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Genome-wide Association Mapping of Fusarium Head Blight Resistance and Agromorphological Traits in Barley Landraces from Ethiopia and Eritrea

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ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is an important disease of barley (*Hordeum vulgare* L.), and other cereals. In barley, the genetic basis of FHB resistance has been intensively studied through linkage mapping that identified several quantitative trait loci (QTL). However, our understanding and application of these QTL in breeding is still limited due to the complex nature and low-to-moderate heritability of FHB resistance. Previous studies used either breeding lines, unimproved varieties, or germplasm selections. Here, we used association mapping in barley landraces to identify QTL associated with FHB severity, deoxynivalenol (DON) concentration and correlated agromorphological traits. Diverse barley landraces (n = 298) from Ethiopia and Eritrea were evaluated for the traits under field conditions for 2 yr (2011–2012) in Crookston, MN, and genotyped with 7842 single nucleotide polymorphism (SNP) markers. Association mapping analysis using a mixed model corrected for pairwise relatedness between individuals identified one common resistance QTL on barley chromosome 2HL significantly associated with both FHB severity and DON concentration and another one on 4HL associated with DON concentration. The QTL identified on 2HL is associated with the row-type locus *Vrs1*. Both of these QTL were not significantly associated with heading date or plant height unlike other QTL reported in previous studies. Thus, the resistant accessions carrying these QTL may be used in breeding programs without the confounding effects from these agromorphological traits. Importantly, these QTL could be new alleles preserved in this unique germplasm, and the linked SNP markers found may be useful in marker-assisted introgression of resistance.

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Abbreviations: BLAST, basic local alignment search tool: BLUP, best linear unbiased predictions: DON, deoxynivalenol: DTH, days to heading: EEBC, Ethiopian and Eritrean Barley Collection: EL, exsertion length: EMMA, efficient mixed model association: FDR, false discovery rate; FHB, Fusarium head blight: GWAS, genome-wide association study: KD, kernel density: LD, linkage disequilibrium: MAF, minor allele frequency: MLM, mixed linear model: NSGC, National Small Grains Collection: PCA, principal component analysis: PH, plant height: QTL, quantitative trait loci: RT, row-type morphology: SA, spike angle: SNP, single nucleotide polymorphism: VIR, N. I. Vavilov Research Institute of Plant Industry: *vrs1*, six-rowed spike 1: *vrs2*, six-rowed spike 2.

Fusarium head blight or scab, caused primarily by *F. graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch.], is a significant disease of wheat (*Triticum aestivum* L.), barley, and other small grain cereals in many countries with temperate and semitropical climates (Parry et al., 1995; Ma et al., 2000; Goswami and Kistler, 2004). Fusarium head blight was first reported in England in 1884 and was considered a major threat to wheat and barley production following the years of its discovery (Stack et al., 2003; Goswami and Kistler, 2004). In the Upper Midwest region of the United States and the Prairie provinces of Canada, epidemics of FHB occurred sporadically during the last century (McMullen et al., 1997; Tekauz et al., 2000). However, starting in 1993, a series of widespread and severe epidemics struck the wheat and barley crops, resulting in losses of more than $3 billion

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during the 1990s (Ward et al., 2008; Windels, 2000). Fusarium head blight also has been a problem in Asia, Europe, and South America in recent years, suggesting that the disease is establishing itself as a perennial problem of economic significance (Parry et al., 1995). In addition to the direct adverse effect of FHB infection on yield and grain quality, most *Fusarium* species causing FHB produce mycotoxins in infected grain (Prom et al., 1999). Deoxynivalenol of one of the most common myotoxins produced by *F. graminearum* and is toxic to humans and other animals (Scott, 1990). Barley grain with DON concentration greater than 0.5 mg Kg^{-1} is not accepted by the malting and brewing industry (Steffenson, 1998). An integrated management approach that combines multiple control strategies, especially host resistance, is needed to reduce the severity of FHB and its detrimental effects (Steffenson, 1998). In barley, the genetic basis of FHB resistance has been intensively studied through QTL mapping. The genetic studies indicated that FHB resistance is a complex quantitative trait with low heritability in elite germplasm and highly influenced by the environment (Urrea et al., 2002). Fusarium head blight resistance also has a low correlation with DON accumulation, adding an additional challenge in breeding for resistance to this disease (Lamb et al., 2009). So far, no major resistance gene against FHB has been reported. Numerous biparental mapping studies identified minor-effect QTL that act additively to confer resistance to FHB (de la Pena et al., 1999; Zhu et al., 1999; Ma et al., 2000; Dahleen et al., 2003; Mesfin et al., 2003; Canci et al., 2004; Hori et al., 2005, 2006; Horsley et al., 2006; Sato et al., 2008; Lamb et al., 2009; Yu et al., 2010; Dahleen et al., 2012). These studies have described QTL for FHB resistance, DON accumulation, and kernel discoloration across all seven barley chromosomes (Kolb et al., 2001; Massman et al., 2011); however, the most important regions for FHB resistance lie on chromosomes 2H, 4H, and 6H. Several of the FHB resistance QTL identified in biparental studies coincide with QTL for low DON concentration (e.g., Dahleen et al., 2003; Mesfin et al., 2003). Moreover, QTL for low FHB severity and reduced DON concentration are often associated with genes or QTL for various agromorphological traits, including the spike-type locus *Vrs1*, heading date, and plant height (de la Pena et al., 1999; Zhu et al., 1999; Ma et al., 2000; Dahleen et al., 2003, 2012; Mesfin et al., 2003; Hori et al., 2005; Horsley et al., 2006; Lamb et al., 2009). The only resistance QTL reported to be independent of these agronomic traits is the one on chromosome 2HL in PI 643302 (Yu et al., 2010). The resistance locus at the centromeric region of chromosome 2H was frequently detected in most of the biparental mapping studies (de la Pena et al., 1999; Ma et al., 2000; Hori et al., 2006; Sato et al., 2008; Lamb et al., 2009) but is also associated with late heading (e.g., de la Pena et al., 1999). The bin 10 region of chromosome 2H contains QTL that confer reduced FHB severity and DON concentration in mapping populations with barley accessions Zhedar 2, CIho 4196, and Chevron as the resistant parents (Ma et al., 2000; Dahleen et al., 2003; Horsley et al., 2006).

More recently, extensive germplasm screening of 23,255 cultivated (*H. vulgare* ssp. *vulgare*) and wild (*H. vulgare* ssp. *spontaneum*) barley accessions has identified only a few sources of partial resistance to the disease, mostly in two-rowed types (Huang et al., 2013). The authors collated a group of 78 accessions that possessed partial FHB resistance and haplotyped the group along with susceptible controls and other accessions. They reported that resistant QTL haplotypes on chromosomes 2H and 6H are rare in susceptible cultivars and accessions grown in the Upper Midwest region of the United States. Given the economic importance of FHB in barley, additional resistant haplotypes should be identified and incorporated into breeding programs. Currently, genome-wide association study (GWAS) is becoming a preferred statistical genetic tool to identify QTL underlying important agronomic traits, including disease resistance in plants. The GWAS approach was recently employed in contemporary US barley breeding germplasm and identified QTL associated with FHB severity and DON concentration on all chromosomes except 7H (Massman et al., 2011). Previously, Ethiopian barley accessions with a modest level of FHB resistance were identified (Dahl et al., 2009). With respect to the current research, diverse barley landraces, originally collected from both Ethiopia and Eritrea, were assembled from various international genebanks. The germplasm set was evaluated for FHB, DON accumulation, and various agromorphological traits. Additionally, the germplasm was genotyped with the barley iSelect SNP chip that contains a total of 7842 SNP markers. The specific objectives of the study were to (i) assess phenotypic variation for FHB resistance and DON accumulation in the barley landraces, (ii) identify loci conferring FHB resistance and low DON concentration in the germplasm using a GWAS approach, and (iii) examine the association of QTL for FHB resistance and DON accumulation with various agromorphological traits.

MATERIALS AND METHODS

Plant Materials

Two hundred and ninety-eight Ethiopian and Eritrean barley landraces were used in this study and were designated as the Ethiopian and Eritrean Barley Collection (EEBC). To incorporate as much genetic diversity as possible in the GWAS panel, the following criteria were used to select the landraces. More than 3000 Ethiopian and Eritrean landraces are held by the USDA–ARS National Small Grains Collection (NSGC) in Aberdeen, ID (USDA–ARS National Genetic Resources Program, 2009). Of the accessions in this list, a total of 87 landraces were already included in the barley core collection developed by the USDA–ARS, (USDA–ARS National Genetic Resources

Program, 2000) and were therefore also included in our GWAS panel. To further enhance the panel diversity, additional accessions were included with special emphasis on landraces from geographically diverse regions with historically intensive barley cultivation. The number of accessions representing each region was selected based on the area of barley cultivation within the respective areas. When multiple accessions from a single site were available, the final accessions for the panel were arbitrarily selected. In the end, 132 additional accessions were selected from the NSGC along with the 87 from the core collection. These Ethiopian and Eritrean barley landraces were collected during various expeditions to the countries between the 1920s and 1970s (Qualset, 1975). Seed of the NSGC landraces were provided courtesy of Harold E. Bockelman, the NSGC curator. In addition to the NSGC holdings, additional accessions were arbitrarily selected from different genebanks whose expeditions collected germplasm in different regions and eras than those conducted by the USDA. In this regard, 63 and 17 additional Ethiopian and Eritrean landraces were obtained from the N. I. Vavilov Research Institute of Plant Industry (VIR) (courtesy of Igor Loskotov) and the International Center for Agricultural Research in the Dry Areas (ICARDA) (courtesy of Jan Valkoun), respectively, and included in the panel. Passport data (where available) and other details about the accessions were published in Mamo et al. (2014). To obtain genetically pure seed stocks of the landraces, single plant selections were made from each accession and selfed twice in the greenhouse. Then, a large greenhouse increase was made to provide sufficient seed for all subsequent experiments. All plant grow-outs for seed multiplication were done in a greenhouse within the Plant Growth Facility on the Saint Paul campus of the University of Minnesota in 2010 and 2011.

Sample Preparation and DNA Extraction

Seeds from the third selfed generation (S_3) of each landrace were grown in the greenhouse. Two weeks after planting, leaf tissue was collected. Details regarding sample preparation and DNA extraction were reported in Mamo et al. (2014).

Single Nucleotide Polymorphism Genotyping and Genotype Data Analysis

The EEBC landrace samples were genotyped with the barley iSelect SNP chip of the expanded barley SNP marker platform using the Illumina Infinium II assay (Steemers et al., 2006). The barley iSelect SNP chip contains a total of 7842 SNPs that comprise the 2832 existing barley oligonucleotide pooled assay (BOPA1 and BOPA2) SNPs discovered and mapped previously (Close et al., 2009; Muñoz-Amatriaín et al., 2011) plus 5010 new ones developed from next-generation sequencing data (Comadran et al., 2012). The mapping information for the barley iSelect SNPs was from the consensus map by Muñoz-Amatriaín et al. (2014). Data generated by the Infinium assay were visualized and analyzed with the genotyping module of the GenomeStudio data analysis software GSGT (version 1.8.4) (Illumina). After quality evaluations, genotype calls were generated using the GenCall algorithm (version 6.3.0) implemented in the GenomeStudio software.

Phenotypic Evaluation for Fusarium Head Blight Reaction

Fusarium head blight evaluations were conducted at the Northwest Research and Outreach Center in Crookston, MN, during the summers of 2011 and 2012. The experiment was organized in a randomized complete block design with two replications in both years of the study. Accessions were planted in one-row plots in 2011 and in two-row plots in 2012. Multiple replicates of the following controls were included in the nursery: (i) the early, highly susceptible six-rowed accession PI 383933; (ii) the midseason, six-rowed susceptible cultivar Stander (PI 564743) and midseason, two-rowed susceptible line ICB 111809; and (iii) the later-maturing six-rowed partially resistant accession Chevron (PI 38061) and later-maturing two-rowed partially resistant accession CIho 4196.

A mixture of 19 isolates of *F. graminearum* collected from the Red River Valley area of northwest Minnesota was used for inoculum production. Inoculum production and inoculations were made using the grain spawn method (Horsley et al., 2006). When the majority of accessions were at the five-leaf stage of development, grain spawn was scattered over the barley plots at a rate of 5.60 g colonized grain m^{-2} (5.21 g $plot^{-1}$). To enhance ascospore liberation and disease development, the plots were irrigated from overhead sprinklers. Disease severity (percentage of florets infected) was assessed when the accessions were at the soft-dough stage of development. All of the spikes within a plot were considered in aggregate for the disease assays, and the percentage of florets displaying symptoms were visually estimated on a 1 (most resistant)-to-9 (most susceptible) scale in comparison to the sets of standard controls. Disease assessments were made twice during the growing season and three times for late-maturing accessions. Terminal disease severity data recorded at the soft-dough stage of development were used in GWAS.

Phenotypic Evaluation for Agromorphological Traits

Agronomic and morphological traits may influence the amount of FHB severity observed on barley (Steffenson, 1998). To determine this possible association, row-type morphology (RT), days to heading (DTH), plant height (PH), the number of nodes per cm of rachis in the spike (kernel density; KD), spike angle (SA), and exsertion length (EL) of the peduncle were assessed for all of the landraces. Days to heading was recorded as the interval after planting to the time when 50% of the plants in a row had at least 50% of the spike emerged from the boot. Plant height was measured as the distance from the ground to the top of the spike, excluding the awns. As a supplement to the primary data collected from Crookston, PH of the barley landraces also was measured at the University of Minnesota Agricultural Experiment Station at Saint Paul, MN, in 2010. Kernel density was obtained by dividing the number of nodes (attachment points of seeds to rachis) by the length of spike. Mean number of nodes was calculated by counting the number of seeds in one row of the spike and multiplying by the row type of the landrace (i.e., two or six) on three randomly selected spikes. Spike length was the distance from the bottom of the lowest floret to the top of the highest floret, excluding the awns. Spike angle was estimated at maturity on a 1-to-3 scale

where spikes bending less than 45° from vertical were scored as 1, those bending from about 45 to 90° from vertical scored as 2, and those bending greater than 90° from vertical scored as 3. Exsertion (or peduncle) length was rated as the length from the flag leaf auricle to the bottom of the lowest floret of the spike. Each accession was harvested at maturity and then dried in an oven at 35°C for 2 d before threshing and cleaning the seed. Deoxynivalenol concentration in the grain samples was analyzed using gas chromatography–mass spectrometry (Mirocha et al., 1998; Fuentes et al., 2005) in the Mycotoxin Diagnostic Laboratory at the University of Minnesota. All marker and phenotypic data used in these studies are available at http://triticeaetoolbox.org/barley/.

Statistical Analysis of Phenotypic Traits

Unbiased mean estimates for each trait and an analysis of variance were generated through the restricted maximum likelihood method in SAS (SAS Institute, 2008). Heritability (h^2), defined here as the reproducibility of the observed phenotype in germplasm across experiments, was estimated based on entry means using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$, or $h^2 = \sigma_g^2/[(\sigma_g^2) + (\sigma_y^2/y) + (\sigma_e^2/ry)]$, where σ_g^2 and σ_e^2 are the genotypic and error variance, respectively, r is the number of replications, and y is the number of years the trial was conducted. Components of variance for calculating heritability were generated by the PROC VARCOMP procedure in SAS. Pearson correlation coefficients also were calculated to assess the phenotypic correlation among traits.

Population Structure

Population structure can be present in germplasm panels assembled from diverse geographic regions and during different eras. To account for this effect and decrease the number of false positives in association mapping analyses, the most likely number of subpopulations in the panel was investigated. The clustering program STRUCTURE (version 2.3.4) (Pritchard et al., 2000; Falush et al., 2003) was used to estimate the membership probability of each barley landrace to a number of hypothetical subpopulations. Details regarding the methods used for analysis of population structure in the EEBC was previously reported (Mamo et al., 2014).

Linkage Disequilibrium

Linkage disequilibrium (LD) between markers was measured using Haploview v4.2 (Barrett et al., 2005). In cases where multiple SNP markers mapping to the same location or region were found significantly associated with a trait, LD, as measured by r^2 among the neighboring markers, was calculated and presented as a heat map.

Association Mapping

A mixed linear model (MLM), with efficient mixed model association (EMMA) method (Kang et al., 2008) implemented in the GWAS package rrBLUP (version 4.1) in software R (version 2.15.2) (R Core Team, 2013), was used for association analysis between 5269 SNP markers and FHB severity, DON concentration, and agromorphological traits in Ethiopian and Eritrean barley landraces. The MLM equation used by the rrBLUP package is based on the mixed model (Yu et al., 2005) $\mathbf{y} = \mathbf{X}\beta + \mathbf{u} + \mathbf{e}$, where $\mathbf{y}$ is a vector of phenotypic observation, $\mathbf{X}$ is a vector of SNP marker genotypes, β is a coefficient of the SNP marker effect being estimated, $\mathbf{u}$ is a vector of random (polygene background) effects (effect of individual relatedness estimated as pairwise kinship coefficients), and $\mathbf{e}$ is a vector of residual effects. In the MLM, marker effect ($\mathbf{X}\beta$) represents fixed effect and relative kinship and residuals represent random effects. Variances of the random effects are given as $\text{Var}(\mathbf{u}) = 2\mathbf{K}V_g$ and $\text{Var}(\mathbf{e}) = V_R$, where $\mathbf{K}$ is an $n \times n$ matrix of kinship inferred from genotypes based on the proportion of shared allele values between a pair of individuals (n is number of individuals in the association mapping panel), V_g is the genetic variance, and V_R is the residual variance. The rrBLUP package in R for GWAS incorporates methods for obtaining best linear unbiased estimates of β (fixed effect) and best linear unbiased predictions (BLUP) of $\mathbf{u}$ (random effects) as originally developed (Kang et al., 2008; Yu et al., 2005). The EMMA association analysis method then fitted each SNP into the MLM individually and generated marker scores [$-\log_{10}$(P-values)].

The rrBLUP GWAS function incorporates a package known as QVALUE (Storey and Tibshirani, 2003) for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) (Benjamini and Hochberg, 1995) and handles permutations of q-value (an FDR-adjusted P-value) for each test internally. After correction for multiple testing, markers with a q-value < 0.05 were considered as truly significant associations. Then, the FDR adjusted marker scores were outputted from which P-values were calculated.

Genome-wide association mapping was first conducted with the phenotype data of individual years separately, designated as EEBC-11 (for 2011), EEBC-12 (for 2012), and then with the combined overall entry means of 2 yr, designated as EEBC-C (2011 and 2012), to identify QTL for FHB severity, DON concentration, and related agromorphological traits. These analyses also allowed assessments for the reproducibility of any given QTL region. Next, GWAS for FHB severity and DON concentration was performed with the phenotype data of two-rowed (2R, 110 landraces) and six-rowed germplasm (6R, 180 landraces) separately for each year (designated as EEBC-2R-11, EEBC-2R-12, EEBC-6R-11, and EEBC-6R-12) and mean of the 2 yr combined (EEBC-2R-C and EEBC-6R-C) to reveal possible QTL confounded with row type. To further reduce possible false positives and highlight truly significant associations, only markers found significantly associated with FHB severity or DON concentration in two or more datasets of the mapping panel are reported. Significant associations found in only two-rowed or six-rowed germplasm sets are included as supplemental information. With the exception of RT and KD, none of the agromorphological traits had coincident QTL with FHB severity or DON concentration. Thus, SNP markers significantly associated with DTH, PH, and EL are only presented as supplemental information.

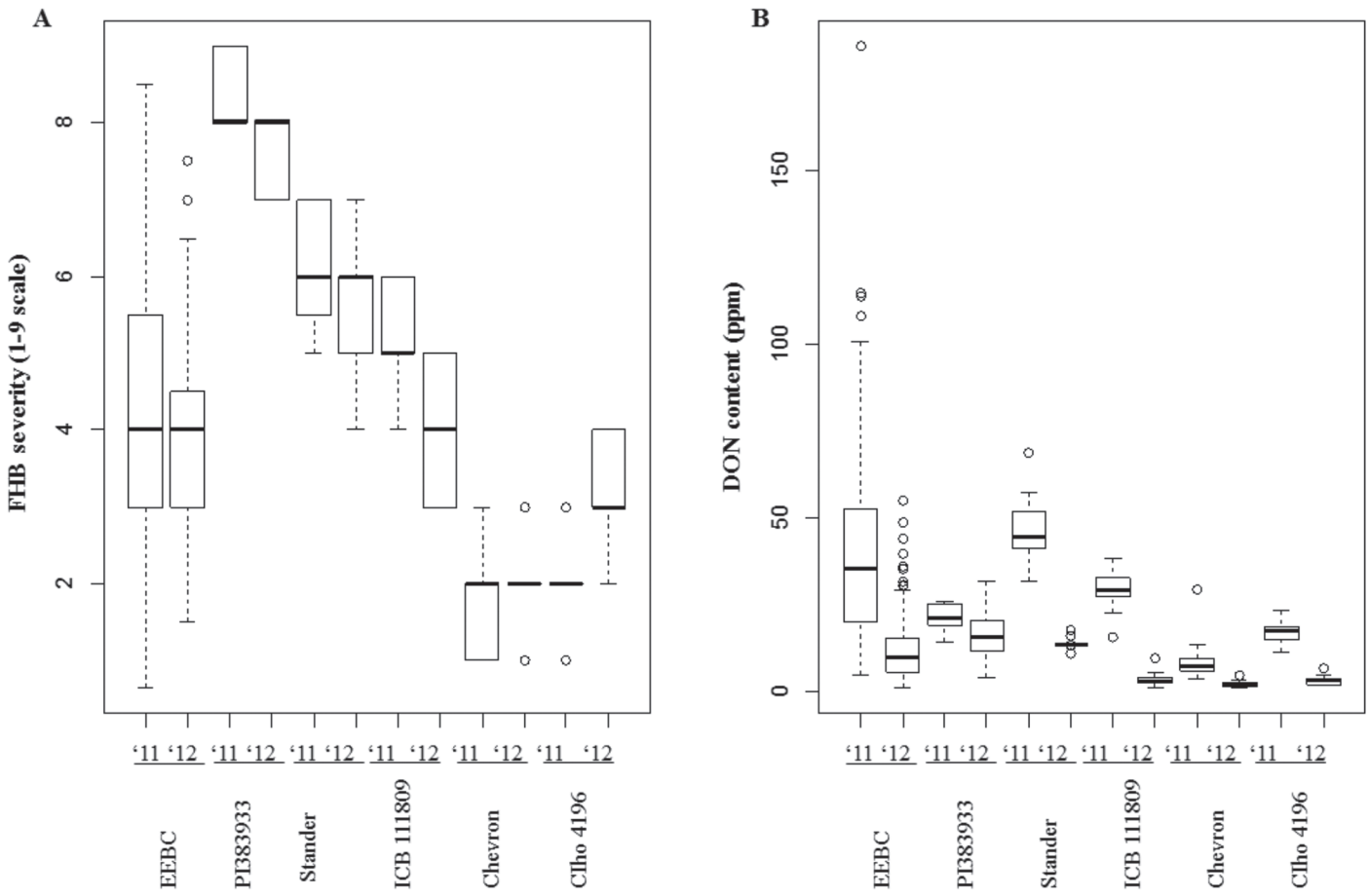

Figure 1. Boxplots of Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, and associated agromorphological traits in the Ethiopian and Eritrean Barley Collection (EEBC) and controls evaluated at Crookston, MN in 2011 and 2012. PI 383933, cultivar Stander and ICB 111809 are the susceptible controls and cultivar Chevron and CIho 4196 are the resistant controls. (A) Fusarium head blight severity data on scale of 1 (most resistant) to 9 (most susceptible) of EEBC germplasm and controls in 2011 and 2012. (B) DON concentration data (mg Kg^{-1}) of EEBC germplasm and controls in 2011 and 2012. (C) Fusarium head blight severity data of two-rowed and six-rowed EEBC germplasm and controls in 2011 and 2012. (D) DON concentration data (mg Kg^{-1}) of two-rowed and six-rowed EEBC germplasm and controls in 2011 and 2012. (E) Agromorphological traits of EEBC germplasm in 2011 and 2012 (DTH, days to heading in days; PH, plant height in cm; KD, kernel density in number of nodes per cm of rachis; SA, spike angle on scale of 1 to 3; EL, exsertion length in cm). Five statistics (bars) are represented in each boxplot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Data points positioned outside this range and depicted as circles are outliers.

RESULTS

Phenotypic Variation for Fusarium Head Blight Reaction and Correlated Traits

Infection in the FHB nurseries was high and uniform in both years of the study, allowing for the clear differentiation of materials with varying levels of resistance. The infection level in 2011 was slightly higher and more variable than in 2012. The highly susceptible and susceptible six-rowed accessions of PI 383933 and Stander had mean FHB severities (1–9 scale) ranging from 7.67 ± 0.47 (2012) to 8.45 ± 0.50 (2011) and from 5.64 ± 0.83 (2011) to 6.18 ± 0.83 (2012), respectively (Fig. 1A). Mean FHB severity of the two-rowed susceptible line ICB 111809 was lower than the six-rowed susceptible controls and ranged from 4.00 ± 0.82 in 2012 to 5.18 ± 0.72 in 2011. The partially resistant six-rowed and two-rowed barley accessions of Chevron and CIho 4196 had mean FHB severities ranging from 1.73 ± 0.62 in 2011 to 2.00 ± 0.43 in 2012 and from 2.09 ± 0.51 in 2011 to 3.22 ± 0.79 in 2012, respectively. The EEBC germplasm exhibited a higher mean and level of variation for FHB severity in 2011 (4.23 ± 1.68) compared to 2012 (4.02 ± 1.12), which was consistent with the disease severity of most controls.

The controls and EEBC accessions exhibited marked differences in DON concentration between the 2 yr of the study, with most having lower DON levels in 2012 (Fig. 1B). The highly susceptible and susceptible six-rowed accessions of PI 383933 and Stander had a mean DON concentration ranging from 16.66 ± 7.27 (2012) to 21.58 ± 3.88 mg Kg^{-1} (2011) and from 13.98 ± 1.58 (2012) to 47.23 ± 9.46 mg Kg^{-1} (2011), respectively. Mean DON concentration of the two-rowed susceptible line ICB 111809 ranged from 3.84 ± 2.35 (2012) to 29.19 ± 6.00 mg Kg^{-1} (2011). The partially resistant six-rowed and

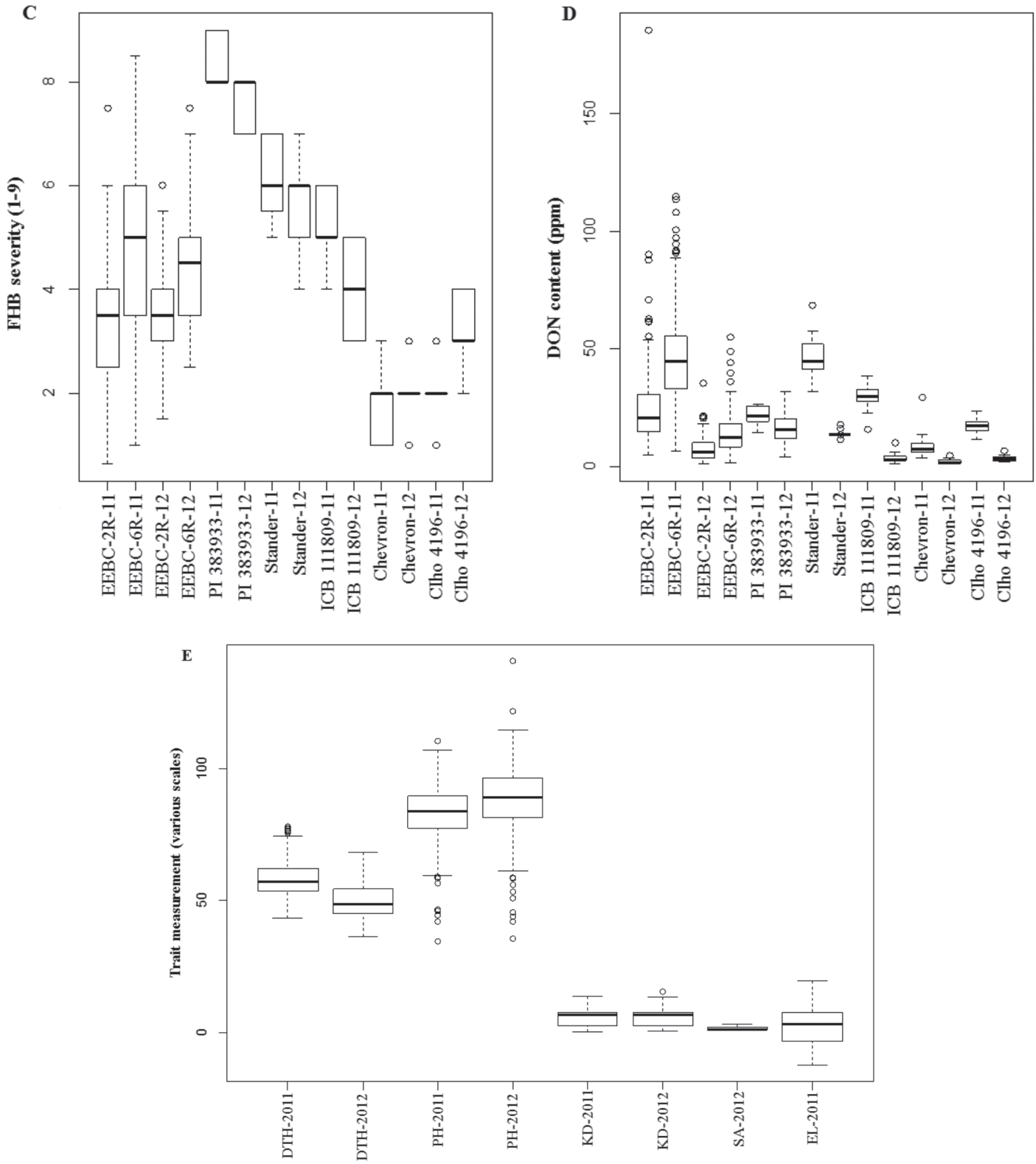

Figure 1. Continued.

two-rowed barley accessions of Chevron and CIho 4196 had mean DON concentrations ranging from 2.19 ± 1.01 (2012) to 9.42 ± 6.81 mg Kg^{-1} (2011) and from 2.37 ± 1.11 (2012) to 10.51 ± 7.07 mg Kg^{-1} (2011), respectively. The EEBC germplasm had a mean DON concentration ranging from 11.62 ± 8.14 (2012) to 39.20 ± 24.42 mg Kg^{-1} (2011). In 2012, warm weather hastened senescence of the germplasm (harvested ~1 wk earlier) and may have contributed to the lower DON concentrations observed. The lower mean precipitation (15% less) in 2012 in the period from planting to harvest maturity may also have played a role in the reduced DON concentration.

Table 1. Components of variance and heritability of resistance to Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, and various agromorphological traits (days to heading, plant height, kernel density, spike angle, and spike exsertion length) in the Ethiopian and Eritrean Barley Collection.

Trait	Variance components			Heritability
	Genotype	Year	Residuals	
FHB	2.83	0.03	0.02	0.99
DON	75.42	272.34	206.18	0.29
Days to heading	48.93	34.48	10.79	0.71
Plant height	170.21	16.96	19.60	0.93
Kernel density	7.02	0.0038	0.64	0.98
Spike angle	0.54	na†	−0.03	1.00
Exsertion length	27.84	na	27.18	0.67

† na, not applicable.

Table 2. Pearson correlation coefficients for Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, and various agromorphological traits (days to heading, plant height, kernel density, and spike exsertion length) in the Ethiopian and Eritrean Barley Collection evaluated at Crookston, MN, in 2011.

Trait†	RT	FHB	DON	DTH	PH	KD	EL
RT	–‡						
FHB	0.41**	–					
DON	0.38**	0.30**	–				
DTH	0.30**	−0.41**	0.19**	–			
PH	−0.13**	−0.30**	−0.01	0.07	–		
KD	0.92**	0.50**	0.36**	0.22**	−0.19**	–	
EL	−0.32**	0.17**	−0.17**	−0.62**	0.41**	−0.30**	–

** Significant at the 0.01 probability level. The rest are not significantly different from zero at this probability level.

† RT, row type; FHB, Fusarium head blight severity; DON, deoxynivalenol concentration; DTH, days to heading; PH, plant height; KD, kernel density; EL, exsertion length.

‡ –, perfect correlation (r = 1.00).

Two-rowed barleys usually sustain lower infection levels of FHB than six-rowed barleys (Zhou et al., 1991; Choo et al., 2004). To assess whether this same trend occurs in the EEBC germplasm, the 110 two-rowed accessions were analyzed separately from the 180 six-rowed accessions. Eight of the EEBC accessions exhibited an irregular row-type morphology and two were segregating for row type. As expected, two-rowed accessions had lower mean FHB severities (3.40 ± 1.16 and 3.47 ± 0.94 for 2011 and 2012, respectively) than six-rowed accessions (4.77 ± 10.74 and 4.37 ± 1.07 for 2011 and 2012, respectively) in both years (Fig. 1C). The same trend was observed for mean DON concentration with two-rowed types having 27.47 ± 23.34 and 7.47 ± 5.47 mg Kg^{-1} and six-rowed types having 46.59 ± 22.32 and 14.24 ± 8.48 mg Kg^{-1} for 2011 and 2012, respectively (Fig. 1D). Heritability was 0.99 for FHB severity and 0.29 for DON concentration (Table 1).

Agromorphological traits (DTH, PH, KD, SA, and EL) previously reported to possibly impact the level of FHB in barley also were investigated in the field. The traits showed a low-to-moderate degree of variation in the EEBC germplasm (Fig. 1E). The germplasm had a mean DTH ranging from 49.81 ± 6.91 d in 2012 to 58.31 ± 7.74 d in 2011. Mean PH of the test entries ranged from 82.27 ± 11.02 cm in 2011 to 87.57 ± 13.12 cm in 2012. Mean KD ranged from 5.58 ± 2.64 nodes cm^{-1} of rachis in 2011 to 5.78 ± 2.76 nodes cm^{-1} of rachis in 2012. The means for SA and EL were 1.51 ± 0.68 and 2.25 ± 7.11 cm, respectively. Heritability estimates were 0.71 for DTH, 0.93 for PH, 0.98 for KD, 1.00 for SA, and 0.67 for EL (Table 1).

Correlation of Fusarium Head Blight, Deoxynivalenol, and Associated Agromorphological Traits

Correlation analysis was conducted to determine the degree of association between the disease and toxin phenotypes (FHB and DON) and various agromorphological traits (RT, DTH, PH, KD, SA, and EL). Significantly positive or significantly negative correlations were detected between FHB severity, DON concentration, and the agromorphological traits in 2011 (when all traits except SA were measured) (Table 2). Correlation analysis for FHB severity, DON concentration, DTH, PH, and KD between the 2011 and 2012 datasets revealed a number of significant positive correlations (Table 3).

Marker Data

The platform used for genotyping the GWAS panel consisted of 7842 SNPs of the barley iSelect SNP chip. After quality evaluations during genotype calling, 85% (6702 out of 7842) of the SNPs generated reliable allele data with a 95% call rate for all accessions. Nearly 99% of these SNPs (6615 out of 6702) had a >95% call frequency. Filtering based on minor allele frequency (MAF) resulted in 5269 SNPs with a MAF >5%, which were subsequently used for association analyses. Of these, 72.46% (3818 out of 5269) had known map positions on the seven barley chromosomes spanning 1112.71 cM with the remaining set (1451 SNPs) yet to be mapped. Both mapped and unmapped markers were used for running GWAS. Of the mapped SNPs, 38.6% (1473 out of 3818) had unique map positions and the rest had redundant map locations. The average density of SNP markers across the genome using all mapped SNPs and unique SNPs was 3.43 and 1.32 SNP markers per cM, respectively. Gaps between SNP markers ranged from 0 to 9.32 cM with a mean of 0.77 cM. Only eight gaps of >5 cM were identified: two each on chromosomes 1H and 7H and one each on chromosomes 2H, 3H, 5H, and 6H.

Population Structure

STRUCTURE and principal component analysis (PCA) were implemented for population structure analysis. From the STRUCTURE analysis, six subpopulations were delineated (Mamo et al., 2014). However, no clear

Table 3. Pearson correlation coefficients for Fusarium head blight severity, deoxynivalenol concentration, days to heading, plant height, and kernel density data in the Ethiopian and Eritrean Barley Collection evaluated at Crookston, MN in 2011 and 2012.

Trait†	FHB11	FHB12	DON11	DON12	DTH11	DTH12	PH11	PH12	KD11	KD12
FHB11	–									
FHB12	0.61**	–								
DON11	0.29**	0.26**	–							
DON12	0.43**	0.46**	0.48**	–						
DTH11	−0.38**	−0.17**	0.24**	−0.004	–					
DHT12	−0.30**	−0.19**	0.29**	0.01	0.76**	–				
PH11	−0.33**	−0.33**	−0.03	−0.10	0.11	0.09	–			
PH12	−0.19**	−0.25**	0.001	−0.15	0.10	0.07	0.55**	–		
KD11	0.53**	0.48**	0.37**	0.44**	0.21**	0.17**	−0.19**	−0.06	–	
KD12	0.48**	0.47**	0.36**	0.43**	0.19**	0.18**	−0.14	−0.03	0.88**	–

** Significant at the 0.01 probability level. The rest are not significantly different from zero at this probability level.

† FHB, Fusarium head blight severity in 2011 and 2012; DON, deoxynivalenol concentration in 2011 and 2012; DTH, days to heading in 2011 and 2012; PH, plant height in 2011 and 2012; and KD, kernel density in 2011 and 2012.

clustering of two- vs. six-rowed groups was obtained as is often found in advanced breeding germplasm (Pasam et al., 2012). Two-rowed landraces were grouped into subpopulations 1, 2, 4, and 5, whereas six-rowed ones were grouped into subpopulations 1, 3, and 6. Moreover, no distinct clustering was found for accessions from the different countries of Ethiopia (269 accessions) and Eritrea (29 accessions). Accessions from the ICARDA genebank were mainly grouped into subpopulations 3 and 4, and those from the VIR genebank were mainly clustered in subpopulations 1, 3, 4, and 6. Subpopulations 1, 2, 5, and 6 were mainly composed of landraces from the NSGC genebank. Subpopulations 3 and 6 were the most genetically heterogeneous as revealed by the highest expected heterozygosity values between individuals in clusters (Mamo, 2013). Based on the PCA analysis, there was no readily apparent population structure differentiating the germplasm (Supplemental Fig. S1). Results for PC1, PC2, and PC3 account, respectively, for 26.05, 7.19, and 3.76% of the variation among the lines. The only apparent clustering in the PCA plots was for the landraces that were obtained from the NSGC genebank. Thus, there is a lack of apparent population structure in the association mapping panel. Therefore, the possible presence of any sample structure was corrected for using the pairwise relatedness between individuals through application of the kinship model.

Genome-Wide Association Mapping of Barley Spike Morphology

To analyze the reliability of the GWAS model for localizing true marker–trait associations and evaluating mapping resolution within the germplasm panel, GWAS was conducted on the spike morphology trait of row type. This trait is mainly controlled by the well-characterized Mendelian gene *Vrs1*, located on chromosome 2H, as well as other genes (Komatsuda et al., 2007; Ramsay et al., 2011). The GWAS model identified 14 SNP markers significantly associated with the row-type locus: eight on chromosome 2HL, one each on chromosomes 4H and 5H, and four unmapped markers (Table 4; Supplemental Table S2; Mamo et al., 2014). Basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) database (Acland et al., 2014) with sequences of the significant SNP markers on chromosome 2H identified the coding sequences of the *H. vulgare* homeodomain-leucine zipper protein (*Vrs1* gene) with ≥99% identity, indicating that the SNP markers are likely located within the coding region of the gene. The significant SNPs on chromosome 2HL were mapped within a short distance (4.03 cM) of each other and are most likely detecting the same *Vrs1* locus. Linkage disequilibrium as measured by r^2 ranged from 0.00 (between SCRI_RS_171032 and 12_30897) to 0.99 (between 12_30897 and SCRI_RS_196853) (Mamo et al., 2014). Linkage disequilibrium between the most significant marker (12_30896) and the rest of the markers ranged from 0.10 (with SCRI_RS_221886) to 0.88 (with 11_20340), indicating that some of the markers are in high LD with the underlying gene controlling spike morphology.

The significant marker on 4H (Supplemental Table S2) maps to the reported position of INTERMEDIUM-C (*Int-c*) (Cuesta-Marcos et al., 2010), a gene that influences both the fertility of the lateral spikelets on the inflorescence and the amount of the basal branching in barley (Ramsay et al., 2011). The one significant marker on chromosome 5H is most likely detecting the six-rowed spike 2 (*vrs2*) or the intermedium spike-b (*int-b*) locus (Lundqvist and Franckowiak, 1997; Mamo et al., 2014). Our results are in close agreement with previous studies (Cuesta-Marcos et al., 2010). This GWAS analysis indicates the strong power of the association mapping model to account for pairwise relatedness and achieve good mapping resolution within the germplasm panel. Accordingly, the association mapping model was used for marker–trait associations of other phenotypes.

Table 4. Single nucleotide polymorphism (SNP) markers significantly associated with Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, row-type morphology (RT), and kernel density (KD) in the Ethiopian and Eritrean Barley Collection evaluated at Crookston, MN, in 2011 and 2012.

			q-value for FHB[§]								
SNP marker	Chromosome[†]	Position[‡]	EEBC-C	EEBC-11	EEBC-12	EEBC-2R-C	EEBC-2R-11	EEBC-2R-12	EEBC-6R-C	EEBC-6R-11	EEBC-6R-12
11_20340	2H	90.99	1.44×10^{-8}[¶]	2.14×10^{-8}	8.51×10^{-8}	*0.07*	*0.04*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	9.88×10^{-8}	1.62×10^{-8}	1.95×10^{-6}	*1.00*	*1.00*	*1.00*	*0.66*	*0.49*	*0.71*
SCRI_RS_72983	Unknown	Unknown	*2.06×10^{-5}*	*5.02×10^{-5}*	1.114×10^{-5}	*0.73*	*0.89*	*0.08*	*1.00*	*1.00*	*1.00*
			q-value for DON[§]								
SNP marker	Chromosome[†]	Position[‡]	EEBC-C	EEBC-11	EEBC-12	EEBC-2R-C	EEBC-2R-11	EEBC-2R-12	EEBC-6R-C	EEBC-6R-11	EEBC-6R-12
SCRI_RS_171032	2H	90.64	9.04×10^{-6}[¶]	*9.54×10^{-5}*	*6.80×10^{-4}*	*3.12×10^{-4}*	1.74×10^{-4}	*1.19×10^{-3}*	*1.00*	*1.00*	*1.00*
12_30901	2H	90.99	3.39×10^{-6}	*5.69×10^{-5}*	*1.22×10^{-4}*	6.73×10^{-5}	2.11×10^{-4}	*4.61×10^{-4}*	*1.00*	*1.00*	*1.00*
11_20340	2H	90.99	4.21×10^{-6}	*8.71×10^{-4}*	1.85×10^{-5}	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*	*1.00*
12_30896	2H	91.09	*4.55×10^{-5}*	*9.04×10^{-3}*	2.93×10^{-5}	*1.00*	*1.00*	*1.00*	*0.90*	*0.89*	*0.42*
12_11485	4H	12.91	*7.93×10^{-4}*	*0.03*	2.45×10^{-8}	*0.42*	*0.88*	*0.02*	7.50×10^{-6}	*1.18×10^{-3}*	1.47×10^{-5}
SCRI_RS_148392	4H	65.52	7.74×10^{-6}	*0.01*	3.26×10^{-8}	*0.02*	*0.18*	*0.01*	3.23×10^{-4}	*0.04*	4.86×10^{-6}
SCRI_RS_157650	4H	65.52	*1.77×10^{-4}*	*0.06*	1.22×10^{-6}	*0.03*	*0.21*	*0.03*	*3.05×10^{-3}*	*0.14*	*7.02×10^{-5}*
SCRI_RS_165473	Unknown	Unknown	7.75×10^{-7}	4.54×10^{-6}	*8.38×10^{-5}*	6.70×10^{-5}	2.94×10^{-4}	*1.67×10^{-4}*	*1.00*	*1.00*	*1.00*
SCRI_RS_72983	Unknown	Unknown	1.06×10^{-6}	*1.83×10^{-5}*	*7.09×10^{-5}*	1.51×10^{-5}	7.36×10^{-5}	*1.43×10^{-4}*	*1.00*	*1.00*	*1.00*
			q-value for RT[#]								
SNP marker	Chromosome[†]	Position[‡]	EEBC								
11_20781	2H	88.04	6.79×10^{-6}[¶]								
SCRI_RS_171032	2H	90.64	1.63×10^{-11}								
11_20340	2H	90.99	9.30×10^{-53}								
12_30901	2H	90.99	3.78×10^{-20}								
12_30896	2H	91.09	1.52×10^{-55}								
SCRI_RS_196853	2H	91.09	2.30×10^{-8}								
12_30897	2H	91.09	4.89×10^{-8}								
SCRI_RS_221886	2H	92.07	7.66×10^{-5}								
SCRI_RS_165473	Unknown	Unknown	1.97×10^{-21}								
SCRI_RS_72983	Unknown	Unknown	8.15×10^{-20}								
SCRI_RS_181051	Unknown	Unknown	7.91×10^{-5}								
SCRI_RS_237894	Unknown	Unknown	1.09×10^{-4}								

(cont'd)

Table 4. Continued.

SNP marker	Chromosome†	Position‡	*q*-value for KD†† EEBC-C
11_20781	2H	88.04	2.61×10^{-6}¶
SCRI_RS_171032	2H	90.64	3.56×10^{-10}
11_20340	2H	90.99	1.58×10^{-46}
12_30901	2H	90.99	1.55×10^{-16}
12_30896	2H	91.09	7.68×10^{-47}
12_30897	2H	91.09	1.40×10^{-10}
SCRI_RS_196853	2H	91.09	1.56×10^{-10}
SCRI_RS_221886	2H	92.07	2.34×10^{-5}
SCRI_RS_165473	Unknown	Unknown	4.20×10^{-20}
SCRI_RS_72983	Unknown	Unknown	1.23×10^{-19}

† Note: some markers have not been mapped.

‡ Genetic position (in cM) of marker on chromosome. Note: some markers have not been mapped to a chromosomal position.

§ EEBC-C, Ethiopian and Eritrean Barley Collection with combined data of 2011 and 2012; EEBC-11, test from 2011 that includes data from the entire collection; EEBC-12, test from 2012 that includes data from the entire collection; EEBC-2R-C, combined 2-yr (2011 and 2012) data of two-rowed accessions only; EEBC-2R-11, 2011 data of two-rowed accessions only; EEBC-2R-12, 2012 data of two-rowed accessions only; EEBC-6R-C, combined 2-yr data of six-rowed accessions; EEBC-6R-11, 2011 data of six-rowed accessions only; EEBC-6R-12, 2012 data of six-rowed accessions only.

¶ *q*-value (multiple testing corrected *P*-value) in exponential expression. Nonsignificant *q*-values are presented for comparison purposes and are indicated in italics.

EEBC, comprises 110 two-rowed and 180 six-rowed germplasm.

†† EEBC-C, combined 2-yr data of the entire collection.

Genome-Wide Association Mapping of Fusarium Head Blight Severity

Using the combined overall entry means of 2 yr (EEBC-C), two SNP markers were found significantly associated with FHB severity (Table 4; Fig. 2A; Supplemental Table S1). The markers (11_20340 and 12_30896) were located on chromosome 2HL within a very narrow interval of 0.10 cM and were likely detecting the same QTL. Indeed, LD between the markers was very high (r^2 = 0.88), indicating that they were associated with the same QTL. The most significant marker was 11_20340 (Table 4; Fig. 2A). Quantitative trait loci associated with SNP markers were named according to the following convention: *Rfg-*, indicating the pathogen abbreviation of reaction to *F. graminearum*; *qtl-*, indicating the quantitative nature of the trait; *2H-*, indicating the chromosome on which the marker lies; and 11_20340, indicating the name of the most significant marker associated with the trait. Thus, the resistance QTL on chromosome 2HL was designated *Rfg-qtl-2H_11_20340*. The two markers identified in the combined dataset also were significant in individual years (Table 4). The *Rfg-qtl-2H_11_20340* also is associated with the row-type locus *Vrs1*. Genome-wide association study scans for marker associations across datasets for individual years are presented in Supplemental Fig. S2A,B.

Genome-Wide Association Mapping of Deoxynivalenol Concentration

In the combined dataset (EEBC-C), six SNP markers were found significantly associated with DON concentration (Table 4; Fig. 2B; Supplemental Table S1). Three of the markers were located on chromosome 2HL, one on chromosome 4HL, and the other two were unmapped (Table 4). The three markers on chromosome 2HL were located within a narrow genetic distance of 0.35 cM and were likely detecting the same QTL. With respect to LD, marker SCRI_RS_171032 was in moderately high LD with the other two associated markers 12_30901 and 11_20340 (r^2 = 0.55 and 0.53, respectively). The 12_30901 and 11_20340 markers had the same exact map location, but the LD between them was lower (r^2 = 0.39). The most significant SNP identified was SCRI_RS_171032, and thus the QTL was designated *DON-qtl-2H_SCRI_RS_171032*. This QTL coincides with *Rfg-qtl-2H_11_20340* described above for FHB severity and also the row-type locus *Vrs1*. The *DON-qtl-SCRI_RS_171032* QTL also was associated with DON concentration within the 2012 DON dataset (EEBC-12) as the other marker (11_20340) representing the same QTL was significant in both the EEBC-C and EEBC-12 datasets (Table 4; Fig. 2C). Another marker (12_30896) mapping 0.10 cM proximal to 11_20340 also was significant in the EEBC-12 dataset (Table 4) and was probably detecting the same QTL. Marker 12_30896 was in high LD with marker 11_20340 (r^2 = 0.88). On

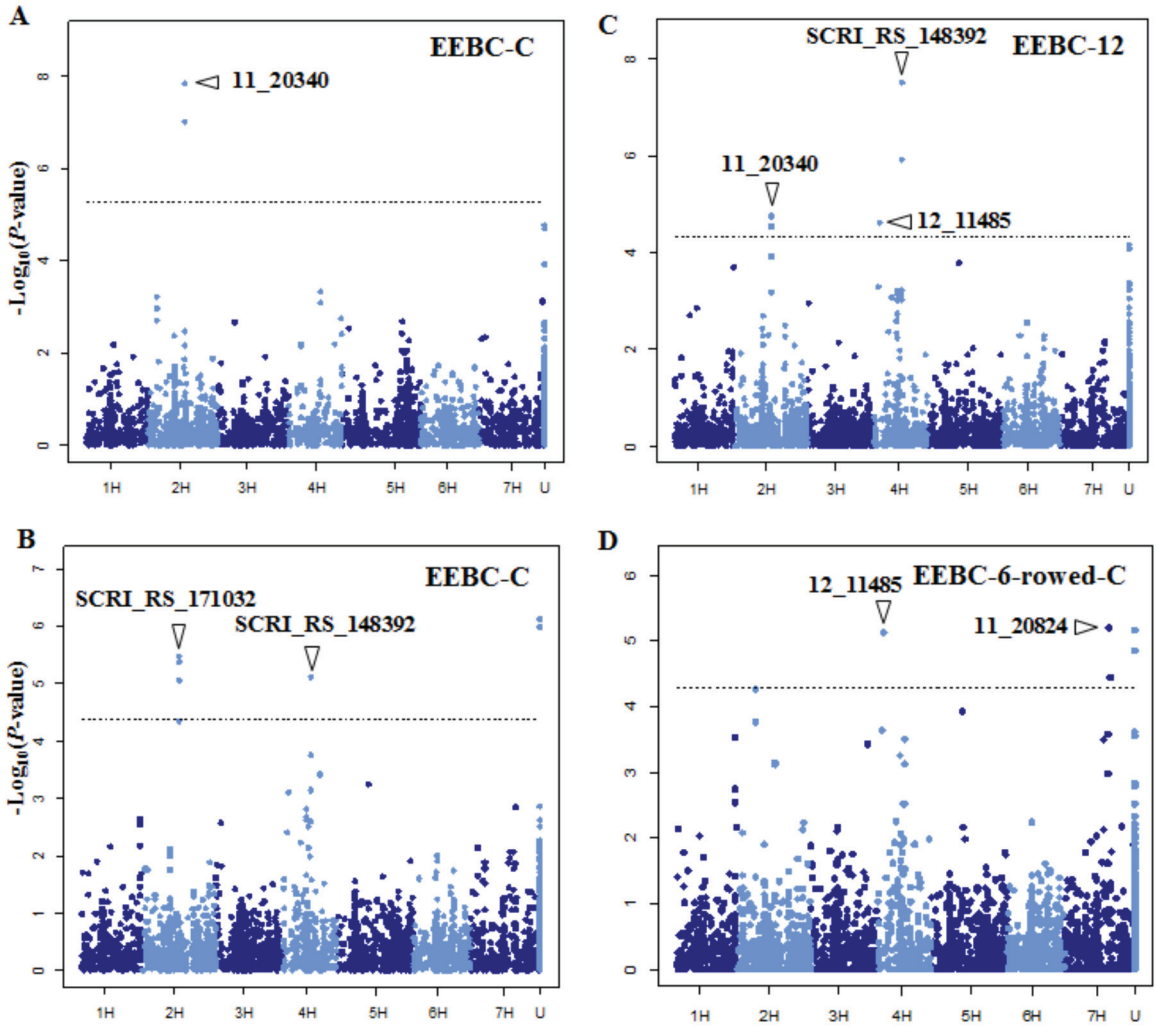

Figure 2. Genome-wide association scan for marker associations with Fusarium head blight (FHB) severity, deoxynivalenol (DON) concentration, and kernel density (KD) in the Ethiopian and Eritrean Barley Collection (EEBC) evaluated at Crookston, MN in 2011 and 2012. Scans are shown for (A) FHB severity for the whole set of landrace germplasm combined over 2 yr (EEBC-C); (B) DON concentration for the whole set of landrace germplasm combined over 2 yr (EEBC-C); (C) DON concentration for the individual year of 2012 (EEBC-12); (D) combined DON concentration in the six-rowed germplasm set (EEBC-6-rowed-C) only; (E) DON concentration in the six-rowed germplasm set in 2012 (EEBC-6-rowed-12) only; (F) combined DON concentration in the two-rowed germplasm set (EEBC-2-rowed-C) only; (G) DON concentration in the two-rowed germplasm set in 2011 (EEBC-2-rowed-11) only; and (H) kernel density for the whole set of landrace germplasm combined over 2 yr (KD-EEBC-C). Vertical axis represents $\log_{10}$(*P*-values) of the marker–trait associations after correction for multiple testing, and the horizontal axis represents the relative chromosomal position of the single nucleotide polymorphism (SNP) markers across the genome. The black horizontal dotted lines show the *P*-value corresponding to a false discovery rate (FDR) of 0.05. The SNP markers with peaks above this threshold level were considered as significantly associated. The most significant marker for each trait or analysis panel is marked with arrows. The full names of two of the most significant markers indicated in (G) on chromosome 2HL are SCRI_RS_130072 and SCRI_RS_139737.

chromosome 4HL, one marker (SCRI_RS_148392) was found significantly associated with DON concentration in the EEBC-C dataset (Table 4; Fig. 2B). This marker, plus another one (SCRI_RS_157650) with the exact map location, also were found significant in the EEBC-12 dataset (Table 4; Fig. 2B,C). Linkage disequilibrium between these markers was high (r^2 = 0.84), indicating that both were likely detecting the same QTL for DON. Since SCRI_RS_148392 was the most significant marker identified, the QTL was designated as *DON-qtl-4H-SCRI_RS_148392* (65.52 cM). On chromosome 4HS, one marker (12_11485) was significantly associated with DON concentration in the EEBC-12 DON dataset (Table 4; Fig. 2C), and the QTL associated with it was designated as *DON-qtl-4H-12_11485* (12.91 cM). Two other significantly associated markers (SCRI_RS_165473 and SCRI_RS_72983) in the EEBC-C dataset have not been assigned map locations (Table 4; Fig. 2B). Marker SCRI_RS_165473 also was significant in the 2011 DON dataset (EEBC-11) (Table 4). No other significant associations were detected in the EEBC-11 dataset (Supplemental Fig. S3A).

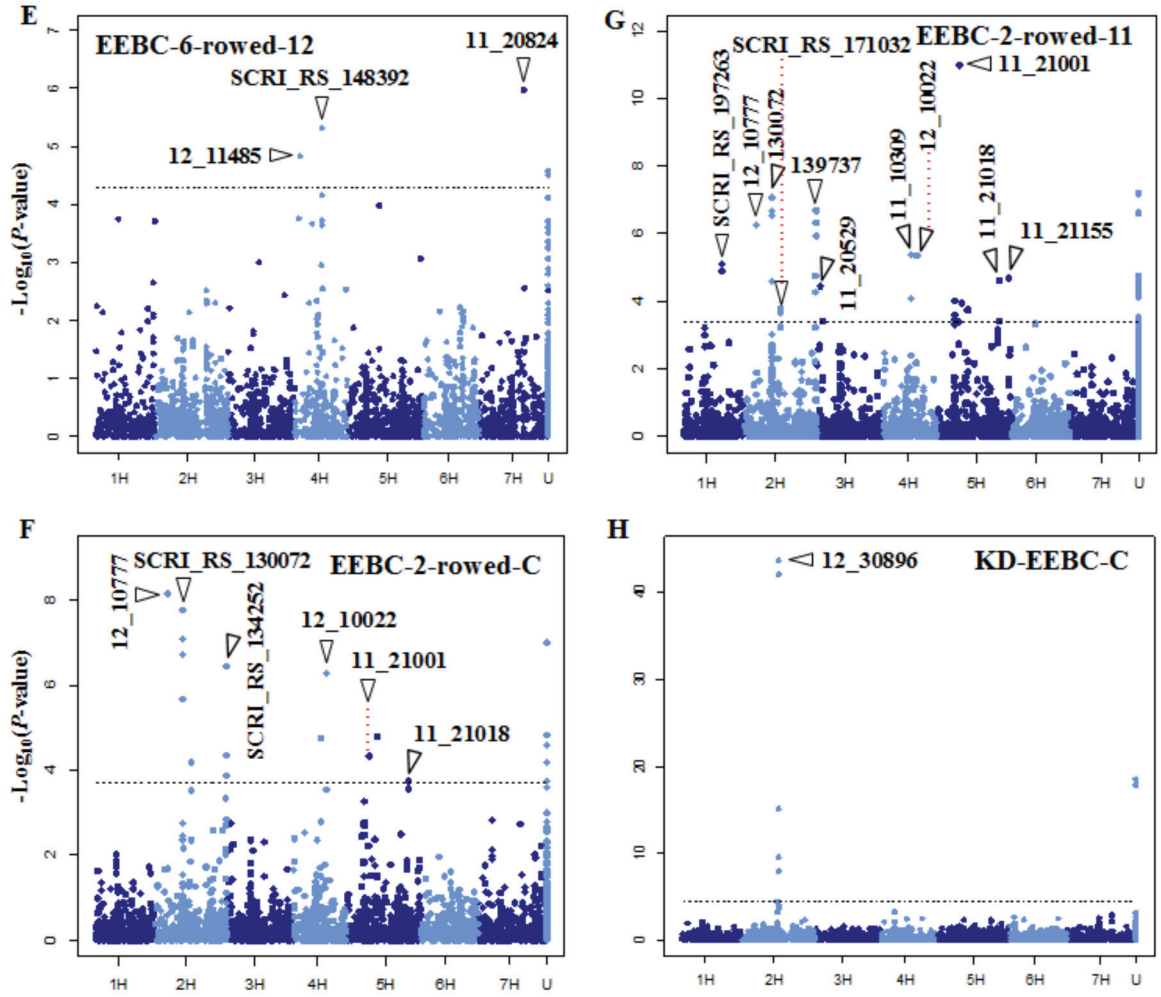

Figure 2. Continued.

Row-type morphology is a prominent trait delineating barley accessions in population structure-based analyses. It also may influence the level of FHB, due either to the underlying genes having a pleiotropic effect or close linkage to genes controlling the FHB reaction. Not surprisingly, the two markers on chromosome 2HL significantly associated with FHB resistance and DON accumulation also were associated with the row-type locus *Vrs1*. The SNP markers associated with *Rfg-qtl-2H_11_20340* and *DON-qtl-SCRI_RS_171032* were two of the most significant markers associated with the row-type trait (Table 4). The consistent significance of these markers suggests that the QTL for FHB resistance and DON accumulation coincide with the spike-type locus *Vrs1* as reported in other studies. To further investigate the possible relationship of row type with FHB severity and DON concentration, GWAS for the two traits were conducted separately for the two- and six-rowed germplasm datasets.

For FHB severity, three mapped markers (one each on chromosomes 1H, 4H, and 7H) and three unmapped markers were significantly associated in the two-rowed germplasm datasets of the combined 2-yr data or the 2012 set (Supplemental Table S1; Supplemental Fig. S2C,E). No significant markers were identified in the separate two-rowed lines of the 2011 FHB severity dataset or any datasets of the six-rowed lines (Supplemental Table S1; Supplemental Figs. S2D,G–H). The 2HL markers (11_20340 and 12_30896) significantly associated with the row-type *Vrs1* locus and the unmapped marker (SCRI_RS_72983) significantly associated with FHB severity in the whole-germplasm set were not significant in the separate two- or six-rowed germplasm datasets (Table 4; Supplemental Table S1). For DON concentration, the four SNP markers (SCRI_RS_171032 and 12_30901 on chromosome 2HL and the unmapped markers SCRI_RS_165473 and SCRI_RS_72983) found significantly associated with DON concentration in the EEBC-C DON dataset were significant in one of the separate two-rowed germplasm datasets (Table 4; Supplemental Table S1). These markers were not significant in any dataset of the separate six-rowed lines (Table 4). On the other hand, marker 12_11485 on 4HS, identified initially in the EEBC-12 DON dataset, also was significant in two of the separate six-rowed germplasm DON datasets (Table 4; Fig. 2D,E; Supplemental

Table S1). Similarly, the SNP marker on chromosome 4HL (SCRI_RS_148392) found significantly associated with DON concentration in the EEBC-C and EEBC-12 DON datasets was significant in two of the separate six-rowed germplasm datasets (Table 4; Fig. 2D,E; Supplemental Table S1). In addition, 43 markers belonging to fifteen different genomic locations and ten other unmapped markers were found significantly associated with DON concentration exclusively in the separate two- and six-rowed germplasm sets (Supplemental Table S1). The description of results for these significant associations is presented as supplemental information (Supplemental Text S1).

Genome-Wide Association Mapping of Correlated Agromorphological Traits

In addition to spike type, FHB severity and DON concentration also may be influenced by other agromorphological traits. Thus, GWAS was conducted on DTH, PH, KD, SA, and EL to investigate whether QTL underlying correlated agromorphological traits map at a coincident location with QTL for FHB resistance and DON accumulation.

Days to Heading

Three markers on chromosome 2HS and six markers of unknown map location were significantly associated with DTH in the EEBC-C and EEBC-11 datasets (Supplemental Table S2; Supplemental Fig. S4A). The same three markers on chromosome 2HS also were significantly associated with DTH in the EEBC-11 dataset (Supplemental Table S2; Supplemental Fig. S5A). In the EEBC-12 dataset, strong but nonsignificant signals appeared on chromosome 1HL (Supplemental Fig. S5B). None of these markers lie at a coincident location with QTL conferring lower FHB severity or DON concentration. The description of results for the SNP markers significantly associated with DTH is presented as supplemental information (Supplemental Text S2).

Plant Height

No significant marker associations were detected for PH in any of the datasets from the Crookston location (Supplemental Table S2; Supplemental Fig. S5C). However, six markers were found significantly associated with PH at Saint Paul (another location where the barley germplasm was evaluated for morphological traits): four on chromosome 1H, one on 5H, and one unmapped (Supplemental Table S2; Supplemental Fig. S4B). None of these markers were associated with QTL identified for low FHB severity or DON concentration. The description of results for the SNP markers significantly associated with PH at Saint Paul is presented as supplemental information (Supplemental Text S2).

Kernel Density

A dense arrangement of nodes (seeds) along the rachis may facilitate the spread of FHB in spikes, leading to higher FHB severities. To investigate whether QTL underlying KD coincide with QTL for FHB severity and DON concentration, GWAS was conducted for the trait. Based on the EEBC-C dataset, 10 markers were found significantly associated with KD: eight on chromosome 2HL and two that were unmapped (Table 4; Fig. 2H; Supplemental Table S2; Supplemental Fig. S4C). Of the eight significant markers on 2HL, marker 12_30896 had the highest significance level; thus, the QTL was designated as *KD-qtl-2H-12_30896*. Seven of the markers mapped within a 1.43 cM genetic interval and one (11_20781) was 3.10 cM away; thus, it is likely that all eight markers are detecting the same QTL. In fact, the same eight markers were significantly associated with the spike morphology trait of *Vrs1* (see above). This QTL (*KD-qtl-2H-12_30896*) maps to a coincident location with the one(s) associated with row-type morphology, FHB severity, and DON concentration (Table 4; Fig. 2A,B,H). Actually, these four traits share some significantly associated markers in common (Table 4).

Spike Angle

Upright spikes may tend to hold water longer (especially in crevices) than those with a nodding profile, possibly leading to greater FHB infection. To assess whether any significant effect could be detected for this trait and if such identified QTL were coincident for those controlling FHB severity and DON concentration, GWAS was conducted for SA. No significantly associated markers were detected in any dataset for SA (data not shown).

Exsertion Length

The extent to which the spike extends above the flag leaf sheath may influence FHB development as the leaf sheath retains moisture around the nonemerged portions of the spike, thereby facilitating greater infection. Thus, GWAS was conducted for EL, and a significantly associated marker of unknown map position was detected (Supplemental Table S2). This marker was not significantly associated with any of the other traits.

Allele Effect of Significant Markers on Trait

The allele frequency and allelic effect (trait means) of each QTL identified in the combined dataset or two different subsets of data in reducing the levels of FHB severity and DON accumulation were estimated (Table 5). The positive allele A of the FHB QTL *Rfg-qtl-2H_11_20340* associated with both disease severity and DON concentration gave a mean disease severity of 2.21 in two-rowed accessions. The corresponding B allele gave a mean disease severity of 4.76 in the six-rowed germplasm set. However, the most common allele B of this QTL resulted in a mean DON concentration of 16.93 and 22.44 mg Kg^{-1} in two-rowed and six-rowed accessions, respectively. In addition to marker 11_20340, another SNP marker

Table 5. Trait means for each allele at chromosome positions significantly associated with Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration identified in the combined dataset or two different sub-sets of data in the Ethiopian and Eritrean Barley Collection.

Germplasm	Trait	QTL or marker	Position (cM)	Allele	FHB	DON	Allele frequency†	Identified in‡
Whole set	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	A	4.37	28.54	0.61	EEBC-C, EEBC-11 and EEBC-12
Whole set	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	B	3.53	17.24	0.39	
Two-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	A	2.21	25.10	0.06	
Two-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	B	3.45	16.93	0.94	
Six-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	A	4.48	28.70	0.95	
Six-rowed	FHB + DON	*Rfg-qtl-2H_11_20340*	2H 90.99–91.09	B	4.76	22.44	0.05	
Whole set	DON	*12_30901*	2H 90.99	A	3.36	14.29	0.20	EEBC-C
Whole set	DON	*12_30901*	2H 90.99	B	4.26	26.86	0.80	
Two-rowed	DON	*12_30901*	2H 90.99	A	3.35	14.18	0.51	
Two-rowed	DON	*12_30901*	2H 90.99	B	3.50	21.43	0.49	
Six-rowed	DON	*12_30901*	2H 90.99	A	ND§	ND	0.01	
Six-rowed	DON	*12_30901*	2H 90.99	B	4.51	28.40	0.99	
Whole set	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	A	4.02	24.03	0.51	
Whole set	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	4.26	24.33	0.49	
Two-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	A	3.41	16.87	0.52	
Two-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	3.50	17.12	0.48	
Six-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	A	4.42	28.38	0.50	
Six-rowed	DON	*DON-qtl-2H_SCRI_RS_171032*	2H 90.64	B	4.82	28.47	0.50	
Whole set	DON	*DON-qtl-2H-12_10777*	2H 30.36	A	4.17	22.44	0.70	EEBC-2R-C, EEBC-2R-11 and EEBC-2R-12
Whole set	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	3.82	28.32	0.30	
Two-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	A	3.42	14.63	0.79	
Two-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	3.37	29.16	0.21	
Six-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	A	4.78	28.58	0.64	
Six-rowed	DON	*DON-qtl-2H-12_10777*	2H 30.36	B	4.01	28.30	0.36	
Whole set	DON	*DON-qtl-4H-12_11485*	4H 12.91	A	4.19	12.82	0.71	EEBC-12, EEBC-6R-C, and EEBC-6R-12
Whole set	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	4.93	18.49	0.29	
Two-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	A	3.22	16.53	0.65	
Two-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	3.71	18.45	0.35	
Six-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	A	4.16	26.71	0.74	
Six-rowed	DON	*DON-qtl-4H-12_11485*	4H 12.91	B	5.63	33.53	0.26	
Whole set	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	A	4.8	28.7	0.33	EEBC-C, EEBC-12, EEBC-6R-C and EEBC-6R-12
Whole set	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	3.7	21.88	0.67	
Two-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	A	3.78	20.21	0.28	
Two-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	3.27	16.14	0.72	
Six-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	A	5.28	32.82	0.37	
Six-rowed	DON	*DON-qtl-4H- SCRI_RS_148392*	4H 65.52	B	4.04	25.87	0.63	
Whole set	DON	*DON-qtl-5H-11_21001*	5H 55.83	A	4.12	24.52	0.75	EEBC-2R-C and EEBC-2R-11
Whole set	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	3.91	22.81	0.25	
Two-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	A	3.31	16.31	0.75	
Two-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	3.68	20.05	0.25	
Six-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	A	4.63	29.49	0.75	
Six-rowed	DON	*DON-qtl-5H-11_21001*	5H 55.83	B	4.18	24.98	0.25	
Whole set	DON	*DON-qtl-7H-11_20824*	7H 107.49	A	4.05	23.88	0.81	EEBC-6R-C and EEBC-6R-12
Whole set	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	4.19	25.15	0.19	
Two-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	A	3.23	16.30	0.65	
Two-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	3.72	19.03	0.35	
Six-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	A	4.43	27.12	0.92	
Six-rowed	DON	*DON-qtl-7H-11_20824*	7H 107.49	B	5.52	42.68	0.08	
Whole set	DON	*SCRI_RS_165473*	Unknown	A	3.34	14.25	0.21	EEBC-C, EEBC-11, EEBC-2R-C EEBC-2R-11
Whole set	DON	*SCRI_RS_165473*	Unknown	B	4.24	26.85	0.79	
Two-rowed	DON	*SCRI_RS_165473*	Unknown	A	3.33	14.17	0.52	
Two-rowed	DON	*SCRI_RS_165473*	Unknown	B	3.49	21.50	0.48	
Six-rowed	DON	*SCRI_RS_165473*	Unknown	A	5.00	18.82	0.01	
Six-rowed	DON	*SCRI_RS_165473*	Unknown	B	4.49	28.35	0.99	

(cont'd)

Table 5. Continued.

Germplasm	Trait	QTL or marker	Position (cM)	Allele	FHB	DON	Allele frequency†	Identified in‡
Whole set	FHB + DON	*SCRI_RS_72983*	Unknown	A	3.26	13.89	0.10	EEBC-12 for FHB, EEBC-C, EEBC-2R-C and EEBC-2R-11
Whole set	FHB + DON	*SCRI_RS_72983*	Unknown	B	4.28	26.81	0.90	
Two-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	A	3.27	13.87	0.25	
Two-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	B	3.57	21.39	0.75	
Six-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	A	ND	ND	0.00	
Six-rowed	FHB + DON	*SCRI_RS_72983*	Unknown	B	4.52	28.40	1.00	

† Resistant allele frequency within analysis set; calculated for the most significant marker where more than marker was significant.

‡ EEBC-C, combined data of 2011 and 2012 of the entire collection; EEBC-11, test from 2011 that includes data from the entire collection; EEBC-12, test from 2012 that includes data from the entire collection; EEBC-2R-C, combined two year (2011 and 2012) data of two-rowed accessions only; EEBC-2R-11, 2011 data of two-rowed accessions only; EEBC-2R-12, 2012 data of two-rowed accessions only; EEBC-6R-C, combined two year data of six-rowed accessions only; EEBC-6R-11, 2011 data of six-rowed accessions only; EEBC-6R-12, 2012 data of six-rowed accessions only.

§ ND, not determined. There were no six-rowed germplasm accessions with the BB allele. One accession that had the BB allele did not yield phenotype data.

with the same map position (12_30901) was significantly associated with DON concentration detecting the QTL *Rfg-qtl-2H_11_20340*. The A allele of marker 12_30901 is associated with low disease severity and DON concentration unlike the A allele of marker 11_20340, which was associated with higher values of these traits. Ninety-seven percent (56 out of 58) of accessions with allele A, and 23% (53 out of 232) of accessions with allele B for marker 12_30901 were two-rowed. Seventy-seven percent (179 out of 232) of accessions with allele B at this marker were six-rowed, that is, 99% (179 out of 180) of the six-rowed accessions had allele B and developed higher FHB severity and DON concentration. This suggests that SNP markers with the same genetic map position that were significantly associated with DON concentration may have alleles with negative correlation as to their effect on the expression of the trait. In addition to the major QTL on chromosome 2HL, QTL for DON concentration also were detected on chromosomes 2HS (*DON-qtl-2H-12_10777*), 4HS (*DON-qtl-4H-12_11485*), 4HL (*DON-qtl-4H-SCRI_RS_148392*), 5HL (*DON-qtl-5H-11_21001*), and 7HL (*DON-qtl-7H-11_20824*). These QTL have alleles with variable effects on FHB severity and DON concentration. Of these, *DON-qtl-4H- SCRI_RS_148392* was the only QTL identified in the combined germplasm set of two- and six-rowed types. Two-rowed accessions with the more frequent positive allele B at *DON-qtl-4H-SCRI_RS_148392* exhibited the lowest mean disease severity and DON concentration of 3.27 and 16.14 mg Kg^{-1}, respectively.

DISCUSSION

Fusarium head blight is a devastating disease of barley causing significant yield and quality losses in many parts of the world. In this study, a diverse panel of Ethiopian and Eritrean barley landrace germplasm was used for whole-genome association mapping to identify QTL associated with FHB resistance, DON accumulation, and related agromorphological traits. Key elements for successful GWAS include high diversity in the association mapping panel, robust quality phenotypic data, and full accounting for population structure (Myles et al., 2009). Except for DON concentration, the traits had heritability values of 0.67 and higher. An unusually high heritability of 0.99 was obtained for FHB severity. This was likely due to three major factors. First, FHB severity was scored on a 1-to-9 scale based on an aggregate of spikes within a row for each accession. This resulted in a narrower and more consistent spread of data across seasons and replications compared to the more commonly used method of calculating the percentage of FHB severity in 10 to 20 arbitrarily selected spikes from a row (Massman et al., 2011). Second, the term heritability is loosely applied here as the presented data are actually assessing the reproducibility of FHB severity readings across experiments. Third, the environmental factors across seasons apparently did not unduly affect the development of FHB.

The EEBC germplasm exhibited a higher level of variation for FHB severity and DON concentration, more so for the latter, in 2011 than in 2012 (Fig. 1A,B). The explanation for these differences lie in the very nature of the FHB disease itself. The environment (mainly moisture and temperature) and associated developmental conditions such as heading time can markedly influence FHB development. In 2011, rainfall was about 15% higher than in 2012 and therefore likely favored additional infection periods for disease development (Tekauz et al., 2000). The two seasons had very similar mean, minimum, and maximum temperatures during the critical growth stages (i.e., heading to late milk) of the germplasm, suggesting that temperature was not a major factor contributing to the differences in disease levels. The time of heading can influence the level of FHB and consequently DON concentration in barley (Urrea et al., 2002). The average heading date in the germplasm was about 7 d earlier in 2012 than in 2011 (Fig. 1E).

The two-rowed and six-rowed EEBC germplasm sets exhibited differences in FHB severity and DON accumulation in both years of the study with the former having lower levels of both traits than the latter (Fig. 1C,D). The differences were very obvious in 2012 for disease severity

and in both years for DON concentration. Spike density may have influenced initial infection and subsequent DON accumulation in this study. Two-rowed EEBC genotypes had longer, more lax spikes. This architecture might alter the spike microenvironment making it less favorable for fungal infection and growth or for reducing the chances for lateral kernel-to-kernel spread. Alternatively, two-rowed germplasm may have a level of basal FHB resistance not present in six-rowed germplasm. This is in accordance with previous studies showing that lower FHB levels are usually found in two-rowed accessions (Zhou et al., 1991; Steffenson et al., 1996; Choo et al., 2004).

Using GWAS with a widely used statistical model that successfully accounts for pairwise relatedness (Kang et al., 2008), a QTL on chromosome 2HL (*Rfg-qtl-2H_11_20340*) was identified for FHB severity and DON accumulation in Ethiopian and Eritrean landrace germplasm (Table 5). This QTL likely coincides with a common FHB resistance QTL detected in biparental mapping studies (de la Pena et al., 1999; Ma et al., 2000; Hori et al., 2006; Lamb et al., 2009; Sato et al., 2008), which lies in the centromeric region of chromosome 2H (bins 8–10) based on the comparative Steptoe/Morex bin map (http://barleygenomics.wsu.edu) and the consensus map of SNP markers used in this study (Muñoz-Amatriaín et al., 2014). In the EEBC, the *Rfg-qtl-2H_11_20340* QTL was detected consistently across years for FHB severity and in the overall mean of 2 yr for DON concentration, indicating that it has a sound genetic basis and is less affected by the environment. This QTL also was significantly associated with row type, detecting the *Vrs1* allele controlling spike row type. The two-rowed spike type governed by the *Vrs1* allele is generally less conducive to disease development and likely contributed to the association of FHB resistance in this region of chromosome 2H (Huang et al., 2013).

In the current study, the *Rfg-qtl-2H_11_20340* QTL shared the same common SNP markers detecting the *Vrs1* locus, suggesting the possibility of linkage or pleiotropy. Several mapping studies found that the two-rowed (i.e., *Vrs1*) trait coincided with QTL conferring FHB resistance and DON concentration (de La Pena et al., 1999; Zhu et al., 1999; Mesfin et al., 2003). It remains to be resolved whether this colocalization of the *Vrs1* locus and QTL for FHB and DON levels is due to linkage or perhaps pleiotropy of the *Vrs1* locus. Some biparental studies have suggested close linkage between *Vrs1* and FHB resistance QTL on chromosome 2H bin10 (Mesfin et al., 2003; Horsley et al., 2006). On the contrary, other studies have suggested a possible pleiotropic effect of *Vrs1* on FHB resistance QTL in this region as detected in two-rowed and six-rowed crosses (Sato et al., 2008). Given the presumably high resolution of GWAS, it is possible that the common FHB and DON concentration QTL detected in this region in the EEBC germplasm is a pleiotropic effect of the *Vrs1* locus. The fact that sequences of some of the SNP markers significantly associated with FHB and DON concentration were identified from within the coding region of the *Vrs1* locus with high sequence identity supports this hypothesis.

The *Rfg-qtl-2H_11_20340* QTL detected in EEBC germplasm may still represent a unique allele for FHB resistance. In previous studies, FHB resistance QTL at the centromeric region of chromosome 2HL were identified using biparental mapping of barley accessions from other countries. To resolve whether the identified QTL are novel or not, additional genetic tests should be conducted. Huang et al. (2013) studied the haplotype diversity of multiple barley accessions with partial resistance and found distinct haplotypes at the FHB resistance QTL on chromosome 2H bins 8 and 10. However, only two barley accessions from Ethiopia (not among the EEBC accessions used in our study) were included in this analysis. Similar analyses should be conducted in the future with some of the germplasm used in the current study to identify the haplotype structure of this candidate region in Ethiopian and Eritrean barley landraces. This may provide valuable information as to whether such germplasm contains FHB resistance haplotypes different from other sources of resistance.

The FHB QTL *Rfg-qtl-2H_11_20340* was detected by two SNP markers (11_20340 and 12_30896) in the combined dataset, but not in the separate two- or six-rowed datasets. However, the AA-AA haplotype for the two markers was predominately found in six-rowed accessions: 93% (168 out of 180) of six-rowed accessions had the AA-AA haplotype. On the other hand, 94% (103/110) of the two-rowed accessions had the BB-BB haplotype at these markers. The A allele was associated with generally higher levels of FHB and DON concentration, whereas the B allele was associated with generally lower levels (Table 5). In addition to marker 11_20340, another SNP marker (12_30901) with the same map position was significantly associated with DON concentration detecting the QTL *Rfg-qtl-2H_11_20340*. The positive allele A of the 12_30901 marker had a low frequency and provided a mean reduction in disease severity and DON concentration of 21.14 and 46.80%, respectively, compared to accessions without the allele in the entire germplasm panel (Table 5).

In addition to the most common QTL on chromosome 2HL, QTL for DON concentration also were detected on chromosome 2HS (*DON-qtl-2H-12_10777*), the short (*DON-qtl-4H-12_11485*) and long arms of chromosome 4H (*DON-qtl-4H-SCRI_RS_148392*), and chromosomes 5HL (*DON-qtl-5H-11_21001*) and 7HL (*DON-qtl-7H-11_20824*) (Table 5). These QTL have alleles with variable effects on FHB severity and DON concentration. Except for *DON-qtl-4H- SCRI_RS_148392*, the remaining QTL were identified in a limited germplasm set, and no QTL for FHB severity were identified in these regions. This indicates that these QTL may have a minor effect on DON accumulation and are probably affected by the environment.

The *DON-qtl-4H-SCRI_RS_148392* QTL detected on chromosome 4HL mapped approximately to bin 7 where FHB resistance QTL were previously identified (Hori et al., 2006; Sato et al., 2008). This suggests that this QTL is possibly the same or an allele of these QTL. *DON-qtl-4H-SCRI_RS_148392* also was detected by another marker (SCRI_RS_157650) sharing the same exact chromosomal position. Allele A of the SCRI_RS_157650 marker contributed to lower FHB severity and DON concentration unlike the allele A of marker SCRI_RS_148392 that contributed to an increase in disease severity and DON accumulation. The SCRI_RS_157650 marker was discovered in the 2012 data set where DON concentration was relatively low and appeared to have a negative correlation with marker SCRI_RS_148392 in allele effect.

In addition to row type, heading date and other agromorphological traits may affect the level of FHB severity (de La Pena et al., 1999). In most of the biparental mapping studies cited above, the centromeric region of chromosome 2H (especially bin 8) was consistently associated with FHB resistance, DON accumulation, and heading date. However, no significant marker associated with DTH was detected in this region in the current study. Likewise, no FHB severity or DON concentration QTL coincident with PH were detected in this study. Among the other agromorphologic traits correlated with disease, a QTL for KD mapped to the same chromosome 2HL location as *Rfg-qtl-2H_11_20340*, sharing identical significant markers. This was likely due to the confounding effect of the row-type trait as the same QTL also coincided with the *Vrs1* locus. In addition to the markers on chromosome 2HL, two unmapped markers (SCRI_RS_165473 and SCRI_RS_72983) were significantly associated with FHB severity, DON concentration, RT, and KD. These unmapped markers are probably associated with one of the same described QTL or perhaps other ones at a unique genomic location. Future research will be done to map such SNP markers significantly associated with the important phenotypic traits considered in this investigation.

In summary, one common QTL conferring low FHB severity and DON concentration and a separate QTL conferring low DON were identified through whole-genome association mapping in barley landraces from Ethiopia and Eritrea. The common QTL identified for FHB and DON on chromosome 2HL appeared to be linked with or a pleotropic effect of the row-type locus *Vrs1*. The other QTL identified for DON in the combined dataset and again in a separate dataset was located on chromosome 4HL. These two QTL (the 2HL and 4HL) mapped to genomic regions where QTL for FHB resistance were previously reported in other studies. Thus, they are not likely unique QTL. Interestingly, both of these QTL were not associated with heading date or plant height, unlike the case in other previously published studies. Thus, resistant germplasm carrying this QTL may be used in breeding programs without the confounding effects from heading date or plant height. Importantly, these QTL could well be new alleles preserved in the unique East African germplasm used in this study. Barley accessions used in previous mapping studies were either breeding lines, unimproved varieties, or raw germplasm selections from other countries. To gain a better understanding of the genetics of FHB resistance in the Ethiopian and Eritrean barley germplasm, more tests should be done to validate the consistency of the QTL detected in this study, primarily those on chromosomes 2HL and 4HL. This could be done through other studies including biparental mapping or allele sequencing of putative QTL regions. It also is a common practice to evaluate materials for 3 to 4 yr to identify reliable sources of FHB resistance given the inherent genotype-by-environment interactions of the pathosystem. Additional barley landraces from Ethiopia should be tested over several years and at multiple locations to obtain more rigorous phenotype data for association genetics.

Supplemental Information Available

Supplemental material is available at https:www.crops.org/publications/cs.

Supplemental Text S1. Genome-wide association mapping of deoxynivalenol (DON) concentration in separate two- or six-rowed germplasm sets.

Supplemental Text S2. Genome-wide association mapping of days to heading and plant height.

Supplemental Table S1. SNP markers significantly associated with Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration in the Ethiopian and Eritrean Barley Collection (EEBC).

Supplemental Table S2. SNP markers significantly associated with row-type morphology (RT), days to heading (DTH), plant height (PH), kernel density (KD), spike angle (SA), and spike exsertion length (EL) in the Ethiopian and Eritrean Barley Collection (EEBC).

Supplemental Fig. S1. Principal component analysis of the Ethiopian and Eritrean Barley Collection (EEBC).

Supplemental Fig. S2. Genome-wide association scan for marker associations with Fusarium head blight (FHB) severity in the Ethiopian and Eritrean Barley Collection (EEBC) evaluated at Crookston, MN, in 2011 and 2012.

Supplemental Fig. S3. Genome-wide association scan for marker associations with deoxynivalenol (DON) concentration in the Ethiopian and Eritrean Barley Collection (EEBC) evaluated at Crookston, MN, in 2011 and 2012.

Supplemental Fig. S4. Genome-wide association scan for marker associations with the agromorphological traits of days to heading, plant height, and kernel density in the Ethiopian and Eritrean Barley Collection (EEBC) evaluated at Crookston, MN in 2011 and 2012 and also plant height at Saint Paul, MN, in 2010.

Supplemental Fig. S5. Genome-wide association scan for marker associations with the agromorphological traits of days to heading and plant height in the Ethiopian and Eritrean Barley Collection (EEBC) evaluated at Crookston, MN, in 2011 and 2012.

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