Detection of molecular markers associated with yield and yield components in durum wheat (Triticum turgidum L. var. durum) under saline conditions. Markers for yield in durum wheat

Dura S., Duwayri M., Nachit M.M.

in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 209-221

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

Markers for yield in durum wheat

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

[1] National Center for Agricultural Research and Extension, Biotechnology Department, Amman, Jordan
[2] University of Jordan, Department of Horticulture and Crop Science, Amman, Jordan
[3] International Centre for Agricultural Research in the Dry Areas (ICARDA), Morocco

Abstract. Durum wheat is one of the most important staple food crops grown mainly in the Mediterranean region where its productivity is drastically affected by salinity. The study objectives were to identify markers associated with grain yield and its related traits under saline conditions. A population of 114 F_8 recombinant inbred lines (RILs) was derived by single-seed descent from a cross between Belikh2 (salinity tolerant variety) and Omrabi5 (less salinity tolerant) was grown under non-saline and saline conditions in a glasshouse. Phenotypic data of the RILs and parental lines were measured for fifteen agronomic traits. Association of 48 SSR loci covering all 14 chromosomes with fifteen agronomic traits was analyzed with a mixed linear model. A total of 28 SSR loci were significantly associated with these traits. Under saline condition, 13 markers were associated with phenological traits while 19 markers were associated with yield and yield components. Marker alleles from Belikh2 were associated with a positive effect for the majority of markers associated with yield and yield components. Under saline condition, four markers (*Xwmc182*, *Xwmc388, Xwmc398*, and *Xbarc61*) were closely linked with grain yield, located on 3A, 3B, 4B, 5A, 6B, and 7A. These markers could be used for marker-assisted selection in durum wheat breeding under saline conditions.

Keywords. Keywords Association mapping – Durum wheat – Marker-assisted selection – Salinity tolerance – SSR.

Détection de marqueurs moléculaires associés au rendement et à ses composantes chez le blé dur (Triticum turgidum L. var dur.) sous conditions de stress salin. Marqueurs du rendement chez le blé dur

Résumé. *Le blé dur est une des cultures vivrières de base les plus importantes, cultivées principalement dans la région méditerranéenne où sa productivité est très affectée par la salinité. Cette étude avait pour objectif d'identifier des marqueurs associés au rendement en grains et à ses caractères corrélés dans des conditions de salinités. Une population de 114 lignées recombinantes (RIL) F8, issue de la descendance mono-graine d'un croisement entre Belikh2 (variété tolérante à la salinité) et Omrabi5 (moins tolérante à la salinité), a été cultivée en serre en conditions non salines et salines. Les données phénotypiques des RIL et des lignées parentales ont été mesurées pour quinze caractères agronomiques. L'association de 48 loci SSR, couvrant l'ensemble des 14 chromosomes, avec quinze caractères agronomiques a été analysée à l'aide d'un modèle linéaire mixte. Au total, 28 loci SSR étaient significativement associés à ces caractères. Dans des conditions de salinité, 13 marqueurs étaient associés à des caractères phénologiques alors que 19 marqueurs étaient associés au rendement et à ses composantes. Les allèles des marqueurs obtenus de Belikh2 étaient associés avec un effet positif pour la majorité des marqueurs associés au rendement et à ses composantes. Sous des conditions de salinité, quatre marqueurs (Xwmc182, Xwmc388, Xwmc398, et Xbarc61), situés sur les chromosomes 3A, 3B, 4B, 5A, 6B et 7A, étaient étroitement associés à la production de grain. Ces marqueurs pourraient être utilisés pour la sélection assistée par marqueurs dans l'amélioration du blé dur dans des conditions de salinité.*

Mots-clés. *Cartographie d'association – Blé dur – Sélection assistée par marqueurs – Tolérance à la salinité – SSR.*

I – Introduction

Salinity is one of the most serious abiotic stresses limiting crop production globally and has become more serious in recent years. It is estimated to affect nearly one-fifth of the world's irrigated land and causes ten million irrigated hectares to be abandoned each year (Flowers and Yeo, 1995). Although durum wheat cultivars are more salt sensitive than bread wheat and may yield less when grown in saline soils, the usual high price of durum wheat in the international market can bring a better return to farmers than bread wheat and other crops (Lindsay *et al*., 2004). Improving the salinity tolerance of durum wheat and increasing its productivity has been an important objective in wheat breeding programs. Salinity tolerance reflects the ability of a genotype to grow and yield well in a saline environment. It is generally measured as the relative biomass production or relative yield under saline and non-saline conditions (Munns, 2002).

Similar to other agronomical traits, breeding for salinity tolerance requires (a) economic justification, (b) genotypic variation, (c) a rapid and reliable selection method, and (d) understanding of genetic control. The first two criteria are satisfied, but the third and fourth criteria require further work. The current situation is that salinity tolerance is difficult to assess in the field due to spatial and temporal variation, although alternative screening methods have been developed (Munns and James, 2003), they are generally time-consuming, expensive (Lindsay *et al*., 2004) and require validation in the field. Salinity tolerance remains complex both physiologically and genetically (Koyama *et al*., 2001; Colmer *et al*., 2005; Munns and Tester, 2008; Genc *et al*., 2010). Pyramiding of salinity tolerance traits into breeding programs using association mapping and subsequent marker-assisted selection (MAS) have a great potential to accelerate the breeding process.

In the last decade, markers associated with salinity tolerance have been mapped in rice (Gong *et al*., 1999; Koyama *et al*., 2001; Lin *et al*., 2004), barley (Mano and Takeda, 1997; Xue *et al*., 2009) and soybean (Lee et al., 2004). In wheat, differences in salinity tolerance including physiological and agronomical response have been reported, but few researches have been done in genetic analysis. Lindsay et al. (2004) identified markers linked to salt tolerance at seedling stage in durum wheat. Although identification of the markers associated with salt tolerance in terms of yield at late growth stage is particularly important, few relevant studies have been done to date. Dura et al. (2013) identified markers linked to drought tolerance using recombinant inbred lines of durum wheat, derived from a cross between Omrabi5 and Belikh2 parents. Omrabi5 durum cultivar combines drought tolerance with yield and yield stability and Belikh2 was developed for saline areas (Dura *et al*., 2011). In the present study, the same mapping population was grown under non-saline and saline glasshouse conditions to (1) identify markers associated with salinity tolerance traits, (2) understand the relationships among these traits and (3) determine their genetic value for marker-assisted selection.

II – Material and methods

1. Plant material

The plant material used in this study was a population which originated from a cross between Omrabi5 with Belikh2. The population consisted of 114 F8 single seed descent recombinant inbred lines (RILs) developed in 2005 by the durum wheat breeding program at the International Center for Agricultural Research in the Dry Areas (ICARDA). Omrabi5 and Belikh2 are durum wheat cultivars developed for the Mediterranean conditions (Nachit, 1998). Omrabi5 was developed from a cross between the Middle East landrace Haurani and the improved cultivar Jori-C69, while Belikh2 (Cr/Stk) was bred at ICARDA for saline area. Omrabi5 was released in Jordan, Turkey, Algeria, Morocco, Iran and Iraq for commercial production; it combines drought tolerance with yield and yield stability, whereas Belikh2 was released in Lebanon and Syria.

2. Glasshouse experiment

The experiment was conducted in a glasshouse of the University of Jordan in 2007 using 114 RILs and the parental genotypes tested under two salinity levels with three replications. Plastic pots were filled with washed sandy soil, each containing 10 kg soil (dry wt. basis). The seeds were germinated in transplanting trays. After 10 days at two leaf stage, seedlings were transferred into each pot at a rate of three seedlings per pot. Seedlings were watered initially with tap water (0.2 mM NaCl), and then quarter strength Hoagland nutrient solution was introduced two days after transplanting and increased to full strength at three weeks after transplanting. The salinity concentration was increased gradually in aliquots of 10 mM NaCl every day until the required concentration of 100 mM NaCl was reached. Salinity treatments were begun 14 days after the start of the experiment.

The following traits were recorded on three plants of each pot. Days to heading (DH) was recorded as the number of days from emergence to the day when half of the spikes have 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 discoloured 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. Spike length (SL) was measured from the base to the top of the spike excluding the awns. Awns length (AL) was measured from the top of the spike to the top of awns. Number of tillers (NT) and number of fertile tillers (NFT) was counted. Main spike weight (WS), number of grains per plant (NG) and number of spikelets per spike (NSS) were counted. Thousand-grain weight (TGW) was measured by weighing grains taken from the plant and converted to the weight of 1000 grains. Biological yield (BY) was measured as the weight of aboveground dry matter (straw + grain). Grain yield was measured as the weight of grain harvested from the plant. Straw yield was calculated as the difference between biological yield and grain yield. The design used was a Complete Randomized Design (CRD) with three replications.

3. Molecular analysis

The following studies were conducted on plant materials grown in 2007 at ICARDA, Aleppo, Syria using ICARDA durum wheat MAS lab.

The DNA was extracted using SDS method from 3-5 gm leaf tissue of each RIL seedling eight-weeks after sowing according to the protocol developed at ICARDA durum wheat MAS lab (Nachit *et al*., 2001) and quantified by the spectrophotometer.

Wheat microsatellites *wmc* (wheat microsatellite) and *barc* (Beltsville agriculture research center) were used as described by Nachit *et al*. (2001). The parents were screened using 300 primer pairs of SSRs out of which 48 (15%) were polymorphic. The Polymerase Chain Reaction (PCR) amplification was carried out in Eppendorff thermal cycle, in a 7.5 µl reaction mixture. Each reaction contained 10 X Taq polymerase buffer, 200 µM of each dNTPs, 0.5 µM of each of the two primers, 1 U Taq polymerase, and 20 ng of genomic DNA as template. Amplifications were performed as follows: 94 °C for 5 min, 35 cycles of (94 °C 1 min, 63-56 °C 1 min, 72 °C 1 min), 72 °C for 5 min. PCR products were mixed with loading buffer, 5-10 µl of mixture was denaturated and loaded into wells in 0.4 mm thick 15% acrylamide gel resolved at constant power (30 w) in 1 X TBE running buffer for 15 min to one hour depending on size of the primer pairs of SSRs. Bands were visualized by silver-staining method as described by Nachit *et al*. (2001).

4. Statistical analysis and association mapping

The statistical analysis was performed using the MIXED procedure of the SAS statistical package (SAS, 1998). Pearson's correlations between phenotypic traits were calculated using SPSS 17.0 statistical software. Forty-eight SSR markers covering the whole durum AB genome were used.

Because of the low number of molecular markers probed in this study to utilize for genetic mapping, we have opted for association mapping between molecular markers and traits. We have a mixed linear model (MLM) within the program TASSEL version 2.0.1 (http://www.maizegenetics.net) where the marker was considered as a fixed-effects factor and the lines of the population considered as a random-effects factor (Kennedy *et al*., 1992). Significance of associations between loci and traits 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 within a chromosome. The additive effects of the markers were estimated using Genstat (Version11).

III – Results

1. Phenotypic

A total of 114 lines and their parents (Omrabi5 and Belikh2) were investigated under salinity stress and normal conditions. The grand means and ranges of measured fifteen agronomic traits for the parent and RIL population are presented in Table 1. The two parents showed the great difference in all fifteen traits. The values of fifteen agronomic traits showed more reduction in Omrabi5 than in Belikh2 when the plants were exposed to salinity stress, which was consistent with the fact that Belikh2 is a well-known salt-tolerant genotype. On an average of all RIL, each value of 15 agronomic traits was obviously reduced under salinity stress relative to the control.

The phenotypic distributions of all examined traits for the RIL displayed a continuous normal pattern. Obviously, these traits were quantitatively inherited. In addition, transgressive segregation in both directions was observed for all traits (Table 1) under both the control and salinity stress.

Significant correlations ($P < 0.05$) were observed between GY and WS, TGW, NG, BY, and SY, irrespective of the control and salinity conditions. However, there was no significant correlation between GY and DH, PL, SL, AL, NT, NFT and NSS in both conditions. GY was positively correlated with PH, PL, SL, AW, NFT, TGW, NG, BY, and SY, and negatively correlated with DH, DM, NT, and NS under salinity stress (Tables 2 and 3).

2. Marker-trait association

A total of 28 SSR markers for 15 agronomic traits were located on all 14 chromosomes of durum wheat (Tables 4 and 5); being 15 and 13 markers under control and salinity stress, respectively. Only markers significant at the multiple testing-corrected significance levels for at least one trait are presented in Tables 4 and 5.

3. Phenological traits

For DH, one significant marker (Xwmc177) was detected both for the control and salt stress located on chromosome 2A accounted for 10.5, 31.0% of the total DH variation, respectively. There was one marker, Xwmc24 on chromosome 1A under the saline condition accounted for 1.6% of the phenotypic variation. All these markers had alleles from parent Belikh2 (Tables 4 and 5). One significant marker (Xwmc617 for DM was detected under the control and salinity stress conditions and mapped on chromosomes 4A, and 4B accounted for 2.6, 3.1% of the phenotypic variation, respectively. Three other markers (Xbarc61, Xbarc353, Xbarc1025) located on chromosomes 2A, 4B, and 7A were detected under the control condition accounted for 2.6, 2.3, and 3.4% of the DM variation, whereas another marker; Xwmc626 ($P < 0.01$) was found under salinity stress accounted for 5.1% of the total variation. All of these markers except; Xbarc61 had alleles from Belikh2 (Tables 4 and 5). Two significant markers; Xwmc177, and Xwmc617 influencing PH were detected under the two environments (control and salt stress).

Table 1. Mean performance, standard deviations, and ranges of traits under the control (S1) and salinity (S2) conditions for the parents and RILs.

Trait	Belikh 2S1 Mean ± SD	Omrabi 5S1 Mean ± SD	RILs Sl Mean ± SD	Range	Belikh 2S2 Mean ± SD	Omrabi 5S2 Mean ± SD	RILs S2 Mean ± SD	Range
DH Days to Heading	111.7 ±3.9	93.5 ±1.4	114.5 ±13.7	92.0-160.0	83.8 ±1.6	81.2 ±2.6	102.7 ±13.4	82.0-150.0
DM Days to Maturity	152.0 ±8.3	114.2 ±1.7	157.8 ±24.6	115.0-201.5	119.5 ±3.1	101.8 ±2.6	122.9 ±16.1	102.0-145.5
PH Plant height (cm)	71.2 ±3.6	78.4 ±3.2	79.5 ±14.1	55.4-108.5	60.8 ±4.6	30.0 ±6.3	62.6 ±12.1	35.6-89.5
PL Peduncle length (cm)	22.5 ±2.7	32.3 ±4.1	17.9 ±7.3	1.2-36.9	9.4 ±2.0	1.8 ±0.4	9.5 ±5.4	0.0-26.8
SL Spike length (cm)	9.8 ±0.50	7.0 ±0.4	9.5 ±1.3	7.0-15.5	8.7 ±0.60	6.0 ±0.04	8.0 ±0.9	5.7-11.8
AL Awns length (cm)	14.5 ±0.92	12.0 ±0.69	13.9 ±1.9	9.6-21.5	11.0 ±0.72	7.2 ±0.7	10.9 ±1.7	7.0-15.5
NT Tillers plant^{-1} No	7.3 ±0.41	5.8 ±0.5	5.7 ±1.6	3.0-13.0	5.3 ±1.0	1.1 ±0.2	4.1 ±1.4	1.0-8.0
NFT Fertile til-lers plant^{-1}No.	7.3 ±0.42	5.7 ±0.5	5.1 ±1.8	2.0-13.0	4.3 ±0.76	2.0 ±0.6	3.8 ±1.0	2.0-8.0
WS Spike Weight (g)	2.6 ±0.61	2.8 ±0.4	2.4 ±0.5	1.4-4.2	2.4 ±0.41	1.4 ±0.5	2.1 ±0.5	1.2-3.5
NG Grains plant^{-1}No.	127.3 ±31.8	122.8 ±20.8	122.6 ±28.4	82.1-227.0	90.7 ±22.0	30.9 ±6.3	91.1 ±25.9	48.0-187.0
NSS Spikelets spike^{-1}No.	25.7 ±1.3	21.5 ±1.2	24.7 ±2.2	20.0-30.0	24.5 ±1.5	14.5 ±2.6	20.7 ±2.4	15.0-26.0
TGW 1000-grainweight (g)	56.9 ±8.1	47.4 ±4.8	59.0 ±6.7	54.8-67.7	52.1 ±10.4	12.1 ±4.8	41.2 ±14.7	15.8-79.6
BY Biological yield (g plant^{-1})	15.9 ±1.7	15.8 ±2.0	14.8 ±2.7	9.8-24.1	8.9 ±2.3	1.7 ±0.44	6.7 ±1.6	0.6-11.5
SY Straw yield (g plant^{-1})	8.4 ±1.4	7.8 ±2.0	8.4 ±2.3	4.4-15.1	7.1 ±1.8	1.5 ±0.46	5.5 ±1.6	0.5-10.2
GY Grain yield (g plant^{-1})	7.5 ±0.62	7.9 ±1.1	6.4 ±1.0	4.1-9.4	1.9 ±0.45	0.12 ±0.02	1.2 ±0.3	0.1-1.2

Table 2. 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), weight of spike (WS), number of spikelets per spike (NSS), 1000-grain weight (TGW), number of grains per plant (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under the control conditions.

	DM	PH	PL	SL	AL	NT	NFT	NSS	TGY	GW	NG	BY	SY	GY
DH	.47	-15	-.28	.01	-.23	.27**	.15	.23	-.16	.29**	.23*	-.15	-.13	-.12
DM		-.02	-.20*	-.01	-.01	.78**	-.05	.10	-.06	-.20*	-.18*	-.17	-.17	-.007
PH			.53**	.01	.43**	-.15	-.28**	-.04	-.06	-.05	-.02	.05	.03	.07
PL				-.06	.55**	-.13	-.16	-.15	.17	.08	-.05	.08	.07	.09
SL					.08	.22*	.14	.13	.01	.07	.06	.13	.10	.18
AL						.02	-.15	-.10	.07	.03	-.03	.03	.02	.03
NT							.39**	-.01	-.15	-.12	-.03	-.09	-.08	-.05
NFT								.07	.14	.006	-.12	.16	.15	.08
WS								.01	.10	.18*	.10	.03	-.007	.22*
NSS									-.05	-.06	-.04	.03	.05	-.12
TGW										.59**	-.11	.20*	.17	.18*
NG												.08	.02	.31**
BY													.98**	.21*
SY														.02

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

Table 3. 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), weight of spike (WS), number of spikelets per spike (NSS), 1000-grain weight (TGW), number of grains per spike (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under the saline conditions.

	DM	PH	PL	SL	AL	NT	NFT	WS	NSS	TGY	NG	BY	SY	GY
DH	.78 **	-.12	-.39 **	.23 *	-.24 **	.21 *	.28 **	-.12	.23 *	-.06	-.34 **	-.17	-.14	-.13
DM		-.14	-.42 **	.20 *	-.21 *	.09	.20 *	-.18 *	.319 **	-.032	-.28 **	-.14	-.09	-.20 *
PH			.58 **	-.15	.29 **	-.20 *	-.25 **	.04	.11	.17	-.07	.12	.06	.18*
PL				-.29 **	.36 **	-.10	-.21 **	.20 *	-.11	.10	.03	.031	.009	.06
SL					-.14	.18 *	.19	.17	.30 **	.01	.05	-.027	-.042	.025
AL						-.18 *	-.29 *	.27 **	-.14	.09	.15	.118	.068	.170
NT							.75 **	-.10	-.01	.03	-.09	.134	.115	.101
NFT								-.17	.05	.02	-.06	.24 **	.25 **	.08
WS									-.031	.21 *	.13	.14	.08	.19 *
NSS										-.04	-.07	.004	.03	-.06
TGY											-.11	.45 **	.36 **	.41 **
NG												.45 **	.41 **	.28 **
BY													.938 **	.57 **
SY														.25 **

These markers, located on chromosomes 2A, 4A, and 4B, accounted for 4.4-13.7% of the total phenotypic variation, with positive alleles coming from parent Belikh2. Under the control condition another marker; *Xbar61* ($P < 0.001$), located on chromosome 4B, accounted for 3.2% of PH variation. For salinity, marker; *Xwmc182* ($P < 0.019$), detected on 3B and 6B, accounted for 3.2% of the total variation, with positive alleles also coming from Belikh2 (Tables 4 and 5).

Three significant markers for PL were detected. Of them, one marker (Xwmc625) was mapped on chromosome 3B under both conditions accounted for 2.4% of the total phenotypic variation and its positive alleles came from Belikh2. Under salt stress, three markers were mapped on chromosomes 2B, 3B, 5B, 7B accounted for 2.4-5.4% of PL variation, and their positive alleles also came from Belikh2 (Tables 4 and 5). For SL three significant markers were found. Only one marker; Xwmc488 was mapped on chromosome 7A under the control condition accounted for 5.3% of the total phenotypic variation. Under salinity stress, two markers were mapped on chromosomes 3B, 4A, and 4B accounted for 2.4-8.6% of the SL variation. The alleles of these markers; which increased SL, came from Belikh2 (Tables 4 and 5). For AL only one significant marker; Xwmc625 ($P < 0.045$) was detected under the saline stress. This marker was located on chromosome 3B accounted for 3.4% of the total phenotypic variation and its positive alleles came from parent Belikh2 (Tables 4 and 5).

4. Yield components

For TN, three significant markers were detected. Under control condition, two markers; *Xwmc667*, and *Xbarc353,* located on chromosome 2A accounted for 3.5, 4.0% of the total phenotypic variation, respectively. For salinity, only one marker; *Xbarc100,* on 2B and 5A accounted for 6.4% of the total variation. The positive alleles also came from Belikh2 (Tables 4 and 5). Only two significant markers for FTN were detected. One off them, *Xwmc426,on 7B,* accounted for 6.6% of the total phenotypic variation. For salinity, another marker (*Xbarc100*) was mapped on 2B and 5A and accounted for 3.3. All of these markers had alleles from Belikh2 (Tables 4 and 5). Six markers were detected for WS. Of them, four were mapped on chromosomes 2A, 3B, 4B, 6B, 7A, and 7B under the control condition accounted for 1.8-3.9%. Under salinity stress, two markers (*Xwmc182*, *Xbarc70*) mapped on chromosomes 3B, 4B, and 6B, explaining 3.6 and 4.9% of the phenotypic variation, respectively. The positive alleles came from Belikh2 (Tables 4 and 5).

5. Yield components

For TN, three significant markers were detected. Under control condition, two markers; *Xwmc667*, and *Xbarc353* were located on chromosome 2A accounted for 3.5, 4.0% of the total phenotypic variation, respectively. For salinity, only one marker; *Xbarc100* detected on 2B, and 5A accounted for 6.4% of the total variation. The positive alleles also came from Belikh2 (Tables 4 and 5). However, two significant markers for FTN were found. Of them, one marker (*Xwmc426*) accounted for 6.6% of the total phenotypic variation and located on chromosome 7B under the control condition. For salinity, another marker (*Xbarc100*) was mapped on 2B and 5A and accounted for 3.3. All of these markers had alleles from Belikh2 (Tables 4 and 5). Six markers were detected for WS. Of them, four markers were mapped on chromosomes 2A, 3B, 4B, 6B, 7A, and 7B under the control condition accounted for 1.8-3.9%. Under salinity stress, two markers (*Xwmc182*, *Xbarc70*) were mapped on chromosomes 3B, 4B, and 6B, explaining 3.6 and 4.9% of the phenotypic variation, respectively. The positive alleles came from Belikh2 (Tables 4 and 5).

Only one marker (*Xwmc597*) was detected in both environments for NSS, being located on chromosomes 1B, 2B, 3B, 4A, and 6B. Under salinity condition, another marker was detected on chromosome 7B accounted for 7.0% of the NSS variation. All of these markers had alleles from Belikh2 (Tables 4 and 5). For TGW, only one significant marker (*Xbarc32*) was found in salinity environment accounted for 9.1% of the total phenotypic variation and located on chromosomes 5B, and 7B and its alleles came from Belikh2 (Tables 4 and 5). Under salinity condition, two

markers were located on chromosomes 1A and 3B accounted for 2.8, and 5.9% of the total variation respectively, and its positive alleles came from Belikh2 (Tables 4 and 5). Five genomic regions related to NG were detected. Of them, three markers were mapped on chromosomes 4A, 4B, 5A, 5B, and 7B under the control condition accounted for 2.8-5.9% of the total phenotypic variation, whereas other markers (*Xwmc398*, *Xbarc315*) were found under salinity stress, being mapped on chromosomes 4A, 6B, and 7B accounted for 2.6, and 5.2% of the phenotypic variation, respectively. The positive alleles are from Belikh2 (Tables 4 and 5).

Table 4. 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), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spiklets per spike (NSS), number of grains per plant (NG), biological yield (BY) and straw yield (SY) under control condition.

Trait	Locus	df_Marker	F_Marker [a]	P_Marker	Marker effect [b]	Allele [c]
DH	WMC177@2Abp190	1	30.34	0.00**	10.5	Blk
DM	WMC617@4B4Abp200	2	8.87	0.00**	2.6	Blk
DM	BARC61@4Bbp150	2	4.44	0.013*	2.3	Mrb
DM	BARC353@2Abp205	1	4.00	0.047*	3.4	Blk
DM	BARC1025@7Abp125	1	8.05	0.005**	5.2	Blk
PH	WMC177@2Abp190	1	12.79	0.000**	4.4	Blk
PH	WMC617@4B4Abp200	2	3.88	0.023*	13.7	Mrb
PH	BARC61@4Bbp150	2	7.21	0.001**	3.2	Blk
PL	WMC625@3Bbp110	1	6.90	0.009**	2.4	Blk
SL	WMC488@7Abp120	1	4.72	0.032*	5.3	Blk
NT	BARC353@2Abp205	1	7.00	0.009**	4.0	Blk
NT	WMC667@2Abp110	1	4.24	0.041*	3.5	Mrb
NFT	WMC426@7Bbp210	2	4.65	0.011*	6.6	Blk
WS	WMC177@2Abp190	1	3.95	0.049*	3.9	Blk
WS	WMC603@7Abp95	2	3.31	0.040*	2.5	Mrb
WS	BARC1025@7Abp125	1	4.15	0.044*	1.8	Mrb
WS	WMC218@7Bbp110	1	3.93	0.049*	3.3	Blk
NSS	WMC597@1B2B3B4A6Bbp240	2	4.41	0.014*	2.7	Blk
NG	WMC617@4B4Abp200	2	4.14	0.018*	5.9	Blk
NG	BARC32@5B7Bbp135	2	3.94	0.022*	2.8	Mrb
NG	WMC475@5A7Bbp125	1	6.58	0.011*	4.2	Blk
BY	WMC617@4B4Abp200	2	4.22	0.017*	5.7	Blk
BY	BARC32@5B7Bbp135	2	3.24	0.042*	3.3	Blk
BY	WMC475@5A7Bbp125	1	4.24	0.041*	2.8	Blk
SY	WMC475@5A7Bbp125	1	5.24	0.024*	4.8	Blk

*a Only markers significant at the multiple testing-corrected significance level $\alpha_c = 0.05$ for at least one trait are shown. *, ** indicate significance at $\alpha_c = 0.05, 0.01$ 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.

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), awn length (AL), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spikelets per spike (NSS), thousand grain weight (TGW), number of grains per spike (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under salinity condition.

Trait	Locus	Df	F[a]	P	E[b]	Allele[c]
DH	WMC177@2Abp190	1	13.5986	0.000**	31.0	Blk
DH	WMC24@1Abp125	1	6.2468	0.0139*	1.6	Blk
DM	WMC617@4B4Abp200	2	6.4589	0.0023**	3.1	Blk
DM	WMC626@7Abp180	1	6.7748	0.0105*	5.1	Blk
PH	WMC177@2Abp190	1	10.787	0.0014**	4.4	Blk
PH	WMC182@3B6Bbp160	1	5.6152	0.0195*	3.2	Blk
PH	WMC617@4B4Abp200	2	3.2302	0.0437*	13.7	Mrb
PL	BARC32@5B7Bbp135	2	3.4317	0.0359*	2.6	Mrb
PL	BARC114@2Bbp130	1	5.2301	0.0241*	5.4	Blk
PL	WMC625@3Bbp110	1	4.9527	0.0281*	2.4	Blk
SL	WMC617@4B4Abp200	2	3.8406	0.0247*	2.4	Blk
SL	BARC344@3Bbp240	1	4.8399	0.0299*	8.6	Blk
AL	WMC625@3Bbp110	1	4.0904	0.0456*	3.4	Blk
NT	BARC100@2B5Albp140	1	7.3021	0.008**	6.4	Blk
NFT	BARC100@2B5Albp140	1	6.0286	0.0157*	3.3	Blk
WS	WMC182@3B6Bbp160	2	3.4352	0.0357*	3.6	Blk
WS	BARC70@4Bbp240	1	4.0537	0.0466*	4.9	Blk
NSS	WMC662@7Bbp190	2	3.4547	0.0355*	5.2	Blk
NSS	WMC597@1B2B3B4A6Bbp240	2	3.1269	0.0478*	7.0	Blk
TGW	BARC32@5B7Bbp135	2	4.3779	0.0148*	9.1	Blk
NG	WMC398@6Bbp90	2	3.9572	0.0222*	2.8	Blk
NG	BARC315@4A7Bbp75	1	6.8057	0.0104*	5.9	Mrb
BY	WMC398@6Bbp90	2	4.0111	0.0211*	2.5	Blk
BY	BARC315@4A7Bbp75	1	6.0583	0.0154*	2.6	Blk
SY	BARC59@4Bbp185	2	3.425	0.0362*	2.2	Blk
SY	WMC475@5A7Bbp125	1	8.3393	0.0047**	4.8	Blk
GY	WMC182@3B6Bbp160	2	3.6303	0.0298*	2.7	Blk
GY	WMC388@3A5A7Abp150	1	7.6316	0.0067**	4.6	Blk
GY	WMC398@6Bbp90	2	3.257	0.0426*	2.3	Blk
GY	BARC61@4Bbp150	2	4.7463	0.0106*	6.1	Blk

6. Yield

Five significant markers for BY were identified. Under the control condition, three markers (*Xwmc617*; P < 0.017, *Xwmc475*; P < 0.042, *Xbarc32*; P < 0.043), being mapped on chromosomes 4A, 4B, 5A, 5B, and 7B accounted for 5.7, 2.8, and 3.3% of the total phenotypic variation, respectively, and their positive alleles are from Belikh2. Other two markers (*Xwmc388*, *Xwmc398*) were identified on chromosomes 4A, 6B, and 7B accounted for 2.5, and 2.6% of the BY variation under saline condition. The positive alleles also are from Bekih2 (Tables 4 and 5). Only one significant marker; *Xwmc475* for SY was identified in both environments accounted for 4.8% of the total SY variation. There was other one marker (*Xbarc59*) on chromosome 4B under the salinity accounted for 2.2% of the total variation. The positive alleles also are from Bekih2 (Tables

4 and 5). Four associated markers for GY were detected under salinity stress. Each of them, accounted for 2.3-6.1% of the total phenotypic variation, and the alleles from parent Belikh2 could increase GY, being *Xbarc61* and *Xwmc388* under salinity condition (Tables 4 and 5).

IV – Discussion

The two parents (Belikh2 and Omrabi5) differed significantly in the measured traits when they were exposed to non- and salinity stresses. Dura *et al*. (2011) found that germination percentage, seedling growth, vegetative growth, grain production, exclusion of Na+ and Cl-, and K+/Na+ ratio were higher in Belikh2 than Omrabi5 under saline conditions. The means of all RILs were close to the mid-parental values for all traits in both treatments (Table 1). Although phenotypic distribution of RILs was normal, transgressive segregation was also observed in both directions for all traits (Table 1). In the past decade, few genetic and molecular analyses were conducted and a small number of QTLs were mapped in durum wheat (Genc *et al*., 2010). Some reports showed that there was a large genotypic diversity for wheat in salinity tolerance (Koyama et al., 2001; Lindsay *et al*., 2004; Genc *et al*., 2010; Dura *et al*., 2011). Ma *et al*. (2004) found markers controlling salt tolerance at germination stage on homologous chromosomes 3, 4, 5 and 7 and at seedling stage on homologous chromosome 1 and 3 in bread wheat. Few research of such molecular analysis has been done under field condition. However, Quarrie *et al*. (2005) detected 7 markers controlling grain yield at mature stage under saline condition using field irrigated with saline water. In this study, a total of 28 markers for the examined fifteen traits were detected under the two treatments. It is suggested that different markers or alleles at the same locus are responsible for genetic variation under diverse environment conditions. The results were consistent with the study of Austin and Lee (1998) in which QTL was analyzed under stress and non-stress environments. The case was same for the markers controlling DH, DM, PH, PL, and NS (Tables 4 and 5).

The results suggested that these markers were stable and not greatly influenced by environments. Most of the detected markers locations were mapped on the same region of chromosomes 3B, 4A, and 7B which accounted for 3.1-6.8% of the total phenotypic traits (Tables 4 and 5). Moreover, these markers represented 73.7% of the total markers found under salt stress, and all their positive alleles came from Belikh2. The results indicated that this region of chromosomes 3B, 4A, and 7B and its homologous are important for salt tolerance in durum wheat. It may be assumed that there is a QTL cluster for salt tolerance in the region of chromosome 3B, 4A, and 7B and its homologous (Tables 4 and 5), and thus the region may be used as an important target for improving salt tolerance of durum wheat.

Under normal (non-stress) environment, one significant marker (Xbarc353) associated with DM and NT were mapped on chromosome 2A and its alleles with positive effect coming from Belikh2, supported by significantly positive correlation (r = 0.78**) between the two traits. Similar results were found for DH, DM, PH, NG, WS and BY in the markers; Xbarc1025, Xbarc61, Xwmc177, Xbarc1025, Xwmc617, and Xbarc32 on chromosomes 2A, 4A, 4B, 5A, 7A, and 7B (Tables 2 and 3). However, these markers were not found under salt stress. It may be assumed that the genes in these regions, controlling DH, DM, PH, NG, WS, and BY are expressed normally under the condition without salt stress, while their expression is greatly inhibited when the plants are exposed to salt stress. Moreover it was found that the markers detected under no salinity differed markedly from those detected under salt stress, and there were many markers which are co-located or tightly linked with these agronomic traits.

Salinity tolerance genes are located throughout the genome and are genotype dependent. In this study, it was found that for grain yield four markers were derived from Belikh2. However, some of the markers were also derived from sensitive parent of the population. This study confirms that salinity tolerance is a quantitative trait and that apparently sensitive parents may contain alleles for tolerance, which may not be found in the tolerant parent. It can be concluded that the sensitive

parent Omrabi5 may contain some tolerance alleles that when combined with alleles from tolerant parents can results in increased level of tolerance.

Under normal (non-stress) environment, one significant marker (Xbarc353) associated with DM and NT were mapped on chromosome 2A and its alleles with positive effect coming from Belikh2, supported by significantly positive correlation (r = 0.78**) between the two traits. Similar results were found for DH, DM, PH, NG, WS and BY in the markers; Xbarc1025, Xbarc61, Xwmc177, Xbarc1025, Xwmc617, and Xbarc32 on chromosomes 2A, 4A, 4B, 5A, 7A, and 7B (Tables 2 and 3). However, these markers were not found under salt stress. It may be assumed that the genes in these regions, controlling DH, DM, PH, NG, WS, and BY are expressed normally under the condition without salt stress, while their expression is greatly inhibited when the plants are exposed to salt stress. Moreover it was found that the markers detected under no salinity differed markedly from those detected under salt stress, and there were many markers which are co-located or tightly linked with these agronomic traits.

Salinity tolerance genes are located throughout the genome and are genotype dependent. In this study, it was found that for grain yield four markers were derived from Belikh2. However, some of the markers were also derived from sensitive parent of the population. This study confirms that salinity tolerance is a quantitative trait and that apparently sensitive parents may contain alleles for tolerance, which may not be found in the tolerant parent. It can be concluded that the sensitive parent Omrabi5 may contain some tolerance alleles that when combined with alleles from tolerant parents can results in increased level of tolerance.

V – Conclusion

Molecular markers closely linked to genes of agronomic importance traits have been demonstrated to be useful tools for indirect selection in durum wheat breeding programs (Nachit *et al*., 1998). Further investigations for salinity 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 salinity 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 genes, which are involved in the control of the phenotypic variation in quantitatively inherited traits related to salinity tolerance. Compared with conventional methods, QTLs and molecular markers provide breeders new alternatives for selection. Marker-assisted selection can accelerate breeding by reducing the time to develop new cultivars (Landjeva *et al*., 2007). Further research is needed on molecular markers and QTL mapping to screen potential genotypes for salinity tolerance in wheat.

Acknowledgments

The authors are grateful to ICARDA durum wheat MAS laboratories for their technical support and Dr. Ayed Alabdullat who provided helpful comments on the manuscript

References

Austin D.F., Lee M., 1998. Detection of quantitative loci for grain yield and yield components in maize across generations in stress and no stress environments. *Crop Sci*., 38, pp.1296-1308.

Colmer T.D., Munns R., Flowers T.J., 2005. "Improving salt tolerance of wheat and barley: future prospects". *Australian J. Exp. Agricult.,* 45, pp.1425-1443.

Dura S., Duwayri M.A., Nachit M.M., 2011. Effect of different salinity levels on growth, yield and physiology on durum wheat (*Triticum turgidum* var. *durum*). *Jordan J. Agric. Sci.,* 7, pp. 518-528.

Dura S., Duwayri M.A., Amri A., Nachit M.M., 2013. Detection of molecular markers associated with yield and yield components in durum wheat (*Triticum turgidum* var. *durum*) under drought conditions. *African J. Agric. Res*., 8, 211-2128.

Flowers T.J., Yeo A.R., 1995. Breeding for salinity resistance in crop plants: Where next?. *Australian J. Plant Physiol*., 22, pp. 875–884.

Genc Y., Oldach K., Verbyla A.P., Lott G., Hassan M., Tester M., Wallwork H., McDonald G.K., 2010. Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. *Theor. Appl. Genet*., 121, 877-894.

Gong J. M., He P., Qian Q., Shen L.S., Zhu L.H., 1999. Identification of salt tolerance QTL in rice. *China Sci. Bull*., 44, pp. 68-71.

Kennedy B.W., Quinton M., Vanarendonk J.A.M., 1992. "Estimation of effects of single genes on quantitative traits". *J. Anim. Sci*., 70, 2000-2012.

Koyama M.L., Levesley A., Koebner R.M.D., Flowers, T.J., Yeo A.R., 2001. Quantitative trait loci for competent physiological traits determining salt tolerance in rice. *Plant Physiology,* 125, pp. 406-422.

Lee G.J., Boerma H.R., Villagarcia M.R., Zhou X., Carter T.E. Jr. Li Z., Gibbs M.O., 2004. A major QTL conditioning salt tolerance in S-100 soybean and descendent cultivars. *Theor. Appl. Genet*., 109, pp. 1610-1619.

Landjeva S., Korzun V., Borner A., 2007."Molecular markers: Actual and potential contributions to wheat genome characterization and breeding. *Euphytica,* 156, pp. 271-296.

Lindsay M.P., Lagudah E.S., Hare R.A., Munns R., 2004. A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. *Functional Plant Biology,* 31, pp. 1105-1114.

Lin H.X., Zhu M.Z., Yano M.; Gao J.P., Liang Z.W., Su W.A., Hu X.H., Ren Z.H., Chao D.Y., 2004. QTLs for Na+ and K+ uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet.,* 108, pp. 253-260.

Mano Y., Takeda K., 1997."Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica,* 94, pp. 263-272.

Mohan M., Nair S., Bhagwat A., Krishna T.G., Yano M., Bhatia C.R., Sasaki T., 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed*., 3, pp. 87-103.

Munns R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*., 25, pp. 239-250.

Munns R., Rawson H.M., 1999. Effect of salinity on salt accumulation and reproductive development in the apical meristem of wheat and barley. *Australian J. Plant Physiol*., 26, pp. 459-464.

Munns R., Tester M., 2008. Mechanisms of salinity tolerance. *Annual Rev. Plant Biology,* 59, pp. 651-681.

Nachit M.M., 1998. Association of grain yield in dryland and carbon isotope discrimination with molecular markers in durum (*Triticum turgidum* L. var. *durum*). In: *Proc. 9th Intl. Wheat Genet. Symp.* Saskatoon, Saskatchewan, Canada, pp. 218-223

Nachit M.M., Elouafi I., Pagnotta M.A., Saleh A., Iacono E., Labhilili M., Asbati A., Azrak M., Hazzam H., Benscher D., Khairallah M., Ribaut J.M., Tanzarella O.A., Porceddu E., Sorrels M.E., 2001. Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). *Theor. Appl. Genet*., 102, pp. 177-186.

SAS Institute, 1998. SAS language guide for personal computers, release 6.03 edition. SAS Institute Inc., Cary, NC, USA.

Xue D., Huang Y., Zhang X., Wei K., Westcott S., Li C., Chen M., Zhang G., Lance R., 2009. Identification of QTLs associated with salinity tolerance at late growth stage in barley. *Euphytica,* 169, pp. 187-196.