

QTLs for yield and adaptation to reduced water and nitrogen inputs in durum wheat

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ABSTRACT

A population of 181 recombinant inbred lines (RILs) derived from the cross Meridiano × Claudio was evaluated in five field trials varying for water and nitrogen regimes. A preliminary analysis carried out with 213 SSR and DArT markers evidenced two major QTL clusters for grain yield, yield components and several other traits on chrs. 4B and 5A. Favourable alleles were inherited from the parental cultivar Claudio at the 4B QTL region and from Meridiano at the 5A QTL. Additional markers with highly significant effects on grain yield were found on chrs. 2B, 3B, 5B and 7B, with favourable alleles contributed by both parents, thus accounting for the wide transgressive segregation observed among the RILs. Grain yield of the RILs was highly predictable based on the SSR alleles present at four key-chromosome regions.

INTRODUCTION

A prerequisite for the use of more sustainable agricultural practices is the release of cultivars (cvs.) requiring lower water and nitrogen (N) inputs; therefore, the improvement of water- and N-use efficiency is an important goal for improving grain yield and reducing N pollution. Durum wheat (*Triticum durum* Desf.) is a cereal of primary importance cultivated across a number of macro-environments differing in photoperiod and thermo-pluviometric patterns (Leemans and Cramer, 1991; Loss and Siddique, 1994). Moreover, it is prevalently grown under rainfed farming systems in the Mediterranean Basin as well as in other semi-arid regions of Asia, America and Australia. Utilization of genomic tools can greatly facilitate breeding procedures aimed at enhancing crop sustainability, yield potential and yield stability.

As compared to hexaploid wheat, durum wheat underwent a more limited selection until 1960, when multi-environment testing for wide adaptation was first adopted. The genetic gains recently obtained in grain yield potential of durum wheat have been mainly attributed to a balanced improvement of fertility concomitant to a general increase in total biomass, with the harvest index remaining practically unchanged (Pfeiffer et al., 2000). The molecular tools necessary to identify the quantitative trait loci (QTLs) governing grain yield, yield components, yield stability, and genotype × environment (G × E) interaction are now

available for most of crops, including wheat (Varshney et al., 2007; Bagge et al., 2007).

The purpose of this research was to investigate the genetic basis of yield and other agronomic traits under different water and N regimes in a durum RIL population obtained from the cross between two elite cultivars.

MATERIALS AND METHODS

A population of 181 RILs were developed by Società Produttori Sementi (Bologna, Italy) from the cross between the two Italian elite durum wheat cvs. Meridiano (Simeto/WB881//Duilio/F21) and Claudio (CIMMYT' selection/Durango//IS139b/Grazia), both characterized by high yield potential and stability. Both cvs. are currently cultivated extensively across Southern Europe. Meridiano is early to medium-early heading, whereas Claudio is medium-early to medium-late depending on the environments. Seeds of the RILs were bulked in the F₇ generation.

During the 2007 season, the RILs were tested using an augmented field design including three replicated checks (the parents and the widely grown CIMMYT's cv. Yavaros 79) in northern Mexico (Obregon, CIMMYT's primary yield testing location, 27° 24' N, 109° 56' W), southern Italy (Lucera, Foggia, 41° 33' N, 15° 22' E) and northern Italy (Cadriano, Bologna, 44° 39' N, 11° 29' E). Three field trials were conducted at Obregon, under full irrigation conditions (OB-FI), reduced irrigation (OB-RI) and low N supply (OB-LN), while the two field trials that were carried out in Italy (Lucera: LU and Cadriano: CD) were managed under rainfed conditions adopting normal rates of N fertilizer. The following traits were considered: heading date (HD, days from sowing), maturity date (MD, days from sowing), plant height (PH, cm), grain yield (GY, t/ha), number of spikes per m² (SPM2, no. m⁻²), number of grains per m² (GRM2, no. m⁻²), grain weight (TKW, g/1000 seeds), test weight (TWT, kg hl⁻¹), flag leaf greenness (SPAD) and NDVI spectro-radiometrical index, both measured at the end of flowering.

The linkage map was obtained based on the wheat simple sequence repeat (SSR) sets available on the GrainGenes database (WMS, WMC, BARC, CFA, CFD probe sets), together with a private WMS set provided by Dr. Martin Ganai, (TraitGenetics, Gatersleben, Germany) and a set of DArT markers generated with the durum DArT v. 2.0 *PsfI* (*TaqI*) service by Triticarte Pty Ltd, (Yarralumla, Australia). A provisional linkage map was constructed with 414 markers (98 SSRs and 316

DArT markers), grouped at high LOD threshold ($LOD \geq 6$) into 41 linkage groups, covering a total of 1856 cM. Linkage groups were identified using JoinMap 4.0 (Stam, 1993). Marker ordering and mapping (Haldane's function) was performed for each linkage group using a combination of Record (van Os et al., 2005) and JoinMap 4.0 mapping programs. A subset of 213 markers selected for high informativeness and even distribution (intermarker distance ≥ 3 cM) was used for QTL analysis. Composite interval mapping was carried out for each environment using the adjusted data from the five field trials. Windows QTL Cartographer v. 2.5 was used (<http://statgen.ncsu.edu>). The results of a preliminary analysis using the "model 6 standard analysis" with up to 5 control markers and a window size of 10 cM are reported. A LOD threshold of 2.5 was used for QTL declaration.

RESULTS AND DISCUSSION

In 2007, the RILs were tested under environments highly differentiated as to photoperiod and temperature conditions during the wheat growth. As shown in Table 1, the mean length of the RIL crop cycle (see HD and MD) in the five field trials ranged from a minimum of ca. 120 days in Obregon to a maximum of more than 200 days in northern Italy. Mean yield of the RILs ranged from a minimum of 1.71 ton/ha in OB-RI to a maximum of 6.28 ton/ha in northern Italy. As shown in Table 1, the RILs showed a wide range of variation for all the considered agronomic traits. A substantial transgressive segregation was observed for most traits, particularly for HD and MD in Obregon.

A preliminary QTL analysis pointed out the presence of QTLs ($LOD \geq 2.5$) for the investigated traits in all chr. groups. Major QTLs ($LOD \geq 3$ and $R^2 \geq 10\%$) for HD and MD were detected only on chrs. 4B and 5A. Both QTLs showed a large interaction with the environment. Additionally, minor QTLs were found on chrs. 3B and 7A.

Several QTLs were found for PH and for two important agronomic traits in durum: TKW and TWT. QTLs for PH were mapped on chrs. 2B, 3B, 4B, 5A, 6A, 6B and 7B. QTLs for TKW were evidenced on chrs. 2A, 2B, 3A, 4B, 5A and 6A. Most of the QTLs for TWT overlapped those for TKW, except for one QTL on chr. 2B.

GY was controlled by the major QTL for adaptation on chr. 5A. However, the second main QTL cluster on chr. 4B for adaptation and for agronomic traits such as TKW and TWT did not have any noticeable effect on GY. Additional QTLs for GY were identified on chr. 3A (CD env.) and on chr. 2A, 2B, 5B, 6A and 7B (LU environment).

Chr. 5A harboured two regions carrying QTLs for PH, GY, TKW and TWT. The first QTL region was detected on the centromeric region of 5AL chr. arm, in an 11-cM interval between *Xcfa2121*, *Xbarc40* and *Xgwm156*. Within this interval, a QTL for GY was found in LU ($R^2 = 8.5\%$) and two QTLs for TKW and TWT ($R^2 = 4.2$ and 5.4% , respectively) in CD. The second, most important, 5A QTL cluster for HD, GY and related traits was positioned in the central region of the chr. arm, identified by *Xwmc388.2* and *Xgwm1570*. The features of this second 5A QTL cluster are reported in Table 2 on a single-environment basis. The interval of significance ($LOD \geq 3$) of all the QTLs detected in this chr. region is comprised within a 10 cM interval. This region strongly influenced the crop cycle and GY in all the three field trials carried out in Obregon. It also indicates that QTLs with strong effects on GY under favourable conditions can be as important under water or N limiting conditions, partly supporting the observation that genetic gains from selection under favourable conditions can result in gains in stressed environments. The allele carried by Claudio considerably delayed HD (difference between the homozygous alleles comprised between 10 and 18 days) in Obregon and negatively impacted GY, TKW and TWT (Table 2). This allelic difference in HD under field conditions, with Claudio carrying the late allele, was also detectable in southern and northern Italy, but the allelic effects on HD were much lower, reaching the minimum in CD, northern Italy (with less than 2 days difference between the homozygous alleles), where the QTL showed no effect on GY. The position of both QTL clusters found on chr. 5AL is mostly overlapping with the two main QTL regions affecting response to photo-thermal conditions and thus earliness in wheat (Hanocq et al., 2007). In particular, the putative genetic position of the major QTL cluster on the central region of chr. 5AL overlaps with the location of *VRN-1*, one of the major genes controlling vernalization requirement in wheat (Yan et al., 2003).

These results highlight that variation at a limited number of key chr. regions largely influence phenology and broad adaptability of tetraploid wheat, similarly to what has been observed also in hexaploid wheat (Cockram et al., 2007; Maccaferri et al., 2008), and that large genetic variation for adaptation, yield potential and yield components is present in the elite durum wheat germplasm. Up to now, this variation has been genetically explored to a limited extent.

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Table 1. Mean phenotypic values of the 181 durum wheat Meridiano × Claudio RILs and their range of variation across the five environments tested in 2007.

		OB-FI ^a		OB-RI		OB-LN		LU		CD	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
HD	days	90	75 - 106	79	71 - 104	83	58 - 99	133	125 - 140	168	159 - 172
MD	days	129	117 - 139	115	100 - 130	119	103 - 133	-	-	208	201 - 214
PH	cm	112	90 - 129	75	51 - 99	100	84 - 118	90	73 - 113	92	78 - 111
GY	ton/ha	5.35	2.14 - 8.00	1.71	0.08 - 3.39	4.04	2.39 - 5.48	5.50	2.93 - 7.52	6.28	2.12 - 8.37
SPM2	no. / m ²	420	277 - 601	156	50 - 270	287	152 - 389	471	243 - 698	420	290 - 632
TKW	g	40.3	21.4 - 55.9	40.8	25.3 - 48.8	51.1	42.1 - 59.7	44.7	31.9 - 59.5	39.3	30.4 - 48.5
GRM2	no.(×1000)/m ²	13.2	6.4 - 22.8	4.1	0.03 - 9.7	7.9	4.9 - 10.7	12.5	5.4 - 17.2	16.1	5.6 - 21.9
TWT	kg/hl	79.5	70.0 - 84.4	78.3	76.0 - 82.0	80.8	77.0 - 83.7	81.0	75.1 - 84.8	75.0	68.0 - 79.9
SPAD	units	54.7	43.6 - 63.4	60.1	56.5 - 66.8	40.2	27.8 - 49.0	-	-	49.5	40.6 - 56.4
NDVI	units	0.90	0.77 - 0.98	0.45	0.82 - 1.08	0.84	0.11 - 0.99	0.89	0.85 - 0.92	0.88	0.82 - 0.93

a) OB-FI: Obregon, full irrigation; OB-RI: Obregon, reduced irrigation; OB-LN: Obregon, low nitrogen; LU: Lucera, Italy; CD: Cadriano, Italy.

Table 2. Single-environment features of the QTL cluster on chr. 5AL. QTL peaks were comprised within a 10 cM interval between *Xwmc388.2* and *Xgwm1570*. Additive effect is reported as half of the difference between the mean phenotypic values of the RILs carrying the Claudio and Meridiano allele.

	OB-FI			OB-RI			OB-LN			LU			CD		
	LOD	R ²	<i>a</i>	LOD	R ²	<i>a</i>	LOD	R ²	<i>a</i>	LOD	R ²	<i>a</i>	LOD	R ²	<i>a</i>
HD	45.3	80.0	+8.7	18.5	40.7	+4.9	34.5	76.9	+9.2	12.2	23.8	+1.6	7.7	17.2	+0.9
MD	22.0	61.4	+4.2	33.7	67.2	+7.5	4.2	12.8	+2.6	-	-	-	2.1	5.3	+0.6
PH	4.0	8.8	-2.0	5.2	13.1	-2.7	7.9	17.6	-2.6	-	-	-	-	-	-
GY	25.1	62.2	-1.2	23.4	55.7	-0.7	11.4	24.1	-0.3	5.2	10.4	-0.2	-	-	-
SPM2	3.4	8.1	-16.9	3.4	8.3	-17.0	-	-	-	-	-	-	-	-	-
TKW	12.9	30.2	-4.1	11.9	32.3	-2.4	-	-	-	-	-	-	-	-	-
GRM2	-	-	-	19.1	41.2	-1376	6.5	15.1	-388	-	-	-	-	-	-
TWT	14.1	33.5	-1.6	-	-	-	-	-	-	-	-	-	-	-	-
SPAD	5.9	14.3	1.4	-	-	-	-	-	-	-	-	-	-	-	-
NDVI	9.3	19.5	0.017	-	-	-	-	-	-	-	-	-	-	-	-

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