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Mining the global diversity of barley for Fusarium resistance using leaf and spike inoculations

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Abstract *Fusarium graminearum* is a devastating fungal pathogen that causes significant yield and quality losses in cereals. We utilized a diversity set of barley (140 genotypes) to explore vital resistance alleles against this aggressive pathogen. The resistance assessment was carried out on spikes and leaves via artificial inoculations under control conditions. The phenotypic data was subjected to genome-wide association analysis using a genetic map based on DArT and SNP markers. This analysis revealed eleven and nine marker trait associations for leaf disease scoring (LDS) and spike disease scoring (SDS), respectively. The strongest QTL for LDS was found on chromosome 1H where a minor allele of wild origin decreased disease symptoms by 78%. The major QTL allele for SDS was linked with marker locus SCRI_RS174710 on chromosome 5H. In addition, four favorable epistatic interactions effects were found in decreasing disease symptoms. Overall, three QTL were common for LDS and SDS, which indicates a partial genetic relatedness of these resistances in barley. The QTL alleles for LDS and SDS will help to establish organ specific resistances in cultivated barley.

Keywords *Fusarium graminearum* · *Triticum aestivum* · Resistance QTL · GWAS · Leaf and spike disease symptoms

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Introduction

Fusarium head blight (FHB) is one the most devastating disease of cereal crops. It is caused by *Fusarium graminearum* that primarily attacks the developing spikes and the vegetative parts like stem and leaves, which results in significant yield losses (Boutigny et al. 2011). It has been reported an estimated yield lose of around 70 million tons in barley, with a raw commodity value of $122 million in the 1993 epidemic alone (Steffenson 1998; Bai and Shaner 2004). In addition, this pathogen produces fungal toxins (mycotoxins) like deoxynivalenol (DON) in

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seeds as well as in the vegetative tissues which seriously affect the quality of grain and fodder for human and livestock consumption, respectively (Goswami and Kistler 2004; Liddell 2003). Therefore, development of resistant cultivars to *F. graminearum* is essential for sustainable crop production and biosafety of cereal crops.

Fusarium head blight is an intensively studied disease among cereal crops like wheat and barley. The previous work on the genetic analysis found quantitative inheritance of FHB resistance and identified associated quantitative trait locus (QTL) in barley. For instance, de la Pena et al. (1999) identified ten and four QTL for FHB severity and low DON content using a barley population of $F_{4:7}$ lines, respectively. Likewise, Mesfin et al. (2003) detected three distinct QTL regions on chromosome 2H associated with FHB resistance; two of these regions were associated with resistance to DON accumulation. Horsley et al. (2006) identified two QTL controlling FHB resistance and plant height simultaneously, and one QTL for DON accumulation in seeds. In another study, Massman et al. (2011) compared FHB QTL locations from seven barley resistant cultivars, of which most of the cultivars showed two FHB resistance QTL on the long arm of chromosome 2H. By utilizing genome-wide association studies, Mamo and Steffenson (2015) detected two major QTL related to FHB severity and DON concentration in a barley diversity panel comprising landraces from Ethiopia and Eritrea.

These reports suggest that most of the previous studies were focused on the resistance assessment in the infected spikes and seeds (Osborne and Jin 2002; Singh et al. 2008; Griffey et al. 2009). Notably, the resistance against *Fusarium* sp. is conditioned by multiple morphological and physiological components across the plant development (Zhu et al. 1999; Horsley et al. 2006). Recently, Buerstmayr and Buerstmayr (2016) studied semidwarfing alleles reduced height (Rht)-D1b and Rht-B1b for Fusarium severity. They found that the reduction of plant height and a high proportion of retained anthers were associated with increased FHB severity. These morphological and physiological processes are usually controlled by a network of genes expressing at a certain development stage. In addition, barley is utilized as fodder crop in many parts of the world like United State of America, China, Poland, Ethiopia, Nigeria, South Africa etc. Hence, it is necessary to assess Fusarium resistance early on vegetative parts as well as later during the spike development to explore resistance scenario at whole plant level. For Fusarium resistance on leaf, a detached leaf assay was used by Diamond and Cooke (1999), Browne and Cooke (2005) and Browne et al. (2005) to study the partial disease resistance (PDR) components in commercial cultivars and germplasm of soft red winter wheat having a range of FHB resistance. Several PDR components were found to be significantly correlated with whole plant reactions in a mist-irrigated field trails via artificial inoculations. They concluded that this method of assay can be used as a pre field-screening tool, as it offers the advantages in controlling the conditions, requires relatively little space, can be more readily repeated and individual measurement of a number of PDR components can be taken rather than just disease incidence or severity alone. Murakami and Ban (2005) reported that an oval lesion resulted from a spore suspension of *F. graminearum* 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 toxin. Using wounded wheat leaves, their bioassay system was able to detect differences in disease reaction between resistant and susceptible cultivars. Kumar et al. (2011) used the same method and showed that 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. This report suggests that measuring these PDR components to identify genotypes may have potential to complement FHB resistance. Although, different and more precise methods of Fusarium resistance assessment are reported, but their utility in identifying essential resistant QTL alleles remained enigmatic among the barley diverse genetic resources.

In the present study, we utilized barley natural diversity comprising of wild accessions, landraces and cultivars to assess resistance against *F. graminearum* at vegetative (on leaf) and reproductive (on spike) developmental stages. A genome-wide association mapping strategy was employed to identify QTL alleles and putative epistatic interaction for Fusarium resistance, and to compare the genetic inheritance of Fusarium resistances using detached leaf assay and spike infections. The identification of new QTL alleles

in the present study will help to establish genotypes carrying leaf and/or spike resistances to *F. graminearum* in barley.

Materials and methods

Plant material

A diversity set of 140 barley genotypes comprising of wild accessions (108), landraces (7) and cultivars (25) across the world were utilized in the present study. The details of these genotypes together with their geographic distribution can be found in Table 1.

Phenotypic evaluation

Leaf disease scoring (LDS)

The seeds of individual genotypes were grown in pots (five plants per pot) in five replications inside the greenhouse in a completely randomized design under control conditions with 16 h light at 24 ± 2 °C. At the fifth leaf stage (seedling stage) the third fully expanded leaf was cut into five sections each 2 cm. These pieces were washed in Ethylalcohol 75% for 3 s, then in distilled water three times, and cultured in petri-dishes including kinetin agar media (6 g Agar–Agar/l + 10 mg Kinetin/l, Browne et al. 2005). Then, each piece of leaf was inoculated with suspension of 10^4 conidia ml^{-1}/leaf section of *F. graminearum* isolate FG 5.1. Finally, petri-dishes were incubated in growth chamber at 12 h light and temperature 24 °C. The disease scoring was performed 7 days after inoculation by taking 25 disease scores (5 replicates × 5 sections) from each genotype using image analysis software for plant disease quantification (APS Assess software, American Phytopathological Society). This disease analysis software calculates the whole area of the leaf and the infected area gives % infection percentage. The *F. graminearum* isolate FG 5.1 was collected from natural field conditions and multiplied through single spore cultures in laboratory for artificial inoculations in the present study.

Spike disease scoring (SDS)

The same plants grown in five replications under glass house conditions were further inoculated at the anthesis stage of each spike by injecting the spore suspension of *F. graminearum* isolate FG 5.1 (10^3 conidia ml^{-1}/floret). The genotypes were inoculated individually at the anthesis stage by selecting five central florets of the main spike. The disease symptoms were scored individually at the maturity stage by a visual scoring scale from 0 to 9 (0 = no bleached spikes and 9 = completely bleached, Osborne and Jin 2002).

Genotyping using DArT and SNP arrays

One hundred twenty-four accessions from the total of 140 accessions were genotyped using diversity array technology (DArT) and gene-specific marker systems. This genotyping resulted in a total of 895 polymorphic DArT markers across the genome. The chromosomal positions of the DArT markers are according to Wenzl et al. (2004). In addition, a sub-population comprising 50 diverse genotypes was genotyped using Illumina 9 K iSelect SNP chip at TraitGenetics (TraitGenetics GmbH, Seeland OT Gatersleben, Germany). The SNP genotyping resulted in 5892 polymorphic markers after passing the criteria of minor allele frequency. The positions of these SNP markers were according to Comadran et al. (2012).

Structure analysis and relatedness relationships

Principal component analysis (PCA) was carried out by using SAS 9.2 program PROC PRINCOMP, according to Price et al. (2006) to study the population structure. The significance for PCA was evaluated using method by Franklin et al. (1995).

The relative kinship coefficients (K-matrix) among all pairs of accessions were calculated using 895 DArT and 5892 SNP markers by "SPAGeDi-1.3d" Software (Hardy and Vekemans 2002).

Genome-wide association analysis (GWAS)

A Mixed Linear Model (MLM) was implemented which is comparable to the GRAMMAR estimation method described by Aulchenko et al. (2007). This technique was used with PCA and kinship for full experimental design, like years, treatments. This analysis was done using SAS Software Version 9.2 to conduct the association analysis and to identify the DArT and SNP markers associated with both the

Table 1 List consists of 140 accessions of the studied barley population

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

Table 1 continued

No.	Accession	Type	Origin	No.	Accession	Type	Origin	No.	Accession	Type	Origin
45	ICB180857	*Hsp*	JOR	92	ICB181436	*Hsp*	PAL	139	Scarlett	*Hv*(C)	GER
46	ICB180862	*Hsp*	UNK	93	ICB181442	*Hsp*	PAL	140	Thuringia	*Hv*(C)	GER
47	ICB180867	*Hsp*	AFG	94	ICB181448	*Hsp*	PAL				

Hsp Hordeum vulgare ssp. *spontaneum*, *Hv Hordeum vulgare* ssp. *vulgare*, *L* Landrace, *C* Cultivar, *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, *CRO* Croatia, *GER* Germany

scorings separately 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 statistical models were:

$$Y_{jkmn} = \mu + M_{\mathrm{j}} + \Sigma PCA_k + A_m(M_{\mathrm{j}})K_n + Y_i * A_m(M_{\mathrm{j}})K_n + \varepsilon_{jkmn}$$

where μ is the general mean, M_j is the fixed effect of j-th marker, PCA_k is the fixed effect of k-th subgroup of the population structure (PC values), $A_m(M_{\mathrm{j}})K_n$ is the random effect of m-th accession nested the j-th marker associated with n-th kinship coefficient. ε_{ijkmn} is the error.

In addition, p-values of significant markers were corrected using probability of false discovery rate (PFDR) using PROC MULTTEST in SAS according to Benjamini and Yekutieli (2005). This procedure was repeated until no marker could be detected, which led to a reduction of significant markers and thereby a reduced number of false positive QTL.

Epistatic interaction effects

Epistatic interactions between all marker pairs were tested with SAS procedure MIXED (SAS ver. 9.2, SAS Institute 2008) using the following mixed hierarchical model:

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

where 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 < 0.001) and have been calculated by PROC MULTTEST procedure in SAS.

Results

Phenotypic evaluation of leaf disease scoring (LDS) and spike disease scoring (SDS)

The analysis of variance showed significant variation for LDS and SDS among the barley diversity set where each trait was found heritable across the replications (Table 2, Fig. 1). The mean comparison and the frequency distribution of LDS and SDS are presented in Fig. 2. A slightly higher mean (32.68%) was found in LDS as compared to SDS (25.58%). Based on frequency distribution, SDS showed more variation which ranged from around 8 to 56% as compared to LDS.

To see the relationship between LDS and SDS disease scores, we plotted a scatter plot presented in Fig. 3. This analysis revealed a significant but modest correlation ($r = 0.24^{**}$) between LDS and SDS.

Population structure and linkage disequilibrium

Principal component analysis (PCA) was used to analyze population structure by using genotypic data of 895 DArT markers. The resulted population structure is shown in Fig. 4. It revealed that the first dimension (PC1) accounted for 11.68% of the total variance. The second dimension (PC2) summarized

Table 2 Analysis of variance of leaf and spike disease scores of *F. graminarum* among barley diversity set

Trait	S.V	D.F	MS	Sign.	Heritability (%)
Leaves disease scoring (LDS)	Genotypes	139	1430.47	***	84.36
	Error	1224	140.65		
	LSmean	32.68			
Spikes disease scoring (SDS)	Genotypes	139	1515.71	***	96.12
	Error	1228	20.89		
	LSmean	25.58			

S.V source of variance, *D.F* degree of freedom, *MS* sum of mean squares, *Sign* level of significance ***P $\leq$ 0.001

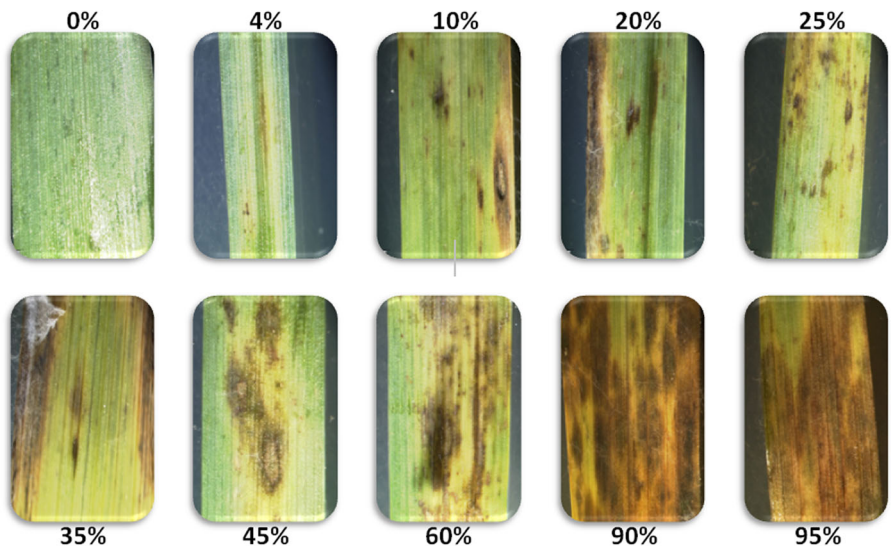

Fig. 1 Percent disease infections of *Fusarium graminearum* isolate FG 5.1 on barley leaves scored by APS software

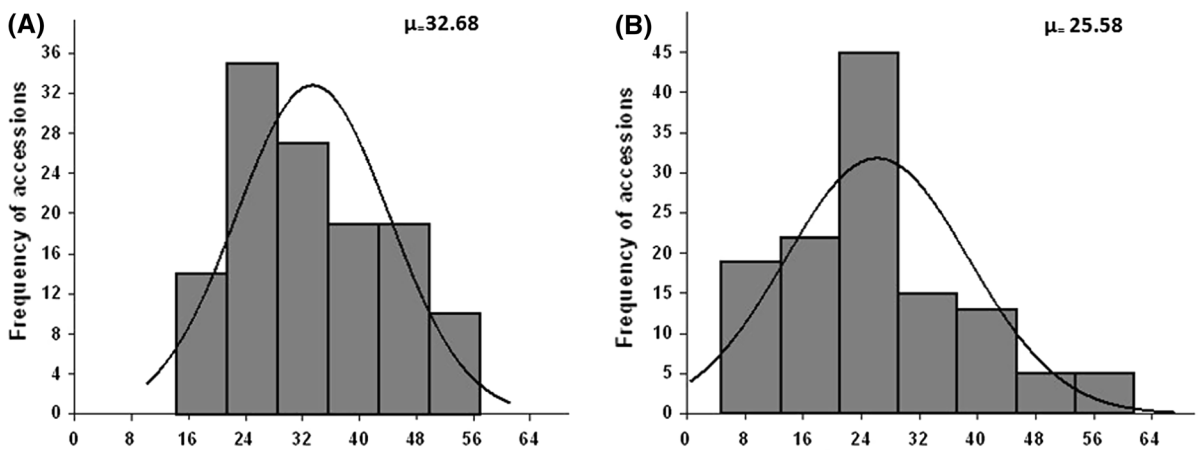

Fig. 2 Frequency distribution of barley diversity set for **a** leaves disease scoring and **b** spikes disease scoring. Horizontal axis represents disease scores in percent

4.57% of the variation. According to this analysis, the population was structured, with three major clusters: first one included the accessions from Uzbekistan (UZB), Palestine (PAL), Libya (LBY), Iraq (IRQ), Syria (SYR), Tajikistan (TJK) and Iran (IRN). Second cluster consists of accessions coming from Russia

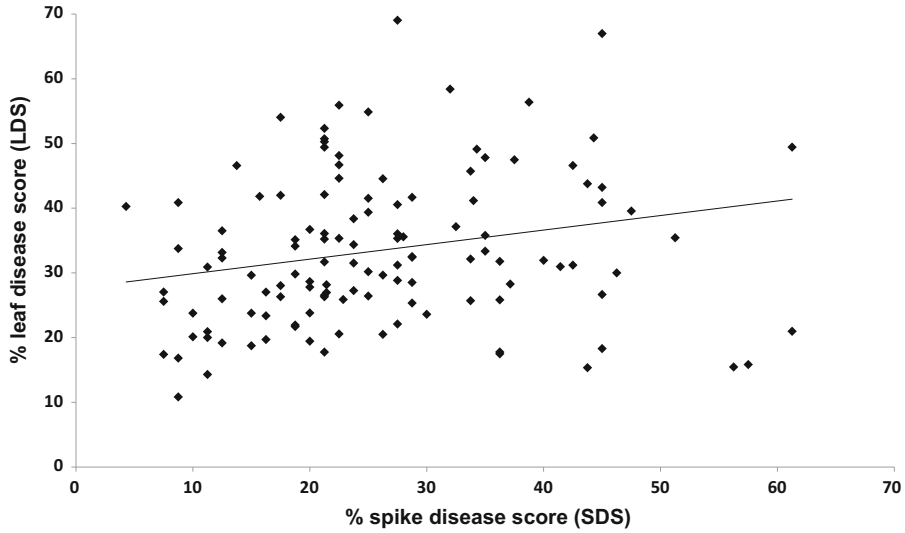

Fig. 3 Scatter plot shows the correlation between percent leaf disease score (LDS) and percent spike disease score (SDS)

(RUS), Afghanistan (AFG), Pakistan (PAK), Jordan (JOR), Palestine (PAL), Syria (SYR), Iraq (IRQ), Turkey (TUR) and Turkmenistan (TKM). However, third cluster consists of the varieties which came from Germany (GER) and one wild barley accession originating from Syria (SYR). The first significant three PCs were used in the association mapping analysis as a structure matrix (Q-matrix). By another SNP marker group using 5892 markers, the first PC1 accounted for 51.56% of total variance.

The squared allele-frequency correlations r^2, representing linkage disequilibrium (LD) were assessed for 23 251 combinations of DArT markers across chromosomes 1H (4691), 2H (5042), 3H (3916), 4H (562), 5H (2556), 6H (3081), 7H (3403). In the structured population all intra-chromosomal loci pairs were in LD with $P < 0.01$, considering all 124 genotypes, the r^2 values for intra-chromosomal pairs of loci ranged from 0.0 to 1.0. The pattern of LD decay plot across the whole genome, a loose curve that fitted the r^2 estimates did not reach the level of 0.1

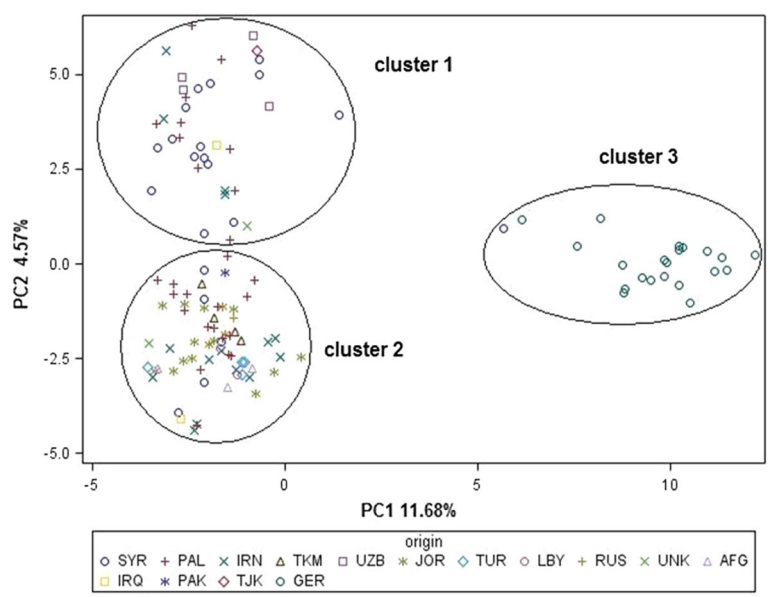

Fig. 4 Population structure of the diversity set achieved by principal component analysis using 895 DArT markers. The genotypes details can be found in Table 1

represented in Fig. S1. This figure also presented low or no LD decay (< 1 cM).

QTL analysis

Leaves disease scoring

GWAS revealed a total of eleven marker associations with LDS. These marker loci were distributed across chromosomes 1H to 7H (Table 3). These QTL effects were significant as marker main effect and ranged from chromosomes. All QTL had main effects of reducing means if infection percentage varied from (6.6 to 29.01%). The strongest QTL Qlds.1Ha was found on chromosome 1H which explained 45.69% of genetic variance (R^2). The QTL was associated with SNP marker locus SCRI_RS_239784 at position 30.45 cM.

Spikes disease scoring

Marker association with SDS revealed nine QTL which were located on 1H, 4H, 5H, 6H and 7H (Table 3). These QTL were significant as marker main effect and ranged from 7.85 to 27.45%. The strongest QTL based on the coefficient of explained genetic variance for SDS was associated to SNP marker loci SCRI_RS_239784 and SCRI_RS_174710. Notably, the SNP marker locus SCRI_RS_239784 on chromosome 1H was common between LDS and SDS.

Epistatic interaction effect

Digenic epistatic interaction played an important role in the determination of complex resistances. In the present study, we found four epistatic interaction effects for LDS and SDS (Table 4). For LDS, the strongest interaction effect was calculated between SNP marker loci BOPA1_2065-3135 (4H) and SCRI_RS_239784 (1H) where the combination of M1*M2 resulted in the lowest disease infection (24.97%). Notably, the marker locus SCRI_RS_239784 also revealed significant interaction with marker locus BOPA1_1272-459 (7H) for SDS where the disease score was reduced by 20.14%. The SNP markers, SCRI_RS_239784 and BOPA1_1272-459 were associated with QTL for LDS and SDS as marker main effect, thus revealing a QTL by QTL interaction in the present analysis.

Discussion

The current research on the resistance development against *F. graminearum* was focused primarily on its infections on the spike—spikelets and grains, which is termed as Fusarium Head Blight (FHB). It was evident that disease scoring for FHB later in the development on growing spike was not an easy task among the cereal crops. In addition, transcriptional activity of essential resistance genes against FHB may be missing or low in later stages of development. This scenario may cast a shadow on the identification of essential genes needed for the development of stable and effective resistance against this cereal menace. Secondly, most studies on FHB were performed in cultivar gene pool where essential disease resistance genes may be lost due to intensive breeding and directional selection for yield. The major aim of the present study was to screen barley natural diversity for Fusarium resistance using an additional disease scoring method on leaf early in the development along with the disease scoring on spike to detect new resistance alleles that can establish broad-spectrum resistance against this pathogen.

The phenotypic evaluation using both methods of disease scoring revealed significant variation of resistance response among the population. We found 18 resistant genotypes showing less than 20% disease scoring in the detached leave assay whereas 48 genotypes showed less than 20% disease symptoms when employed disease observation using 0–9 scale. One reason behind this difference may be the genetic inheritance of genotypes for their resistance in leaf and spikes. However, it is likely that these differences may be due to the use of two different systems for quantification of disease symptoms. For instance, a computer mediated disease scoring method was utilized for leaf disease symptoms which appear to deliver more precise phenotypic evaluation as compared to visual observation on spikes. This variation is similar to Ūsele et al. (2013) who found significant differences for FHB between barley genotypes. Kumar et al. (2011) found an inconsistent relationship among barley partial disease resistance components based on detached leaf assay of which several were poorly correlated with symptoms on spikes.

The genotypic characterization of the present population found three sub-clusters suggesting a structured population of barley. In this classification

Table 3 Summary of QTL for LDS and SDS detected as markers main effect

Trait	QTL[a]	Marker	Chr[b]	Pos[c]	Flanking	ProbF[d]	Sign[e]	R^{2f}	M_0^g	M_1^h	Diff[i]
LDS	Qlds.1Ha	SCRI_RS_239784	1H	30.45	30.45	1.0E-06	***	45.69	56.76	27.75	29.01
	Qlds.1Hb	bPb-5290	1H	64.89	64.89–67.88	0.0002	***	3.58	35.93	27.13	8.8
	Qlds.2H	bPb-5991	2H	14.40	14.40–15.76	0.0003	***	1.72	35.90	27.87	8.03
	Qlds.3Ha	BOPA1_76-1059	3H	109.77	109.77	0.0033	**	21.53	37.86	27.12	10.74
	Qlds.3Hb	bPb-8621	3H	140.29	140.29	0.0008	***	11.29	37.86	30.73	7.13
	Qlds.4H	SCRI_RS_181886	4H	52.69	52.69	0.0002	***	30.57	40.76	26.31	14.45
	Qlds.5Ha	bPb-7407	5H	16.91	16.91–18.03	0.0010	***	10.12	36.58	29.59	6.99
	Qlds.5Hb	bPb-2273	5H	43.50	43.50–45.58	0.0021	**	10.03	41.76	32.30	9.46
	Qlds.6H	bPb-3554	6H	19.42	19.42–20.46	0.0013	**	8.26	35.91	29.31	6.6
	Qlds.7Ha	BOPA2_12_11499	7H	74.58	74.58	0.0004	***	27.72	42.42	27.37	15.05
	Qlds.7Hb	bPb-1770	7H	84.95	84.95–87.39	0.0001	***	4.41	36.07	26.88	9.19
	Qsds.1H	SCRI_RS_239784	1H	30.45	30.45	6.8E-06	***	35.26	50.19	22.74	27.45
SDS	Qsds.4H	BOPA1_6249-572	4H	50.85	50.85	4.5E-04	***	22.97	17.91	28.71	10.8
	Qsds.5Ha	SCRI_RS_219574	5H	44.24	43.96–46.59	5.0E-04	***	22.74	35.61	21.93	13.68
	Qsds.5Hb	SCRI_RS_161118	5H	71.67	71.67	1.8E-03	***	23.59	29.57	18.60	10.97
	Qsds.5Hc	SCRI_RS_174710	5H	121.74	121.74	8.2E-06	***	39.24	41.54	21.45	20.09
	Qsds.6H	bPb-0245	6H	40.08	40.08	0.0018	**	8.52	21.84	29.69	7.85
	Qsds.7Ha	bPb-6747	7H	35.22	35.22–38.70	0.0015	**	10.32	27.95	18.97	8.98
	Qsds.7Hb	SCRI_RS_150783	7H	48.30	48.30	3.0E-04	***	29.12	30.51	18.65	11.86
	Qsds.7Hc	BOPA1_1272-459	7H	74.43	74.43	5.3E-04	***	26.85	34.01	21.21	12.8

[a]QTL names consist of a letters of ''Q'', the trait abbreviation LDS or SDS, the chromosomal location and a sequential character to discriminate two or more QTL per chromosome

[b]*Chr* chromosome, [c]*Pos* the position in cM, [d]*ProbF* probability value, [e]*Sign* Significant of each QTL where **, *** represent significance level at 0.01 and 0.001, respectively

[f]R^2 refers to the explained genetic variance for each QTL

[g,h]M_0, M_1 allelic means of infection percentage of marker allele 0 (absence) and maker allele 1 (presence)

[i]Diff refers to the differences between the means of M_0 and M_1

the genotypes clustered together with respect to their genotypic background as well as according to their geographical origin except few accessions from Syria, Libya and Iraq. Population based linkage disequilibrium (LD) 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 linked to phenotypes of interest. In this study, we employed a high resolution genetic map comprising of DArT and SNP markers, where markers are well distributed across the chromosomes ranging from genetic distances of 1.42 to 3.5 cM. 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 share more or less similar LD blocks across the genome. 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. This could be an explanative reason for the higher LD decay observed in the present study which suggests that the wild accession in this association panel may be useful for the resolution of association mapping.

The present GWAS analysis found eleven and nine QTL effects for leaf and spike disease scoring,

Table 4 Digenic epistatic interactions effects for LDS and SDS across the barley genome

Trait	Effect	Marker 1			Marker 2			PropF	Lsmeans of digenic interaction			
		Marker	Chr.	Pos.	Marker	Chr.	Pos.		$M1_{M0}$/ $M2_{M0}$	$M1_{M1}$/ $M2_{M0}$	$M1_{M0}$/ $M2_{M1}$	$M1_{M1}$/ $M2_{M1}$
LDS	BOPA1_2065-3135* SCRI_RS_239784	BOPA1_2065-3135	4H	14.95	SCRI_RS_239784	1H	30.45	1.06E-08	35.07	67.60	24.97	30.39
	bPb-6135* bPb-5290	bPb-6135	5H	115.34	bPb-5290	1H	64.89	1.99E-06	43.50	26.16	32.70	25.56
SDS	bPb-2971* bPb-0245	bPb-2971	2H	131.49	bPb-0245	6H	40.08	9.99E-06	20.41	60.26	30.37	28.07
	BOPA1_1272-459* SCRI_RS_239784	BOPA1_1272-459	7H	74.43	SCRI_RS_239784	1H	30.45	3.19E-07	45.90	58.77	31.85	20.14

All epistatic effects were significant at ≤ 0.001. M1and M2 refers to marker 1 and marker 2, respectively where M_0 and M_1 refer to the present and absent alleles at a given marker, respectively

respectively. More QTLs were found for leaf disease scoring as compared to spike disease scoring. It may happen because precise disease symptoms quantification on leaves sections was conducted using APS Assess software in comparison to visual scoring on spikes. The second reason behind this difference may be due to the presence of more active genes in the early plant development. The most significant QTL for leaf disease scoring were found on chromosome 1H and 7H. Notably, the strongest QTL effect for leaf and spike disease scoring was common and linked to SNP marker SCRI_RS_239784 on chromosome 1H at position 30.45 cM. This QTL seems similar to previously identified QTL by Dahleen et al. (2012), who used immunosorbent assay (ELISA) for FHB resistance in the doubled haploid lines from Zhedar 2/ND9712//Foster under ten environments. In addition, the chromosome 7H carried QTL for leaf and spike disease resistances at positions 74.58 and 74.43 cM, respectively. As these QTL effects showed association to adjacent SNP markers, these QTL effects may underlie one common genetic factor. However, it is likely that with two different disease scoring methods even tightly linked markers showed variations in their significances. A major QTL Qsds.5Hc for SDS on chromosome 5H, appeared to be a new QTL for Fusarium resistance in barley.

Epistasis is an important genetic component underlying quantitative trait variation. One major difficulty in developing a powerful statistical approach for mapping QTL with epistatic effects is the treatment of many parameters for multiple QTL. In the present study we employed a mixed model in testing for the epistatic interaction which detected altogether four pairs of interaction effects. These effects were mainly QTL*marker or QTL*QTL interactions, because associated markers were also significant as marker main effect. LDS had two different epistatic effects (both marker*QTL epistatic interaction) suggesting, two out of eleven LDS QTL revealed epistasis. SDS revealed almost similar number of epistatic interactions but both with marker*QTL and QTL*QTL interactions effects. Ma et al. (2006) studied the epistatic effect for FHB resistance in wheat, the analysis resolved nine pairs of AA interactions involving 17 different loci that explained 26% of phenotypic variation, whereas only seven QTL identified as main effect QTL explained $\approx$ 24.8% phenotypic variation. This indicates that genetic effect of AA epistasis was equally important as that of QTL main effect. Hence, the identification of epistatic effects in the present study suggests that the resistance against *F. graminearum* seems complex and may be controlled by a network of genes or due to pleitropic effect of genes (related to morphological and physiological parameters) which may be associated with such type of resistances indirectly. Recently, Buerstmayr and Buerstmayr (2016) found a marked difference of semi-dwarfing alleles *Rht-D1b* and *Rht-B1b* for FHB severity. Steffenson (2002) found that QTL associated with resistance are usually associated with agronomic and morphological traits such as late heading, plant height, lax spike and two-rowed spike. Yang et al. (2005) reported that five QTL were pleiotropic and found to be associated with component of FHB resistance in wheat. Additional studies have found that FHB is conditioned by many genes which are distributed throughout the genome (de la Pena et al. 1999; Ma et al. 2000; Kolb et al. 2001; Mesfin et al. 2003; Dahleen et al. 2012).

Taken together, the present study showed a successful utility of leaf and spike disease assessment in the genetic analysis of resistance against *F. graminearum*. The weak correlation of leaf and spike disease scores suggests partly independent genetic relatedness of resistance mechanisms to Fusarium in two different organs. Therefore, the use of artificial inoculation on leaf and spike seems demanding for precise evaluation of resistance and the identification of resistance QTL early and late in plant development. But, to claim something significant for practical Fusarium resistance breeding, field evaluations are needed among the selected resistant and susceptible genotypes of the barley diversity-set. In this regard, it is worthy to mention that the resistance QTL alleles detected in the present study are tightly linked with corresponding DArT and SNP markers, which can directly be employed for future work on Fusarium resistance in barley through marker assisted selection.

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