ORIGINAL RESEARCH

Association Mapping Reveals Novel Stem Rust Resistance Loci in Durum Wheat at the Seedling Stage

Tesfaye Letta,* Pablo Olivera, Marco Maccaferri, Yue Jin, Karim Ammar, Ayele Badebo, Silvio Salvi, Enrico Noli, Jose Crossa, and Roberto Tuberosa

Abstract

Wheat stem rust rapidly evolves new virulence to resistance genes. Recently emerged races in East Africa, such as TTKSK (or Ug99), possess broad virulence to durum cultivars, and only a limited number of genes provide resistance. An association mapping (AM) study conducted on 183 durum wheat accessions has allowed us to identify 41 quantitative trait loci (QTLs; determination coefficient [R^2] values from 1.1 to 23.1%) for seedling resistance to one or more of four highly virulent stem rust races: TRTTF, TTTTF, TTKSK (Ug99), and JRCQC, two of which (TRTTF and JRCQC) were isolated from Ethiopia. Among these loci, 24 are novel, while the remaining 17 overlapped with loci previously shown to provide field resistance in Ethiopia and/or chromosome regions known to harbor designated stem rust resistance designated loci (*Sr*). The identified loci were either effective against multiple races or race specific, particularly for race JRCQC. Our results highlight that stem rust resistance in durum wheat is governed in part by loci for resistance across multiple races, and in part by race-specific ones (23 and 18, respectively). Collectively, these results provide useful information to improve the effectiveness of marker-assisted selection towards the release of durum wheat cultivars with durable stem rust resistance.

DURUM WHEAT [*Triticum turgidum* ssp. *durum* (Desf.) Husn.] is an important crop in the Mediterranean Basin, particularly in West Asia and North Africa, where durum wheat is grown annually on >13 million hectares. Mediterranean countries account for approximately 75% of global worldwide durum wheat production. In Sub-Saharan Africa, Ethiopia is the largest wheat-growing country and is one of the centers of diversity for tetraploid wheat. Durum wheat represents approximately 40% of the total wheat area in Ethiopia, with a tendency to increase in response to the growing internal demand for pasta.

Durum production and kernel quality can be negatively affected by rust diseases (Singh et al., 2005). Historically, stem rust infections of *Puccinia graminis* Pers. f. sp. *tritici*, (*Pgt*) have caused severe losses to wheat production (McIntosh and Brown, 1997; Eversmeyer and Kramer, 2000; Singh et al., 2011). While >50 *Sr* loci have been identified in cultivated wheat and wild relatives, only a few of them remained effective against the newly emerged races in East Africa, including TTKSK = Ug99 (Pretorius et al.,

T. Letta, M. Maccaferri, S. Salvi, E. Noli, and R. Tuberosa, DipSA, Univ. of Bologna, Viale Fanin 44, 40127 Bologna, Italy; P. Olivera and Y. Jin, USDA-ARS, Cereal Disease Lab., 1551 Lindig St., St. Paul, MN 55108, USA; K. Ammar and J. Crossa, CIMMYT, Carretera Mexico-Veracruz KM. 45, 56130 Texcoco, Mexico; A. Badebo, Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia; and T. Letta, Sinana Agricultural Research Center, Bale-Robe, Ethiopia. Received 9 Aug. 2013. *Corresponding author (tesfayeletta@gmail.com).

Abbreviations: AM, association mapping; APR, adult plant resistance; DArT, Diversity Array Technology marker; FDR, false discovery rate; h^2, broad-sense heritability; GLM, general linear model; IT, seedling infection type; **K**, kinship matrix; LD, linkage disequilibrium; MLM, mixed linear model; Q, population structure covariate; QTL, quantitative trait locus; R^2, determination coefficient; r^2, marker pair-wise linkage disequilibrium estimate; SNP, single nucleotide polymorphism; *Sr*, stem rust resistance designated (loci); SSR, simple sequence repeat (marker); STS, sequence tagged site (marker).

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2000) and its variants, and even fewer are effective against the durum-specific Ethiopian races (Admassu et al., 2009; Rouse et al., 2012). In Ethiopia, Ug99 was, in fact, added to previously existing races, several of them specifically virulent on durum wheat (Admassu and Fekadu, 2005; Haile et al., 2012). Two such races, typed as TRTTF and JRCQC, have a combined virulence on stem rust resistance genes *Sr9e* and *Sr13* (Olivera et al., 2012). Virulence on *Sr13* appears to be widespread in Ethiopia (Admassu et al., 2009). Very limited effective resistance (5.2%) to races TRTTF and JRCQC was found in a highly diverse collection of 996 tetraploid genotypes evaluated for field reaction at the Debre Zeit Research Center in Ethiopia (Olivera et al., 2012). Therefore, the combination of Ug99 with *Sr9e*- and *Sr13*-virulent Ethiopian races represents a major threat to the viability of the Ethiopian durum wheat production. Achieving higher and more durable stem rust resistance requires the characterization of the genetic basis underlying the resistance present in improved germplasm or in exotic sources used for introgression in breeding. Only then can breeding programs develop strategies to preemptively counter the emerging new virulence types in the pathogen populations. Whereas field resistance is the ultimate goal sought in breeding programs, seedling tests are a good complement for resistance characterization, as they allow screening a large number of lines for reaction to multiple races, one race at a time, in relatively short periods and with modest space requirements. Seedling screening provides information for postulating the presence of designated loci based on the series of available races and/or the presence of novel loci, avoiding the confounding effects of having several races acting at the same time, as is usually the case in field experiments.

Marker-based approaches can be used to identify genes and QTLs governing the disease responses. Until recently, the standard approach is to use biparental mapping populations to relate phenotypic information to genotypic data obtained from molecular markers to determine the number and chromosomal location of resistance loci (Maccaferri et al., 2008; Simons et al., 2011; Singh et al., 2013). An alternative to the use of biparental mapping is AM or linkage disequilibrium (LD)-based mapping, in which genotype–phenotype relationships are explored in germplasm collections or natural populations (Flint-Garcia et al., 2003; Zhu et al., 2008). Since its first use in plants a decade ago, AM has been used in many crops due to advances in high-throughput genotyping technologies, increased interest in identifying useful and/or novel alleles, and improvements in statistical methods (Gupta et al., 2005; Yu et al., 2006; Zhu et al,. 2008). In both tetraploid and hexaploid wheat, AM has already proven to be an effective strategy to identify marker-trait associations for agronomically valuable traits (Breseghello and Sorrells, 2006; Maccaferri et al., 2010, 2011) and response to diseases (Adhikari et al., 2012; Kollers et al., 2013), including resistance to stem rust (Yu et al., 2011) and leaf rust (Maccaferri et al., 2010) in durum wheat. Linkage disequilibrium in the elite durum wheat germplasm utilized herein ranges from 5 to 10 cM (Maccaferri et al., 2005, 2011), similarly to observations performed in elite hexaploid wheat (Zhang et al., 2010), thus enabling a whole-genome scan analysis for marker-trait associations with a relatively modest number of markers as compared with species with lower LD.

The objectives of this study were to carry out a genome-wide search in durum wheat for resistance loci to *Pgt* races TRTTF, TTTTF, TTKSK, and JRCQC at the seedling stage, and the identification of genomic regions suitable for marker-assisted selection and further genetic dissection.

Materials and Methods

Plant Materials

One-hundred-eighty-three accessions from different durum wheat-growing regions of Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), the Southwestern United States, and Mexico already used in previous AM analysis for stem rust resistance under field conditions (Letta et al., 2013) were analyzed in this study. A detailed description of the accessions at the molecular and phenotypic level is reported in Maccaferri et al. (2006 and 2010).

Pathogen Races

The AM panel was evaluated for reaction to four *Pgt* races: TRTTF, TTTTF, TTKSK, and JRCQC. The race designation is based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs et al., 1993), modified to further delineate races in the TTKS lineage (Jin et al., 2008). These races were selected based on their differential virulence pattern and/or importance for durum wheat. Race TTKSK (Ug99) has a wide virulence spectrum and is rapidly evolving in East Africa. Race TTTTF is the most widely virulent race known in the United States, producing high infection types (ITs) on the majority of stem rust differential lines (Jin et al., 2007). Races TRTTF and JRCQC, both present in Ethiopia, possess a virulence combination that overcomes both the resistance genes *Sr13* and *Sr9e*, two genes present at high frequency in durum wheat (Klindworth et al., 2007). Information about the stem rust isolates used in the disease phenotyping tests is summarized in Table 1.

Inoculation, Incubation, and Disease Assessment

The AM panel was evaluated under controlled conditions using a completely randomized design with two replications (over time) for each of the four races. Five to six seedlings per line were inoculated on the fully expanded primary leaves 8 to 9 d after planting. This work was conducted at the Cereal Disease Laboratory, St. Paul, MN, and the experimental procedures in inoculation and disease assessment were performed as described by Jin et al. (2007). Wheat cultivar McNair 701 (CItr 15288) was used as susceptible control. Plants were evaluated for their ITs 14 d postinoculation using the 0 to 4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2, or X are considered as low ITs, and ITs of 3 or 4 are considered as high ITs. Lines giving variable

Table 1. Origin and virulence properties of the *Puccinia graminis* f. sp. *tritici* races used to evaluate the durum panel.

Race	Isolate	Origin	Avirulence	Virulence	IT on *Sr13*†
TRTTF	06YEM34–1	Yemen	*Sr8a 24 31*	*Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 Sr21 30 36 38 McN*	3+
TTTTF	01MN84A-1-2	United States	*Sr24 31*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 Sr17 21 30 36 38 McN*	2
TTKSK (Ug99)	04KEN156/04	Kenya	*Sr24 36 Tmp*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 Sr17 21 30 31 38 McN*	22–
JRCQC	09ETH08-3	Ethiopia	*Sr5 7b 8a 36 9b 30 Tmp 24 31 38*	*Sr21 9e 11 6 9g 17 9a 9d McN*	3

†Infection types (IT) observed on seedlings at 14 d postinoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types of 1, 2, or X are considered as a low IT, and ITs of 3 or higher are considered as a high IT.

reactions between experiments were repeated again to confirm the most likely reactions.

Statistical Analysis

Stakman's ITs were converted to a linear scale using a conversion algorithm proposed by Zhang et al. (2011). Briefly, ITs are converted as follows: 0, 1^-, 1, 1^+, 2^-, 2, 2^+, 3^-, 3, and 3^+ are coded as 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9, respectively. The symbol for hypersensitive flecks (;) is converted to 0, and IT 4 is converted to 9. Special annotation codes C and N are ignored. Double minus and double plus annotations are converted to single minus and single plus, respectively. Complex ranges such as ;12^+ are first collapsed to ;2^+. Then the first and last ITs of the ranges are converted and averaged; with the first IT double-weighted because the most prevalent IT is listed first. Infection types X^-, X, and X^+ are converted to linearized scores of 4, 5, and 6, respectively. These linearized 0-to-9 scale values were used for subsequent statistical analysis.

The heritability (h^2) of linearized IT responses was calculated for each of the four races on a mean basis across two replications according to the following: $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E/r)$ where r = number of replicates, σ^2_G = genotypic component of the MS among accessions, and $\sigma^2_E = MS_{error}$, with MS indicating the mean square values as from the ANOVA results.

The dendrogram analysis was performed using NTSYS-pc software v. 2.0 (Rohlf 1997) and was based on the virulence phenotypes (ITs estimated with a 0 to 4 scale) of the races; the distances among races have been computed using the standardized Manhattan distances ("city-block" method).

Molecular Profiling

Genomic DNA extraction and other molecular procedures were performed as described in Maccaferri et al. (2010). The accessions were profiled with 350 simple sequence repeat (SSR) loci, 900 Diversity Array Technology (DArT) markers, and three additional sequence tagged site (STS) markers including those previously reported to be associated to major stem rust resistance genes (Yu et al., 2010).

The choice of SSR marker loci, fluorescent PCR amplification, and polyacrylamide-gel electrophoresis were performed as detailed in Letta et al. (2013). Genotype alleles of the 183 Durum panel accessions were scored using founder genotypes as an allele reference set. The DArT marker genotypes were obtained as reported in Letta et al. (2013).

Association Mapping

Genome-wide AM was performed with software and analysis parameter settings as described in Letta et al. (2013), with minor modifications. Briefly, only markers with non-rare alleles (frequency > 0.10) were considered for the LD and marker-trait association analysis. Rare alleles and data points showing residual allelic heterogeneity within accessions were considered as missing data. Association mapping tests were performed with the molecular data produced from 323 SSRs and STSs, plus 538 DArT markers for which both map position on a durum-specific consensus map (described in Letta et al., 2013) and the genotype score of the 183 durum panel accessions were already available.

The genetic structure of the panel has been investigated with a combination of model- and distance-based analyses using the software programs STRUCTURE v. 2 (Pritchard et al., 2000) and NTSYS-pc v. 2 (Rohlf, 1997). Details for structure and kinship analysis were reported in Maccaferri et al. (2010, 2011). TASSEL (http://www.maizegenetics. net, verified 12 Dec. 2013) was used to estimate the LD parameter D' and marker pair-wise linkage disequilibrium estimate (r^2) values as a function of the corresponding intermarker distances, and the comparison-wise significance was computed with 10,000 permutations. The r^2 parameter was estimated for all loci on the same chromosome and compared based on the genetic distances measured in cM. If all pairs of adjacent loci within a given chromosomal region were in LD ($r^2 \geq 0.4$ and highly significant LD P values), then the region was referred to as a LD block.

Marker-Phenotype Association Analysis

Genome-wide scan for loci governing stem rust resistance at the seedling stage was conducted using phenotypic data converted to a linear scale. The AM analysis was performed with TASSEL, ver. 2.1 (www. maizegenetics.net, verified 12 Dec. 2013; Yu et al., 2006). The 323 SSRs and STSs and 538 DArT markers were tested for significance of marker-trait associations under (i) the fixed general linear model (GLM) including the Q population structure results as covariates (Q GLM), (ii) the mixed linear model (MLM) including the Q population structure results plus the **K** kinship matrix (Q + **K** MLM).

For GLM analysis, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on a permutation test (10,000 permutations). In the MLM analysis, experiment-wise significance was inspected using the false

discovery rate (FDR) approach according to Storey and Tibshirani (2003) and implemented in *Qvalue* program.

Multiple adjacent co-segregating significant markers were assigned to a unique QTL region on meeting the following conditions: <20 cM of intermarker genetic distance, presence of significant and strong LD among the markers (with R^2 values ≥ 0.4) within the QTL region, and consistency of allelic effects across significant markers.

To estimate the cumulative effect of the markers that were significant in the single-marker analysis association tests, several multiple regressions considering the markers that were significant at the experiment-wise (or genome-wise) level (FDR approach, $P \leq 0.05$) only and markers significant at both the experiment-wise ($P \leq 0.05$) and marker-wise ($P \leq 0.01$) levels were performed for each race. The number of markers included in the multiple regressions varied from three to four when considering the experiment-wise significant markers and from 15 to 20 markers when considering both the significant experiment-wise and the marker-wise highly significant markers. Finally, all significant markers (41 in total) were used in a multiple regression analysis for the responses to all the four races.

Results

Seedling Evaluations

Seedling ITs for each of the 183 durum accessions listed by accession code and sorted by population structures are presented in Supplemental Tables S1 and S2, respectively. The ITs frequency distribution presented in Fig. 1 depicts a continuous variation for all four races, with that for JRCQC being skewed toward susceptibility scores (3 and 4).

The ANOVA for stem rust seedling response showed highly significant differences ($P \leq 0.0001$) among races and accessions with highly significant effects of subgroups of accessions and subgroup × race interaction (results not reported). The highly variable classification and ranking of the accessions (Supplemental Table S1) based on their responses to the different races supports the significance of the race × accession interaction. Heritability of the linearized IT values was high for all four races, ranging from $h^2 = 93.0\%$ for race TTTTF to $h^2 = 98.9\%$ for TTKSK.

The frequencies of accessions categorized as resistant, susceptible, and heterogeneous in their reaction to the four races varied markedly depending on the race (Table 2). Seedling resistance to TRTTF, TTTTF, TTKSK, and JRCQC was observed in 149 (81.4%), 117 (63.9%), 106 (57.9%), and 87 (47.5%) accessions, respectively (Table 2). Sixty-six (36.1%) accessions were resistant (IT = 0 to 2) to all four races.

Figure 2 reports the pattern of diversity among the four stem rust races based on the unweighted pair-group method with arithmetic mean-cluster analysis of the avirulence or virulence patterns on the 183 durum accessions. The dendrogram clearly shows that the races grouped into three well-distinct groups with TTTTF and TTKSK that clearly clustered together while TRTTF and JRCQC showed independent virulence patterns.

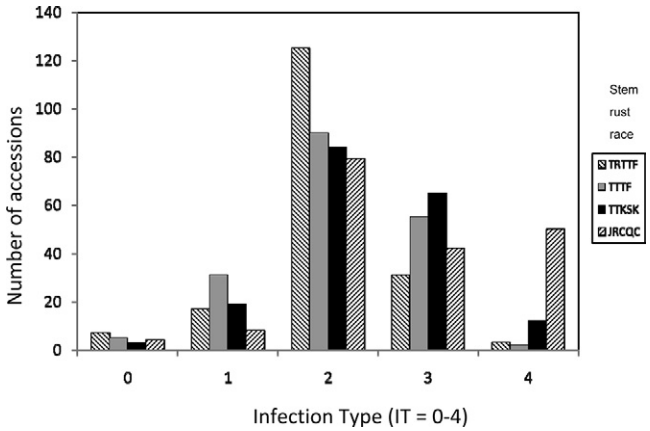

Figure 1. Frequency distribution of infection types (ITs) of 183 durum genotypes challenged at the seedling stage with four stem rust races. The ITs were assigned according to Long and Kolmer (1989) with resistant reactions indicated by ITs between 0-2 and susceptible reactions indicated by ITs of 3 or 4.

Highly significant correlations of ITs among genotypes were observed for all the four races. In particular, relatively high *r*-values were observed for the pair-correlations of TTKSK vs. TTTTF (0.72), TTTTF vs. JRCQC (0.57), TRTTF vs. TTTTF (0.51), and TTKSK vs. TRTTF (0.46). The correlation of ITs between JRCQC and TTKSK or JRCQC and TRTTF was rather weak (0.36 and 0.15, respectively).

Relationship between Population Structure and Seedling Response to Stem Rust

The genetic relationships among the accessions were investigated using both a genetic-similarity and a model-based Bayesian clustering method, and the results have been reported elsewhere (Maccaferri et al., 2006, 2011; Letta et al., 2013). Both methods pointed out that the minimum and optimum number of hypothetically well-distinct subgroups present in the panel was equal to five, corresponding to clearly distinct breeding lineages (from S1 to S5). Each subgroup contains 11, 55, 26, 56, and 35 accessions, respectively. The differences for seedling stem rust response among the five subgroups were highly significant ($P \leq 0.001$, data not shown). The coefficients of membership to the five main subgroups as estimated with STRUCTURE were used to assess the effect of population structure to single race responses by means of multiple regression. The percentage of phenotypic variation accounted for by population structure ranged from a minimum of 9.09% for response to race TTKSK to a maximum of 12.15% for response to race JRCQC.

The percentage of resistant and susceptible accessions for each of the five main subgroups is reported in Table 3. This clearly shows that all five subgroups included accessions with different responses, thus indicating that all subgroups are equally informative for AM purposes.

The complete dataset of seedling phenotypic response and population structure membership coefficients for each

Table 2. Numbers and frequencies of infection types (ITs) and resistant, susceptible, and heterogeneous reactions of the 183 durum genotypes included in the association mapping panel to four races of *Puccinia graminis* f. sp. *tritici* and combined reactions to all races.

	TRTTF		TTTTF		TTKSK (Ug99)		JRCQC		All races	
IT† or reaction	Lines	%	Lines	%	Lines	%	Lines	%	Lines	%
"0" or ";"	10	5.5	3	1.6	2	1.1	4	2.2	0	0
"1"	1	0.5	10	5.5	1	0.5	5	2.7	0	0
"2" or "23" or "X"	138	75.4	104	56.8	103	56.3	78	42.6	66	36.1
Resistant reaction	149	81.4	117	63.9	106	57.9	87	47.5	66	36.1
"3"	22	12.0	47	25.7	53	29.0	39	21.3	10	5.5
"4"	4	2.2	2	1.1	13	7.1	50	27.3	2	1.1
Susceptible reaction	26	14.2	49	26.8	66	36.1	89	48.6	12	6.6
Heterogeneous‡	8	4.4	17	9.3	11	6.0	7	3.8	1	0.5

†Infection types observed on seedlings at 14 d postinoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types of ;, 1, 2, or X are considered a low IT, and ITs of 3 or higher are considered a high IT.

‡Accessions that contained both resistant and susceptible plants.

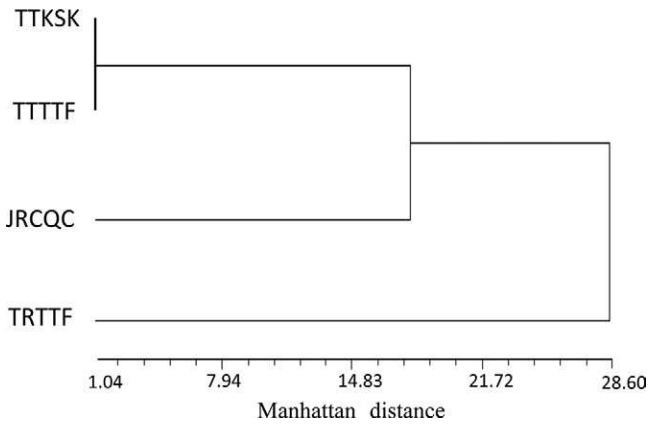

Figure 2. An unweighted pair-group method with arithmetic mean dendrogram of the four stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) races used to characterize the durum germplasm collection. The dendrogram is based on the races' virulence phenotypes (infection types estimated with a 0–4 scale). Distances were computed using the standardized Manhattan distances (city-block method).

of the 183 accessions included in the association panel is reported as Supplemental Table S1.

The ranking values of the four races, on the basis of their frequencies of avirulence or virulence interactions considering the germplasm collection as a whole (with TRTTF showing the highest degree of avirulent interactions, followed by TTTTF, TTKSK, and finally JRCQC, which showed the highest frequency of virulent interactions), was roughly confirmed when considering each of the five different subgroups of germplasm accessions separately (Table 3). One exception was observed for the race virulence spectrum to accessions of Subgroup 3 (including the Italian and early 1970's CIMMYT germplasm) where race TTTTF showed the highest frequency of avirulence and race TTKSK resulted the most virulent.

Differences among subgroups for frequency of resistance were observed in the proportion of accessions resistant to a given race. For all four races, Subgroup 5 (CIMMYT germplasm of the late 80s, early 90s) had the highest frequency of seedling resistant accessions, mostly scored as IT = 2 (Table 3). On the other hand, Subgroup 1 (ICARDA accessions for rainfed environments), which is also the least represented within the panel, had the highest frequency of susceptible accessions, except when considering TRTTF, for which only Subgroup 3 showed a higher frequency of susceptible accessions. Overall, more accessions in Subgroups 4 and 5 showed resistance to all four races than in the other subgroups.

Association Mapping for Seedling Response to Stem Rust

Association mapping revealed multiple putative QTLs for stem rust resistance to the four races (Table 4). In total, 41 distinct QTLs represented by either single markers or sets of closely linked markers, were found to be significantly associated to the seedling responses to the four tested races under the Q + **K** MLM models, with 15, 20, 19, and 19 QTLs for the response to TRTTF (marker R^2 from 1.13 to 8.34%), TTTTF (R^2 from 1.92 to 17.64%), TTKSK (R^2 from 1.75 to 23.12%), and JRCQC (R^2 from 1.51 to 15.33%), respectively (Table 4). All these regions identified with the Q + **K** MLM showed significant effects also with the Q GLM model. In some cases, the presence of a QTL was evidenced by multiple significant associations at linked SSR and DArT markers within 10 cM, as estimated from the durum consensus map and LD r^2 values higher than 0.4 in most cases (results not reported). Using a more stringent model, including the FDR multiple testing correction and Q + **K** MLM model, the number of chromosomal regions (QTLs) that showed significant ($P \leq 0.05$) associations were 4, 3, 4, and 4 for races TRTTF, TTTTF, TTKSK, and JRCQC, respectively (Table 4), while the Q GLM model detected a higher number of significant markers.

Based on the simultaneous fit of the most significant markers found in this study (results reported in Table 5), it is worth noting that as few as three to four markers, that is, those that were significant at the experiment-wise levels according to Table 4, accounted for a rather sizeable portion

Table 3. Numbers and frequencies of reactions of 183 durum genotypes included in the association mapping panel to four races of *Puccinia graminis* f. sp. *tritici* classified by origin subgroups.

Durum panel subgroup†	TRTTF			TTTTF			TTKSK			JRCQC			Across races		
	Res.‡	Sus.§	% Res.¶	Res.	Sus.	% Res.	Res.	Sus.	% Res.	Res.	Sus.	% Res.	Res.	Sus.	% Res.
	No.		Rank	No.		Rank	No.		Rank	No.		Rank	No.		Rank
S1 (11 accessions)															
Lines	9	2	4	5	5	5	3	7	5	2	9	5	2	1	4
%	81.8	18.2		50	50		30	70		18.2	81.8		66.7	33.3	
S2 (55 accessions)															
Lines	42	9	3	41	11	2	30	21	2	23	28	3	22	6	3
%	82.4	17.6		78.8	21.2		58.8	41.2		45.1	54.9		78.6	21.4	
S3 (26 accessions)															
Lines	14	8	5	17	5	3	12	11	4	16	10	2	8	4	5
%	63.6	36.4		77.3	22.7		52.2	47.8		61.5	38.5		66.7	33.3	
S4 (56 accessions)															
Lines	53	3	2	32	16	4	31	23	3	21	33	4	19	2	2
%	94.6	5.4		66.7	33.3		57.4	42.6		38.9	61.1		90.5	9.5	
S5 (35 accessions)															
Lines	35	0	1	29	5	1	29	5	1	25	9	1	26	0	1
%	100	0		85.3	14.7		85.3	14.7		73.5	26.5		100	0	

†Durum panel subgroups: S1, ICARDA germplasm for dryland areas; S2, ICARDA germplasm for temperate areas; S3, Italian and early 1970's CIMMYT germplasm; S4, CIMMYT germplasm (late 1970 to early 1980); S5, CIMMYT germplasm (late 1980 to early 1990).

‡Resistant accessions: infection types (ITs) equal to ;, 1, 2, or X.

§Susceptible accessions: ITs of 3 or higher.

¶Subgroup rank values based on their percentage of resistant accessions.

of the global phenotypic variation that varied between 29.2 to 46.5%, depending on the stem rust race (TTTTF to JRCQC, respectively). By considering pools of 15 to 20 markers that included both the experiment-wise significant ($P \leq 0.05$) and the marker-wise highly significant ($P \leq 0.01$) markers it was possible to account for a percentage of phenotypic variation ranging from 54.9 (TRTTF race) to 72.3% (JRCQC race). Finally, when considering all markers that showed at least one significant association to any of the four different race responses (41 markers in total, see Table 4), the percentage of phenotypic variation accounted for by the marker genotypes varied between 75.4 (TTTTF) and 80.9 (JRCQC) % of the overall response variation.

The molecular genotypes of the 183 accessions for all the significant markers are reported on Supplemental Tables S3 (experiment-wise significant markers only) and S4 (all significant markers) by sorting the genotypes according to their phenotypic responses (from accessions that showed a completely resistant response against all the four races to the accessions that were completely susceptible). The graphical genotypes of the accessions underlines the remarkable association between marker alleles (associated to partial resistance or susceptibility, respectively) and classes of phenotypic response to the four races (from completely resistant to completely susceptible responses).

The most important region in terms of significance and R^2 effects was observed on chromosome arm 6AL, in a 28.7-cM interval (based on the durum consensus map reported in Letta et al., 2013) harboring four distinct QTLs with R^2 values ranging from 1.51 to 23.12%. Within this wide interval, noticeable associations across the four races were found at the two sites tagged by *CD926040* (143.9 cM on the consensus linkage group) and *barc104* (155.3 cM). These two markers (*CD926040* and *barc104*) showed consistently high R^2 values (from 10.47 to 23.12%) for races TTTTF, TTKSK, and JRCQC. Conversely, *CD926040* and *barc104* showed only a limited effect, though still significant, for race TRTTF (R^2 equal to 3.52 and 2.84% respectively). Supplemental Table S3 highlights the tight association between molecular marker alleles at *CD926040* and *barc104* at chromosome 6AL and TTKSK response. In terms of significance across all four races, apart from the two sites on chromosome arm 6AL, only one QTL on chromosome 5A (*gwm410*) showed significant effects in response to all the four races considered in this study. Two genomic regions were identified on chromosomes 1B (*barc61*) and 2A (*wPt-5839*) that were putatively effective across three races (TRTTF, TTTTF, and TTKSK but not for race JRCQC at both regions). The R^2 values of markers on chromosome 1B ranged from 2.27 to 2.45%, while markers on chromosome 2A explained from 1.60 to 2.44% of the phenotypic variation. On chromosome 3A, marker *wPt-1923* tagged a region significant for TTTTF, TTKSK, and JRCQC with R^2 values from 2.09 to 3.98%.

Race-specific effects ($P < 0.001$) were observed for each race as following: for race TRTTF, putative genomic regions significantly affecting the response were found on chromosomes 2A, 2B, and two regions on chromosome 7A. The region with the largest effect ($R^2 = 8.34\%$) was tagged by *gwm47* on chromosome 2BL. The second and third regions with a sizeable effect to the response to race TRTTF were tagged by markers *wPt-6668* and *gwm344*

Table 4. Most significant markers associated with quantitative trait loci for resistance at seedling stage to stem rust races TRTTF, TTTTF, TTKSK, and JRCQC.

			P value (Q + K model)†				R^2 (%)				Number of significant tests over four races		Effectiveness under field conditions‡ (R^2, %)	Suggestive designated *Sr* locus
Marker	Chr.	cM	TRTTF	TTTTF	TTKSK	JRCQC	TRTTF	TTTTF	TTKSK	JRCQC	Marker-wise tests	Genome-wise tests		
wPt-1876	1B	31.3	–	–	–	0.0033	–	–	–	3.13	1	0	yes (R^2 = 4.6)	*Sr14*
barc61	1B	87.2	0.0306	0.0393	0.0328	–	2.45	2.27	2.41	–	3	0	yes (R^2 = 1.0)	–
barc81	1B	119.9	–	0.0144	0.0131	–	–	2.42	2.37	–	2	0	no	–
wmc44	1B	158.1	–	0.0283	–	0.0093	–	3.88	–	4.86	2	0	no	–
wPt-5839	2A	0	0.0165	0.0098	0.0155	–	1.60	2.44	2.17	–	3	0	no	–
cfa2201	2A	47.8	0.0054	–	0.0137	–	4.25	–	3.50	–	2	0	no	–
wPt-2293	2A	63.7	**1.26×10^{-4}**	–	–	–	**4.20**	–	–	–	1	1	no	–
gwm410	2B	64.1	0.009	–	–	–	3.70	–	–	–	1	0	no	–
gwm47	2B	158.9	**1.81×10^{-5}**	–	–	–	**8.34**	–	–	–	1	1	no	*Sr9/Srweb*
gwm1300	2B	169.1	–	0.0215	0.0041	–	–	3.36	4.66	–	2	0	yes (R^2 = 1.7)	*Srweb*
wmc356	2B	220	–	0.0421	–	0.008	–	2.44	–	4.22	2	0	yes (R^2 = 4.1)	*Sr16/Sr28*
wPt-1923	3A	46.4	–	0.0029	0.017	**6.94×10^{-4}**	–	3.17	2.09	**3.98**	3	1	yes (R^2 = 2.2)	–
gwm1620	3A	83	–	0.0375	–	0.0152	–	2.47	–	3.09	2	0	yes (R^2 = 4.0)	–
wmc264	3A	119.6	0.0217	–	–	0.0322	3.7	–	–	3.49	2	0	no	–
wmc43	3B	57.9	–	0.0127	**0.0017**	–	–	2.75	**4.87**	–	2	1	no	–
wmc418	3B	122	0.0116	0.0143	–	–	3.66	3.56	–	–	2	0	no	–
wPt-9049	3B	182.5	–	–	–	0.0043	–	–	–	2.84	1	0	no	–
barc78	4A	110.6	–	–	–	**6.87×10^{-7}**	–	–	–	**9.36**	1	1	yes (R^2 = 1.9)	–
gwm1084	4B	91.9	–	0.0081	–	–	–	3.67	–	–	1	0	no	–
gwm617	5A	4.4	–	–	0.0024	–	–	–	4.80	–	1	0	no	–
gwm1570	5A	32.6	–	–	–	0.004	–	–	–	2.94	1	0	no	–
barc165	5A	57.1	–	–	–	0.0248	–	–	–	3.50	1	0	no	–
gwm410	5A	120	0.0155	0.0191	0.009	0.0032	2.04	1.92	2.56	3.56	4	0	no	–
wPt-5514	5B	0	–	0.011	0.0067	–	–	2.48	2.89	–	2	0	no	–
gwm234	5B	27.2	–	–	–	0.007	–	–	–	3.51	1	0	no	–
wPt-0566	5B	189.1	0.0415	0.0021	–	–	1.13	3.56	–	–	2	0	no	–
gwm169	6A	126.6	–	0.002	0.0345	–	–	3.68	1.75	–	2	0	yes (R^2 = 1.5)	*Sr26*
gwm427	6A	139.5	–	–	0.0153	0.0323	–	–	2.49	1.51	2	0	yes (R^2 = 3.5)	*Sr13*
CD926040	6A	143.9	0.0087	**3.43×10^{-12}**	**4.17×10^{-16}**	**1.56×10^{-10}**	3.52	**17.64**	**23.12**	**15.33**	4	3	yes (R^2 = 7.1)	*Sr13*
barc104	6A	155.3	0.037	**7.52×10^{-7}**	**3.35×10^{-10}**	**4.02×10^{-6}**	2.84	**11.95**	**17.82**	**10.47**	4	3	yes (R^2 = 4.5)	*Sr13*
wPt-2991	6B	19.4	–	–	–	0.0095	–	–	–	2.4	1	0	no	–
wPt-0470	6B	51.6	–	0.0088	0.0086	–	–	2.45	2.50	–	2	0	no	–
gwm518	6B	84.1	–	–	0.0032	–	–	–	4.31	–	1	0	no	–
gwm816	6B	102.1	–	–	–	0.0307	–	–	–	2.60	1	0	no	–
wPt-6668	7A	32.7	**8.35×10^{-4}**	–	–	–	**2.70**	–	–	–	1	1	yes (R^2 = 5.2)	–

(cont'd)

Table 4. Continued.

Marker	Chr.	cM	*P* value (Q + K model)†				R^2 (%)				Number of significant tests over four races		Effectiveness under field conditions‡ (R^2, %)	Suggestive designated *Sr* locus
			TRTF	TTTF	TTKSK	JRCQC	TRTF	TTTF	TTKSK	JRCQC	Marker-wise tests	Genome-wise tests		
wPt-7188	7A	80.4	0.0056	–	0.0341	–	2.11	–	1.77	–	2	0	no	–
wPt-7299	7A	150.7	–	–	0.0037	–	–	–	3.14	–	1	0	no	*Sr22*
gwm344	7A	241.9	**5.25×10^{-4}**	0.0088	–	–	**5.79**	3.79	–	–	2	1	yes (R^2 = 1.3)	*Sr15*
gwm573	7B	66.6	–	–	–	0.0347	–	–	–	3.18	1	0	yes (R^2 = 3.4)	–
gwm333	7B	99.7	–	–	–	0.0182	–	–	–	3.90	1	0	no	–
wmc517	7B	155.6	–	**1.73×10^{-5}**	**0.0016**	–	–	**8.0**	**4.96**	–	**2**	**2**	yes (R^2 = 2.3)	*Sr17*
Total significant regions marker-wise test			**15**	**20**	**19**	**19**								
Total regions after FDR§ genome-wise test			**4**	**3**	**4**	**4**								

†*P* values of markers that showed significant ($P \leq 0.05$) effects at the genome-wise level are reported in bold.

‡Results reported in Letta et al. (2013).

§False discovery rate.

both on chromosome 7A, with R^2 values of 2.70 and 5.79%, respectively. Marker *wPt-2293* on chromosome 2A tagged an additional region with a sizeable effect (R^2 = 4.20%) on this race. Also in this case, Supplemental Table S3, shows the strong association of marker alleles at *wPt-2293* on chromosome 2A and *gwm47* on chromosome 2BL to the accessions response to TRTTF.

For race TTTTF, marker *wmc517* on chromosome 7B showed a significant effect (R^2 = 8.00%) that was shared with race TTKSK (R^2 = 4.96%). A QTL (R^2 = 4.87 and 2.75%) specific for TTKSK and TTTTF, respectively, was observed on chromosome 3B (*wmc43*).

Remarkably, up to 10 QTLs with high specificity for race JRCQC were identified. These ten QTLs were tagged by *wPt-1876*, *wPt-9049*, *barc78*, *gwm1570*, *barc165*, *gwm234*, *wPt-2991*, *gwm816*, *gwm573*, and *gwm333*. Among those QTLs, the most important in terms of significance and R^2 value was located on chromosome 4A, tagged by *barc78* (R^2 = 9.36%).

A previous study has examined the present collection for resistance to stem rust at the adult stage in the field (Letta et al., 2013) using an inoculum which included three of the four races (TTKSK, TRTTF, and JRCQC) tested herein. Letta et al. (2013) highlighted the presence of 24 QTLs with significant effects in two out of four seasons and 12 QTLs in three out of four seasons. The results of the seedling tests confirm that more than half (15 in total) of the loci reported in Letta et al. (2013) were detectable through AM performed based on the response to *P. graminis* tested at the seedling stage (Table 4). The features of those loci that could be detected both at the seedling stage and under field conditions showed that the majority were effective against two to three races while those that showed a race-specific response were mostly effective against JRCQC and TRTTF, that is, the two Ethiopian races, as expected (Table 4).

More in detail, the most interesting regions based on their effects across both selected races and field trials were those located on chromosomes 1B, 2B, 3A, 6A, 7A, and 7B. The QTLs tagged by *barc61* (1B), *gwm1300*, and *wmc356* (2B), *wPt-1923* (3A), the complex locus tagged by *gwm169*, *gwm427*, *CD926040*, *barc104* (6A), and *wmc517* (7B) were all effective against race TTKSK, present throughout Africa and Ethiopia, as well as other races and in two to four field trials. Three additional QTLs significant for both seedling tests and field trials were mostly shown to be specifically effective for the Ethiopian race JRCQC (*gwm1620* on chromosome 3A, *barc78* on chromosome 4A, and *gwm573* on chromosome 7B). Another QTL highly effective in the field (tagged by *wPt-6668* on chromosome 7A) was shown to be specific for the second Ethiopian race used in this study (TRTTF).

The graphical genotypes of the 183 Durum panel elite accessions herein characterized were inspected for the most significant loci, side to side to their responses across the four tested races (as reported in Supplemental Table S3, accessions clustered based on classes of increasing susceptibility response). In the first group of accessions, classified as resistant to all the four races, up to 32 accessions showed a frequency of cumulated favorable marker alleles equal or higher than 0.60, suggesting that these accessions

Table 5. Percentage of phenotypic variation explained by the simultaneous fit of the most significant markers associated with quantitative trait loci for resistance at seedling stage to stem rust races TRTTF, TTTTF, TTKSK, and JRCQC.

Multiple regression model	TRTTF		TTTTF		TTKSK		JRCQC	
	Markers	R^2	Markers	R^2	Markers	R^2	Markers	R^2
	no.	%	no.	%	no.	%	no.	%
Experiment-wise significant markers†	4 (7 alleles)	32.7	3 (9 alleles)	29.2	4 (9 alleles)	44.5	4 (11 alleles)	46.5
Experiment- and marker-wise significant markers‡	15 (34 alleles)	54.9	20 (49 alleles)	58.4	19 (43 alleles)	63.0	19 (52 alleles)	72.3
All markers§	41 (100 alleles)	77.5	41 (100 alleles)	75.4	41 (100 alleles)	76.7	41 (100 alleles)	80.9

†Marker sets that were significant at the experiment-wise level for each stem rust race.

‡Marker sets that were significant at the experiment- and marker-wise levels for each stem rust race.

§Pool of all markers that were significant (experiment- and marker-wise levels) in any stem rust race tested.

carried at least five to seven favorable alleles across the ten marker loci considered. A few of these accessions with promising seedling response were originated directly or indirectly from the CIMMYT and ICARDA breeding programs. Some accessions from the Italian breeding program were susceptible to TTKSK (Ug99) but showed valuable resistances to the highly virulent JRCQC race and also to TRTTF. Conversely, a high frequency of accessions completely susceptible to the tested races was present in high frequency in the Italian germplasm and in some ICARDA materials including the old Haurani landrace founder.

Discussion

There is a growing interest in applying AM to a wide range of crops to identify loci responsible for quantitatively inherited variation, including durable resistance (Hall et al., 2010; Kollers et al., 2013). Accordingly, a better understanding of the genetic basis underlying the naturally occurring genetic diversity for stem rust response in durum wheat could help to accelerate the progress of enhancing stem rust resistance in this crop while shedding light on the evolution of the host–pathogen relationships. Along this line, the panel of accessions herein evaluated surveys the genetic variation present in elite germplasm pool commonly used by durum breeders, a feature that makes our results more readily transferable to breeding activities.

The survey was performed based on a set of *Pgt* isolates belonging to four races chosen to represent the most virulent, diverse, and aggressive pathotypes challenging durum wheat worldwide, that is, the TTKSK (= Ug99) race now diffused throughout Central and Northeast Africa and Iran in Asia (Singh et al., 2006; SeedQuest, 2008), The North American TTTTF race (Jin et al., 2008) and two recently described and highly virulent Ethiopian races (TRTTF and JRCQC) that overcame some of the few resistance genes effective against Ug99 (Olivera et al., 2012). These four races complement each other in terms of their virulence/avirulence formula, thus providing a nearly complete spectrum of virulence against known resistance loci (Rouse et al., 2012; Pretorius et al., 2012).

Association mapping test identified 41 distinct QTL regions for resistance to stem rust at the seedling stage, a number higher than those reported in both durum and bread wheat for response at the adult plant stage under field conditions (Yu et al., 2011, 2012; Letta et al., 2013). The higher number of significant loci herein reported is in line with the expectation based on: (i) the higher h^2 values of the phenotypes at the seedling stage as compared with the field-based phenotypes, (ii) the satisfactory genome coverage level reached in this study through a combined utilization of different marker classes (SSR and DArT), and (iii) the higher potential of AM as compared with biparental mapping in revealing segregating loci, based on the wider genetic diversity explored (Hall et al., 2010; Sukumaran and Yu, 2014). Only a few mapping studies have reported complete genome-wide surveys of rust resistance at both seedling and adult-plant field conditions and compared the results observed in such conditions (Crossa et al., 2007; Maccaferri et al., 2010; Ingala et al., 2012).

This evaluation at the seedling stage allowed us to identify the most important loci effective under field conditions, as reported in Letta et al. (2013), while providing evidence for the presence of additional loci involved in stem rust response. Overall, these results show that an accurate and targeted screening at the seedling stage is effective in identifying loci for resistance under field conditions.

Significant Markers Tagging Identified *Sr* Genes or Novel Resistance Loci

To determine whether any known resistance gene coincided with the putative genomic regions identified in this study, the current results were compared with previous findings for stem rust resistance in wheat. A number of QTLs identified in this study co-located with previously reported major *Sr* loci (within a 10-cM-wide interval) as well as with QTLs recently identified through linkage mapping in tetraploid wheat (Haile et al., 2012) and AM in hexaploid wheat (Yu et al., 2011, 2012). One QTL tagged by wPt-1876 (chromosome 1B) for response to race JRCQC corresponds to a region previously shown to influence stem rust resistance in two independent field studies (Crossa et al., 2007; Yu et al., 2011). Moreover, this region harbors *Sr14* which appears effective against several stem rust races (Singh et al., 2006). This region did not show significant effects for race TTKSK, in accordance to the *Sr14* seedling IT reported by Jin et al. (2007). The genomic region on the distal part of chromosome 1B, tagged by *wmc44*, and associated with seedling resistance to TTTTF and JRCQC has been shown to harbor genes for multiple diseases: *Lr46/Yr29/Pm39* and a still undesignated gene

for adult plant resistance (APR) to stem rust (Bhavani et al., 2011; Ravi Singh, personal communication, 2013). On chromosome 2A, *cfa2201* and wPt-5839 co-located with the region known to host *Sr38* and *Sr34*, respectively. However, both genes are ineffective against races of the Ug99 lineage (Jin et al., 2007; Singh et al., 2011) and originate from *T. comosum* and *T. ventricosum*, which makes their presence in durum wheat highly unlikely. Consequently, *cfa2201* and wPt-5839 appear to tag new resistance loci. Several *Sr* genes are located on chromosome arm 2BL, including *Sr9*, *Sr16*, *Sr28* (McIntosh et al., 1995) and *SrWeb* (Hiebert et al., 2010). *SrWeb* confers resistance to Ug99, while none of the four alleles of *Sr9* confers resistance to the same race (Jin et al., 2007). Hence, the significant effects detected by *gwm1300* for race TTKSK might be tagging the presence of *SrWeb* (Hiebert et al., 2010) while *gwm47*, detected for race TRTTF, tags potentially new alleles near or at the *Sr9* locus. Additionally, at the end of chromosome 2B, significant effects of *wmc356* on races TTTTF and JRCQC were detected, but it is unlikely that *Sr16* or *Sr28* plays any role since both genes are ineffective to these races. Chromosome arm 3BS harbors several *Sr* genes including *Sr2*, *Sr51*, *Sr12*, and *SrB* (Yu et al., 2009).

Among the >50 *Sr* resistance loci identified so far, *Sr2* has been one of the most widely deployed and has provided durable adult plant rust resistance for more than 50 yr (McIntosh et al., 1995). A previous study suggested that the *Sr2* APR allele is rare in the AM panel considered herein (Letta et al., 2013). Accordingly, none of the markers near the position of *Sr2* showed significant effects (Mago et al., 2010). Based on IT responses, the chances of detecting *Sr2* at the seedling stage are low, being *Sr2* an APR locus. Nevertheless, *Sr2* has been reported to be tightly linked to a specific leaf chlorosis (mosaic) phenotype that was not observed in the panel (Olivera, unpublished results, 2013). However, the genomic region at *wmc43* on chromosome arm 3BS conferring resistance to races TTKSK and TTTTF is in the same region as a previously reported QTL region for field stem rust resistance (Yu et al., 2011).

Four markers that were mapped in a 28.7 cM-wide region (as estimated based on the consensus map reported in Letta et al., 2013) on chromosome arm 6AL with negligible LD as to each other (*gwm169*, *gwm427*, *CD926040*, and *barc104*) were strongly associated with resistance to all stem rust races and showed significant effects in the same region previously reported to harbor genes for stem rust resistance. For instance, *gwm427*, *CD926040*, and *barc104* correspond to the region reported by Simons et al. (2011) and Letta et al. (2013), while *gwm169* co-locates with *Sr26*, a gene effective against Ug99 (Singh et al., 2006) and the Ethiopian races (Badebo and Ammar, unpublished results, 2010). However, *Sr26* has been reported to be present exclusively in bread wheat following an introgression from the wild relative *Thynopirum elongatum*, thus its presence within the elite durum wheat germplasm included in this study is unlikely. The *gwm427-CD926040-barc104*-wide region co-locates with *Sr13* that in tetraploid wheat has been mapped within a 1.2- to 2.8-cM interval, flanked by the EST-derived markers *CD926040* and *BE471213* (Admassu et al., 2011; Dubcovsky et al., 2011; Simons et al., 2011). In our study, *CD926040* showed the largest R^2 value and significance effects for resistance to all four races. *Sr13* is effective against the TTKS complex of *Pgt*, namely TTKSK (Ug99), TTKST, and TTTSK, but its resistance has been overcome by Ethiopian stem rust populations (Admassu et al., 2009) and more specifically by the two recently characterized Ethiopian races (TRTTF and JRCQC) used in this study and collected at the site near Debre Zeit, Ethiopia (Olivera et al., 2012). The strong association between the markers located in the *Sr13* region and resistance to the TRTTF and JRCQC races, as well as to the moderate resistance shown in Debre Zeit field trials, suggests the presence on chromosome arm 6AL of an additional and novel gene closely linked to *Sr13*. Fine mapping and more precise characterization of allelic variation (single nucleotide polymorphism [SNP]-haplotyping) present in the germplasm will help to elucidate the precise genetic basis underlying the chromosome 6AL-related resistance to stem rust in durum wheat. Other *Sr* genes were mapped to chromosome 6A, such as *Sr5* and *Sr8a*, both highly effective for races TRTTF and JRCQC (Yue Jin, personal communication, 2013). However, their mapping locations did not coincide with the 6AL-distal region.

Two significant QTLs for resistance to stem rust were found on chromosome arm 7AL, where *Sr22* and *Sr15* are located. *Sr15* is distally located on chromosome arm 7AL near *gwm344*, while wPt-7299 appears to be linked to *Sr22*. Finally, AM detected a QTL at the distal end of chromosome arm 7BL near *wmc517*, a region known to harbor *Sr17*, a gene linked to *Lr14a* and *Pm5* in bread wheat (Crossa et al., 2007). It is also consistent with a region reported to include a stem rust QTL in the Arina × Forno recombinant inbred line population (Bansal et al., 2008). The majority (30 out of 41) of significant markers tagged regions where no stem rust genes had previously been reported. These regions with significant associations were detected for all chromosome groups except for Group 1. Six of these regions were also relevant for adult-based field resistance in Debre Zeit.

Among the regions for which designated candidate loci could not be envisaged, *cfa2201* and *wPt-5839* co-located in a region on chromosome 2A known to host *Sr38* and *Sr34*, respectively. However, both designated genes are ineffective against the Ug99 race lineage (Jin et al., 2007; Singh et al., 2011) and originate from *T. comosum* and *T. ventricosum*, which makes their presence in durum wheat unlikely. The QTLs on chromosome 3A (*gwm1620* and *wmc264*) mapped where *Sr27* and *Sr35*, both effective against Ug99, have been reported (McIntosh et al., 1995; Singh et al., 2006; Jin et al., 2007). However, as *Sr27* originated from a wheat-rye translocation present mostly in triticale and *Sr35* from *T. monococcum* and then transferred to some tetraploids of Canadian origin, none of which were present in this study or in the pedigree of the accessions of the AM panel, the chromosome 3A-related associations detected herein are likely to involve alternative and unknown loci. Additionally, no *Sr* gene has been

reported on chromosome arm 3BL, and thus the significant effects associated with *wmc418* and *wPt-9049* is likely due to putatively novel loci. Similarly, the QTL on chromosome arm 4AL with a major effect for race JRCQC and the QTL on chromosome 4BL for race TTTTF represent new race-specific loci for stem rust resistance.

The significant markers identified on chromosomes 5A and 5B did not overlap with any reported major *Sr* gene, although QTLs for response to Ug99 have been mapped in similar locations in hexaploid wheat and represent six novel loci for resistance in durum wheat (Yu et al., 2011). Accordingly, all the four significant regions identified on chromosome arm 6BS did not coincide with any of the reported major *Sr* genes, and thus may also represent new *Sr* loci. Additionally, the two genomic regions detected on chromosome 7AS by wPt-6668 and wPt-7188 were not significantly associated with stem rust resistance in previous reports. Although no *Sr* gene has been reported for chromosome arm 7BS, two distinct QTLs were detected for race JRCQC near *gwm573* and *gwm333*, which could thus also be considered as novel *Sr* loci. Following further characterization and validation, diagnostic markers for all these resistance loci reported in this paragraph would be useful for enhancing APR to stem rust, provided they are also associated with broad-range APR.

Reaction of Race-Specific Resistance Genes

All designated genes, except *Sr2* and the recently characterized *Sr55* and *Sr57*, are race specific. In our study, several race-specific QTLs associated to resistance to three or four of the races were detected that, on pyramiding, may reduce susceptibility and enhance durability. Most studies in which several races were used to detect QTLs for resistance have reported either race-specific QTLs (Niks et al., 2000; Zhu et al., 2003) or a combination of broad-spectrum and race-specific QTLs with various effects on resistance (Qi et al., 1999). The results of our study appear even more complex, since it was possible to report the concomitant presence of different categories of loci involved in the stem rust response, such as (i) race-specific QTLs with strong effects on resistance (e.g., the QTL on chromosome 2B tagged by *wPt-2293* and *gwm47* for race TRTTF and the QTL on chromosome 4A tagged by *barc78* for race JRCQC), (ii) QTLs with relatively strong effects that were confirmed across at least two races (e.g., QTL on chromosome 7B tagged by *wmc517* for races TTKSK and TTTTF), (iii) some minor QTLs were effective across all races (QTL on chromosome 5A tagged by *gwm410*) and (iv) the QTL cluster on chromosome 6A tagged by *CD926040* and *barc104* that showed broad spectrum resistance with major effects for races TTKSK, TTTTF, and JRCQC and relatively minor effects for race TRTTF. The greater complexity observed in our study could partly derive from the evaluation of a large number of accessions originated from different genetic backgrounds tested with races characterized by distinct virulence.

Breeding Perspectives and Conclusions

This study clearly shows that the level of seedling resistance to stem rust in elite durum wheat is governed by a relatively high number (41 in total) of QTLs whose effects have been detected across races, as well as in race-specific interactions. Among these, a sizeable portion (73%) are novel chromosome regions for which no previously designated loci have been mapped. Notwithstanding the clearly quantitative and complex base of genetic response to stem rust in the elite materials studied herein, the few (up to 10) marker loci that proved successful in tagging a sizeable portion of the phenotypic variation can be conveniently used for prescreening of the breeding germplasm to be used in crosses specifically designed to improve stem rust resistance.

In the case of the major QTL on chromosome 6A (known to harbor *Sr13*), the results with the single races suggest that the resistance could be more complex than what was expected based on the model of a single gene-for-gene interaction. Selection for markers closely linked to these loci has thus the potential to improve stem rust resistance in pedigree-related breeding materials based on marker haplotyping (Maccaferri et al., 2007; Yu et al., 2010; Haile et al., 2013). Suitable markers are already available for *Sr9* (Tsilo et al., 2007), *Sr13* (Simons et al., 2011), *Sr26* (Mago et al., 2005; Liu et al., 2010), and *Sr25* (Yu et al., 2010). If further confirmed, the QTLs reported here for seedling resistance and the corresponding closely linked molecular markers will contribute to broadening the genetic basis of stem rust resistance, an important goal of durum wheat breeding. Our results indicate the suitability of AM to identify novel sources of stem rust resistance alleles to accelerate durum wheat improvement and cultivar release. Additionally, the results confirm the role of *Sr9*, *Sr13*, and *Sr14* previously described in biparental mapping studies while unveiling the presence of putatively novel loci. Combining the results of this study with those on APR in the field where races such as TTKSK, TRTTF, and JRCQC prevail (Letta et al., 2013) will facilitate the selection of suitable parental lines for further improving stem rust resistance of durum wheat. Notably, some of the durum wheat lines that were tested herein carry resistance to all four *Pgt* races. Interestingly, a number of these accessions characterized by an overall valuable phenotypic response to stem rust and carrying beneficial resistance alleles at different QTLs at the same time have been identified and reported. These accessions would then be useful donor parents in traditional breeding programs, as well as in marker-assisted backcrossing schemes aimed at selecting lines with resistance alleles at different loci in an elite genetic background. Further characterization of sets of near-isogenic lines in different genetic backgrounds would confirm the QTL effects while providing more accurate estimates of allelic effects and their possible epistatic interactions. In the near future, the availability of high-density SNP platforms including thousands of markers will allow for studies with almost complete genome coverage and a much more refined resolution at the haplotype

level (Trebbi et al., 2011; You et al., 2011; van Poecke et al., 2013). The use of the same SNP assays in applied breeding programs will also facilitate the simultaneous selection of multiple beneficial alleles for partial resistance. Finally, the relatively large number and small effects of the QTLs herein described suggest that a more comprehensive selection strategy, such as genomic selection (Heffner et al., 2009; Rutkoski et al., 2011, 2012), may prove more cost-effective than conventional MAS at accumulating beneficial alleles in breeding populations.

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