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Detection of molecular markers associated with yield and yield components in durum wheat (*Triticum turgidum* L. var. *durum* Desf.) under drought conditions

Susan A. M. Dura[1]*, Mahmud A. Duwayri[2] and Miloudi M. Nachit[3]

[1]National Center for Agricultural Research and Extension (NCARE)/Biotechnology Unit, Jordan.
[2]Department of Horticulture and Crop Science, Faculty of Agriculture, University of Jordan, Amman, Jordan.
[3]Biodiversity and Integrated Gene Management Program, ICARDA, P. O. Box 5466, Aleppo, Syria.

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Durum wheat is one of the most important staple food crops grown mainly in the Mediterranean region where its productivity is drastically affected by drought. Detecting the important traits and molecular markers associated with drought and grain yield traits will aid breeders to understand the genetic mechanisms of drought tolerance of durum wheat that will assist in the development of drought tolerant varieties through the use of marker-assisted selection (MAS). The study objectives were to identify putative chromosomes regions affecting grain yield expression under drought conditions. A population of 114 F_8 recombinant inbred lines (RILs), derived by single-seed descent from a cross between "Omrabi 5" (drought tolerant variety) and "Belikh 2" (less drought tolerant), was used for this purpose. Phenotypic data of the RILs and parental lines were measured for 16 traits at Maru Agricultural Research Station (Jordan) for two consecutive seasons; 2006 to 2007 (annual rainfall 268.9 mm) and 2007 to 2008 (annual rainfall 230.6 mm). Marker-trait associations were investigated using a mixed linear model (MLM). A total of 32 simple sequence repeat (SSR) loci were significantly associated with these traits. For phonological traits, 18 markers were associated with morpho-phonological, while 31 markers were associated with yield and its components. Marker alleles from "Omrabi 5" were associated with a positive effect for the majority of traits for yield and its components. Six markers were closely linked with grain yield and these markers could be used for MAS in durum wheat breeding under drought conditions.

Key words: Durum wheat, marker-assisted selection, trait analysis by association, evolution, and linkage (TASSEL), recombinant inbred lines (RILs), simple sequence repeat (SSR), transgressive segregation.

INTRODUCTION

Wheat is the most important and strategic cereal crop for the majority of the world's population. It is the most important staple food crop of about 40% of the world population, occupying 17% of crop acreage worldwide, and providing nearly 55% of the carbohydrates and 20% of the food calories consumed globally (FAO, 2003; Gupta et al., 2008). Developing cultivars with high grain yield for drought-prone environments has been the principal aim of wheat breeding programs worldwide. In the Mediterranean region, it is of special interest because of the low and erratic distribution of rainfall, which has explained as much as 75% of the variation in wheat yield

* Corresponding author. E-mail: susanduraa@yahoo.com. Tel: 0096265345519.

(Blum and Pnuel, 1990). Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is a tetraploid made up of A and B genomes (AABB); it is the main source of semolina for the production of pasta, couscous, burghul and other Mediterranean local end-products (Nachit, 1992). Detecting the important traits and the molecular markers associated with drought tolerance and grain yield traits will aid breeders to understand the genetic mechanisms of drought tolerance of durum wheat that will assist in the development of drought tolerant varieties through the use of marker-assisted selection (MAS).

Association mapping (AM) is a method used to identify association between genotype and phenotype (Yu et al., 2006), and facilitates the identification of agronomically valuable alleles using a reasonably low number of markers to reveal significant marker-trait associations (Breseghello and Sorrells, 2006). However, computational methods have been developed to account for population structure (Pritchard et al., 2000) and implemented in AM studies in a number of crop species including barley (Rostoks et al., 2006), wheat (Parker et al., 1998, 1999; Breseghello and Sorrells, 2006; Crossa et al., 2007; Tommasini et al., 2007) and rice (Agrama et al., 2007).

Most AM studies in wheat have focused on specific chromosomes where major quantitative trait locus (QTL) have previously been reported and there is still a lack of published literature on genome-wide AM studies of important target traits in wheat. Although AM is intrinsically more powerful than genetic linkage mapping because it scrutinizes the results of thousands of generations of recombination and selection (Syvänen, 2005; Cattivelli et al., 2008), most of the data available up to date, on drought tolerance, are based on segregation mapping and QTL analysis. Future studies of AM in crop plants will also elucidate further the structures of plant genomes and will facilitate the use of MAS and map based cloning of genes for difficult traits (Gupta et al., 2005). Therefore, the aim of this study was to determine the number of markers linked with grain yield and its related traits in durum wheat under dry conditions.

MATERIALS AND METHODS

Plant material

The plant material used is a population stemmed from a cross between "Omrabi 5" and "Belikh 2". The population is constituted of 114 single seed descent recombinant inbred lines (RILs) developed by Nachit in 2005 at ICARDA durum wheat breeding program (Nachit, Personal Communication). The "Omrabi 5" durum cultivar is a cross between the landrace "Haurani" and the improved cultivar "Jori-C69"; "Omrabi 5" and "Belikh 2" were developed for the Mediterranean dryland conditions (Nachit, 1998). "Omrabi 5" is released in Jordan, Turkey, Algeria, Iran, and Iraq for commercial production; it combines drought tolerance with high and stable yields. "Belikh 2" is released in Algeria, Lebanon, and Syria; it was bred at ICARDA (Crane/Stork) and developed for rain-fed areas. It is early heading and maturing and shows good protein quality for pasta processing.

Testing environments

The RILs trial was grown at Maru experimental station (NCARE-Jordan) for two consecutive seasons: 2006 to 2007 and 2007 to 2008. Maru station is located in Jordan at 32° 36 N latitude, 35° 40 E longitude and 620 m above sea level, with silt clay soil with an average annual precipitation of 425 mm. The experiment was arranged in an augmented complete block design (Federer, 1956) in six blocks, with 1.5 m between them. Each block consisted of two sub-blocks, with 50 cm in between. Twelve genotypes, grown each in four rows (2.5 m long rows, spaced 0.35 m apart) were tested per each sub-block. Five checks per each block were used: "Omrabi 5", "Belikh 2", "Cham 1", "Korifla", and "Haurani Nawawi". Seeding rate was 120 $kgha^{-1}$. Fertilizers were applied at sowing date at a rate of 60 $kgha^{-1}$ nitrogen units (NH_4^+; ammonium form) and 40 $kgha^{-1}$ H_2PO_4 for both growing seasons and weeds were mechanically controlled.

Data collection

In addition to grain yield, information on adult-plant characters was taken for the two consecutive seasons at Maru experimental station. Days to heading (DH) was recorded as the number of days from emergence, to the day when half of the spikes appeared in 50% of the plants. Days to maturity (DM) was recorded as the number of days from emergence to the day when the peduncle was completely discolored in 90% of the plants. Plant height (PH) was measured at harvest maturity from the ground level to the top of the spikes excluding awns. Peduncle length (PL) was measured from the node to the ligule of the flag leaf as the mean of 10 random spikes. Spike length (SL) was measured from the base to the top of the spike excluding the awns as the mean of 10 random spikes. Awns length (AL) was measured from the top of the spike to the top of awns as the mean of 10 random spikes. Number of tillers (NT) was accounted in two 2.5 m row segments, 35 cm apart and converted to square meters. Number of fertile tillers (NFT) was calculated from the number of grain-bearing culms in two 2.5 m row segments, 35 cm apart and converted to square meters. Main spike weight (SW), number of grains per spike (NGS) and weight of grains per spike (GWS), and number of spikelets per spike (NSS) were determined from the mean of 10 random spikes. 1000 grain weight (TGW) was measured by weighing 200 grains taken randomly from the harvested seeds and converted to the weight of 1000 grains. Biological yield (BY) was measured as the weight of above-ground dry matter (straw + grain) harvested from the inner two 2.5 m row segments, 35 cm apart and converted to $kgha^{-1}$. Grain yield was measured as the weight of grain harvested from the inner two 2.5 m row segments, 35 cm apart and converted to $kgha^{-1}$. Straw yield was calculated as the difference between biological yield and grain yield.

Molecular analysis

DNA extraction

Leaves from seedlings grown in the field were used for DNA extraction. The DNA was extracted following the protocol developed at ICARDA durum wheat MAS laboratory (Nachit et al., 2001) and quantified by the spectrophotometer (S2100 Diode Array Spectrophotometer).

Microsatellites (simple sequence repeat, SSRs)

Wheat microsatellites consortium (WMC) and Beltsville Agriculture Research Center (BARC) were used as described by Nachit et al. (2001). Molecular analysis was conducted at ICARDA durum wheat

Table 1. Monthly minimum, maximum and average temperatures, precipitation and evaporation for Maru experimental station during 2006 to 2007 and 2007 to 2008 growing seasons.

Month	Minimums temperature (°C)		Maximum temperature (°C)		Average temperature (°C)		Precipitation (mm)		Evaporation (mm)	
	2006/2007	2007/2008	2006/2007	2006/2007	2007/2008	2006/2007	2006/2007	2007/2008	2006/2007	2007/2008
October	6.4	7.2	24.6	25.2	15.5	16.1	0.0	0.0	228.9	2456.9
November	5.0	4.4	16.4	14.2	10.7	9.3	0.0	4.3	134.9	80.4
December	3.1	3.7	14.9	12.3	8.7	8.0	20.8	49.8	75.8	69.3
January	3.8	4.6	13.6	11.8	8.4	8.2	62.2	79.1	98.7	88.9
February	6.0	5.3	15.1	10.3	10.2	7.8	102.1	85.1	133.0	78.1
March	6.8	6.0	17.8	21.2	11.9	13.6	51.3	76.9	157.5	187.2
April	9.5	10.5	21.9	27.7	15.2	19.1	23.1	35.4	216.3	245.7
May	15.1	18.1	29.6	33.9	22.0	26.0	9.4	0.0	280.6	355.7
Jun	17.6	19.6	32.0	36.1	24.2	27.9	0.0	0.0	360.4	380.1
Total							268.9	230.6	1686.1	3942.3

MAS laboratory. The parents were screened for polymorphism using 300 SSR primer pairs, out of which 96 SSR gave clear, reproducible and polymorphic amplification products. The polymerase chain reaction (PCR) amplification was carried out in Eppendorff thermal cycle, in a 7.5 µl reaction mixture. Each reaction contained 1X *Taq* polymerase buffer, 200 µM of each dNTPs, 0.5 µM of each of the two primers, 1U *Taq* polymerase, and 20 ng of genomic DNA as a template. Touchdown PCR cycling was performed as follows: an initial denaturation was performed at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing in 1°C incremental steps between 63 and 56°C for 1 min, extension at 72°C for1 min), with a final extension at 72°C for 5 min. PCR products were mixed with loading buffer, 5 to 10 µl of mixture was denaturated and loaded into wells in 0.4 mm thick 15% acrylamide gel (20 × 20 cm) resolved at constant power (30 w) in 1X TBE running buffer for 15 min to 1 h depending on size of the primer pairs of SSRs. Bands were visualized by a silver-staining method as described by Nachit et al. (2001). Dried gels were visually scored and scanned for records.

Statistical analysis and AM

The statistical analysis was performed using the MIXED procedure of the SAS statistical package (SAS, 1998). The broad-sense heritability values (H^2) were calculated as ratios of genetic and phenotypic variances (σ^2_g/σ^2_p), where σ^2_p is the phenotypic variance and was estimated as $\sigma^2_g + \sigma^2_e$, σ^2_g is the genetic variance and σ^2_e is the residual variance. Pearson's correlations between phenotypic traits and phenotypic frequency distribution were performed using SPSS 17.0 statistical software.

Because of the low number of molecular markers probed in this study to construct a genetic map, we have opted for AM between molecular markers and morpho-phonological traits under investigation. We used a mixed linear model (MLM) which is implemented in the software package version 2.0.1 (http://www.maizegenetics.net) where the marker was considered as a fixed-effect factor and the lines of the population considered as a random-effect factor (Kennedy et al., 1992). Significance of associations between marker loci and traits under investigation was based on an F-test, at a level α_c corresponding to α corrected for multiple testing. Corrected significance levels α_c were computed by 1000 permutations. The locations of these markers were identified following the genetic maps carried out at ICARDA durum wheat MAS laboratory (Nachit, Personal Communication).

The least significant difference (LSD), coefficient of variation (CV), and additive effect of the marker were estimated using Genstat (Version 11). In general and with few exceptions, the alleles of "Omrabi 5" (Mrb) had a positive additive effect on increasing trait value, whereas "Belikh 2" (Blk) had a negative one.

RESULTS AND DISCUSSION

Weather conditions

The annual rainfall and its monthly distribution differed from year to year at Maru station, Jordan. The total amount of rainfall was 268.9 and 230.6 mm for Maru in the 2006 to 2007 and 2007 to 2008 growing seasons, respectively (Table 1). In general, rainfall was lower than the long-term average (425 mm) in both growing seasons. In both growing seasons, during the post-anthesis developmental stage, rainfall in the months of April and May was low and much less than the crop requirements. This low rainfall and its poor distribution in both growing seasons affected crop performance; it subjected the crop to severe drought stress, particularly during the grain filling period. However, the months of April and May

Table 2. Combined values of means, standard deviations, and ranges for the agronomic traits of parents and the RIL population.

Trait	Omrabi5	Belikh2	RILs	
	Mean	Mean	Mean ± SD	Range
Days to heading (days)	90.2[zb]	97.4[a]	96.2 ± 0.36	85.0 - 112.0
Days to maturity (days)	128.4[b]	137.6[a]	138.1 ± 0.43	122.0 - 161.0
Plant height (cm)	73.1[a]	62.2[b]	69.3 ± 0.89	40.7 - 100.0
Peduncle length (cm)	13.7[a]	2.4[b]	6.70 ± 0.38	0.0 - 18.6
Spike length (cm)	5.5[a]	7.7[b]	7.3 ± 0.08	3.0 - 10.1
Awns length (cm)	9.7[a]	12.1[b]	11.7 ± 0.36	3.8 - 15.5
Number of tillers (m^{-2})	506.9[a]	319.2[b]	377.5 ± 8.80	120.6 - 810.0
Number of fertile tillers (m^{-2})	345.5[a]	206.6[b]	240.7 ± 65.8	80.6 - 480.5
Spike weight (g)	2.0[a]	1.5[b]	1.93 ± 0.02	0.6 - 4.1
Number of grains $spike^{-1}$	44.0[a]	32.4[b]	37.9 ± 7.0	9.5 - 64.8
Grain weigh $spike^{-1}$ (g)	1.8 [a]	1.2[b]	1.5 ± 0.30	0.5 - 2.6
Number of spikelets/spike	17.2[b]	22.8[a]	21.3 ± 2.3	14.0 - 28.6
Thousand grain weight (g)	48.9[a]	33.8 [b]	40.6 ± 5.5	25.3 - 61.2
Biological yield (kg/ha)	5616.4[a]	4179.7[b]	4385.4 ± 1159.6	1457.6 - 9619.8
Grain yield (kg/ha)	1921.7[a]	1024.6[b]	1278.0 ± 344.1	392.4 - 2809.8
Straw yield (kg/ha)	3694.7[a]	3155.0[b]	3107.4 ± 893.8	882.6 - 7343.8

For each trait, means between the parents in the same row followed by the same letter are not significantly different at $P \geq 0.05$.

2007 to 2008 season were warmer, drier, with more evaporative demand than in the 2006 to 2007 season.

Phenotypic evaluation

A total of 114 lines and their parents ("Omrabi 5" and "Belikh 2") were investigated under drought conditions. The grand means, standard deviation, and ranges of measured 16 agronomic traits for the parents and the RIL population are presented in Table 2. The two parents showed a significant difference in all 16 traits. "Omrabi 5" was earlier in DH, DM and taller than "Belikh 2". Also, "Omrabi 5" showed larger/greater NFT, NGS, GWS, PL, TGW, and yield (grain, biological, and straw) than "Belikh 2". In contrast, "Belikh 2" showed larger NSS, SL, and AL than "Omrabi 5". The largest ranges were shown for PH, NFT, PL, AL, NGS, TGW, BY, and GY (Table 2).

The phenotypic distributions of all examined traits for the lines displayed a continuous normal pattern. Obviously, these traits were quantitatively inherited. In addition, transgressive segregation in both directions was observed for all traits (Figure 1), and the parent "Omrabi 5" transmitted favorable alleles for the majority of the traits. The combined mean, LSD at 5% level, CV (%), and heritability (H^2) for all studied traits are shown in Table 3. High heritability values were shown for DH, DM, PH, PL, SL, AL, NFT, GWS, NGS, SW, NSS, and TGW, moderate values for NT, and BY; and low values fo r GY, and SY (Table 3). Significant positive correlations ($P < 0.01$) were observed between GY and PH, PL, NT, NFT, GWS, NGS, TGW, BY, and SY; and negative correlations between GY and DH, and DM. The strongest positive correlation was between BY and SY ($r = 0.79$), whereas the correlation between GY and SL, AL, NSS, and SW was not significant (Table 4).

Marker-trait association

The trait analysis by association, evolution, and linkage (TASSEL) program was used to detect associations between markers and phenotypic data by using a MLM of the combined values for the two growing seasons. A total of 32 SSR markers showed significant associations with 16 agronomic traits on all 14 chromosomes of durum wheat (Table 5). The significantly associated markers with these traits are identified as putative QTLs. The detected QTL individually accounted for 2.41 to 7.42% of the phenotypic variation. The positive effects of the linked markers originated from the parent "Omrabi 5" (increasing effects of QTL); whereas the markers with negative effects originated from the parent "Belikh 2". A list of the putative markers loci along with their df, F-test, ρ-values association, and additive effects are presented in Table 5. Most of the significantly associated markers with the examined traits were mapped on Chromosome 2A. The results indicated that this region of Chromosome 2A is important for drought tolerance in durum wheat. It may be assumed that there is a QTL cluster for drought tolerance in the region of Chromosome 2A (Table 5), and thus the region may be used as an important target for improving

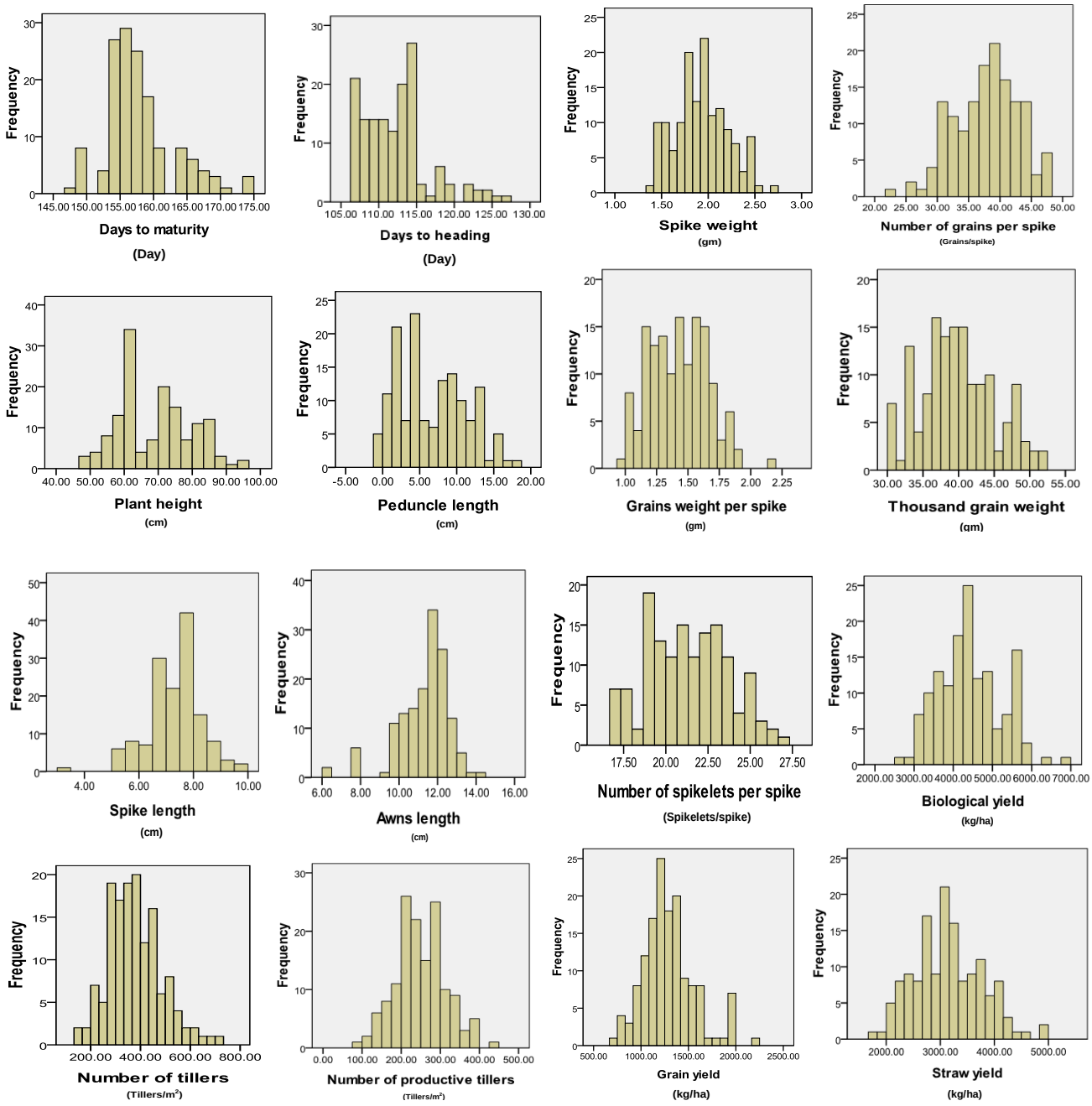

Figure 1. Frequency distributions of different agronomic traits in the Omrabi5/Belikh2 RIL population.

drought tolerance of durum wheat.

Phenological traits

Three markers showed significant association with DH. The *Xwmc177* (P < 0.06) located on Chromosome 2A accounted for 5.98% of the phenotypic variation. In addition, the marker *Xwmc3*88 (P < 0.012) accounted for 2.54% and the marker *Xwmc322* (P < 0.093) accounted for 4.90%; both markers are located on 3B. The added effects of these 3 markers have accounted for 13.45% of the total variation for DH. The significantly associated markers are defined as QTLs and all these QTLs have alleles from "Omrabi 5" (Table 5). Our results are in agreement with the previous studies of Kuchel et al. (2006), Zhang et al. (2009), and Maccaferri et al. (2008) for the QTLs locations on Chromosomes 2A and 3B.

However, these QTLs locations were not detected by Quarrie et al. (2006), and Rebetzke et al. (2008) who have

Table 3. The traits LSD at 5% level, coefficient of variation (CV, %), and heritability (H^2) over two seasons.

Parameter	LSD (5%)	CV (%)	H^2
DH	1.11	0.79	93.4
DM	2.37	1.20	80.6
PH	4.24	5.38	89.9
PL	2.15	26.96	91.9
SL	1.65	4.96	96.1
AL	0.98	7.64	96.4
NT	28.6	9.43	71.7
NFT	41.57	12.16	86.9
NSS	0.87	3.75	92.7
TGW	4.68	10.15	85.6
SW	1.26	5.98	85.2
GWS	0.23	14.96	81.2
NGS	4.45	10.29	87.8
BY	857.87	15.91	65.4
SY	663.50	17.14	54.6
GY	250.64	16.71	59.0

worked on bread wheat.

Five significantly associated with DM were identified; these markers are localized on Chromosomes 2A, 3A, 5A, 5B, 6A, and 6B. Their contributions ranged from 2.54 to 5.75% of the total variation. All of these markers, except for *Xbarc309,* had alleles from "Omrabi 5" (Table 5). The results of the present study agree with previous study of Merza et al. (2006) for the location of QTLs for DM on Chromosomes 2A and 6B, except for the QTL on 1B which was not detected in our study.

Morphological traits

For PH, two significantly associated markers were detected. The marker *Xwmc177* (P < 0.095) located on Chromosome 2A accounted for 2.49% of the phenotypic variation. In addition, the marker, *Xwmc24* (P < 0.098) located on Chromosome 1A accounted for 2.44% of the phenotypic variation. The total contribution of both markers was 4.93%. The positive alleles for this trait are from "Omrabi 5" (Table 5). Our results are in agreement with the findings of Yao et al. (2009) who mapped one QTL for PH on Chromosome 2A. Maccaferri et al. (2008) and Rebetzke et al. (2008) localized the PH on 1B (homologous to 1A); and Marza et al. (2006) on 2B (homologous to 2A). In addition to this, Maccaferri et al. (2008), Rebetzke et al. (2008), Quarrie et al. (2005; 2006), Zhang et al. (2008), and Marza et al. (2006) detected PH-QTLs on other Chromosomes: 3A, 3B, 4B , 5A, 6A, 7A, and 7B.

Five putative markers were significantly associated with PL. These markers were detected on Chromosomes 1B, 2B, 3A, 3B, 4A, 5A, 6A, 6B, and 7A. Their contributions for the phenotypic variation ranged from 3.05 to 4.95%. Similarly to plant height, "Omrabi 5" alleles have contributed to an increased PL (Table 5).

Concerning the AL, 6 significantly associated markers were detected. These markers were located on Chromosomes 1B, 2A, 4A, 4B, 5A, 5B, and 7B. Their contribution range was from 2.73 to 6.06%. All of these markers originated from "Omrabi 5", except *Xbarc32* was from "Belikh 2" (Table 5).

Yield components

In this study, five significantly associated markers with SL were localized on chromosomes 1B, 4B, and 7B and their contributions ranged from 2.42 to 3.37%. All of these markers are from "Omrabi 5", except, *Xwmc415* and *Xwmc617* are from "Belikh 2" (Table 5). All QTLs identified in this study were mapped earlier by Merza et al. (2006) who identified the location of QTLs on Chromosomes 1A, 1B, 2B, 3B, 4B, 5B, 7A, and 7B.

The NT was found to be significantly associated with two markers: *Xwmc35* (P < 0.013) and *Xbarc100* (P < 0.031), located on Chromosomes 2A, and 2B and accounted for 5.83 and 4.1% of the phenotypic variation, respectively. The alleles of these markers, which increased NT, originated from "Omrabi 5" (Table 5). Our results were in disagreement with Kumar et al. (2007) and Kaur et al. (2008) that worked on bread wheat, identified three QTLs for NT on Chromosomes 3A, 7A, and 7B. On the other hand, four different markers for NFT were detected on Chromosomes 4A, 4B, 5A, 5B, and 7A and their contributions ranged from 2.51 to 3.83%. All positive alleles originated from "Omrabi 5" (Table 5). These detected markers were not found in the previous studies (Quarrie et al., 2005; Marza et al., 2006).

For the SW, 4 markers showed significant association. These markers were located on Chromosomes 1A, 1BL, 1BS, 2A, 2B, 4B, 6B, and 7B. Their contributions ranged from 2.95 to 4.44% of the total WS variation. All of these markers originated from "Omrabi 5", except *Xwmc419* and *Xwmc716* are from "Belikh 2" (Table 5). The results of the present study confirm the locations of QTLs for SW detected by Campbell et al. (1999) on Chromosomes 2A, and 2B; Pushpendra et al. (2007) on Chromosome 2B; and Sun et al. (2009) on Chromosome 2A, and disagree with the locus found by Quarrie et al. (2006) on locus 7A. However, in our study the locus for SW was found on 7B the homologous to 7A.

For NGS, 4 significantly associated markers were detected (*Xbarc59*, *Xwmc264*, *Xwmc476*, *Xwmc617*) contributed to NGS total variation ranging from 2.64 to 7.17%. Their chromosomal locations are on 2A, 3A, 4A, 4B, 5BL, and 7B

.The alleles with positive effects for NGS originated from "Omrabi 5" (Table 5). These detected marker locations are in agreement with previous studies for NGS except, the markers identified on 3A, 4A, and 5B. In addition, the NGS

Table 4. Simple phenotypic correlation coefficients between days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), awns length (AL), number of tillers (NT), number of fertile tillers (NFT), spike weight (SW)), number of spikelets spike^{-1} (NSS), thousand grain weight (TGW), grain weight spike^{-1} (GWS), number of grains spike^{-1} (NGS), biological yield (BY), straw yield (SY), and grain yield (GY).

	DH	DM	PH	PL	SL	AL	NT	NFT	SW	NSS	TGW	GWS	NGS	BY	SY	GY
DH	1.00	0.79**	-0.26**	-0.60**	0.46**	-0.11	-0.20*	-0.32**	0.14	0.39**	-0.02	-0.01	-0.13	-0.06	0.07	-0.37**
DM			-0.33**	-0.57**	0.43**	0.02	-0.11	-0.25**	0.11	0.28**	-0.08	-0.03	-0.07	-0.16	-0.04	-0.44**
PH				0.68**	-0.07	0.18	0.05	0.05	0.07	0.03	0.29**	0.13	-0.13	-0.37**	0.36**	0.29**
PL					-0.36**	0.25**	0.00	0.09	0.09	-0.29**	0.24**	0.15	-0.05	0.16	0.08	0.31**
SL						0.08	0.18	-0.03	0.26**	0.55**	0.06	0.22*	0.09	0.18	0.25**	-0.06
AL							0.03	0.06	0.08	0.04	0.09	0.08	-0.02	-0.01	-0.01	0.00
NT								0.81**	-0.12	0.14	-0.26**	-0.06	0.16	0.25**	0.19*	0.33**
NFT									-0.12	0.05	-0.29**	-0.07	0.17	0.30**	0.22*	0.42**
SW										0.16	0.08	0.20*	0.08	-0.07	-0.06	-0.09
NSS											-0.14	0.24*	0.35**	0.17	0.21*	0.03
TGW												0.46**	-0.30**	0.02	0.01	0.61**
GWS													0.56**	0.03	-0.03	0.19*
NGS														0.05	-0.02	0.22*
BY															0.97**	0.79**
SY																0.62**
GY																1.00

*, Correlation is significant at the 0.05 level. **, Correlation is significant at the 0.01 level.

localized on 3A, 4A, and 5B were also detected by Merza et al. (2006) and Quarrie et al. (2005, 2006) on the homologous Chromosomes 3B, 4B, and 5A. Moreover, Marza et al. (2006), Quarrie et al. (2005; 2006), Kumar et al. (2007), and Yao et al. (2009) detected other QTLs on 1A, 1B, 2B, 3B, 4B, 5A, 6A, 7A, and 7B.

Four significantly associated markers with GWS were detected with contributions ranging from 2.41 to 4.15%. The positive parental allele contribution for grain weight per spike originated from "Omrabi 5". Their chromosomal locations were on 2A, 2B, 5AL, 6A, 6B, and 7B. Concerning the NSS, 5 significantly associated markers were detected. Their contributions ranged from 2.41 to 3.58%. The alleles of these markers, which increased NSS came from "Omrabi 5" and their chromosome locations are on 2A, 2B, 3B, 4A, 5AL, and 7A (Table 5). Kumar et al. (2007) and Yao et al. (2009) did not detect the QTLs on 3B, 5A, and 7A.

For TGW, 3 significantly associated markers were detected. The marker, *Xwmc322* ($P < 0.016$) located on Chromosomes 3A, and 3B, accounting for 5.57% of the phenotypic variation, and its positive alleles came from "Omrabi 5". Another marker, *Xwmc475* ($P < 0.070$) was identified on Chromosomes 5A, and 7B. This marker accounted for 2.99% of the TGW variation, and its positive alleles also came from "Omrabi 5", whereas, the third marker, *Xbarc100* ($P < 0.027$) was detected on Chromosomes 2A, 2B, and 5AL accounting for 4.31% of the phenotypic variation, and its alleles came from "Omrabi 5" (Table 5). The total contribution of the 3 markers was 12.87%. These markers are located on Chromosomes 2A, 3A, and 7B and confirm the QTLs location detected by Sun et al. (2009), and Yao et al. (2009).

Yield

For BY, 3 putative markers *Xwmc476*, *Xwmc716*, and *Xbarc59* were detected, which are located on Chromosomes 7B, 1A, and 5BL, respectively. Their contributions ranged from 2.51 to 7.42% and its alleles with a positive effect coming from

Table 5. Comparison-wise ρ-values association of SSR loci for days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), awns length (AL), number of tillers (NT), number of fertile tillers (NFT), spike weight (SW), number of grains spike^{-1} (NGS), grain weight spike^{-1} (GWS), number of spikelets spike^{-1} (NSS), thousand grain weight (TGW), biological yield (BY), grain yield (GY) and straw yield (SY).

Trait	Locus	Site	p - marker[a]	R^2 - marker (%)	Effect of marker[b]	Allele[c]
DH	*XwmcC177*	2A	0.006***	5.98	3.875	Mrb
DH	*Xwmc322*	3B	0.093*	2.54	3.347	Mrb
DH	*Xwmc388*	3A, 5A, 6A, 6B, 7A	0.012**	4.90	2.847	Mrb
DM	*Xbarc309*	2A	0.093*	2.54	-0.557	Blk
DM	*Xbarc353*	2A, 6A	0.035**	3.97	0.435	Mrb
DM	*Xwmc264*	2A, 3A	0.011**	5.75	2.654	Mrb
DM	*Xwmc398*	6A, 6B	0.062*	3.35	4.602	Mrb
DM	*Xwmc415*	5A, 5B	0.068*	3.21	2.983	Mrb
PH	*Xwmc177*	2A	0.095*	2.49	1.937	Mrb
PH	*Xwmc24*	1A	0.098*	2.55	3.069	Mrb
PL	*Xwmc35*	2A, 2B	0.055*	3.52	4.955	Mrb
PL	*Xwmc311*	7B	0.026**	4.32	1.072	Mrb
PL	*Xwmc388*	3A, 5A, 6A, 6B, 7A	0.060*	3.15	1.814	Mrb
PL	*Xwmc597*	1B, 2B, 3B, 4A, 6B	0.064*	3.05	1.544	Mrb
PL	*Xwmc607*	7A	0.018**	4.95	0.928	Mrb
SL	*Xbarc32*	5BL, 7BS, 7BL	0.064*	2.93	-0.247	Blk
SL	*Xbarc60*	1B, 4BL	0.088*	2.42	0.224	Mrb
SL	*Xbarc8*	1BL	0.063*	3.37	0.057	Mrb
SL	*Xwmc626*	1B	0.068*	2.82	1.055	Mrb
AL	*Xbarc343*	4A	0.071*	2.73	1.223	Mrb
AL	*Xbarc8*	1BL	0.050*	4.02	0.428	Mrb
AL	*Xwmc177*	2A	0.008***	6.06	0.066	Mrb
AL	*Xwmc415*	5A, 5B	0.060*	3.23	-0.824	Blk
AL	*Xwmc475*	5A, 7B	0.061*	3.05	3.687	Mrb
AL	*Xwmc617*	4B, 4A	0.046**	3.80	-0.492	Blk
NT	*Xbarc100*	2A, 2B, 5AL	0.031**	4.10	1.013	Mrb
NT	*Xwmc35*	2A, 2B	0.013**	5.83	0.055	Mrb
NFT	*Xwmc405*	5B, 7A	0.090*	2.56	0.214	Mrb
NFT	*Xwmc415*	5A, 5B	0.091*	2.76	0.0015	Mrb
NFT	*Xwmc617*	4B, 4A	0.047**	3.83	1.016	Mrb
NFT	*Xwmc727*	5A	0.096*	2.51	-0.059	Blk
SW	*Xbarc61*	1BS, 1BL	0.060*	2.95	0.218	Mrb
SW	*Xwmc311*	7B	0.019**	4.44	2.005	Mrb
SW	*Xwmc35*	2A, 2B	0.045**	3.27	0.053	Mrb
SW	*Xwmc419*	1B, 4B, 6B	0.055*	3.30	-0.084	Blk
SW	*Xwmc716*	1A	0.022**	4.31	-0.007	Blk
NGS	*Xbarc59*	5BL	0.004***	7.17	0.053	Mrb
NGS	*Xwmc264*	2A, 3A	0.089*	2.64	0.084	Mrb
NGS	*Xwmc476*	7B	0.085*	2.82	0.320	Mrb
NGS	*Xwmc617*	4B, 4A	0.098*	2.67	6.91	Mrb
GWS	*Xbarc100*	2A, 2B, 5AL	0.065*	2.41	16.347	Mrb
GWS	*Xwmc177*	2A	0.019**	4.15	4.885	Mrb
GWS	*Xwmc398*	6A, 6B	0.060*	2.69	7.563	Mrb
GWS	*Xwmc475*	5A, 7B	0.035**	3.30	-0.320	Blk
NSS	*Xbarc100*	2A, 2B, 5AL	0.096*	2.41	6.916	Mrb
NSS	*Xbarc1025*	7A	0.064*	3.02	12.337	Mrb
NSS	*Xbarc343*	4A	0.037**	3.58	15.6	Mrb
NSS	*Xbarc344*	3B	0.089*	2.64	2.32	Mrb

Table 5. Contd.

NSS	*Xwmc607*	7A	0.075*	2.82	5.94	Mrb
TGW	*Xbarc100*	2A, 2B, 5AL	0.027**	4.31	3.07	Mrb
TGW	*Xwmc322*	3B	0.016**	5.57	-1.495	Blk
TGW	*Xwmc475*	5A, 7B	0.070*	2.99	1.621	Mrb
BY	*Xbarc59*	5BL	0.004***	7.42	1.249	Mrb
BY	*Xwmc476*	7B	0.083*	2.86	0.114	Mrb
BY	*Xwmc716*	1A	0.094*	2.51	-2.448	Blk
GY	*Xbarc100*	2A, 2B, 5AL	0.012**	5.44	0.296	Mrb
GY	*Xbarc59*	5BL	0.080*	2.79	1.153	Mrb
GY	*Xbarc70*	4AL, 7AL	0.094*	2.64	3.069	Mrb
GY	*Xwmc322*	3B	0.011**	6.11	4.955	Mrb
GY	*Xwmc388*	3A, 5A, 6A, 6B, 7A	0.052*	3.28	-5.381	Blk
GY	*Xwmc398*	6A, 6B	0.064*	3.01	-4.149	Blk
SY	*Xwmc388*	3A, 5A, 6A, 6B, 7A	0.017**	5.04	18.01	Mrb
SY	*Xwmc453*	2A, 2B	0.062*	3.23	-2.653	Blk
SY	*Xwmc617*	4B, 4A	0.032**	4.44	8.175	Mrb

[a]Only markers significant at the multiple testing-corrected significance level α_c = 0.05 for at least one trait are shown. *, **, and *** indicate significance at α_c = 0.1, 0.05, 0.01 respectively. [b]Positive and negative values indicate that MRBmrabi5 and Belikh2 alleles increased the phenotypic values, respectively. [c] Mrb and Blk indicate Omrabi5 and Belikh2, respectively.

"Omrabi 5" (Table 5). As for GY, 6 markers were significantly associated. Their contributions ranged from 2.64 to 6.11%. All these QTLs, except *Xwmc617*, had their alleles from parent "Omrabi 5" (Table 5). These markers are located on 2A, 2B, 3A, 3B, 4AL, 5AL, 6A, 6B, 7A, and 7B.

Concerning the SY, 3 markers showed significant associations. Their contributions were 3.23, 4.44, and 5.04% of the total SY variation, respectively and are on Chromosomes 2A, 2B, 3A, 4A, 4B, 5A, 6A, 6B. The total contribution of the 3 markers was 12.71%. The positive alleles were contributed by "Omrabi 5" (Table 5). In the present study, the markers controlling yield (BY; GY; SY) were spread over the 14 durum chromosomes, and these locations were also identified by others (Quarrie et al., 2005, 2006; Marza et al., 2006; Kumar et al., 2007; Kuchel et al., 2007; Cuthbert et al., 2008; Maccaferri et al., 2008).

Co-located QTLs

Since the yield traits are correlated, association-trait analysis was also conducted to detect co-located markers. Out of 32 significantly associated markers detected, 56% of these markers were contributing for more than one trait. Some markers were found to be linked to different traits on Chromosomes 1A (*Xwmc716)*, 1B (*Xbarc8*), 2A (*Xwmc35, Xwmc177*, *Xwmc264, Xbarc100*), 2B (*Xwmc35, Xbarc100*), 3A (*Xwmc322, Xwmc388*), 3B (*Xwmc322*), 4A (*Xbarc343*), 4B (*Xwmc617*), 5A [3A (*Xwmc388, Xwmc415,Xwmc475, Xbarc100*)], 5B (*Xwmc415*), 6A (*Xwmc388*, *Xwmc398*), 6B (*Xwmc388*, *Xwmc398*), 7A (*Xwmc388, Xwmc607*), and 7B (*Xwmc322*, *Xwmc475, Xwmc476*) (Table 5). The number of associated markers for a trait ranged from 1 (SW and BY; SL and AL; DM and NGS; AL and NSS; TGW and GWS; PL and NSS; PL and SW; NGS and BY) to 5 (NT, TGW, NSS, GWS and GY) (Table 5). Five significantly associated markers for GY (*Xbarc59*, P < 0.279; *Xbarc100*, P < 0.014; *Xwmc322*, P < 0.061; *Xwmc388*, P < 0.032; *Xwmc398*, P < 0.030), could represent co-located QTLs with other traits [(NT, NSS, TGW, and GWS); (NGS, and BY); (TGW, and DH) (DH, PL, and SY); and (GWS, and DM)], respectively (Table 5).

Source of alleles for drought tolerance

Drought tolerance genes are located throughout the genome and are genotype dependent. In this study, it was found that for grain yield, 4 significantly associated markers were derived from "Omrabi 5". However, some of the markers were also derived from a drought sensitive parent of the population. Börner et al. (2002) and Huang et al. (2003) found similar results for other traits of interest. These findings further confirm that drought tolerance is a quantitative trait and that apparently sensitive parents may contain alleles for tolerance, which may not be found in the tolerant parents. It can be concluded that the sensitive parent "Belikh 2" may contain some tolerance alleles that when combined with alleles from tolerant parents can result in an increased level of tolerance.

Perspectives for MAS

Molecular markers, closely linked to genes of agronomic important traits, have been identified to be useful markers

for an indirect selection in durum wheat breeding programs (Nachit, 1998). MAS is time efficient (Landjeva et al., 2007), non-destructive and depending on associated relationships, characterized by low selection error (Nachit et al., 2000).

Further investigations for drought tolerance will be required to establish the importance of the identified genomic regions in other backgrounds. In addition, field evaluation is required to establish the effectiveness of the drought screening system in modeling water responses and in evaluating the stability of QTLs across environments (Mohan et al., 1997). Our results indicate the existence of markers/genes, which are involved in the control of the phenotypic variation in quantitatively inherited traits related to drought tolerance.

Further research is needed on molecular markers and QTLs mapping to screen potential genotypes for drought tolerance in durum wheat. The current study represents potential targets for MAS of grain yield improvement in durum wheat. These were *Xbarc59, Xbarc70, Xbarc100, Xwmc322, Xwmc388,* and *Xwmc398.*

Conclusions

This study has shown the importance of using molecular markers to identify traits and markers for further improving durum performance under drought conditions. AM revealed the presence of a number of significantly associated markers with grain yield and its related traits. Several of these markers overlapped with QTLs mapped in other durum and bread wheat mapping populations. In addition, we are working on developing genetic linkage maps with marker saturated chromosomes.

The comparative analysis carried out, considering the result from QTL analysis obtained so far in durum wheat and bread wheat, provide further supporting evidence for the relative importance of the identified markers, chromosome regions, and the potential value for the application of MAS to the most agronomic traits of interest in durum wheat.

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