	X	1	1	1	1	1	1	1	X	1	1	1
20	222651	-	1	X	1	1	1	X	1	1	1	1	1	1	1	1
21	222475	-	1	1	1	1	X	1	1	0	1	1	1	X	X	1
22	223262	-	X	X	1	1	X	0	0	1	1	1	1	X	0	1
23	223028	-	1	1	1	X	0	0	1	1	1	X	1	0	0	0
24	221908	-	0	0	X	0	0	1	1	X	0	0	X	1	1	1
25	221193	-	1	1	X	1	1	1	1	1	1	1	X	1	1	1
Total of favorable QTLs			13	13	10	16	11	11	12	13	16	14	9	14	11	10
Total of the most relevant QTLs			5	6	2	6	5	6	4	5	6	6	2	7	4	3

Each marker was scored for each sample as 0, 1, and X, whereas 0 for absent, 1 for present, and x stands for missing data. The favorable QTLs were detected for LDS= Leaves disease scoring, LDSI= leaves disease scoring isolate, MILDS= Marker*isolate interaction within leaves disease scoring. *, means the most relevant QTL with studied trait.

Table (13) Relationship between promising accessions with lower infection percentage over two years and favorable QTLs detected for SDS.

Favorable QTL			Accessions						
No.	Marker name	Chr.	Danubia	ICB180051	Oberpfälzer	Voldagsen	ICB181186	ICB180303	ICB181500
Means of infection %			7.00	8.50	8.75	8.75	8.75	9.00	9.75
1	bPb-9881*	1H	0	1	1	1	1	1	1
2	bPb-3056	2H	0	0	0	0	0	0	0
3	bPb-6881	2H	0	0	0	0	0	0	0
4	bPb-6568	5H	X	1	1	1	0	0	0
5	bPb-6124	5H	1	0	1	1	1	0	1
6	bPb-0730*	6H	1	1	1	1	0	0	1
7	bPb-9051*	6H	1	1	0	1	0	1	1
8	bPb-4379*	6H	1	1	1	X	0	X	1
9	bPb-9285*	6H	1	0	0	0	0	0	0
10	bPb-2058	6H	0	X	0	0	1	1	1
11	bPb-0522*	6H	X	0	0	0	0	0	0
12	bPb-6661*	6H	X	1	1	1	1	0	0
13	bPb-1336*	7H	1	0	1	1	0	0	1
14	bPb-3091*	-	1	0	1	1	0	1	0
15	bPb-5057	-	1	1	1	1	1	1	X
16	bPb-5669*	-	1	1	1	1	1	1	1
17	bPb-5727	-	X	0	1	1	1	1	0
18	bPb-5935	-	1	1	0	0	0	0	1
19	bPb-1084*	-	1	1	1	1	1	1	1
20	221193	-	X	0	X	X	1	1	1
21	221054*	-	X	0	0	1	0	0	0
22	224171*	-	0	1	1	0	0	1	0
Total of favorable QTLs			11	10	13	13	9	10	11
Total of the most relevant QTLs			8	8	9	9	4	6	7

Each marker was scored for each sample as 0, 1, and x, whereas 0 for absent, 1 for Present, and x stands for missing data. The favorable QTLs were detected for SDS= Spikes disease scoring, SDSI= Spikes disease scoring isolate, MISDS= Marker*isolate interaction within spikes disease scoring. *, means the most relevant QTL with studied trait.

3.10 Real time PCR (qPCR)

For validation of spikes disease scoring (SDS), real time polymerase chain reaction (qPCR) was conducted for 9 random samples (DNA extracted from infected seeds) from the population with different SDS. Results from qPCR compared with SDS for 9 samples as shown in table (14). **Fig. 25** shows the amplification plot for qPCR reaction (efficiency was 90.42%). This reaction conducted for to see if the spikes score scale 0-9 which done for the studied population or not. Pearson correlation coefficients (r) conducted between DNA fungus concentration and spikes disease scoring, r value was 0.893 and also highly significant at 1 % level. These results supported the validity of this score.

Table: (14) qPCR test results for 9 random Accessions from structured barley population efficiency is 90.42%

Accession	concentration of fungus DNA on sample pg/mg dry weight	SDS
ICB180013	1588,7088	5
ICB180063	1583,193	5
ICB180215	20,4816	0
ICB180231	1767,5067	6
ICB180303	180,735	1
ICB180802	906,5933	3
ICB180857	230,95	1
ICB180994	929,067	6
ICB181331	1429,963	4

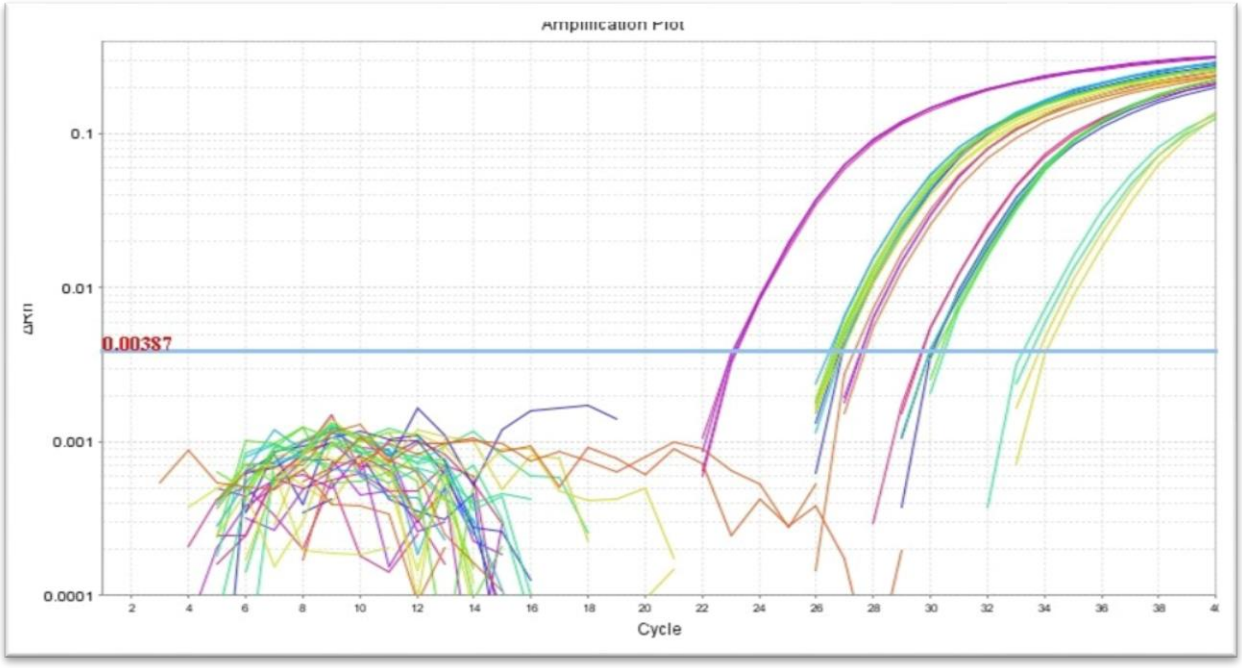


Fig. (26) The amplification plot for qPCR reaction (efficiency was 90.42%).

4. Discussion

The major challenge for Association Mapping is to ensure any marker trait associations are genetically significant and not the result of spurious associations due to population structure and/or relatedness (Mamidi et al. 2011). The results present a striking demonstration of the potential effect of population structure in causing an elevated false positive rate in AM. To overcome this problem, linear models with fixed effects for subpopulations (Breseghello and Sorrells 2006) or a logistic regression-ratio test (Prichard et al. 2000, Thornsberry et al. 2001) can be employed. Association mapping identifies (QTLs) by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008).

Fusarium head blight disease cause significant yield losses and quality reductions (Goswami and Kistler 2004, Liddell 2003). Breeding for FHB resistance has been difficult, because resistance to FHB is conditioned by many genes distributed throughout the genome (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Kolb et al. 2001; Mesfin et al. 2003). In addition, quantitative trait loci (QTLs) associated with resistance are often inconsistently detected among environments and are usually associated with agronomic and morphological traits such as late heading, plant height, lax spike and two-rowed spike (Steffenson 2002).

This study has been carried out in green-house during three seasons 2009, 2010 and 2011, in field-potted in 2011 at Bonn University, Germany. In this study used a structured barley population consists of 124 accessions came from different origins. Association mapping analysis was applied using 895 DArT markers to identify favorable QTLs that related to FHB disease tolerance. Association analysis done by using QK mixed-model approach, which proposed by Yu et al. (2006) that promises to correct for linkage disequilibrium (LD) caused by population structure and relatedness relationship. The validity of this approach has to be evaluated in breeding germplasm of autogamous species, because the population structure is presumably high and levels of relatedness relationship are diverse (Garris et al. 2005).
In the following discussion, the phenotypic variation and QTL-results have been presented.

4.1 Phenotypic traits

In the present study 140 accessions were evaluated under FHB disease stress. The studied traits were, leaves disease scoring (LDS), visual scoring (VS), spikes disease scoring (SDS) for two different isolates from *Fusarium graminarum* and Heading date (HD).

In general, over two years and two different isolates from fungus for LDS; 18 different accessions had lower than 20% infection; Candice, Camelot, Heines Hanna, Thurigina, Danubia, ICB181174, Alpine Pfauengerste, Cheri, ICB181436, ICB180217, ICB180231, Otis, Ackermanns Bavaria, Ingrid, Voldagsen, Heidesandgerste, ICB181168 and ICB181454, with infection percentages; 13.74, 13.79, 15.74, 16.56, 16.88, 16.97, 17.52, 18.04, 18.18, 18.37, 18.50, 18.62, 18.83, 18.98, 19.16, 19.59, 19.65 and 19.97 %respectively. These accessions have favorable QTLs ranged from 9 for Voldagsen to 16 for Otis, for the most relevant QTLs the number were ranged from 2-7 for Voldagsen and Heidesandgerste, respectively, which detected to LDS, LDSI, and MILDS. The relationship among barley partial disease resistance (PDR) components was inconsistent and several were poorly correlated based on the detached leaf assay Kumar et al. (2011).

Over two years and two different isolates from fungus for SDS; 48 accessions had lower than 20% infection percentage, 9 from this group were lower than 10% infection percentage; Danubia, Ackermanns Bavaria, Scarlett, ICB180051, Oberpfälzer, Voldagsen, ICB181186, ICB180303 and ICB181500 with infection percentages; 7, 8.34, 8.5, 8.5, 8.75, 8.75, 8.75, 9 and 9.75% respectively. These accessions were having promising QTLs ranged from 9 for ICB181186 to 13 for Oberpfälzer, the most relevant QTLs for the same group ranged from 4 to 9 for the ICB181186 and Oberpfälzer, which detected to LSDS, SDSI, and MISDS. Usele et al. (2011) also found significant differences between 126 spring barley varieties and lines for resistance to FHB, also reported that 15 genotypes with FHB severity lower than 10%.

Over two year's experimental work, 2 accessions had good results against two isolates from *Fusarium graminarium* fungus and these results were low also over two different ways of disease scoring. These two were Danubia and Ackermanns Bavaria with LDS 16.88, 18.83% and with SDS 7, 8.34% respectively. Danubia cultivar was having 18 promising QTLs (10 LDS QTLs+ 8 SDS QTLs) and 10 relevant QTLs (2 LDS QTLs+ 8 SDS QTLs). These results showed significant different between the ability of two isolates of *Fusarium graminarium* for the disease incidence within the studied population. Goswami and Kistler (2005) also reported significant variation among the strains of *F. graminearum* species in their ability to cause FHB on wheat. The precise assessment of cultivar resistance to FHB would undoubtedly require the involvement of different *Fusarium* strains applied under different environmental conditions (Šíp et al. 2011).

4.2 Correlations between studied traits

For more Information on association of leaves disease scoring and spikes disease scoring, both with heading date trait could be useful in selection of FHB tolerant/resistant genotypes. Correlation analysis was done for all scores over two years under green-house in a structured barley population under disease infection stress. In this study, positive correlations found between VS 2010 and LDS 2010 and 2011, leaves and spikes disease scoring were correlated positively in year 2011 for both isolates. This result supports the idea of using two different types of scoring for assessment fusarium head blight disease in barley.

The correlations between most PDR components in the detached leaf assay and FHB field severity ratings assessed were non-significant in Kumar et al. (2011) study. Brown and Cooke (2005) similarly reported that some detached leaf assay-derived PDR components were not effective in identifying wheat lines with high levels of whole-plant FHB resistance.

Negative significant correlations were found between HD and SDS over two years and isolates, this means lower SDS correlated with late heading (Steffenson et al. 1996).

4.3 Population structure and linkage disequilibrium

Population structure analysis which done for the studied population, by using principal component analysis PCA. Fig. 12 showed a structure shape for the population by dividing the population onto 3 clusters, first one included the accessions came from Uzbekistan, Palestine, Libya, Iraq, Syria, Tajikistan and Iran. Second cluster consists of accessions came from Russia, Afghanistan, Pakistan, Jordan, Palestine, Syria, Iraq, Turkey and Turkmenistan. However, third cluster consists of the varieties which came from Germany and one wild type from Syria. In this classification the accessions which they came from same geographical region came together in same cluster except few accessions from Syria, Libya and Iraq.

Fig. 13 represents the pattern of LD in a structured barley population in the seven chromosomes and the whole genome, r^2 values ranged from 0.0 to 0.3. However, the chromosomes recorded higher r^2 values of 0.6, 0.8, 0.9 and 0.1. Thus, the higher r^2 values observed in most of loci came from the German cultivars. The r^2 values of 1.0 indicate that the two markers provide identical information. The result showed that loess LD curve that fitted the r^2 estimates did not reach the baseline. The two explainable reasons for this phenomenon could be (i) Marker density and, (ii) type of population. Chen et al. (2012) earlier reported that LD decay distance determines the marker density needed to effectively associate genotypes with traits and influences the precision of association mapping. Thus, low marker density could result in the inability to detect putative

markers that are link to phenotypes of interest. In this study, the chromosomes are well covered by DArT markers between distances of 1.42 to 3.5 cM. Haixia et al. (2012) observed similar trend with respect to the LD decay in chromosome 3B of wheat. The effects of population structure might also influence the magnitude and pattern of LD (Ostrowski et al. 2006 and Rostoks et al. 2006). The studied population constitutes mainly the wild types that shares more or less similar LD blocks across the genome. The homology observed in most of the genomic region shows that that the wild types most have evolved from similar co-ancestry, therefore there was little or no decay in the population over the years. Thus, given rise to the straight loess curve observed in this study. Morrell et al. (2005) reported that wild barley has remarkably low levels of LD for a self-pollinating plant; the extent of LD in cultivated barley is much greater. This could be an explanative reason for the low LD decay observed. The wild forms panel has high resolution for association mapping.

4.4 QTLs identification

Breeding for FHB tolerance in barley is one of the most important objectives of plant breeders focusing on this crop to minimize the yield losses resulting from disease incidence. In the past, plant breeders dealt with FHB in cereals through field observations and standard breeding practices. The evolution to molecular breeding has yielded a deeper understanding of the interacting quantitative trait loci (QTLs) of the FHB tolerance/resistance and related traits such as heading date, plant height etc., exposed underlying genetic variation useful in marker-assisted breeding (de la Pena et al., 1999; Zhu et al., 1999; Ma et al., 2000; Mesfin et al., 2003; Dahleen et al., 2011). Mapping quantitative trait loci (QTL) in a structured barley population had accessions from different origins, allows the detection of chromosome segments controlling trait of interest and related traits (Massman et al. 2011).

1) Leaves Disease Scoring (LDS)

Seven QTLs were identified to be associated with LDS, 3 located in 2H, 2 in 7H, one in 5H and one in 6H. Five QTLs were in 7.59, 70.81 cM on chromosome 2H, 115.64 cM on 5H, 38.04 on 6H and 123.08 on 7H, they were the responsible of improving this trait by reducing the infection percentage with 7.29, 7.44, 7.46, 7.66 and 5.98 respectively. The other two were increasing infection percentage with 10.74 and 9.26. In the same region of 70.81 cM on 2H (Massman et al. 2012) identified a FHB QTL.

There are no studies worked befor for detection QTLs for LDS only for assessment the partial disease resistance (PDR).

2) Leaves Disease scoring Isolate (LDSI)

Ten QTLs were detected to LDSI in the whole genome except two chromosomes 2H and 7H. Only one from this group in 90.22 cM on chromosome 4H was increasing the infection percentage with 11.14. The nines were 2 in 59.67, 63.32 cM on chromosome 1H, 50.43 cM on 3H, 56.76, 76.76, 120.44, 139.48 cM on 5H and 60.92, 143.80 cM on 6H. They had highly significant effect on this trait with 5.95, 5.92, 8.18, 7.16, 7.39, 7.07, 6.70, 7.47 and 7.00 respectively. (Massman et al., 2012) identified a FHB QTL region on Bin 5-7 on chromosome 6H, in same region the present study detected a QTL (bPb-3746) which also associated to LDSI trait.

3) Visual disease Scoring (VS)

Only two QTLs were identified to be associated with VS in 66.55, 148.34 cM on chromosome 3H, both had highly significant effect for decreasing the infection percentage with 12.91 and 9.61 respectively. Another marker (bPb-3843) so near to second marker, found in 3H on 148 cM associated to the interaction between marker and isolates within LDS. The reasons for lower numbers of detected QTLs can back to, using highly significant level 0.001 and also, this trait measured for one time at 2010.

4) Spikes Disease Scoring (SDS)

Six QTLs were detected to SDS, 4 from this group were decreasing the infection percentage with 7.06, 5.60, 9.09 and 6.93 in 141.81, 70.80, 70.57, 122.08 cM on chromosomes 7H, 2H and 6H respectively. On the other hand two QTLs were increasing the infection percentage with 7.91 and 9.66 in 190.97, 133.40 cM on chromosomes 5H and 7H respectively.

The marker bPb-3056 was on the same position with marker bPb-6881 in 70.81 on 2H, which was detected to LDS trait, this mean there are a genetic association between this two traits on this study. In the same position Dahleen et al. (2012) detected a QTL associated to FHB resistance and HD in the same time. Hori et al. (2006), Sato et al. (2008) and Massman et al. (2012) detected on chromosome 6H a QTL region Bin 6-8 associated to FHB resistance, marker bPb-9051was in same area for this region. Massman et al. (2012) also reported, important resistance QTL is either linked to the vrs1 (row type) (chromosome 2H bin 10) gene or is a pleiotropic effect of this locus. However, Dahleen et al. (2012) reported that the identity of specific genes associated with FHB resistance is generally unknown.

5) Spikes Disease scoring Isolate (SDSI)

Six QTLs were identified to be associated to this trait, 3 located in chromosome 6H, they were in 134.62, 68.20, 142.51 cM and one on 1H in 7.21, these 4 were responsible of improving this trait by reducing the infection percentage with 7.73, 7.14, 6.43 and 8.97 respectively. The other two were in 133.4 and 21.54 cM on 7H and 5H; they were increasing infection percentage with 6.24 and 4.43. Previous and recent studies for Hori et al. (2006), Sato et al. (2008) and Massman et al. (2012) detected on chromosome 6H a QTL region Bin 6-8 associated to FHB resistance, marker bPb-0730 located in the same region.

6) Heading date (HD)

Only one QTL in 122.08 cM on chromosome 6H was detected to HD trait and this marker working by decreasing the trait with 4.84. Lowest QTL numbers for this trait can be due to using highly significant level 0.001 and the trait recorded for one year 2011. The positive result from this QTL was in the same chromosomal position there are another QTL bPb-4379, which identified to be associated with SDS. This means in this position have a pleiotropic effect on HD and SDS. Dahleen et al., 2003 reported that the *vrs1* gene that determines the two-rowed spike-type in the marker interval M4E3-9 to MWG581 on chromosome 2H was linked in coupling with FHB resistance and late HD alleles. Dahleen et al. 2012 confirmed the genetic association between HD and FHB resistance on chromosome 2H.

7) Marker Isolates interaction within Leaves Disease Scoring (MILDS)

Among 7 QTLs found interacted with the markers within LDS, five of them were detected befor for LDS and LDSI. Only two markers in 1H (bPb-5339) on 76.8 cM and 3H (bPb-3843) on 148 cM were only detected to MILDS. Zhu et al. (1999) detected FHB QTL in bin 13 on 3H in same region of (bPb-3843).

8) Marker Isolates interaction within Spikes Disease Scoring (MSLDS)

Three markers on 6H detected for MISDS, two of them were having a main effect for SDSI only one (bPb-6661) on 28.8 cM only detected for MISDS.

4.5 Epistatic analysis reveals interactions contributing to FHB

Epistasis, an important genetic component underlying quantitative trait variation. One major difficulty in developing a powerful statistical approach for mapping QTLs with epistatic effects is the treatment of many parameters for multiple QTLs involved in the statistical model. In the

present study, a mixed model was tested for the epistatic interaction between all DArT markers and studied traits (Sayed et al. 2012).

Interactions were detected between 11 pairs of QTLs for FHB resistance/tolerance and one interaction for heading date, which involved 24 QTLs on 6 chromosomes (Table 11; Fig. 21, 22, 23 and 24). These results indicated that barley FHB resistance/tolerance is a complicated trait and may be controlled by a complicated gene network.

1) Epistatic effects for leaves disease scoring (LDS)

Leaves disease scoring is one of two different disease scoring used in this study for assessment the FHB resistance/tolerance in structured barley population. This trait had 4 different epistatic effects, the means of these interaction presents in table (11), varied from 28.07 - 56.39. The favorable epistatic effect was (bPb-6408* bPb-4285) in the interaction ($M1_{M1}/M2_{M1}$) with mean 28.07. These 4 epistatic effects were having different affect on the trait ranged from ≥ 0 - < 40 to ≥ 60 %. In the epistatic interaction effect (bPb-9486* bPb-9912); the second marker bPb-9912 have been detected as a main effect for LDS trait. The best means of interactions found in $M1_{M1}/M2_{M1}$ and $M1_{M0}/M2_{M1}$ with 28.07 and 29.54, respectively. On chromosome 7H on 68.20 cM a QTL was detected as a main effect for SDSI, on 68.53 cM another QTL bPb-3722 was sharing in the epistatic interaction (103087*bPb-3722); this means there are a genetic correlation between LDS and SDS in this study.

2) *Epistatic effects for spikes disease scoring (SDS)*

Previous work for resistance to FHB mentioned that, the resistance in wheat and barley to FHB is considered to be a quantitative trait; therefore it is likely controlled by several genes (Buerstmayr et al. 1999; Bai&Shaner, 2004). In this study, 4 epistatic effects were detected for spikes disease scoring (FHB). One of these effects was in chromosome 2H have higher F-value and also strong affect on the trait more than 60%. The favorable means of interactions found in $M1_{M0}/M2_{M1}$ and $M1_{M1}/M2_{M1}$ with 18.44 and 18.68, respectively. In the epistatic effect (bPb-7067* bPb-8823); the marker bPb-8823 on 133.4 cM on 7H have befor main effect on two traits SDS and SDSI. The marker bPb-3574 which located on 2H on 49.03 cM and sharing in the interaction effect (bPb-3574* bPb-8530), this marker also detected to LDS in this study. Also another marker bPb-1420 on 5H 138.99 cM, sharing in the epistatic effect (bPb-6347* bPb-1420), on so close chromosome region 139.48 cM, marker bPb-7277 had a main effect for LDSI. These results also support the genetic correlation between LDS and SDS even if it with only one

marker. These results were in matching with phenotypic correlations in 2011 results and support it, which showed correlation between LDS and SDS.

3) Epistatic effects for leaves disease scoring isolate (LDSI)

Three epistatic interactions were detected for LDSI trait. First interaction (bPb-5755* bPb-6069) was having lower infection percentage mean (16.67) when the interaction the allele 1 is presences in both markers. The third interaction (bPb-6727*222522) also have lower interaction means with 18.91 for the interaction $M1_{M0}/M2_{M1}$. So near to marker bPb-6069 on chromosome 6H on 26.51 cM another marker (bPb-6661 on 28.8 cM), was detected as a main effect for MISDS trait. Also, very close to marker bPb-6727 (6H on 134.08 cM) another marker (bPb-9285 on 134.6 cM) was associated to SDSI and MISDS traits as a main effect. This makes a genetic interaction relationship between the QTLs which have main effects and other markers for improving traits under study.

From previous works, Ma et al. (2006) studied the epistatic effect for FHB resistance in wheat, the analysis resolved 9 pairs of AA interactions involving 17 different loci that explained 26% of phenotypic variation, whereas only 7 QTLs, identified as main effect QTLs, explained ≈24.8% phenotypic variation. This indicates that genetic effect of AA epistasis is equally important as that of QTL main effect.

4) Epistatic effects for Heading date 2011 (HD)

Only one epistatic interaction effect was detected (bPb-2976* bPb-3598), the interaction means $M1_{M1}/M2_{M1}$ was 82.11. In the marker bPb-3598 position on chromosome 2H on 7.59 cM another marker (bPb-6466) with main effect for 2 traits LDS and MILDS, this result indicated to genetic correlation between LDS and HD, previously in this study found a genetic correlation between SDS and HD.

von Korff et al. (2010) identified strongest effect for HD between the markers GBM1035[2HS] on 27 cM and Ebmac415[2HL] on 146 cM and between HVM13[4HS] located on 55 cM and Vrn-H1[5HL] in the region of 125 cM, the majority of significant interactions for HD were detected for the marker GBM1035 on chromosome 2H adjacent to the major photoperiod response gene Ppd-H1 on 41 cM.

5. Summary

Fusarium Head Blight (FHB) is a historically devastating disease of wheat, barley, maize and other cereal crops across the world. FHB is a preharvest disease; it reduces kernel weight, cause significant yield losses and quality reductions. Breeding resistant cultivars could be an effective strategy to manage FHB in barley; several genetic mapping studies have shown that resistance to FHB are conditioned by many genes distributed throughout the genome. Association mapping of a trait is to identify chromosomal regions that contain genes affecting the trait. The discovery of dense polymorphic markers covering the entire genome provides us an opportunity to localize these regions by trying to find the markers closest to the genes of interest.

The objectives of this study were:

1. The main goal of this research was to apply association mapping approaches to identify DArT markers associated with fusarium head blight disease tolerance in a structured barley population.
2. Investigation of new sources for barley genotypes those have tolerant against *Fusaruim.*
3. Evaluation of leaves disease scoring efficiency as a disease assessment method.
4. Identification of promising tolerance QTLs in barley against fusarium head blight.

In the present study, 108 accessions of wild barley (*H. vulgare ssp. spontaneum*) and 7 landraces (*H. vulgare ssp. vulgare*) from the ICBB core collection (gene banks in Gatersleben and Braunschweig). 21 spring barley cultivars representative for the breeding pool of spring barley (*H. vulgare ssp. vulgare*) in the North Rhine Westphalia (NRW), Germany, (Reetz and Léon 2004) and 4 common cultivars (Scarlett, Lerche, Barke and Thuringia). The seeds of these cultivars were provided by the Institute of Crop Science and Resource Conservation (INRES), chair of plant breeding.

The experiments were carried out in glass green-house during 2009, 2010 and 2011 and also in field-potted in 2011 at INRES, chair of Plant Pathology, Faculty of Agriculture, Rheinische Friedrich-Wilhelms-University of Bonn. The experiment in 2009 was conducted in autumn season using 30 barley genotypes consists of 21 spring barley cultivars, 7 Landraces and 2 common German cultivar Scarlett and Barke. The experiment was arranged in a completely randomized design using 5 replications. In 2010 and 2011 the experiments were conducted in winter/spring season using 140 barley accessions. The experiment was arranged in a completely randomized design using 5 replications. Field-potted experiment was carried out using 32 Barley genotypes consisting of 21 spring barley cultivars, 7 Landraces and 4 common German cultivars

(Scarlett, Lerche, Barke and Thuringia), during spring/summer season 2011, the experiment was arranged in a randomized block design with 5 replications.

Two different isolates from *Fusarium graminearum* were used in green-house and field-potted experiments; first isolate (FG 5.1) was used in 2009, 2010 and 2011 and second isolate (FG 5.3) was used only in 2011 experiments. First isolate was FG 5.1 and second isolate was FG 5.3. Both isolates were from the Institute of Crop Science and Resource Conservation, Chair of Plant Pathology, University of Bonn, Germany. For assessment FHB disease infection in the studied population, two different evaluations were conducted. First was leaves evaluation; when plants reached the fifth leaf, an experiment was carried out for leaves evaluation by cutting third leaf from each plant and dividing it into 5 pieces (2 cm). These pieces were washed in Ethyl alcohol 75% for 3 seconds, then in distilled water three times, and cultured it in petri dichs included kinetin agar media (6g Agar-Agar/l + 10mg Kinetin/l). Then, each leaf piece was inoculated with 10^4 conidia mL^{-1}/piece of *Fusarium graminearum*. Finally, the petri dichs were incubated in growth chamber at 12/12 light/darkness and temperature 24°C. Second one was spikes evaluation; at anthesis, 5 central florets from the main spike of each plant with were inoculated with ca. 10^3 conidia/floret, then covered with paper bag for each spike (for 24 h). At the plant maturity time, spikes were harvested by hand and the spike was placed into the paper envelopes in order to avoid the loss of grains. In field-potted the same evaluation have been done for leaves; spikes evaluation were done by inoculated each pot at anthesis with 100 mL spore suspension (10^5 conidia mL^{-1}). The plants were covered with big plastic bag for each pot (for 24 h), to ensure high relative humidity for optimum infection conditions.

Phenotypic data measurements were; leaves disease scoring (LDS) seven days after inoculation, the disease symptoms were scored using scale from 0 to 9; (0 = immunity to 9 = very susceptible), for each part from five parts of third cut leaf. In 2009, visual scoring (VS) was carried out without monitoring leaves, while in 2010 and 2011 all inoculated leaf parts were monitored using electronic microscope, for assessment disease infection, image analysis software for plant disease quantification (APS Assess) was used for evaluation of the images (this program calculates the whole area of the leaf and the infected area gives % infection percentage). Spikes disease scoring (SDS), the symptoms were visible to assess, disease severity was assessed as the percentages of bleached spikes, using a nine-class rating scale from 0 to 9 (0 = no bleached spikes and 9 = completely bleached). Heading date (HD) was scored in 2011 only, and was calculated in green-house as days from planting (10^{th} February) to anthesis. In field-potted calculated from date of sowing (23^{rd} May) to 50% of spikes were fully emerged from the boot in each pot.

In parallel, DNA has been extracted from 10 mg freeze drying of each accession by using "Kit" procedure according DNeasy Plant Handbook 07/2006. The produced DNA of the accessions was sent to Australia and genotyped by using 1081 DArT markers (YarralumlaACT, Australia). The phenotypic data were analyzed each season separately as one way ANOVA using Proc GLM procedure and the Pearson correlation coefficients (r) between traits under disease infection condition were calculated by SAS version 9.2 (SAS institute 2008). PCA was carried out by using SAS 9.2 program PROC PRINCOMP, for study of the population structure. The significance for PCA was evaluated using Franklin et al. (1995) method. The relative kinship coefficients (K matrix) among all pairs of accessions were calculated using 895 DArT markers data by "SPAGeDi-1.3d" Software to calculate the pair-wise kinship coefficients for all accessions. The association analysis was performed in mixed linear model (MLM) including PCA values and K matrix. All studied traits LDS, VS, SDS and HD were exhibited highly significantly differences in three years. Results from combined means in two years and two isolates showed that; 18 accessions had lower than 20% infection percentage for LDS and 48 accessions had lower than 20% infection percentage, 9 from this group were lower than 10% infection percentage for SDS. Positive and significant phenotypic correlation has been recorded between LDS and SDS and negative correlation also have been recorded between SDS and HD in 2011. According to PCA results, the population was structured with three major clusters. The first five PCs were significant, the first three used in the association mapping analysis as Q matrix. Seventy three markers were correlated significantly with all studied traits and covered the whole genome of the studied population. Different QTLs have been identified for LDS, LDSI, SDS, SDSI, VS, HD, MILDS and MISDS. Some of these QTLs were identified in two traits; marker bPb-0522 detected to SDSI and MISDS. Few of this QTLs were identified for two traits in the same/nearly position on the chromosome; in the position 122.08 cM on chromosome 6H two markers (bPb-4379 and bPB-3375) were identified to be associated with SDS and HD. Also in chromosome 2H on 70.8 cM two markers detected to LDS and SDS. In this study a mixed model was tested for the epistatic interaction between all DArT markers and studied traits. Interactions were detected between 11 pairs of QTLs for FHB resistance/tolerance and one interaction for heading date, which involved 24 QTLs on 6 chromosomes. The results indicated that barley FHB resistance/tolerance is a complicated trait and may be controlled by a complicated gene network.

6. References

Abdurakhmonov IY and Abdukarimov A (2008) Application of Association mapping to understanding the genetic diversity of plant germplasm resources. International journal of plant genomics Article ID 574927: 1-18

Arthur JC (1891) Wheat scab. Indiana Agricultural Experimental Station Bulletin 36, 129-32

Asfaw Z, von Bothmer R (1990) Hybridization between landrace varieties of Ethiopian barley (*Hordeum vulgare* subsp. *vulgare*) and the progenitor of barley (*H. vulgare subsp. spontaneum*). Hereditas 112: 57-64

Atwell S et al. (2010) Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465, 627–631

Bai G and Shaner G (1994) Scab of wheat: Prospects for control. Plant Disease 78: 760-766

Bai G and Shaner G (2004) Management and resistance in wheat and barley to fusarium head blight. Annu. Rev. Phytopathol. 2004. 42:135–61

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

Bai G, Plattner R, Desjardins A, and Kolb F (2001) Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breeding 120:1-6

Balasubramanian S et al. (2009) QTL mapping in new Arabidopsis thaliana advanced intercross-recombinant inbred lines. PLoS ONE 4, e4318

Benjamini Y, Yekutieli D (2005) Quantitative trait Loci analysis using the false discovery rate. Genetics 171: 783-790

Benson J, Guedira GB, Murphy JP, and Sneller C (2012) Population Structure, Linkage Disequilibrium, and Genetic Diversity in Soft Winter Wheat Enriched for Fusarium Head Blight Resistance. The Plant Genome 5:71–80

Bergelson J and Roux F (2010) Towards identifying genes underlying ecologically relevant traits in Arabidopsis thaliana. Nat. Rev. Genet. 11, 867–879

Betchel DB, Kaleikan LA, Gaines RL, Seitz LM (1985) The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chemistry 62: 191-197

Bhatty RS (1999) The potential of hull-less barley. Cereal Chemistry, 76 (5) (1999), pp. 589–599

Bilgi B and Çelik S (2004) Solubility and emulsifying properties of barley protein concentrate. European Food Research and Technology, 218 pp. 437–441

Börner A, Buck-Sorlin GH, Hayes PM, Malyshev S, Korzun V (2002) Molecular mapping of major genes and quantitative trait loci determining flowering time in response to photoperiod in barley. Plant Breeding 121,129-132

Bothmer von R (1992) the wild species of *Hordeum*. In Shewry PR (ed) Barley genetics, biochemistry, molecular biology and biotechnology. Wallingford, CAB International, pp 2-17

Boutigny AL, Ward TJ, Van Coller GJ , Flett B, Lamprecht SC, O'Donnell K and Viljoen A (2011) Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genetics and Biology 48 914–920

Brągoszewska HB, Heller-Uszyńska K, Wenzl P, Uszyński, Andrzej K. G and Rakoczy T. M. 2009. DArT markers for the rye genome - genetic diversity and mapping. BMC Genomics 2009, 10:578 doi:10.1186/1471-2164-10-578

Breseghello F, Sorrells ME (2006) Association mapping of kernel Size and Milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165 – 1177

Browne RA and Cooke BM (2005) Resistance of wheat to Fusarium spp. in an in vitro seed germination assay and preliminary investigations into the relationship with fusarium head blight resistance. Euphytica, 141, 23–32

Browne RA, Murphy JP, Cooke BM, Devaney D, Walsh EJ, Griffey CA, Hancock JA, Harrison SA, Hart P, Kolb FL, Mckendry AL, Milus EA, Sneller C and Van Sanford DA (2005) Evaluation of components of fusarium head blight resistance in soft red winter wheat germplasm using a detached leaf assay. Plant Dis., 89, 404–411

Buerstmayr H, Lemmens M, Berlakovich S, and Ruckenbauer P (1999) Combiningability of resistance to head blight caused by *Fusarium culmorum* in the F1 of a seven parent diallel of winter wheat. Euphytica 110:199-206

Caldwell KS, Russell J, Langridge P, and Powell W (2006) "Extreme population dependent linkage disequilibrium detected in an inbreeding plant species, *Hordeum vulgare*," Genetics, vol. 172, No. 1: 557-567

Canci PC, Nduulu LM, Muehlbauer GJ, Dill-Macky R, Rasmusson DC and Smith KP (2004) Validation of quantitative trait loci for Fusarium head blight and kernel discoloration in barley. Mol Bre 14: 91–104, 2004

Chen F, Prehn D, Hayes PM, Mulrooney D, Corey A, Vivar H (1994) Mapping genes for resistance to barley stripe rust (*Puccinia striiformis f. sp. hordei*). Theor Appl Genet 88:215–219

Chen FQ, Hayes PM (1989) A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using invitro floret and tiller culture. Theor Appl Genet 77: 701–704

Chen X, Min D, Yasir TA, Hu Y-G (2012) Genetic Diversity, population structure and linkage disequilibrium in elite Chinese winter wheat investigated with SSR markers. PLoS ONE 7(9): e44510. doi:10.1371/journal.pone.0044510

Cowger C and Arellano C (2012) *Fusarium graminearum* infection and deoxynivalenol concentrations during development of wheat spikes. Phytopathology "First Look" paper. http://dx.doi.org/10.1094/PHYTO-03-12-0054-R • posted 12/19/2012

Dahleen LS, Morgan W , Mittal S, Bregitzwer P, Brown RH and Hill NS (2012) Quantitative trait loci (QTL) for Fusarium ELISA compared to QTL for Fusarium head blight resistance and deoxynivalenol content in barley. Plant Breeding 131, 237—243

Dahleen LS, Agrama HA, Horsley RD, Steffenson BJ, Schwarz PB, Mesfin A, and ranckowiak JD (2003) Identification of QTL associated with Fusarium head blight resistance in Zhedar 2 barley. Theor. Appl. Genet. 108:95–104

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

Diamond H and Cooke BM (1999) Towards the development of a novel in vitro strategy for early screening of Fusarium ear blight resistance in adult winter wheat plants. Eur. J. Plant Pathol., 105, 363–372

Dill-Macky R (2003) Inoculation methods and evaluation of Fusarium head blight resistance in wheat. See Ref. 62, pp. 184– 210

DNeasy® Plant Handbook July 2006. www1.qiagen.com/HB/DNeasy96Plant

Ehrenreich IM et al. (2009) Candidate gene association mapping of Arabidopsis flowering time. Genetics 183, 325–335

Ellis RP, Forster BP, Robinson D, Handley LL, Gordon DC, Russell JR, and Powell W (2000) Wild barley: a source of genes for crop improvement in the 21st century? Journal of Exp. Botany, Vol. 51, No. 342: 9 – 17

Fang WY, Chi Y and Jun-Liang Y (1997) Sources of resistance to head scab in Triticum. Euphytica, 94, 31-36

Feng Z, Lei Z and Hong-Wen D (2009) A PCA-based method for ancestral informative markers selection in structured populations. Sci China C Life Sci. October; 52(10): 972–976

Fisher RA (1918) The correlations between relatives on the supposition of Mendelian inheritance. Trans R Soc Edinb 52:399-433

Franklin SB, Gibson DJ, Robertson PA, Pohlmann JT and Fralish JS (1995) Parallel analysis: a method for determining significant principal components. Journal of Vegetation Science 6: 99-106

Garris AJ, McCouch SR, Kresovich S (2003) Population structure and itseffect on haplotype diversity and linkage disequilibrium surrounding the xa5 locus of rice (*Oryza sativa* L.) Genetics 165: 759-769

Garris AJ, Thomas HT, Coburn J, Kresovich S, McCouch S (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics, 169: 1631 – 1638

Gebhardt C, Ballvora A, Walkemeier B, Oberhagemann P, Schüler K (2004) Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. Mol Breed 13:93–102

Goswami RS and Kistler HC (2005) Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology, 95: 1397–1404

Goswami R, Kistler H (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5, 515–525

Graner A, Jahoor A, Schondelmaier J, Siedler H and Pillen K et al. (1991) Construction of an RFLP map of barley. Theor Appl Genet 83: 250-256

Griffey CA, Thomason WE, Pitman RM, Beahm BR, Paling JJ, Chen J, Gundrum PG, Fanelli JK, Kenner JC, Dunaway DW, Brooks WS, Aughn ME, Hokanson EG, Behl HD, Corbin RA, Hall MD, Liu S, Custis JT, Waldenmaier CM, Starner DE, Gulick SA, Ashburn SR, Jones Jr EH, Whitt DL, Bockelman HE, Souza EJ, Brown-Guedira GL, Kolmer JA, Long DL, Jin Y, Chen X, and Cambron SE (2009) Registration of '5205' Wheat. Journal of Plant Registrations, Vol. 3, No. 3, Sep

Haixia Yu, Deng Z, Xiang C and Tian J (2012) Analysis of diversity and linkage disequilibrium mapping of agronomic traits on B-genome of wheat. Journal of Genomics, 1

Hardy OJ and Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2: 618-620

Harlan J and Zohary D (1966) Distribution of wild wheat and barley. Science 153: 1074-80

Harlan JR (1976) Barley *Hordeum vulgare* (Gramineae--Triticinae) In Evolution in Crop Plants, ed N W Simmonds Longman, London, pp 93-8

Hayes PM, Castro A, Marquez-Cedillo L, Corey A, Henson C, Jones B et al (2000) A summary of published barley QTL reports. (http://barleyworld.org/northamericanbarley/qtlsummary.php)

Horsley RD, Schmierer D, Maier C, Kudrna D, Urrea CA, Steffenson BJ, Schwarz PB, Franckowiak JD, Green MJ, Zhang B, and Kleinhofs A (2006) Identification of QTLs associated with Fusarium head blight resistance in barley accession CIho 4196. Crop Sci. 46, 145—156

http://www.faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Hussain SS (2006) Barley genetics and genomics: a review. Proc Pakistan Acad Sci 43:63-84

Iwata H, Uga Y, Yoshioka Y (2007) Bayesian association mapping of multiple quantitative traits loci and its application to the analysis of genetic variation among *Oryza sativa* L. germplasms. Theor Appl Genet. 114: 1437-1449

Jaccoud D, Peng K, Feinstein D, Kilian A: Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research 2001, 29:e25

Kantartzi SK, and Stewart JMcD (2008) Association analysis of fiber traits in *Gossypium arboreum* accessions. Plant Breeding, 1-7

Kilian A (2004) Methods for genotyping by hybridisation analyses, United States Patent number 6,713,258 B2

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R and Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86: 705-712

Kloth KJ, Thoen MPM, Bouwmeester HJ, Jongsma MA and Dicke M (2012) Association mapping of plant resistance to insects. Trends in Plant Science May 2012, Vol. 17, No. 5

Köksel H, Edney MJ and Özkaya B (1999) Barley bulgur: effect of processing and cooking on chemical composition. Journal of Cereal Science, 29 pp. 185–190

Kolb FL, Bai GH, Muehlbauer GJ, Anderson JA, Smith KP, Fedak G (2001) Host plant resistance genes for Fusarium head blight: mapping and manipulation with molecular markers. Crop Sci 41:611–619

Komatsuda T, Li WB, Takaiwa F, Oka S (1999) High resolution map around the vrs1 locus controlling two- and six-rowed spike in barley, *Hordeum vulgare*. Genome 42: 248-253

Kopecky D, Bartos J, Lukaszewski JA, Baird HJ, Cernoch V, Kölliker R, Rognli OA, Blois, Vanessa HC, Lübberstedt T, Studer B, Shaw P, Dolezel J and Kilian A (2009)

Development and mapping of DArT markers within the *Festuca - Lolium* complex. BMC Genomics 2009, 10:473 doi:10.1186/1471-2164-10-473

Kraakman ATW (2005) Mapping of yield, yield stability, yield adaptability and other traits in barley using linkage disequilibrium mapping and linkage analysis. PhD thesis Wageningen University, The Netherlands

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, Van Eeuwijk FA (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics168: 435–446

Kumar K, Xi K, Turkingtont K, Tekauz A, Helm JH and Tewari JP (2011) Evaluation of a detached leaf assay to measure fusarium head blight resistance components in barley. Can. J. Plant Pathol. 33(3): 364–374

Lamb KE, Jose LG-H, Zhang B, Green M, Neate SM, Paul BS and Horsley RD (2009) Identification of QTL Conferring Resistance to Fusarium Head Blight Resistancein the Breeding Line C93-3230-24. Crop Sci. 49:1675–1680 (2009)

Lande R and Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743-756

Legge WG (1999) Breeding for head blight resistance in barley. In Canadian workshop on Fusarium head blight, pp. 59–66. Winnipeg, Manit

Lezar S, Myburg AA, Berger DK, Wingfield MJ, Wingfield BD (2004) Development and assessment of microarray-based DNA fingerprinting in Eucalyptus grandis. Theor. Appl. Genet. 109: 1329 – 1336

Li JZ, Huang XQ, Heinrichs F, Ganal MW, and Röder MS (2006) Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross population of spring barley. Genome, vol. 49, No. 5: 454 - 466

Liddell C (2003) Systematics of *Fusarium graminearum* species and allies associated with Fusarium head blight. In: Leonard, K., Bushnel, W. (Eds.), Fusarium Head Blight of Wheat and Barley. APS Press, Minnesota, USA, pp. 35–43

Lu WZ, Cheng SH, Wang YZ (2001) Wheat scab research in China. Scientific publication Ltd, Beijing, pp 229. ISBN 7-03-009199-X

Ma Z, Steffenson BJ, Prom LK, Lapitan NLV (2000) Mapping of quantitative trait loci for Fusarium head blight resistance in barley. Phytopathology 90:1079–1088

Ma H-X, Bai G-H, Zhang X and Lu W-Z (2006) Main effects, epistasis, and environmental interactions of quantitative trait loci for fusarium head blight resistance in a recombinant inbred population. Phytopathology 96:534-541.

Mace ES, Xia L, Jordan DR, Halloran K, Parh DK, Huttner E, Wenzl P and Kilian A (2008) DArT markers: diversity analyses and mapping in *Sorghum bicolor*. BMC Genomics, doi:10.1186/1471-2164-9-26

Malysheva-Otto VL, Martin W, Ganal MW, and Röder MS (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). BMC Genetics 7: 6 1-14

Mamidi S, Chikara S, Goos RJ, Hyten DL, Annam D, Moghaddam SM, Lee RK, Cregan PB and McClean PE (2011) Genome-wide association analysis identifies candidate genes associated with iron deficiency chlorosis in soybean. The Plant Genome 4:154–164

Massman J, Cooper B, Horsley R, Neate S, Dill-Macky R, Chao S, Dong Y, Schwarz P, Muehlbauer GJ and Smith KP (2011) Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. Mol Breeding (2011) 27:439–454

Mesfin A, Smith KP, Dill-Macky R, Evans CK, Waugh R, Gustus CD, and Maeuhlbauer GJ (2003) Quantitative trait loci for Fusarium head blight resistance in barley detected in a two-rowed by six-rowed population. Crop Sci. 43:307–318

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

Mesterhazy A, Bartok T, Mirocha CG and Komoroczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding 118:97-110

Mohamed NEM (2009) Association mapping for drought stress related traits in a structured population with wild and cultivated barley. PhD Thesis, Bonn University

Morrell PL, Toleno DM, Lundy KE, and Clegg MT (2005) "Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare ssp. spontaneum*) despite high rates of self-fertilization," Proceedings of National Academy of Science U S A, vol. 102, No. 7: 2442 - 2447

Murakami J and Ban T (2005) Development of novel bioassay system for FHB molecular interaction. In Proceedings of the 4th Canadian Workshop on fusarium head blight. 1–3 Nov. 2005, Ottawa Congress Centre, Ottawa, ON. p. 62

Nduulu LM, Mesfin A, Muehlbauer GJ, and Smith KP (2003) Fine mapping of Fusarium head blight resistance and heading date QTL in barley. p. 30. In S.M. Canty, J. Lewis, L. Siler, and R.W. Ward (ed.) Proc. 2003 National Fusarium Head Blight Forum, Bloomington, MN. 13–15 Dec. 2003. U.S. Wheat & Scab Initiative, East Lansing, MI

Nei M (1972) Genetic distance between populations. Am. Nat. 106:283– 292. doi:10.1086/282771

Nevo E (1992) Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. In: Barley, genetics, biochemtry, molecular biology and biotechology (ed. R. Shewry). C. A. B. International, Wallindford, Oxon, UK CAB International: 19-43

Osborne L and Jin Y (2002) Epidemiological studies on fusarium head blight of wheat in south Dakota for 2002 (2002) National Fusarium Head Blight Forum Proceedings

Ostrowski MF, David J, Santoni S, et al. (11 co-authors) (2006) Evidence for a large-scale population structure among accessions of Arabidopsis thaliana: possible causes and consequences for the distribution of linkage disequilibrium. Mol Ecol. 15:1507–1517.

Price AL et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38, 904–909

Pritchard JK, Stephens M, Rosenberg NA, and Donnelly P (2000) Association mapping in structured populations. Am. J. Hum. Gen. 67, 170 – 181

Pritchard JK, Wen W (2007) Documentation for structure Software, version 2.2. Department of Human Genetics, University of Chicago, Chicago

Prom LK, Steffenson BJ, Salas B, Mos J, Fetch, Jr TG, and Casper HH (1996) Evaluation of selected barley accessions for resistance to Fusarium head blight and deoxynivalenol concentration. p. 764–766. In G. Scoles and B. Rossnagel (ed.) Proc. of the Fifth Int. Oat Conf. and VII Int. Barley Symp., Saskatoon, Canada. 30 Jul.–6 Aug. 1996. University Extension Press, Saskatoon, SK, Canada

Qi X, Stam P, and Lindhout P, (1998) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet 96: 376-384

Rafalski A, and Morgante M (2004) Corn and humans: recombination and linkage disequilibrium in two genomes of similar size. Trends in Genetics, vol. 20, No. 2, 103-111

Reetz T, Leon J (2004) Die Erhaltung der genetischen Diversität bei Getreide. Auswahl einer Gersten Core-Collection aufgrund geographischer Herkunft, Abstammung, Morphologie, Qualität, Anbaubedeutung und DANN Markeranalysen. Institut für Pflanzenbau Professur für Speziellen Pflanzenbau und Pflanzenzüchtung

Reich DE, Cargill M, Bolk S et al. (2001) Linkage disequilibrium in the human genome. Nature, vol. 411, No. 6834: 199–204

Romagosa I, Han F, Ullrich SE, Hayes PM, and Wesenberg DM (1999) Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. Molecular Breeding, vol. 5, No. 2, 143 -152

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF, Graner A, Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci USA 103:18656–18661

Saghai Maroof MA, Zhang Q, Biyashev R (1995) Comparison of restriction fragment length polymorphisms in wild and cultivated barley. Genome 38: 298-306

SAS Institute (2008) The SAS system for Windows, release 9.2. SASInstitute, Cary, N.C. USA

Sato K, Hori K and Takeda K (2008) Detection of Fusarium head blight resistance QTLs using five populations of top-cross progeny derived from two-row 3 two-row crosses in barley. Mol Breeding (2008) 22:517–526

Sayed MA, Schumann H, Pillen K, Naz AA and Léon J (2012) AB-QTL analysis reveals new alleles associated to proline accumulation and leaf wilting under drought stress conditions in barley (*Hordeum vulgare* L.). BMC Genetics 2012, 13:61

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

Semagn K, Bjørnstad Å, Skinnes H, Marøy AG, Tarkegne T, William M (2006) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled haploid hexaploid wheat population. Genome 49: 545 – 555

Shin JS, Chao S, Corpuz L and Blake T (1990) A partial map of the barley genome incorporating restriction fragment length polymorphism, polymerase chain reaction, isozyme, and morphological marker loci. Genome 33: 803-810

Schouten HJ, Eric van de Weg W, Carling J, Khan SA, McKay SJ, van Kaauwen MPW, Wittenberg AHJ, Koehorst-van Putten HJJ, Noordijk Y, Gao Z, Rees DJG, Van Dyk MM, Jaccoud D, Considine MJ and Kilian A (2012) Diversity array technology (DArT) markers in apple for genetic linkage maps. [Mol. Breeding 2011, doi: 10. 1007/s11032-011-9579-5], Mol. Breeding, 29: 645-660.

Simko I, Costanzo S, Haynes KG, Christ BJ, Jones RW (2004) Linkage disequilibrium mapping of a Verticillium dahliae resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach. Theor Appl Genet 108:217–224

Singh AK, Knox RE, Clarke FR, Clarke JM, Somers DJ , Fedak G , Singh A and DePauw R (2008) Fusarium head blight QTL mapping in durum wheat and Triticum carthlicum sources of resistance. The 11th International Wheat Genetics Symposium proceedings

ŠÍP V, CHRPOVÁ J and ŠTOČKOVÁ L (2011) Evaluation of Resistance to Fusarium Head Blightin Wheat Using Different Sources of Inoculum. Czech J. Genet. Plant Breed., 47, (4): 131–139

Sogaard B, and Von-Wettstein-Knowles P (1987) Barley: genes and chromosomes. Carlsberg Res Commun 52: 123-196

Sreenivasulu N, Graner A, Wobus U (2008) "Barley Genomics: An Overview," Inter J Plant Genomics, Article ID 486258, 13 pages

Stack R (1999) Return of an old problem: Fusarium Head Blight of small grains. American Pathology Society. www.scisoc.org APSnet Feature

Steffenson BJ (1998) Fusarium head blight of barley: epidemics, impact, and breeding for resistance. Tech. Q. 35(4):177–84

Steffenson BJ (2002) Fusarium head blight of barley: impact, epidemics, management, and strategies for identifying and utilizing genetic resistance. In: Leonard KL, Bushnell WR (eds) Fusarium head blight of wheat and barley. The American Phytopathological Society, St. Paul, pp 256–258

Steffenson BJ, Olivera P, Roy JK, Jin Y, Smith KP, and Muehlbauer GJ (2007) A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Australian Journal of Agricultural Research, vol. 58, No. 6: 532-544

Steffenson BJ, Prom LK, Salas B, Fetch Jr. TG, Wesenberg DM and Bockelman HE (1996) Severity of Fusarium head blight and concentration of deoxynivalenol in near-isogenic lines of barley differing for several agronomic characters. p. 774-776. In A.E. Slinkard, G.J. Scoles, and B.G. Rossnagel (eds.) Proc. Fifth Int. Oat Conf. & Seventh Int. Barley Genet. Symp., Saskatoon. Univ. of Saskatchewan, Saskatoon

Structure 2.2, April 3, 2007. http://pritch.bsd.uchicago.edu/software

Suárez RC, Giménez MJ, Gutiérrez N, vila CMA ´, Machado A, Huttner E, Ramirez M C, Martín AC, Castillo A, Kilian A, Martin A and Atienza SG (2012) Development of wild barley (Hordeum chilense)-derived DArT markers and their use into genetic and physical mapping . Theor Appl Genet (2012) 124:713–722

Szalma S J, Buckler IV ES, Snook ME, McMullen MD (2005) Association analysis of candidate genes for maysin and chlorogenic acid accumulation in maize silks. Theor Appl Genet 110:1324– 1333

Takeda K and Heta H (1989) Establishing the testing method and a search for the resistant varieties to *Fusarium* head blight in barley. *Jpn. J. Breed.* 39:203–16

Tanksley SD and Nelson JC (1996) Advanced backcross QTLs analysis: a method for the simulation discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the vrs1 locus (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor Appl Genet 104: 54-60

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D et al. (2001) Dwarf8 polymorphisms associate with variation in flowering time. Nat. Genet. 28: 286–289

Toojinda T, Baird E, Booth A, Broers L, Hayes P, Powell W, Thomas W, Vivar H and Young G (1998) Introgression of quantitative loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor Appl Genet 96:123–131

Urrea CA, Horsley RD, and Steffenson BJ (2002) Heritability of Fusarium head blight resistance and deoxynivalenol accumulation from barley accession CIho 4196. Crop Sci. 42:1404–1408

Usele G, Legzdina L and Beinarovica I (2011) Screening results of diverse spring barley (*Hordeum vulgare* L.) varieties and breeding lines for resistance to fusarium head blight. Plant breeding and seed science 64: 147-152 . DOI: 10.2478/v10129-011-0038-6

Voorrips RE (2002) MapChart: software for the graphical representation of linkage maps and QTLs. J of Heredity 93:77–78

Van Sanford D, Anderson J, Campbell K, Costa J, Cregan P, Griffey C, Hayes P and Ward R (2001) Discovery and deployment of molecular markers linked to Fusarium Head Blight resistance: an integrated system for wheat and barley. Crop Sci 41:638-644.

Vanhala TK (2004) Exploring and mapping genetic variation in wild barley, *Hordeum spontaneum*. PhD Thesis Wageningen

Varshney RK and Tuberosa R (2007) Application of linkage disequilibrium and association mapping in crop plants. Genomics Approaches and Platforms, Vol 1: 97 – 119

Von Korff M, Léon J and Pillen K (2010) Detection of epistatic interactions between exotic alleles introgressed from wild barley (*H. vulgare ssp. spontaneum*). Theor Appl Genet 121:1455–1464

Waalwijk C, van der Heide R, de Vries I, van der Lee T, Schoen C, Corainville GC, Häuser-Hahn I, Kastelein P, Köhl J, Lonnet P, Demarquet T and Kema GHJ (2004) Quantitative detection of *Fusarium* species in wheat using TaqMan. Eur J Plant Pathol 110: 481-494

Wang J, McClean PE, Lee R, Goos RJ, and Helms T (2008) Association mapping of iron deficiency chlorosis loci in soybean (*Glycine max* L. Merr.) advanced breeding lines. Theor. Appl. Genet. 116: 777 – 787

Waugh R, Bonar N, Baird E, Thomas B, Graner A et al. (1997) Homology of AFLP products in three mapping populations of barley. Mol. Gen. Genet. 255: 311-321

Webster J and Weber WS (2007). Introduction to Fungi. Cambridge: Cambridge University Press.

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhof A and Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley, Proceedings of the National Academy of Sciences USA, vol 101: 9915-9920

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna´ J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206

Wittenberg AHJ, van der Lee T, Cayla C, Kilian A, Visser RGF & Schouten HJ (2005) Validation of the high-throughput marker technology DArT using the model plant Arabidopsis thaliana. Molecular Genetics and Genomics (2005) 274: 30–39

Xia L, Peng K, Yang S, Wenzl P, Carmen de Vicente M, Fregene M and Kilian A (2005) 'DArT for highthroughput genotyping of Cassava (*Manihot esculenta*) and its wild relatives', Theoretical and Applied Genetics, vol 110: 1092-1098

Xu X and Nicholson P (2009) Community Ecology of Fungal Pathogens Causing Wheat Head Blight. Annual Review of Phytopathology, 47: 83-103

Yin X, Stam P, Dourleijn CJ, and Kropff MJ (1999) AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor Appl Genet 99: 244-253

Yu GT, Franckowiak JD, Neate SM, Zhang B, Horsley RD (2010) A native QTL for Fusarium head blight resistance in North American barley (*Hordeum vulgare* L.) independent of height, maturity, and spike type loci. Genome. 2010 Feb;53(2):111-8

Yu J, Pressoir G, Briggs HW, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland Jb, Kresovich S, and Bucker ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Gen., Vol. 38, 2: 203 – 208

Yu JM and Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr. Opin. Biotechnol. 17, 155–160

Zhao K et al. (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet. 3, e4

Zhao K et al. (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet. 3, e4.

Zhou H, Muehlbauer G, Steffenson B (2012) Population structure and linkage disequilibrium in elite barley breeding germplasm from the United States. Zhejiang Univ-Sci B (Biomed & Biotechnol) 13(6):438-451

Zhou M-P, Hayden MJ, Zhang Z-Y, Lu W-Z and Ma H-X (2010) Saturation and mapping of a major Fusarium head blight resistance QTL on chromosome 3BS of Sumai 3 wheat. J Appl Genet 51(1), 2010, pp. 19–25

Zhou W, Kolb F, Bai G, Shaner G, and Domier L (2000) SSR mapping and sub-arm physical location of a major scab resistance QTL in wheat. 2000 National Fusarium Head Blight Forum. Erlanger, KY. 10-12 December 2000. Michigan State University

Zhou X, Chao M, Liang X (1991) Screening and testing of barley varieties for scab resistance. Acta Phytopathol. Sin. 18:261–65

Zhu C et al. (2008) Status and prospects of association mapping in plants. Plant Gen. 1, 5–20.

Zhu H, Gilchrist L, Hayes P, Kleinhofs A, Kudrna D, Liu Z, Prom L, Steffenson B, Toojinda T and 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 (1999) 99:1221–1232

Zohary D (1969) The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the old world. In Ucko PJ, Dimbleby GW (eds) The domestication and exploitation of plants and animals. General Duckworth and Co. Ltd., London, pp 47-66. http://faostat.fao.org

7. List of tables

8. List of figures

9. Abbreviations

Abbreviation	Explanation
AFLP	Amplificated fragment length polymorphism
AM	Association mapping
ANOVA	Analysis of variance
Chr.	Chromosome
cM	centiMorgan
DArT	Diversity array technology
DNA	Deoxyribonucleic acid
et al.	et aleri
F_2	Second generation after a cross
FDR	False discovery rate
GLM	General linear model
HD	Heading date
Hsp	*Hordeum spontaneum*
Hv	*Hordeum vulgare*
K matrix	Kinship matrix
LD	Linkage disequilibrium
LDS	Leaves disease scoring
LDSI	Leaves disease scoring isolate
M*T	Marker- treatment interaction
MCMC	Monte carlo markov chain
MILDS	Marker isolate interaction within leaves disease scoring
MISDS	Marker isolate interaction within Spikes disease scoring
MLM	Mixed linear model
PCA	Principal component analysis
PDR	Partial disease resistance
Pos.	Position
Q matrix	Population structure matrix
qPCR	Real time polymerase chain reaction
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
SDS	Spikes disease scoring
SDSI	Spikes disease scoring isolate
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
VS	Visual leaves disease scoring