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A MULTIDISCIPLINARY APPROACH FOR DISSECTING QTL CONTROLLING HIGH-YIELD AND DROUGHT TOLERANCE-RELATED TRAITS IN DURUM WHEAT

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ABSTRACT

Durum wheat (*Triticum turgidum ssp. durum*) is an economically and nutritionally important cereal crop in the Mediterranean region and its production is largely influenced by environmental stresses, such as drought, salinity, heat and nutrient deficiency. The objective of this study was to dissect quantitative trait loci (QTL) controlling grain yield, yield components and drought tolerance in durum. A molecular genetic linkage map for F2 durum mapping population derived from an intraspecific cross between Baniswif-1 x Sohag-2 was constructed using 114 DNA markers (9 SSRs, 14 SCoTs, 90 AFLPs and 1 RAPDs) distributed over the 14 linkage groups and spanning 2040.9 cM of the durum wheat genome. The size of linkage groups varied greatly from 6.8cM for LG11 to 317.5cM for LG4 with an average length of 145.8cM. Based on the used anchor SSR markers, only eight linkage groups were assigned to chromosomes, where LG1, LG3, LG5, LG6, LG7, LG9, LG13 and LG14 were assigned to chromosomes 1B, 3B, 5B, 6A, 6B, 7A, 3A and 2B, respectively. Single point analysis was used to identify genomic regions controlling eleven morpho-physiological traits related to grain yield, yield components and drought tolerance. A total of 74 QTL were identified for the eleven traits on all linkage groups except (LG10 and LG11). These included 3 QTL for root length (RL), 11 QTL for plant height (PH), 7 QTL for spike length (SL), 3 QTL for number of branches/plant (NBP), 3 QTL for number of spike/plant (NSP), 8 QTL for number of spikelets/spike (NSS), 15 QTL for number of kernel/spike (NKS), 10 QTL for thousand-kernel weight (TKW), 4 QTL for fresh weight (FW), 5 QTL for dry weight (DW) and 5 QTL for total amino acids (TAA). This work represents the first genetic linkage map for durum wheat population derived from an intraspecific cross between 'Baniswif-1' and 'Sohag-2' showing chromosomal regions associated with 11 morpho-physiological traits related to grain yield, yield components and drought tolerance in durum wheat.

KEYWORDS: QTL, Durum, Drought, Yield, Molecular Markers, SCoT, SSR, AFLP

INTRODUCTION

Drought is one of the most common abiotic stresses limiting crops productivity throughout the world. During last decades, drought continues to be a challenge to agricultural scientists in general and to plant breeders in particular. Therefore, breeding and selection for high-yield crops under drought stress is a major objective of crop breeders working under unfavorable environments.

Drought tolerance is a multi-genic trait that manifests at different stages of crop development and affects the plant in different ways. The morpho-physiological traits related to water-use efficiency, kernel characters and yield components have been found to play a major role in drought tolerance in durum wheat (Diab *et al.*, 2007; Kadam *et al.*, 2012; Kumar *et al.*, 2013 and Patil *et al.*, 2013). Understanding the basis of such traits can offer a potential way for selection of promising germplasm based on key-traits associated with high grain yield in dry land (Elouafi and Nachit, 2004).

Durum wheat is an allotetraploid (AABB genome, 2n=4x=28, seven homoeologous groups) with an approximately genome size of 13,000 Mbp.

In contrast to bread wheat, for which several QTL maps have been developed, mapping in durum wheat has been relatively delayed. The first constructed durum map of 65 RILs based on 259 markers was reported by Blanco *et al*. (1998). Then, more than thirteen durum wheat genetic maps have been published (Nachit *et al.* 2001; Elouafi and Nachit 2004; Diab *et al.* 2008; Peleg *et al.* 2008; Zhang *et al.* 2008, Gadaleta *et al.* 2009; Kadam *et al*. 2012; Kumar *et al*. 2013 and Patil *et al.* 2013). Most of these maps were based on breeding lines issued from North American or Italian genetic pools. They contained large gaps partly due to absence of polymorphism between genitors or partly due to the low polymorphism level of the elite pool.

The major molecular markers used for wheat mapping are SSRs and AFLPs. A new type of genetic marker is represented by start codon targeted (SCoT) polymorphism, which is a novel, simple and reliable gene targeted marker technique based on designed primers targeting conserved region surrounding the translation initiation codon ATG (Collard and Mackill, 2009). The major objectives of this work were to construct a QTL map for Egyptian durum wheat through the application of different DNA markers (SSR, SCoT, RAPD and AFLP) and to develop an F_2 segregating population from an intraspecific cross between two durum varieties (Baniswif-1 and Sohag-2) and to tag QTL controlling yield, yield components and drought tolerance-related traits.

MATERIALS AND METHODS

Mapping Population and Genomic DNA Extraction

In order to construct a genetic map, two polymorphic varieties Baniswif-1 and Sohag-2 were selected among the germplasm available at Wheat Research Dept. - Crop Research Institute - ARC - Egypt. These two varieties were used to develop an F_2 mapping population comprising 76 plants. The parents and F_2 individuals were grown in 2009 at one of the Agricultural Genetic Engineering Research Institute (AGERI) experimental fields. The F_2 plants were grown in two replicates in a randomized complete block design. High-quality genomic DNA was extracted from fresh young leaves (100 mg) of the two parents and the 76 F_2 individuals using a DNAeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA).

Morpho-Physiological Traits Measurements

The parents and F2 mapping population were evaluated for eleven morpho-physiological traits related to drought tolerance and high-yield as follows: Root length (RL) was measured as described by Manschadi *et al.* (2006). At 50% maturity, plant height (PH) was measured from the soil surface to the top of the spikes of the main tiller, excluding awns as described by Marza *et al.* (2006). Spike length (SL) was measured from the base of the rachis to the top of the uppermost spikelets excluding the awns as described by Villegas *et al.* (2007). While, the data of Number of spikelets/spike (NSS) and number of kernel/spike (NKS) traits were recorded as described by Nachit *et al*. (1992). Thousand-kernel weight (TKW) was measured as weight in grams of 100 kernels and converted to the weight of 1000 kernels. Moreover, the data of number of branches/plant, number of spike/plant, fresh weight and dry weight were estimated according to Peng *et al*. (2003) and Quarrie *et al*. (2006), respectively. The total amino acids trait was determined by the micro-Kjeldahl technique as described by Levey *et al*, 2000.

Statistics and Normality Test of Traits

The skewness and kurtosis values were calculated for each of the eleven traits to determine if these traits data were normally distributed. The program Windows QTL Cartographer V. 2.5 (Wang *et al*., 2007) was used to analyze the

data and to obtain the mean, variance, standard deviation, coefficient of variation, skewness and kurtosis values for each trait.

Markers Analysis

Fifty-six anchor SSR primer pairs, 26 SCoT primers, 42 RAPD primers and 32 AFLP primer combinations were initially used to screen polymorphism between the two parental varieties (Baniswif-1 and Sohag-2). The polymorphic markers (15 SSR, 10 SCoT, 1 RAPD and 11 AFLP) were subsequently used to analyze the segregation among the 76 individuals of the F_2 mapping population.

Microsatellite Analysis

Anchor microsatellite markers (56 markers) located on chromosomes of the A and B genomes of durum wheat were chosen from GrainGenes database for analysis. SSR analysis was conducted as described by Adawy (2007) and Hussein *et al.* (2006). Analysis of segregation among the 76 F2 individuals was performed using 15 anchor SSR primer pairs that showed a polymorphic pattern between the parents.

SCoT Analysis

SCoT analysis was performed as described by Collard and Mackill (2009). Initially, twenty-six 18-mer SCoT primers were screened aginst the two parental varieties. Ten out of these 26 primers showed polymorphic pattern. Therefore, they were used to survey the segregation among the 76 F2 individuals.

RAPD Analysis

RAPD amplification was carried out as described by Hussein *et al.* (2006). A set of 42 random 10-mer primers were initialy screened aginst the two parents. Only one RAPD primer revealed discernible polymorphism among the two parents. This primer was used to screen segregants in the F2 population.

AFLP Analysis

The AFLP analysis was performed using the AFLP® Analysis System II (Invitrogen, USA) as described by Vos *et al.* (1995). Initially, 32 AFLP primer combinations (PCs) were tested on the two parental varieties. Among these, the best eleven PCs were selected on the basis of the number of bands, clarity of pattern, and distribution on the gel. These were tested again on the parental varieties and the 76 F2 individuals.

Genetic Linkage Map Construction and QTL Detection

The 125 markers that showed polymorphism between the parental varieties were used to construct the genetic linkage map. Chi-squared tests were applied for all markers to evaluate the goodness of fit of observed segregations to specific inheritance ratios. Linkage analysis and map construction were performed using Map Manager QTX V. 1.4 (Manly and Cudmore, 1997). The graphic representation of the linkage groups was created using MapChart 2.1 (Voorrips, 2002). The association between phenotype and genotype was investigated using single point analysis (SPA), using Windows QTL Cartographer V. 2.5 (Wang, *et al*., 2007) at significance levels of 5%, 1%, 0.1% and 0.01%.

RESULTS

Statistics and Normality Test of Traits

The frequency distribution for all traits evaluated in this study approximately fitted normal distribution. The no. of kernel/spike (NKS) was the most variable trait among the 76 plants of the F_2 population, while the least variable trait was

the dry weight (DW) High kurtosis values were observed for no. of kernel/spike (NKS) and total amino acids (TAA) and a large skewness value was obtained for total amino acids (TAA).

Molecular Markers Analysis

Different molecular markers including SSR, SCoT, RAPD and AFLP were employed to identify markers that reveal differences between parents and among the F_2 individuals.

- **Polymorphism as Detected by SSR Analysis**

Fifty six anchor microsatellite primer pairs located on the A and B durum wheat chromosomes were screened against parental genotypes, Baniswif-1 and Sohag-2, in an attempt to detect polymorphic markers. Among these, only 15 SSR primers (26.8%) showed polymorphism between the two parents. Thus, analysis of segregation among the 76 F2 individuals was performed using these 15 SSR polymorphic primers. The 15 SSR primer pairs exhibited 15 SSR alleles (Table 1 and Figure 1). The allele size ranged from 60bp to 550bp.

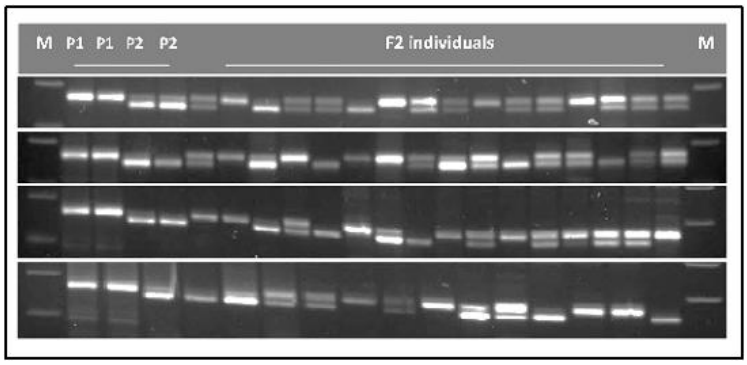


Figure 1: SSR Patterns of the Two Parents and F2 Individuals Derived from the Cross Baniswif -1 and Sohag-2 as Revealed by Primer S8. M is the Standard DNA Marker 100 bp Ladder, P1 (cv. Baniswif -1) and P2 (cv. Sohag-2)

- **Polymorphism as Detected by AFLP Analysis**

Among the 32 AFLP primer combinations that were used to screen the two parents, fifteen AFLP primer combinations (46.8%) showed polymorphism. From these 15 primer combinations, only eleven primer combinations showed high discernible polymorphism between the two parents. Thus, analysis of segregation among the 76 F_2 individuals was performed using these eleven AFLP polymorphic primer combinations (Figure 2). The selected primer combinations, the total number of amplicons, polymorphic amplicons and polymorphism percentage are listed in Table (2). A total of 837 major AFLP bands were observed, 95 of these (11.4%) were polymorphic between the two parents. The number of amplicons/primer combination ranged from 63 (E-ACG/M-CTA) to 95 (E-ACA/M-CTA). While, the number of polymorphic amplicons varied from 6 in primer combination (E-AGG/M-CAG) to 10 in primer combinations (E-AAC/M-CTA, E-ACA/M-CTA, E-ACG/M-CTA, E-AGC/M-CAC and E-AGG/M-CTC). Thus, the average number of polymorphic fragments per combination was 8.6.

Table 1: Primer Code, Primer Name, Primer Sequence, Chromosome and Marker Size as Detected by SSR Analysis

Primer Code	Primer Name	Primer Sequence	Chrom.	Marker Size
S1	Xgwm99	F- AAGATGGACGTATGCATCACA R- GCCATATTTGATGACGCATA	2A	120
S2	Xgwm369	F- CTGCAGGCCATGATGATG R- ACCGTGGGTGTTGTGAGC	3A	150
S3	Xgwm334	F- AATTTCAAAAAGGAGAGAGA R- AACATGTGTTTTTAGCTATC	6A	130

Table 1: Contd.,				
S4	Xgwm210	F- TGCATCAAGAATAGTGTGGAAG R- TGAGAGGAAGGCTCACACCT	2B	120
S5	Xwmc50	F- CTGCCGTCAGGCCAGGCTCACA R- CAACCAGCTAGCTGCCGCCGAA	3A	140
S6	Xwmc505	F-AGGGGAGGAAAACCTTGTAATC R- ACGACCTACGTGGTAGTTCTTG	3A	160
S7	Xwmc596	F- TCAGCAACAAACATGCTCGG R- CCCGTGTAGGCGGTAGCTCTT	7A	150
S8	Xgwm333	F- GCCCGGTCATGTAAAACG R- TTTCAGTTTGCGTTAAGCTTTG	7B	160
S9	Xwmc626	F- AGCCCATAAACATCCAACACGG R- AGGTGGGCTTGGTTACGCTCTC	1B	180
S10	Xgwm181	F- TCATTGGTAATGAGGAGAGA R- GAACCATTCATGTGCATGTC	3B	170
S11	Xcfd39	F- CCACAGCTACATCATCTTTCCTT R- CAAAGTTTGAACAGCAGCCA	4B	180
S12	Xgwm335	F- CGTACTCCACTCCACACGG R- CGGTCCAAGTGCTACCTTTC	5B	200
S13	Xgwm371	F- GACCAAGATATTCAAACTGGCC R- AGCTCAGCTTGCTTGGTACC	5B	200
S14	Xgwm219	F- GATGAGCGACACCTAGCCTC R- GGGGTCCGAGTCCACAAC	6B	550
S15	Xgwm273	F- ATTGGACGGACAGATGCTTT R- AGCAGTGAGGAAGGGGATC	1B	60

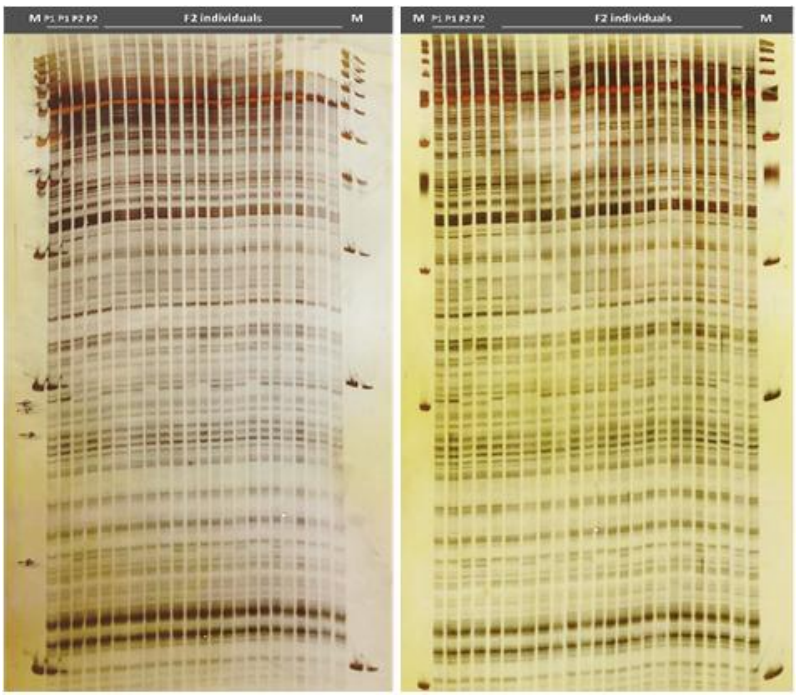


Figure 2: AFLP Patterns of the Two Parents and F2 Individuals Derived from the Cross Baniswif-1 and Sohag-2 as Revealed by Primer Combination 3/6. M is the Standard DNA Marker 100 bp Ladder, P1 (cv. Baniswif -1) and P2 (cv. Sohag-2)

Table 2: Selective Nucleotides of AFLP Primer Combinations, Number of Total Bands, Polymorphic Bands and Polymorphism Percentage

Primer Comb.	Selective Nucleotides		Number of Bands		% of Polymorphism
	*Eco*R1	*Mse*I	Total	Polymorphic	
1/5	AAC	CTA	68	10	14.7
3/5	ACA	CTA	95	10	10.5
3/6	ACA	CTC	67	7	10.4

Table 2:Contd.,

4/4	ACC	CAT	74	9	12.2
4/6	ACC	CTC	78	8	10.3
5/3	ACG	CAG	80	8	10.0
5/5	ACG	CTA	63	10	15.9
6/6	ACT	CTC	70	7	10.0
7/2	AGC	CAC	69	10	14.5
8/3	AGG	CAG	87	6	6.9
8/6	AGG	CTC	86	10	11.6
Total			**837**	**95**	**11.4**
Average			**76.1**	**8.6**	

- **Polymorphism as Detected by SCoT Analysis**

Among 26 SCoT primers initially screened against parental genotypes, only ten SCoT primers (41.7%) showed polymorphism between the two parents and consequently used in the analysis of segregation among the 76 F_2 individuals. These primers amplified a total of 126 amplicons including 112 monomorphic DNA fragments. While, 14 fragments were polymorphic, corresponding to a level of polymorphism of 11.1% (Figure 3 and Table 3).

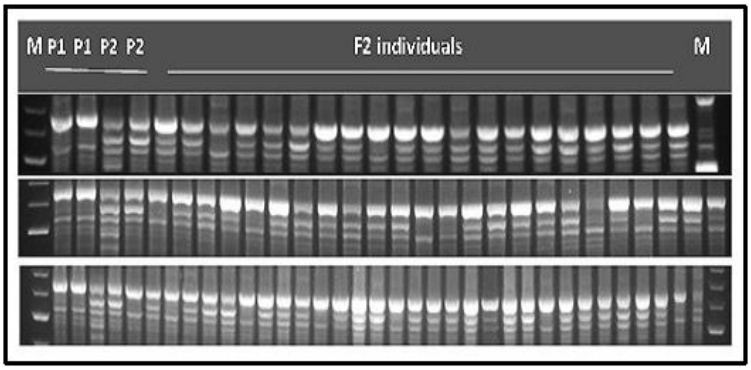


Figure 3: SCoT Patterns of the Two Parents and F2 Individuals Derived from the Cross Baniswif-1 and Sohag-2 as Revealed by Primer SCoT-13. M is the Standard DNA Marker 100 bp Ladder, P1 (cv. Baniswif-1) and P2 (cv. Sohag-2)

- **Polymorphism as Detected by RAPD Analysis**

In the present study, out of 42 decamer random primers initially screened against parental genotypes, only one RAPD decamer primer (2.4%) showed polymorphism between the two parents and used to analyze the segregation among the 76 F_2 individuals. This primer amplified a total of 11 amplicons including 10 monomorphic DNA fragments. While, one fragment was polymorphic, corresponding to a level of polymorphism of 9.1% (Figure 4 and Table 3).

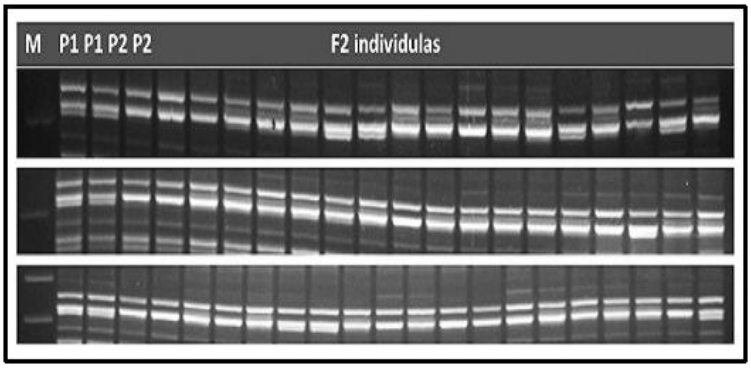


Figure 4: RAPD Patterns of the Two Parents and F2 Individuals Derived from the Cross Baniswif-1 and Sohag-2 as Revealed by Primer OP-C4. M is the Standard DNA Marker 100 bp Ladder, P1 (cv. Baniswif-1) and P2 (cv. Sohag-2)

Table 3: Primer Name, Primer Sequence, Number of Total Bands, Polymorphic Bands and Percentage of Polymorphism as Detected by SCoT and RAPD

Primer Name	Primer Sequence	Number of Bands		% of Polymorphism
		Total	Polymorphic	
SCoT				
SCoT-1	ACGACATGGCGACCACGC	14	1	7.1
SCoT-3	ACGACATGGCGACCCACA	10	1	10.0
SCoT-8	ACAATGGCTACCACTGAG	16	4	25.0
SCoT-11	ACAATGGCTACCACTACC	15	1	6.7
SCoT-13	ACCATGGCTACCACGGCA	9	2	22.2
SCoT-17	CCATGGCTACCACTACCC	12	1	8.3
SCoT-20	CAACAATGGCTACCACGC	10	1	10.0
SCoT-21	CCATGGCTACCACCGGCC	15	1	6.7
SCoT-23	CATGGCTACCACCGGCCC	10	1	10.0
SCoT-26	ACGACATGGCGACCACGC	15	1	6.7
Total		**126**	**14**	**11.1**
Average		**12.6**	**1.4**	
RAPD				
OP-C4	CCGCATCTAC	**11**	**1**	**9.1**

Linkage Analysis and Map Construction

One hundred twenty five loci were used to construct the genetic map. One hundred and forteen genetic loci including (9 SSRs, 90 AFLPs, 1 RAPDs and 14 SCoT) were mapped in 14 linkage groups spanning 2040.9 cM of the durum wheat genome. . The remaining 11 markers were unlinked. The resulting linkage groups were numbered starting with LG1 to LG14. The size of linkage groups varied greatly from 6.8cM for LG11 to 317.5cM for LG4 with an average length of 145.8 cM. The number of markers located on each linkage group also varied from 2 to 17. The distribution of markers, linkage group assignment and map coverage across the 14 durum wheat linkage groups are summarized in Table (4). The main marker type contributing to this linkage map was AFLPs (78.9% AFLPs, 7.9% SSRs, 0.9% RAPDs and 12.3% SCoT). As shown in Table (4) and Figure (5) some linkage groups were composed entirely of AFLPs (LG 8, 10 and 12), whereas, others consisted of AFLPs and SSRs (LG1, 3, 6 and 13), or AFLPs and SCoTs (LG4 and 11). The eleven unlinked markers represented 8.8% of the total markers used to construct the genetic map. Thus, additional markers are needed to provide bridges for joining these unlinked markers.

Table 4: Distribution of Molecular Markers, Assignment and centiMorgan (cM) Coverage across the 14 Linkage Groups of the Genetic Map Used in QTL Mapping

Linkage Groups	AFLP	SCOT	SSR	RAPD	Markers		cM	cM/ Marker
					#	%		
LG 1 (1B)	4	0	2	0	6	5.3	90.9	15.2
LG 2	5	1	0	1	7	6.1	98.5	14.1
LG 3 (3B)	5	0	1	0	6	5.3	124.2	20.7
LG 4	12	2	0	0	14	12.3	317.5	22.7
LG 5 (5B)	14	2	1	0	17	14.9	298.4	17.6
LG 6 (6A)	10	0	1	0	11	9.6	136.3	12.4
LG 7 (6B)	7	3	1	0	11	9.6	199.6	18.1
LG 8	5	0	0	0	5	4.4	95.2	19.0
LG 9 (7A)	6	1	1	0	8	7.0	183	22.9
LG 10	3	0	0	0	3	2.6	40.3	13.4
LG 11	1	1	0	0	2	1.8	6.8	3.4
LG 12	2	0	0	0	2	1.8	29.5	14.8
LG 13 (3A)	6	0	1	0	7	6.1	179.2	25.6
LG 14 (2B)	10	4	1	0	15	13.2	241.5	16.1
Total	**90**	**14**	**9**	**1**	**114**	**100**	**2040.9**	**17.9**
Average	**6.4**	**1.0**	**0.6**	**0.1**	**8.1**		**145.8**	

Table 4: Contd.,								
Genome A	**22**	**1**	**3**	**0**	**26**	**22.8**	**498.5**	**19.2**
Genome B	**40**	**9**	**6**	**0**	**55**	**48.2**	**954.6**	**17.3**
Un-localized	**28**	**4**	**0**	**1**	**33**	**28.9**	**587.8**	**17.8**

Assignment of Linkage Groups to the Chromosomes

All 56 SSR primer pairs assayed in the present study were previously mapped on the durum wheat chromosomes (GrainGenes database; http://www.graingenes.org). Therefore, in the present study these SSR markers were used to assign the linkage groups to specific chromosomes. Only 15 SSRs anchored primers revealed polymorphic patterns between the two parents. Based on the presence of these assigned SSR markers, only eight linkage groups (LG) were assigned to chromosomes, where, LG1, LG3, LG5, LG6, LG7, LG9, LG13 and LG14 were assigned to chromosomes 1B, 3B, 5B, 6A, 6B, 7A, 3A and 2B, respectively. The eight SSR markers used to assign the chromosomes are highlighted in the linkage groups shown in Figure (5). Six SSR assigned markers were unlinked, representing 40% of the total SSR assigned markers used to construct the genetic map.

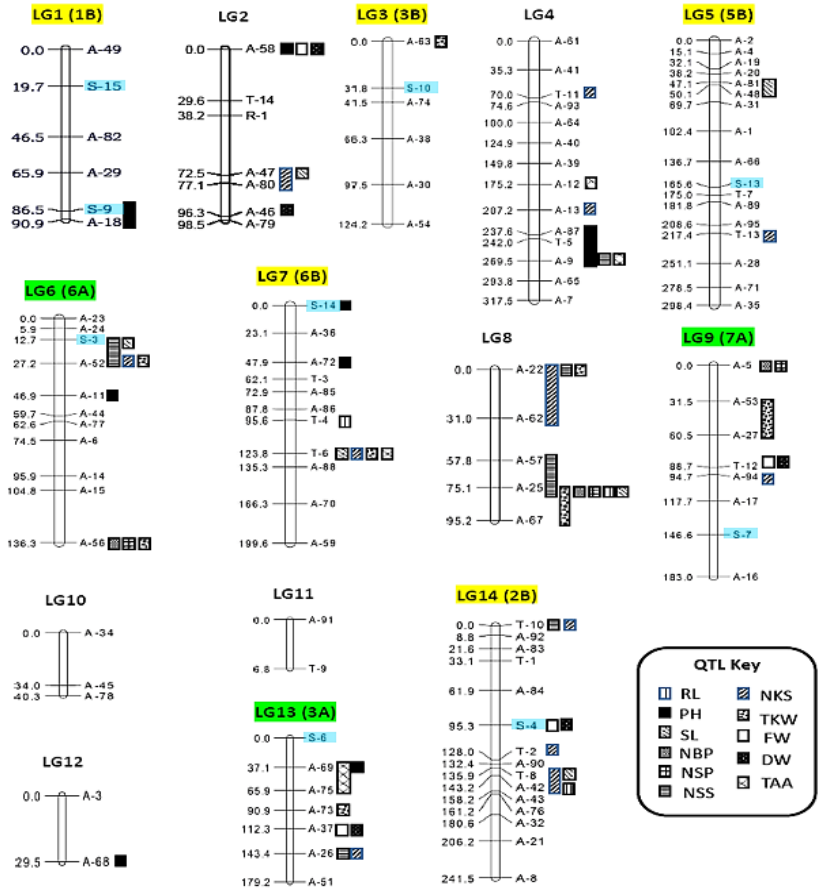


Figure 5: Molecular Linkage Groups of Durum Wheat (Intercross between Baniswif-1 and Sohag-2) Showing Positions of QTL Influencing Root Length, Plant Height, Spike Length, Number of Branches/Plant, Number of Spike/Plant, Number of Spikelets/Spike, Number of Kernel/Spike, Thousand Kernel Weight, Fresh Weight, Dry Weight and Total Amino Acids. Map Distances between Adjacent Markers are in cM

QTL Analysis

A total of 74 QTL with a significance ranging from 0.01% to 5% were identified for the eleven traits on all linkage groups except (LG10 and LG11). These included, 3 QTL for root length, 11 QTL for plant height, 7 QTL for spike length, 3 QTL for number of branches/plant, 3 QTL for number of spike/plant, 8 QTL for number of spikelets/spike, 15 QTL for number of kernel/spike, 10 QTL for thousand kernel weight, 4 QTL for fresh weight, 5 QTL for dry weight and 5

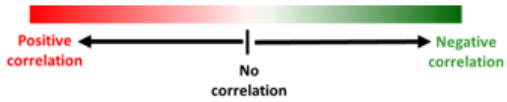
QTL for total amino acids (Figure 5). These 74 QTL were identified by using single-point analysis (SPA) which is the prefered method when the number of markers is not large enough and when complete genetic map is not available (Muhanad, 2003), which is the case in this study where only 114 markers were mapped on the 14 linkage groups.

Correlation between Traits

Correlation coefficient analysis of the eleven traits indicated that highly significant positive correlation found between the number of branches/plant and number of spike/plant (r=0.989, P≤0.01). While, low positive correlation was observed between number of spikelets/spike and total amino acids (r=0.031, P≤0.01). On the other hand, a significant negative correlation between the root length and total amino acids (r= -0.165, P≤0.01) was determined, while, low negative correlation was detected between the plant height and total amino acids (r= -0.046, P≤0.01) (Table 5).

Table 5: Correlation Coefficient among Root Length, Plant Height, Spike Length, Number of Branches/ Plant, Number of Spike/Plant, Number of Spikelets/Spike, Number of Kernel/Spike, Thousand Kernel Weight, Fresh Weight, Dry Weight and Total Amino Acids Traits in F2 Segregating Population

	RL	PH	SL	NBP	NSP	NSS	NKS	TKW	FW	DR
PH	0.167									
SL	0.197	0.457								
NBP	0.461	0.258	0.251							
NSP	0.460	0.219	0.228	0.989						
NSS	0.257	0.525	0.675	0.425	0.404					
NKS	0.184	0.257	0.536	0.344	0.307	0.575				
TKW	0.252	0.324	0.612	0.516	0.519	0.671	0.443			
FW	0.242	0.301	0.189	0.411	0.389	0.340	0.230	0.366		
DR	0.249	0.277	0.159	0.386	0.366	0.315	0.230	0.323	0.988	
TAA	-0.165	-0.046	0.132	0.060	0.084	0.031	0.166	0.113	-0.161	-0.162



DISCUSSIONS

In the present investigation, an F2 segregating population obtained from an intraspecific cross between Baniswif-1 and Souhag-2 was developed in order to identify quantitative trait loci (QTL) controlling yield, yield components and drought tolerance-related traits.

Statistics Analysis and Normality Test

The accurate estimation of QTL positions and its effects requires the normal distribution of residuals in the experimental data. However, the data often contain outliers and influential observations that may seriously affect the estimation of model parameters and can lead to errors in the detection of QTL positions and their predicted effects (Fernandes *et al*., 2007). The statistical analysis of the eleven morpho-physiological traits indicated that these traits continuously segregated and both kurtosis and skewness values suggested that these traits normally distributed which make them suitable for QTL analysis. The recorded traits were classified into three groups: (i) agronomic traits (number of branches/plant, number of spike/plant, number of spikelets/spike, number of kernel/spike and thousand-kernel weight), (ii) morphological traits (root length, plant height and spike length), (iii) Physiological traits included: fresh weight, dry weight and total amino acids.

Comparison with Previously Published Maps

The development of genetic linkage map in durum wheat represents a first step towards the detection of factors/genes controlling the important traits. The first durum map was based on 65 RILs (Blanco *et al*., 1998); and after 3

years the second durum map was developed based on an intraspecific cross Jennah Khetifa x Cham1 with 110 RILS (Nachit *et al*., 2001). The genetic map presented here complements the information of previously published durum maps (Nachit *et al.* 2001; Elouafi and Nachit 2004; Diab *et al.,* 2008; Zhang *et al.,* 2008; Gadaleta *et al.,* 2009; Kadam *et al*., 2012; Kumar *et al*., 2013 and Patil *et al.,* 2013) and adds 14 SCoT markers not mapped before in durum maps. More importantly, this map, for the first time, integrates SCoT marker technology in durum wheat map.

The early maps were based on restriction fragment length polymorphism (RFLP) markers (Blanco *et al*., 1998), while later the polymerase chain reaction (PCR)-based markers became dominant for genetic map construction, e.g. amplified fragment length polymorphisms (AFLPs) (Nachit *et al*., 2001), differentially expressed sequence tags (dESTs) (Diab *et al.,* 2008) and simple sequence repeats (SSRs) (Elouafi and Nachit 2004). Later, High-density genetic map of durum wheat was developed based Diversity Arrays Technology (DArT) markers (Peleg *et al*., 2008). Moreover, single-nucleotide polymorphisms (SNPs) have been included in durum wheat genetic maps (Terracciano *et al*., 2013).

In this investigation, four user-friendly PCR-based marker systems (SSR, SCoT, AFLP and RAPD) were used to generate a genetic linkage map in an intraspecific cross between Baniswif-1 and Sohag-2. In this work, the constructed linkage map contains four marker types that were not mapped collectively in any other durum maps. A genetic map constructed in 14 linkage groups consisted of 114 loci and spanning a total of 2040.9cM with an average distance between loci of 11.1 cM. The longer durum maps constructed by Diab *et al*. (2008) and Nachit *et al*. (2001) are due mainly to the greater number of mapped markers (468 and 306 markers spanning 5672.8cM and 3,597.8cM, respectively). The map constructed in the present study is longer than the maps of Blanco *et al*. (1998) (1352 cM) and Mantovani *et al*. (2008) (2022 cM).

Distribution of Markers among Chromosomes and Genomes

The seven homologous linkage groups of the durum wheat genome varied in the number of markers, marker density, and map length. Total marker number and density was highest in linkage group 5 (5B) (total 17 loci, with 14.9 cM per marker), whereas total map length was the highest (317.5 cM) in linkage group 4. Linkage groups 11 and 12 had the lowest marker number and density (total 2 loci, with 3.4cM and 14.8cM per marker, respectively) and linkage group 11 had the shortest map length (6.8 cM). Differences were also found between the two sub-genomes, with 55 (48.2%) markers mapped to the B genome (average 11 markers per chromosome) and 26 (22.8%) to the A genome (average of 8.6 markers per chromosome). The B-genome skeleton map was denser, with 55 markers that accounted for 954.6cM of genetic distance (17.3cM per marker). The A genome skeleton map spanned 498.5cM with 28 markers (17.8cM per marker). In this respect, this result is in high degree of agreement with Peleg *et al.* (2008) reported results. They also found that the B-genome skeleton map was denser, with 339 (60%) markers mapped to the B genome (average 57 markers per chromosome) and 270 (40%) to the A genome (average of 39 markers per chromosome).

QTL Related to Agronomic Traits

QTL Associated with Number of Branches/Plant

Tillering is one of the most important agronomic traits in cereal crops because tiller number per plant determines the number of spikes or panicles per plant, a key component of grain yield and/or biomass (Li *et al*., 2010). Although a number of QTL controlled tillering were discovered in rice (Liu *et al*., 2009), and barley (Franckowiak *et al*., 2005), only few studies have been carried out in wheat generally and in durum wheat particularly. Therefore, the present study represents the first study successfully identifying 3 significant QTL on 3 linkage groups (6A, 7A and LG8) in Egyptian durum wheat. On the other hand, Li *et al*. (2010) identified 9 and 18 significant QTL across different environments for

tillering in the DH and IF2 bread wheat populations, respectively. Four QTL were common between two populations and a major QTL located on the 5D chromosome.

QTL Associated with Number of Spike/Plant

Spike number is one of the most important yield components and is highly correlated with tillering capacity in wheat (Peng *et al*., 2011). The map constructed in the present investigation detected 3 QTL for number of spike/plant trait on the same position on the same 3 linkage groups (6A, 7A and LG8). While, Peng *et al*. (2003) detected seven QTL for spike number per plant on five chromosomes (1B, 2A, 2B, 5A, 7A). From these results, it is clear that there is a consistent QTL for number of spike/plant on chromosome 7A that could be used for marker assisted selection for this trait. However, more study and investigation are needed to validate the consistency of this QTL.

QTL Associated with Number of Spikelets/Spike

Aruna and Raghaviah (1997) indicated that there is positive and significant association between number of spikelets per spike and grain yield. In this study, eight QTL for number of spikelets/spike were identified on five linkage groups (2B, 3A, 6A, LG4 and LG8). While, Peng *et al*. (2003) identified six QTL for number of spikelets/spike on four chromosomes (1B, 2A, 5A and 6B). On the other hand, Patil *et al*. (2013) successfully detected six QTL on four chromosomes (2A, 4A, 4B and 7A).

QTL Associated with Number of Kernel/Spike

Calderini *et al*. (1999) reported that increased number of kernels per spike is the main yield component that influences the grain yield. In this study, we identified 15 significant QTL on 9 linkage groups (3A, 2B, 5B, 6A, 6B, 7A, LG2, LG4 and LG8). Chromosome 2B (LG14) had the largest QTL number for kernels per spike (4 QTL). In this context, Patil *et al*. (2013) identified only five QTL for kernel per spike on chromosome 1B, 2A, 2B, 4A and 5B explaining up to 11.2 % variation in the trait.

QTL Associated with Thousand-Kernel Weight

Elouafi and Nachit (2004) reported that thousand-kernel weight (TKW) trait is highly related to semolina yield in durum wheat. The TKW in durum varies greatly from 20 to over 60 g. High TKW is desirable for easy processing, milling and semolina traits. It is affected by the environment during grain filling and by the number of heads and the number of fertile florets per spike, and has been strongly correlated with kernel length, width and volume (Schuler *et al*. 1994). The genetics of TKW is unknown, but it has been reported to be highly heritable with high-additive and multigenic effects (Joppa and Williams, 1988). In this study, 10 QTL were identified on six linkage groups (3A, 3B, 6A, 6B, 7A and LG8). This is in a good agreement with Elouafi and Nachit (2004) results, who detected two QTL with epistasic effect on 7AS and 6BS, explaining 30% of the total variation. In addition, they reported that the major TKW-QTL were around the centromere region of 6B.

QTL Related to Phenological Traits

QTL Associated with Root Length

Rooting depth is among the most important traits required to sustain plant function under low water availability conditions. The influence of root architecture on yield and other agronomic traits, especially under stress conditions, has been widely reported in all major crops (Tuberosa *et al*., 2002 and de Dorlodot *et al*., 2007). In this work, three QTL for root length trait were identified on three linkage groups (2B, 6B and LG8). In this respect, Bai *et al*. (2013) identified 43 QTL for root, shoot, and seed traits in bread wheat. Among the 43 QTL, only four QTL associated with root length trait are

located on four chromosomes (6A, 3B, 5B and 4D). While Kadam *et al*. (2012) identified only one QTL for root length (qMRL.4B.1) located on chromosome 4B. In addition, Sharma *et al*. (2011) identified 15 QTL (6 additive and 9 epistatic) for different traits of root length and root weight in 1RS wheat.

QTL Associated with Plant Height

Plant height is an extremely important target trait in modern wheat breeding. Since the ‘‘green revolution’’ in cereals was achieved by reducing plant height, and thus the lodging susceptibility and increase in grain yield. Plant height has shown considerable influence on kernel size in bread and durum wheat (Cuthbert *et al*., 2008). In the current study, 11 QTL for plant height were positioned on seven linkage groups (1B, 3A, 6A, 6B, LG2, LG4 and LG12). In this respect, Kadam *et al*. (2012) identified one major QTL for plant height (qPH.4B.1) on chromosome 4B. This QTL was located in the same marker interval “barc20-gwm368”under drought and control conditions. While, McIntyre *et al*. (2010) have identified a QTL for plant height on chromosome 4B with one of the flanking markers (barc20). This QTL corresponds to the *Rht1b*, a known gene for plant height in wheat (Cadalen *et al*., 1998).

QTL Associated with Spike Length

Ma *et al*. (2007) reported that spike length trait is positively related to number of spikelet per spike. Theoretically, gains in each of these yield components or traits could lead to potential increase in durum wheat yield. In addition, Ul-haq *et al*. (2010) demonstrated that spike length had positive correlation with grain yield. The present investigation identified 7 QTL for spike length located on 6 linkage groups (2B, 5B, 6A, 6B, LG2 and LG8). In this context, Ma *et al*. (2007) identified 10 QTL for SPL that mapped on 1A, 2D, 4A, 5A, 5B, and 7D. Whilst, Marza *et al*. (2006) identified ten QTL for spike length on eight chromosomes (1A, 1B, 2B, 3B, 4B, 5B, 7A and 7B).

QTL Related to Physiological Traits

QTL Associated with Fresh and Dry Weight

Increasing grain yield can be achieved by increasing either the total biomass produced by the crop (bigger plants tend to produce greater yield) or the proportion of the total biomass that is invested in grains (greater harvest index). Thus, a gene that increases yield should do so through one of these two fundamental mechanisms. In this work, 4 QTL for fresh weight and 5 QTL for dry weight were co-localized on the same chromosomal positions on (2B, 3A, 7A and LG2). These results are in partaly agreement with the results of Quarrie *et al*. (2006) who found that QTL for FW and DW were grouped in two regions of 7A: on the short arm co-localizing with the distal QTL cluster for yield around locus Xpsr558, and highly significantly on 7AL co-localizing with the yield QTL cluster, with increasing alleles coming from SQ1.

QTL Associated with Total Amino Acids

The contents of protein and amino acids are the major factors of nutritional quality, and their regulation has increasingly become a major breeding objective. Wheat protein quality is mainly influenced by protein content and the balance of amino acid composition in the wheat (Liu *et al*., 2002 and Li and Zhang, 2000). Several studies on QTL mapping for amino acid contents in rice (Tang, 2007 and Zheng *et al*., 2008) and soybean (Panthee *et al*., 2006) are available. Wang *et al*. (2008) identified 18 chromosomal regions controlling the AAC in rice by using 190 recombinant inbred lines. However, no studies were found for QTL mapping for amino acid contents of bread or durum wheat. Therefore, the present study successfully dissected the genetic basis of total amino acid contents in durum wheat. Five QTL for total amino acids were identified on three linkage groups (3A, 6B and LG4). To our knowledge, this represents the first report of QTL mapping for TAA in Egyptian durum wheat.

Correlation between Traits

Traits that are correlated, as in the case of yield and yield components, are likely to have QTL mapping to similar locations. Common genomic regions for yield and yield components have also been reported in previous studies, *i.e.* co-locations of QTL related to yield and QTL for grain weight (Marza *et al*., 2006) and grain number (Kirigwi *et al*., 2007). The co-location of QTL associated with different traits can be the result of: (1) Two strongly linked genes affecting different traits, (2) One single gene that produces a series of effects in related traits, (3) One gene affecting two or more independent traits, (4) Two linked genes with effects in the same traits (Yang *et al*., 2007). The results obtained from previous studies are in good correspondence with the QTL results obtained from this work as some genomic regions were occupied by overlapped QTL on linkage groups 2, 4, 6, 7, 8, 9, 13 and 14. The linkage groups 6, 7 and 8 showed the most overlapped traits. For example, QTL for spike length, number of kernel/spike, thousand kernel weight and total amino acids were mapped to the same chromosomal location (Marker T-6) on chromosome (6B) at position 123.8cM. This correlation between traits can be interpreted according to their physiological effects to determine their relevance to yield improvement under drought stress.

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