

## Identification of Chromosomal Regions and Genetic Contributions of Genes Controlling Yield and Other Agronomic Traits in Durum Wheat Grown under Different Egyptian Environmental Conditions

<sup>1</sup>Ayman A. Diab, <sup>1</sup>Ashraf H. Fahmy, <sup>1</sup>Osama S. Hassan, <sup>2</sup>M.M. Nachit and <sup>1</sup>Osama A. Momtaz

<sup>1</sup>Agricultural Genetic Engineering Research Institute (AGERI),  
Agricultural Research Center (ARC), 9 Gamaa St., Giza, Egypt

<sup>2</sup>ICARDA, CIMMYT/ICARDA, Durum Improvement Program, P.O. Box 5466, Aleppo, Syria

**Abstract:** A better understanding of the genetics of complex traits, such as yield, may be achieved by using molecular tools. Molecular markers provide a rapid approach to breeding for desired agronomic traits. To use them, it is necessary to determine the linkage between quantitative trait loci (QTLs) and such markers. This study was conducted to estimate the number and effect of alleles and the chromosomal locations of QTLs responsible for yield and some agronomic traits in durum wheat. A recombinant inbred population derived from a cross between two durum (*Triticum turgidum* L. var durum) parents Jennah Khetifa and Cham1 was characterized for molecular markers and traits measured in different Egyptian environments. The environmental and genotypic effects on the measured traits were determined. Single point analysis (using Qgene) and composite interval mapping (using QTL cartographer) were used to identify the genomic regions controlling traits related to yield and other agronomic traits with a threshold of LOD 4. Analysis of QTLs has revealed the approximate location of the significant markers including genes and differentially expressed sequence tags (dESTs) associated with 10 traits related to yield and other agronomic traits across the A and B genomes. One hundred and seventy five markers, including 3 candidate genes and 14 dESTs were associated with QTL for traits related to yield and other agronomic traits either across environments or in individual environment. Significant QTL were identified for biomass, days to heading, days to maturity, glume color, Kernel per spike, kernel weight, lodging, spike per meter square, spikelets per spike and yield. The overall results show that there is a considerable potential for improving durum wheat yield by marker-assisted selection technology.

**Key words:** Durum wheat • yield • agronomic traits • QTL analysis • genetic linkage mapping • dESTs • RFLP • marker-assisted selection

### INTRODUCTION

Crop improvement begins with the selection of parental lines having the desired traits that will meet the objective of the breeder. This is followed by making crosses between the parents to generate a segregating population. Selection of progenies showing the desired traits then commences and continues as the population is advanced from one generation to the next. For simply inherited traits controlled by single genes with major effects, the selection process can be made by traditional breeding methods. However, plant breeders are often confronted with problems when trying to improve a trait

that is controlled by many genes through traditional breeding methods. In durum wheat breeding, most agronomic and yield traits are quantitative traits controlled by many genes each of which has a relatively small effect on the overall phenotype. Quantitative traits are difficult to study because the phenotypes do not give an insight into the genotype. The expression of genes controlling quantitative traits can be greatly influenced by the environment [1]. Consequently, the improvement of polygenic traits by traditional breeding methods is time consuming and the gains are harder to realize. Breeders usually overcome this problem by multi-environmental evaluation of replicated trials to capture the effect of the environment [2].

With the advent of molecular markers, it has become possible to analyze in detail the genetic basis for complex polygenic traits [3]. Because quantitative traits are strongly influenced by environmental factors, deducing their genetic basis usually requires comparing mean trait values in different environments. QTL analysis is a useful approach to discover and dissect complex traits and to identify favorable alleles in diverse germplasm [4]. Systematic studies on QTLs mapping have been conducted in a number of crop species [4-7].

Grain yield in cereals is generally controlled by a number of QTLs and is affected by environmental factors, making it difficult to manipulate and improve breeding programs. Grain yield can be dissected into a number of component traits such as kernel per spike, kernel weight, spike per meter square and spikelets per spike. These component traits are also under QTLs control and the effects of individual QTLs on phenotypic variation are relatively small [8]. Some of them, however, are less environmentally sensitive and have higher heritabilities than grain yield itself [8, 9]. Therefore, while looking for QTLs controlling grain yield, QTLs for yield components should also be determined to provide more useful information.

It is possible to estimate the number of loci controlling genetic variation in a segregating population, using linkage genetic maps and to characterize these loci with regard to map position, gene action, phenotypic effects, pleiotropic effects and epistatic interactions with other QTLs [10].

Durum wheat (*Triticum turgidum* L. var *durum*) is an allotetraploid (genome AABB,  $2n = 4X = 28$ ) with seven homoeologous groups. It is from the staple component of the diets of millions of people and known for its uses in pasta on an international scale; however, it is also an important food for rural communities, forming the basis of couscous, burghul, frike (roasted green grains) and home-baked flat-bread. Durum is the hardest and highest protein wheat grown in the United States and Mediterranean region [11].

The objective of this study was to identify chromosomal locations and genetic contributions of genes controlling yield and some agronomic traits in durum wheat. In this context, previously constructed genetic linkage map containing 468 markers [12] was used to detect QTLs affecting traits related to yield and some other agronomic variation. The results revealed

associations between markers and QTLs under different Egyptian environmental conditions. Locating parts of the genome control QTLs for yield and other agronomic traits in durum wheat promises to enhance durum wheat breeding programs and produce markers and genomes for the strategic improvement by marker-assisted breeding.

## MATERIALS AND METHODS

**Plant materials:** A segregating durum population of 110 F9 Recombinant Inbred Lines (RILs) derived by single-seed descent from the cross ICD-MN91-0012 between Tamgurt (Jennah Khetifa) and Cham 1 was used in this study. The CIMMYT/ICARADA durum-breeding program for Mediterranean dryland has developed the population in 1991 at the Tel Hadya research station (Aleppo province, Syria). Jennah khetifa is a landrace, grown in the dry areas of Algeria and Tunisia that shows specific adaptation to North African continental dryland. Cham 1 is grown for commercial production in several countries of the Mediterranean basin.

**Genetic map:** The map used in this study, previously constructed with data obtained from the RILs, including the data of Nachit *et al.* [13] consisted of 468 loci covering 5672.8 cM [12]. The map consisted of 191 RFLPs, 27 SSRs, 199 AFLPs, 5 SSPs, 9 genes and 37 ESTs (Table 1).

**Experimental design:** The 110 RILs and the two parents were grown in two environments; Sids and New Valley for two successive seasons. New Valley is a research station at southwest Egypt elevated from the sea by 78 meter with annual rain fall of 0.8 mm and the humidity is 31%. Sids is one of the experimental stations for Agricultural Research Center located at the Nile valley elevated from the sea by 22 meter with annual rain fall of 11 mm and 21 % humidity. The field layout was a randomized complete block design with two replications in each environment. The data of the following traits were measured as described by Nachit *et al.* [14]: biomass (Bmass), days to heading (DH), days to maturity (DM), kernel per spike (K/S), kernel weight (KW), lodging (Lod), spike per meter square ( $S\ m^{-2}$ ), spikelet per spike (S/S), Glume color (GC) and yield. The 110 RILs were divided over 6 blocks where 19 test RILs were included in each block, with 5 well-known durum

Table 1: Distribution of molecular markers, assignment and centiMorgan (cM) coverage across the 14 durum A and B genome of the genetic map used in QTL mapping

Chrom-osome	RFLPs	SSRs	AFLPs	SSPs	Genes	ESTs	Markers		cM	cM/Marker
							#	%		
1A	14	2	15	0	0	0	31	6.6	339.7	10.9
1B	26	1	16	3	1	3	50	10.7	448.2	8.9
2A	7	4	5	0	2	5	23	5.0	268.4	11.6
2B	16	2	16	0	1	0	35	7.5	466.1	13.3
3A	12	2	10	0	0	0	24	5.1	305.5	12.7
3B	17	4	19	0	0	1	41	8.8	422.7	10.3
4A	26	2	17	0	0	0	45	9.6	559.5	12.4
4B	8	2	12	0	1	8	31	6.6	484.6	15.6
5A	11	3	3	0	0	1	18	3.8	225.4	12.5
5B	8	1	12	0	1	4	26	5.6	368.7	14.1
6A	14	1	14	1	1	2	33	7.0	494.2	14.9
6B	15	1	13	1	2	3	35	7.5	509.3	14.5
7A	9	2	20	0	0	0	31	6.6	327.5	10.5
7B	8	0	27	0	0	10	45	9.6	453	10.0
Total	191	27	199	5	9	37	468	100	5672.8	12.1

Table 2: Analysis of variance for the 10 traits showing phenotypic means and standard deviation obtained from each environment. Sids: Sids/Egypt; NV: New Valley/Egypt. The environment and genotypic effects are indicated

Trait	Mean				Standard deviation				Environment Effect (F value)	Genotype Effect (F value)
	Sids 98	Sids 99	NV 98	NV 99	Sids 98	Sids 99	NV 98	NV 99		
Biomass	3.5	5.0	6.6	6.1	1.17	0.68	0.87	4.8	34.05***	1.22 <sup>ns</sup>
Days to heading	106.1	101.2	85.8	86.6	7.8	5.3	9.8	6.8	271.06***	7.75***
Days to maturity	148.3	144.4	136.1	136.3	5.7	4.9	6.2	49	6.70**	1.12 <sup>ns</sup>
Glume color	1.39	1.36	1.43	1.42	0.54	0.71	0.57	0.56	1.16 <sup>ns</sup>	47.46***
Kernel per spike	52.8	47.5	54.6	35.6	6.8	7.5	175.6	5.66	4.03**	1.00 <sup>ns</sup>
Kernel weight	49.8	50.5	54.8	47.6	6.1	6.7	6.4	4.8	55.84***	3.06***
Lodging	27.6	50.8	54.1	49.8	22.4	29.4	30.7	32.1	43.32***	6.01***
Spike per meter <sup>2</sup>	396.8	348.3	272.1	251.8	43.8	55.4	24.8	28.4	496.52***	1.78***
Spikelet per spike	21.2	20.4	18.1	17.3	1.88	1.67	1.88	1.69	171.08***	2.70***
Yield	1.06	2.5	1.5	1.3	0.33	0.53	0.35	0.55	386.02***	3.28***

\*\* , \*\*\* Significant at P<0.01, 0.001 respectively or non significant (ns)

genotypes as checks (Omrabi5, Haurani, Korifla, Cham1 and Gidara 2). The field design used was the augmented design [15]. The total number of entries of the whole trial was 142 (including 110 RILs, the 2 parents and the 5 checks repeated in each block).

**Statistical method:** Analysis of variance (ANOVA) was performed using SAS program (SAS Institute 1987, Cary N.C., USA) to determine the significance of variation among the RILs for all the traits measured in each environment. The environment and genotypic effects were also calculated (Table 2).

**QTL analysis:** Genotypic data obtained from the previous study [12, 13] were combined with the phenotypic data measured in this study to identify putative QTLs for biomass (Bmass), days to heading (DH), days to maturity (DM), kernel per spike (K/S), kernel weight (KW), lodging (Lod), spike per meter square (S/m<sup>2</sup>), spikelet per spike (S/S), Glume Color (GC) and yield. Two analytical approaches were employed to identify and validate putative QTLs. First, interval analysis using Qgene program [16] was employed to identify marker intervals on the durum chromosomes that contained QTLs. To identify the appropriate threshold LOD score for declaring a QTL,

given the population size and the number of markers used, a permutation test was first conducted. The putative QTLs were declared significant when the LOD score was  $\geq 4.0$ . The second approach used for QTL identification was the Composite Interval Mapping (CIM) analysis [17] using QTL Cartographer program [18]. This method is a multiple regression procedure adjusting for background effects of markers (co-factors) other than those in the interval being tested. A 1000 permutation test was performed to estimate appropriate significant threshold LOD scores for CIM. A LOD threshold level of 4.0 was used.

## RESULTS

**Trait variation within and across environments:** The trait values for each environment are presented in Table 2. Considering the two environments and two seasons, a significant genotypic effect with significance of ( $P < 0.001$ ) was obtained for days to heading (DH), Glume Color (GC), Kernels Weight (KW), lodging (Lod), spike/square meter (S/M), spikelets/spike (S1/S) and grain yield. However, no significant genotypic effect was observed for biomass (Bmass), days to maturity (DM) and kernel/spike (K/S). Meanwhile, a significant environmental

Table 3: The most significant QTLs detected by the composite interval mapping. A QTL was included in this table if it was associated with the trait with a LOD score  $> 4$  and R2 value  $> 0.05$

Trait	Environment	Chrom-osome	Marker	Position	LOD score	Additive	R2
Biomass	Sids 98	5B	PaggMctg19	276.45	4.3605	0.3058	0.053
		7B	PaagMcga7	391.28	6.0812	0.4319	0.0725
	NV 98	7B	PacgMctg7	446.11	5.5212	-0.3679	0.063
		1B	BM816287a	4.01	4.3873	0.2401	0.0505
		1B	bcd265	426.57	8.3374	-0.2912	0.0879
		2B	bcd718b	414.57	5.05	-0.2491	0.0639
	Sids 99	7A	PaggMcgg13	20.01	4.8664	0.221	0.0512
		5B	PaggMctg19	276.45	7.9998	0.2473	0.1081
		5B	PacgMcgc2	278.96	6.6722	0.2402	0.1019
	NV 99	7B	PacgMcgg6	423.99	4.1888	-0.2105	0.0606
		4A	utv1136a	54.51	4.1427	1.6922	0.0566
		6A	PaccMcga8	114.07	4.9449	1.6392	0.0714
		7A	PaagMcgc3	63.05	4.0114	1.2708	0.0577
		7B	PacgMctg7	452.11	5.4841	-1.8419	0.1036
Days to heading	Sids 98	2A	BM816609	10.01	5.1846	-2.2154	0.0506
		2B	cdo365a	193.62	6.6448	-2.4781	0.0613
		4B	PaggMcag5	169.31	7.1171	2.6176	0.0611
		4B	PaggMcgt5	399.08	7.6862	-2.159	0.0656
	NV 98	2B	cdo365a	185.62	7.6427	-3.6101	0.1027
		6A	PaggMctg9	309.45	4.7448	-3.0882	0.0666
		7B	PaccMcgt8	420.52	6.1644	3.4466	0.0699
	Sids 99	1B	PaagMcac1	46.48	4.4266	-1.5842	0.0693
		1B	PaagMcac4	48.76	5.2426	-1.7435	0.0769
		3B	PaggMctg1	36.7	6.7642	-1.9804	0.0967
		5B	PacgMcgg7	14.01	4.2375	1.7324	0.0699
		6A	cdo786a	368.48	5.0835	2.0429	0.0667
	NV 99	3A	utv920	172.26	5.0978	1.9298	0.0603
		5A	cdo57	18.64	4.6034	1.9795	0.0596
Days to maturity	Sids 98	1B	uaz243	314.86	6.639	-1.8843	0.0868
		7B	BM816624	214.62	6.7337	1.8877	0.0768
		7B	BM816648	227.21	10.9194	2.3984	0.118
		7B	BM817327b	232.97	7.7462	2.0337	0.0868
		7B	HC105A03	237.35	4.0603	1.473	0.0514
	NV 98	6B	PaggMcgg2	272.79	6.278	1.8345	0.0721
		6B	BM816608	328	4.4282	-1.5539	0.0518
	Sids 99	1B	bcd265	428.57	7.8831	1.8734	0.1122
		3A	bcd22	92.39	5.3612	1.4422	0.0694
		3A	gwms155	128.37	6.3941	-1.8362	0.1047

Table 3: Continue

Trait	Environment	Chrom-osome	Marker	Position	LOD score	Additive	R2
Days to maturity	NV 99	2B	PaggMcgt2	78.12	7.3791	22.0914	0.1017
		2B	gwms148	114.69	5.0364	-16.4166	0.076
		4A	PaggMcgt10	110.61	5.0912	15.533	0.0614
		4A	PaccMcat10	127.89	4.536	-22.3252	0.1026
		4B	PaggMcag9	234.91	10.3988	25.9356	0.1666
		7B	utv507b	342.23	4.7208	13.6534	0.0619
		7B	utv507a	358.27	6.0361	16.185	0.0794
Glume color	Sids 98	1A	gwms136	173.25	6.9403	0.1592	0.0727
		5B	LoX11-1	162.98	9.3611	0.2076	0.1024
		5B	bcd450	177.36	5.5942	0.1798	0.0663
		5B	PacgMcag5	302.81	6.4984	0.1951	0.0727
		6A	Gli-A2	164.85	5.5298	0.1599	0.0587
		7B	wg380	322.84	4.1057	-0.152	0.0581
		7B	PaagMcga12	333.34	7.6496	-0.1947	0.0919
	NV 98	7B	utv507b	350.23	4.1113	-0.1503	0.0531
		1A	gwms136	173.25	7.2094	0.1821	0.0917
		7A	gwms332a	72.65	4.1052	0.1498	0.05
		7B	PaagMcga12	333.34	4.5886	-0.1618	0.0612
		7B	utv507b	342.23	5.5969	-0.1758	0.0732
		7B	PacgMctg7	452.11	5.5531	0.1734	0.0669
		Sids 99	4A	mwg634c	413.8	5.6258	-0.1762
	NV 99	1A	gwms136	177.25	5.2728	0.1383	0.0539
		3A	utv920	172.26	5.5689	0.1535	0.0523
		4A	opf(RWA)14	558.26	4.443	-0.1554	0.0518
5B		cdo457a	247.57	5.5951	0.1551	0.0627	
7B		utv507b	342.23	7.5785	-0.2105	0.0968	
7B		PacgMctg7	452.11	5.0401	0.1634	0.0537	
Kernel per spike	Sids 98	1B	bcd200	168.62	9.7707	3.45	0.1522
		1B	bcd1495	179.18	13.4382	4.0104	0.2039
		2A	cdo346	125.05	6.5132	-2.2253	0.0738
		7B	HC105A03	237.35	5.474	2.1439	0.0623
		7B	PaagMctg4	367.8	8.2001	-3.0072	0.104
	NV 98	1B	PaagMcac1	48.48	5.1858	49.9531	0.0637
		3A	PaggMctg3	12.79	9.1175	74.2556	0.121
		3A	cdo534	141.88	8.2819	-92.4643	0.156
		3A	utv920	190.26	5.3712	58.389	0.0649
		5B	PaggMcgt8	344.74	4.7369	44.7664	0.051
	Sids 99	1B	utv111c	87.3	4.1041	2.0485	0.051
		1B	utv1181b	97.43	5.9584	2.2836	0.0715
		7B	BM816953	233.99	4.5643	1.9501	0.0531
7B		BM816620	238.3	4.3961	1.8621	0.0514	
Kernel per spike	NV 99	1A	utv1441e	259.3	8.3676	-2.1999	0.132
		3B	bcd195a	116.68	8.6794	-2.1646	0.1204
		3B	bcd115	135.85	4.1827	-1.8601	0.0688
		3B	gwms247	332.57	5.4637	1.7322	0.0701
		4B	bcd327	417.68	6.1507	-1.8677	0.0778

Table 3: Continue

Trait	Environment	Chrom-osome	Marker	Position	LOD score	Additive	R2
Kernel weight	Sids 98	1A	PaagMcac9	30.58	4.3722	-1.9448	0.0514
		5B	PaagMctg6	89.76	5.7994	1.9417	0.073
		5B	PacgMcgc2	286.96	8.4116	2.4132	0.1275
		6B	PaggMctg8	20.68	4.0045	-1.7073	0.0614
		6B	PacgMctg3	36.72	4.0112	-1.691	0.0631
		6B	utv1469a	314.61	7.8717	-2.2458	0.1032
	NV 98	2A	BM816257	102.47	4.3047	2.4192	0.0696
		2B	PaccMctc6	46.64	5.5198	2.2199	0.073
		5B	PaccMcgt3	215.12	4.0885	1.6647	0.0533
		6B	bcd279a	492.49	6.4083	-2.0304	0.0753
		7B	utv507b	350.23	4.938	-1.8367	0.0647
	Sids 99	1A	wg241	274.61	4.156	1.7589	0.0514
		4A	utv434b	398.72	4.6835	-2.1678	0.0578
		5A	PaccMcat6	128.28	5.7594	1.9716	0.0698
		6A	bcd21	387.01	5.958	-2.0552	0.0778
		7A	PacgMcgg10	71.87	6.4247	2.2527	0.083
	NV 99	3A	utv920	176.26	5.5838	-1.6703	0.0759
		6A	PaccMcga8	114.07	5.5596	1.6591	0.0674
		7B	bcd87	49.61	5.3005	1.6538	0.0838
Lodging	Sids 98	1B	cdo1173	161.69	9.2441	-12.5938	0.1437
		2B	cdo36d	268.34	4.5634	7.5364	0.0719
		4A	bcd348d	183.67	9.248	9.5531	0.1446
		4A	mwg634c	413.8	5.8613	-6.8338	0.0774
		4A	mwg634b	418.65	5.6423	-6.8047	0.0748
	NV 98	5B	PaagMcag5	238.29	4.1183	-5.5185	0.0534
		2B	utv873	287.8	5.1777	-7.8342	0.0521
		4B	PaggMcgt5	405.08	6.9716	9.9379	0.0885
		4B	cdo949	407.67	8.9838	11.2213	0.1047
		4B	bcd327	417.68	4.514	8.6481	0.0596
		6B	PaggMcat5	279.39	5.129	-7.8793	0.0538
	Sids 99	7B	PaccMcgt8	422.52	4.6615	-10.4421	0.0536
		7B	PacgMctg7	452.11	5.0001	9.7977	0.0534
		2B	utv873	287.8	7.856	-9.3973	0.0803
		3B	PaggMctg16	58.74	5.0854	-7.6756	0.0536
Lodging	NV 99	5A	utv624	223.59	4.6806	8.9372	0.0681
		7B	PacgMctg8	72.25	6.2261	-9.2557	0.0578
		3B	PaccMcga6	64.49	6.3844	-10.8786	0.0837
		4B	bcd221b	421.19	4.4553	8.7721	0.0588
Spike per meter square	Sids 98	5A	gwms129b	12.18	5.4593	8.342	0.0504
		7B	PacgMctg8	72.25	8.9934	-12.5192	0.087
		3A	bcd22	92.39	8.2495	14.1439	0.0795
	NV 98	3B	utv1371a	342.54	5.8267	-12.1238	0.056
		4A	utv434c	94.7	6.9591	-12.4974	0.0685
		1A	PaagMctg2	200.1	6.5914	7.702	0.0773
	Sids 98	6A	PaccMcat13	435.95	6.423	-8.8196	0.1051
		7A	PaccMcag9	323.22	5.2628	12.2292	0.0827
		1B	cdo1373b	114.07	7.9301	19.597	0.083
		1B	cdo1373a	249.87	5.8097	-15.8714	0.0611

Table 3: Continue

Trait	Environment	Chrom-osome	Marker	Position	LOD score	Additive	R2
	NV 99	3A	utv920	172.26	11.2101	-22.5841	0.1173
		1B	bcd304	308.12	5.2565	8.2058	0.0689
		1B	uaz243	312.86	4.9058	7.9267	0.0665
		2B	utv873	299.8	4.7464	8.3012	0.0647
		6B	PaggMctg4	402.27	5.6687	-9.898	0.0844
spikelet per Spike	Sids 98	1B	bcd386	187.95	5.7746	0.5422	0.0633
		2A	PaagMcag4	187.05	5.6788	-0.634	0.0742
		4B	PaggMcag5	169.31	4.251	0.5457	0.0502
		5A	gwms126	170.79	5.7132	0.5392	0.0616
	NV 98	1A	utv1699d	62.51	4.0947	-0.5279	0.0547
		2B	PacgMcgc4	352.62	5.34	-0.5624	0.0595
		4A	cdo1312d	302.96	10.0159	0.9648	0.1293
		6A	PaagMctg1	263.16	4.4877	-0.482	0.051
		6B	cdo665c	411.83	7.0907	0.684	0.0842
		2A	gwms47a	34.79	5.0574	0.4321	0.0548
	Sids 99	3B	cdo328	111.04	4.3864	-0.4259	0.0534
		3B	bcd195a	116.68	7.2174	-0.5895	0.083
		3B	PacgMcgc1	399.85	4.7399	0.4912	0.0696
		4B	BM816848	264.46	4.0813	-0.5858	0.0516
		7B	BM816953	233.99	5.2148	0.4631	0.056
		7B	BM816286	239.73	4.3933	0.4364	0.051
		7B	PacgMcgg9	331.65	5.2748	-0.4814	0.062
		7B	PacgMcgc9	331.65	5.2748	-0.4814	0.062
	NV 99	4A	cdo1312d	302.96	4.3645	0.603	0.0533
		6A	PaccMcag4	261.14	4.3118	-0.4679	0.0521
Yield	Sids 98	3B	utv560b	233.57	7.1176	-0.106	0.0749
		5B	BM816242	144.95	4.617	0.1004	0.0539
		5B	cdo457a	261.57	9.472	0.1495	0.1329
		5B	PaccMcat7	264.45	9.4261	0.1577	0.1393
		6A	cdo962	143.87	5.8744	-0.1066	0.0696
		6B	PaagMcga9	155.46	4.9277	0.0977	0.0553
	NV 98	6B	cdo365b	167.51	5.3166	-0.1089	0.0639
		2A	ksud23	134.17	4.9753	0.1498	0.0735
		2A	utv861b	149.29	5.074	-0.1368	0.0778
		4B	LoXmj1	169.88	9.1018	-0.1901	0.1335
		6B	PaggMcgt14	466.16	6.0308	0.1186	0.0797
	Sids 99	1B	cdo1373a	249.87	4.8323	0.1547	0.0601
		5B	PaccMcat7	276.45	4.0112	0.1528	0.0541
	NV 99	7A	PaagMcag1	162.53	6.2903	0.1732	0.0831
		1B	M96856	209.97	6.6648	0.1905	0.0943
		3B	gwms340	328.1	8.365	0.1572	0.1
		7B	BM816571	248.97	5.5785	0.1482	0.0709
7B		PaagMcgc11	291.25	4.3974	-0.1185	0.0546	
		7B	PacgMctg7	452.11	4.0529	-0.1064	0.0504

effect of  $P < 0.01$  was obtained for days to maturity (DM) and kernel/spike (K/S) and  $P < 0.001$  for Biomass (B-mass), days to heading (DH), kernels weight (KW), lodging (Lod), spike/square meter (S/M), spikelets/spike (S1/S) and grain yield. However, Glume Color (GC) did not show a significant environmental effect.

All the traits showed normal distribution and the progeny showed large variation for most of the traits within each environment. For example, biomass values

ranged from 1.15 to 6.24 in Sids 98 and from 2.7 to 6.7 in New Valley. There were also significant differences between environments for all traits except Glume Color (GC) and the trait means presented large differences among environments.

**Identification of QTLs:** The QTL parameters presented in this paper are those generated by QTL cartographer and include the genomic map position of each LOD score peak

Table 4: Differentially expressed sequence tags (dESTs) and candidate genes co-segregating with yield and other agronomic traits in different Egyptian environments

Locus	Gene product	Chr	Associated trait(s)	Environment
BM816287a	Putative protein, Arabidopsis	1B	Biomass	NV 98
M96856	Protein associated with G-Box binding complex	1B	yield	NV 99
BM816609	Aluminum induced protein	2A	Days to heading	Sids 98
BM816257	Actin depolymerizing factor 4	2A	Kernel weight	NV 98
Loxnjt	Lipoxygenase	4B	Yield	NV 98
BM816848	Hypothetical protein	4B	Spikelet per spike	Sids 99
BM816242	Glutathione S-transferase	5B	Yield	Sids 98
Lox11-1	Lipoxygenase	5B	Glume color	Sids 98
BM816608	Glutathione oxidase	6B	Days to maturity	NV 98
BM816624	Glycine dehydrogenase	7B	Days to maturity	Sids 98
BM816648	Arginine 2-monooxygenase	7B	Days to maturity	Sids 98
BM817327b	alpha-glucanotransferase	7B	Days to maturity	Sids 98
HC105A03	No annotation	7B	Days to maturity	Sids 98
BM816953	Nitrilase-like protein	7B	Kernel per spike Spikelet per spike	Sids 99 Sids 99
BM816620	Glycine dehydrogenase	7B	Kernel per spike	Sids 99
BM816286	hypothetical protein	7B	Spikelet per spike	Sids 99
BM816571	Metallothioneine	7B	Yield	NV99

(the QTL), the additive effect on the trait, percentage of the trait variation ( $R^2$ ) explained by the QTL conditioned on the background markers (Table 3). A total of 175 QTLs with LOD score  $\geq 4$  were identified for the 10 traits evaluated in this study. Fourteen QTLs were identified for biomass, 14 for days to heading, 17 for days to maturity, 20 for glume color, 19 for kernel per spike, 19 for kernel weight, 21 for lodging, 13 for spike per meter square, 19 for spikelets per spike, 19 for yield. The number of QTLs identified for each trait varied from 13 to 22 with the phenotypic variation ( $R^2$ ) ranging from 0.05 to 0.2. The highest LOD peak (13.4) of the study was obtained for Kernel per spike on chromosome 1B at Sids 98. QTLs for the measured traits were identified in all environments. For these QTLs, change was observed in the direction of the allele effect across environments.

**Candidate genes and differentially expressed sequence tags (dESTs)**

**co-segregating with traits:** Comparison of genome locations for QTLs of the traits with candidate genes and differentially expressed sequence tags (dESTs) showed coincidences on chromosomes 1B, 2A, 4B, 5A, 6B and 7B (Fig. 1 and Table 4). For the NV 98 environment, 1 gene and 3 dESTs were found to be associated with QTLs for biomass, kernel weight, yield and days to maturity. In the NV 99 environment, 2 QTLs for yield was associated with one candidate gene and one dEST. One candidate

genes and 6 dESTs were associated with QTLs for days to heading, yield, glume color and days to maturity, while 3 EST was associated with spikelet per spike and kernel per spike at Sids 99 environment (Fig. 1 and Table 4).

**Correlation between traits:** Since most of the traits studied in this investigation are related, it is of interest to examine the genetic relationships between them. Some genomic regions were found where QTLs for different traits overlapped. For example, QTLs for days to heading, glume color, kernel per spike, kernel weight and spike per meter square were mapped to approximately the same chromosomal location. Similarly, QTLs for days to maturity and biomass were mapped to identical genomic region and so also were the QTLs for yield and glume color. Also QTLs for kernel per spike and days to maturity were often mapped to the same regions. The loci *utv920* on chromosome 3A and *PaccMctc2* on chromosome 7B showed the most overlapped traits where QTL for days to heading, glume color, kernel per spike, kernel weight and spike per meter square were overlapped at the locus *utv920* and QTLs for biomass glume color and lodging were overlapped at the locus *PaccMctc2* (Fig. 1).

**Consistent QTL in different environment:** Some traits were shown to be consistent and overlapped at the same genomic region in different environments. For example,



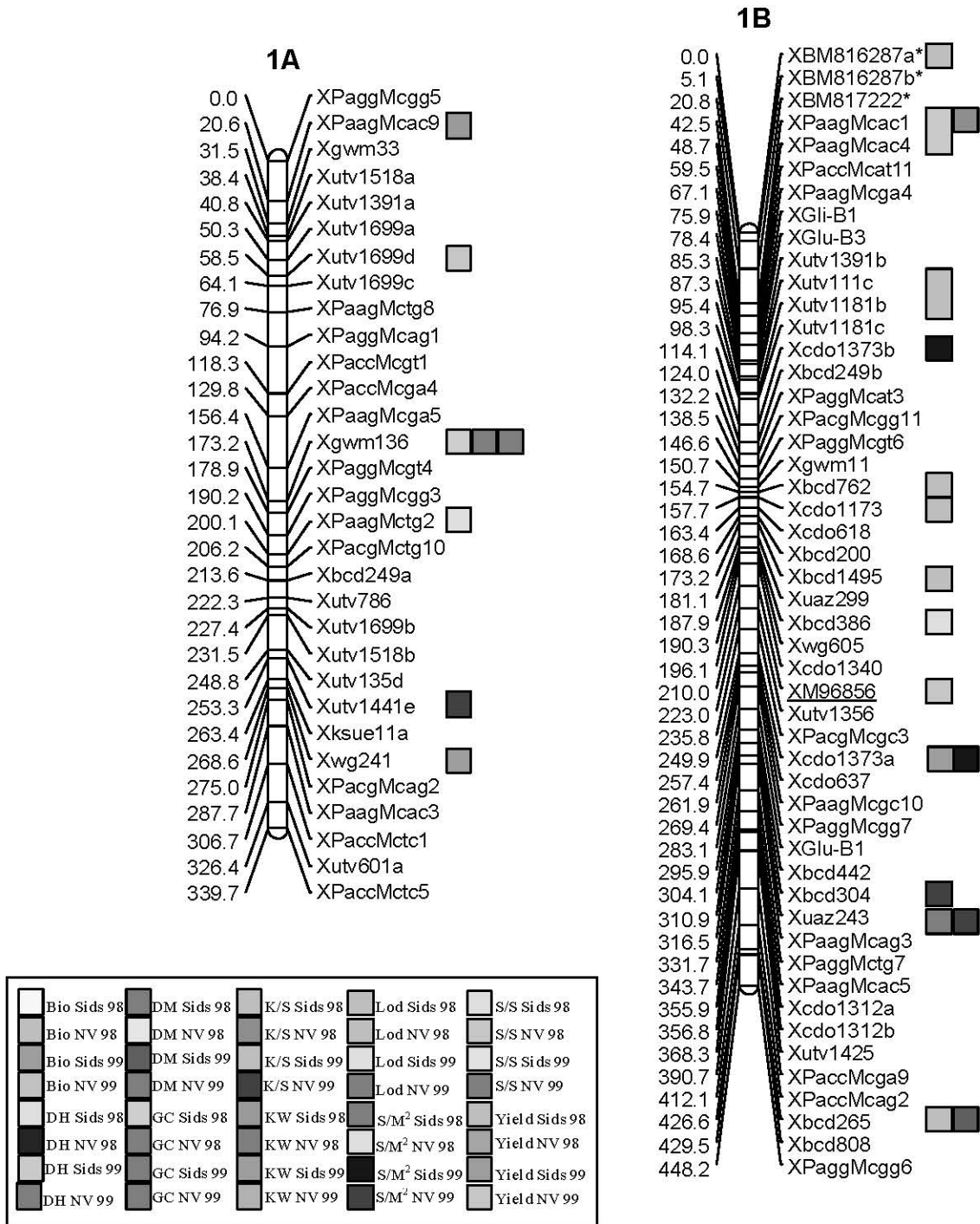


Fig. 1: Continued

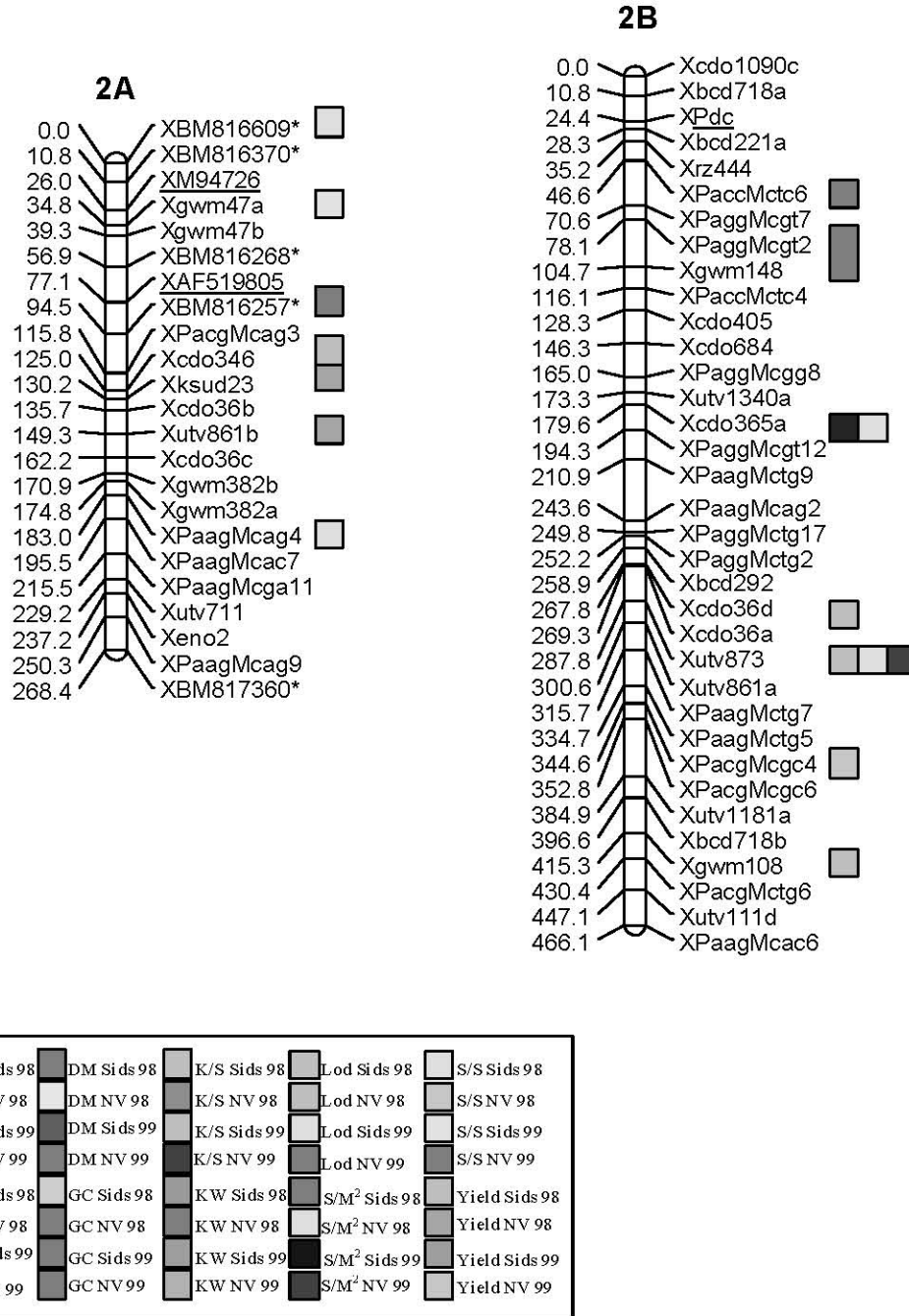


Fig. 1: Continued

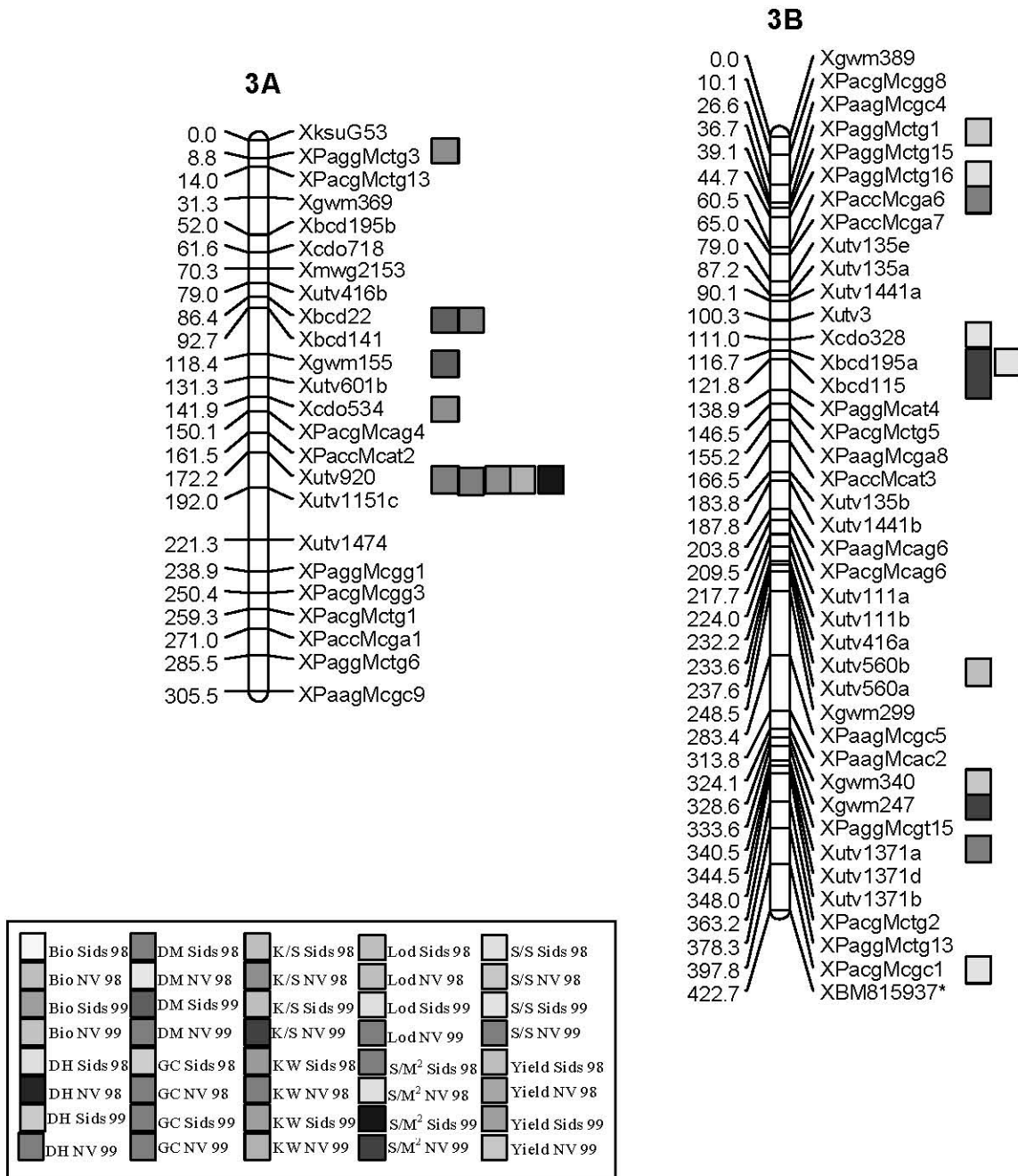


Fig. 1: Continued

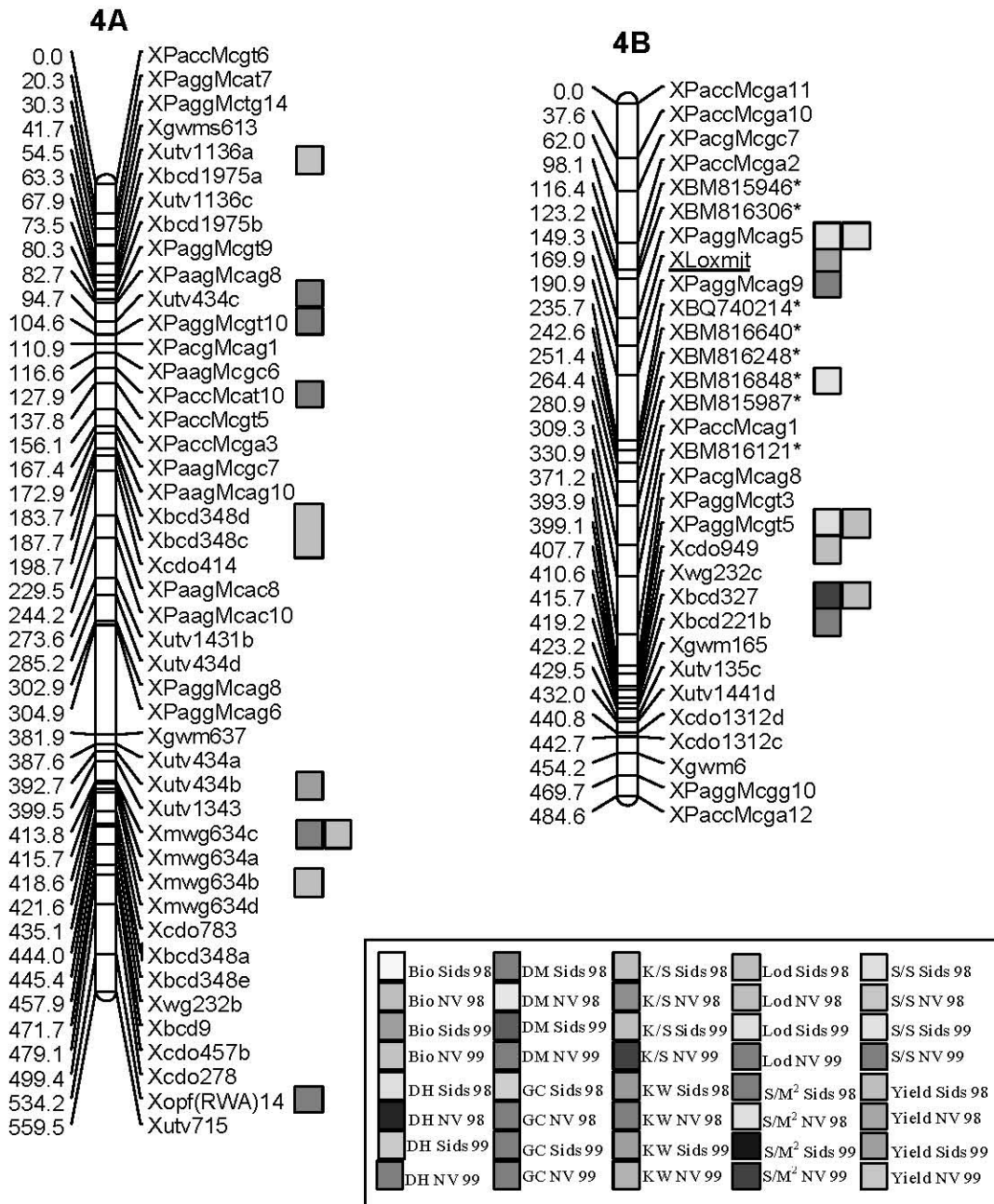


Fig. 1: Continued

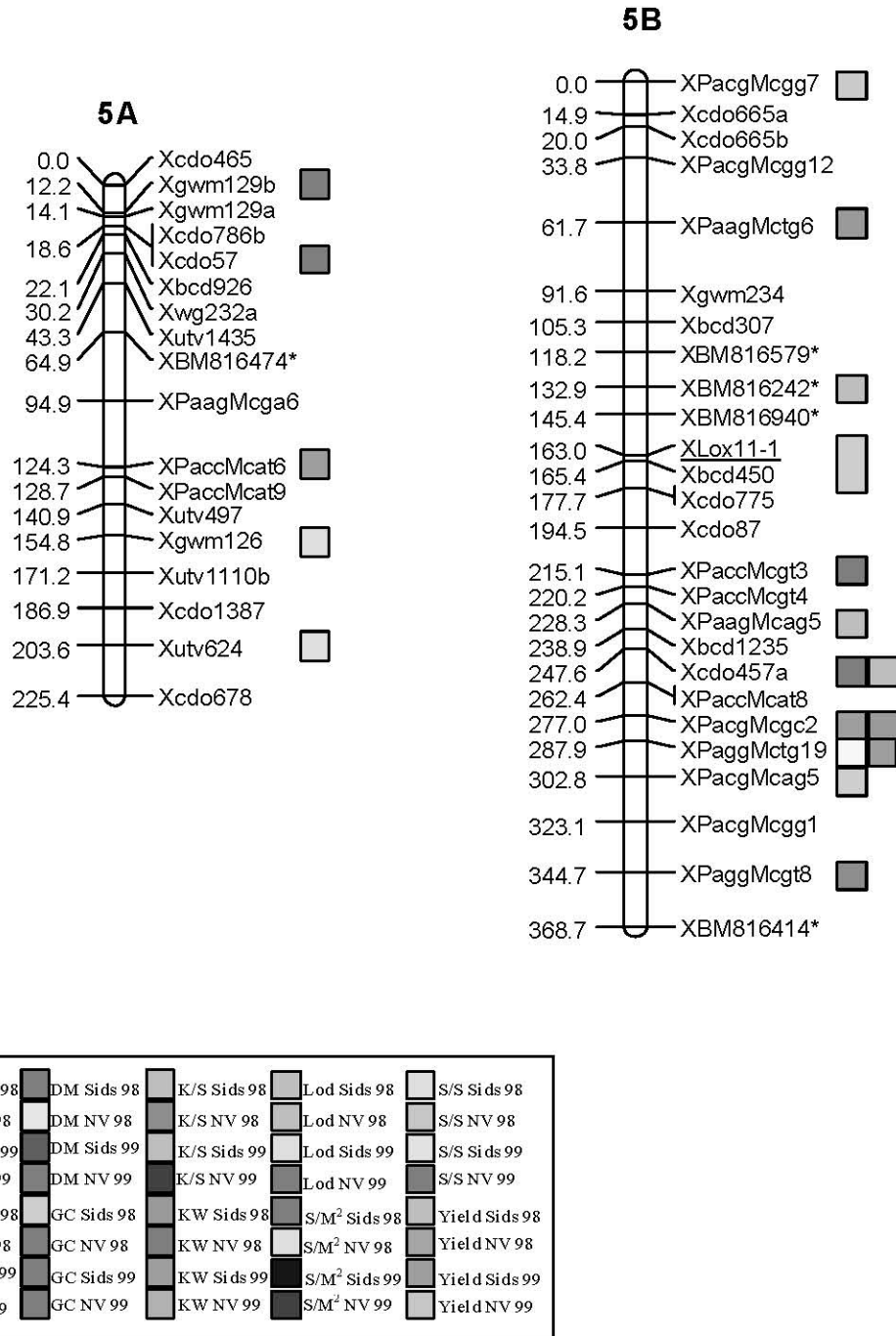


Fig. 1: Continued

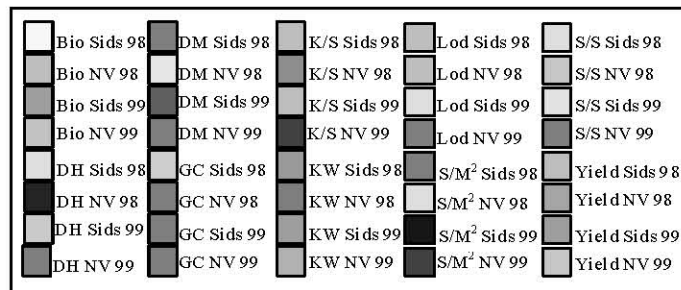
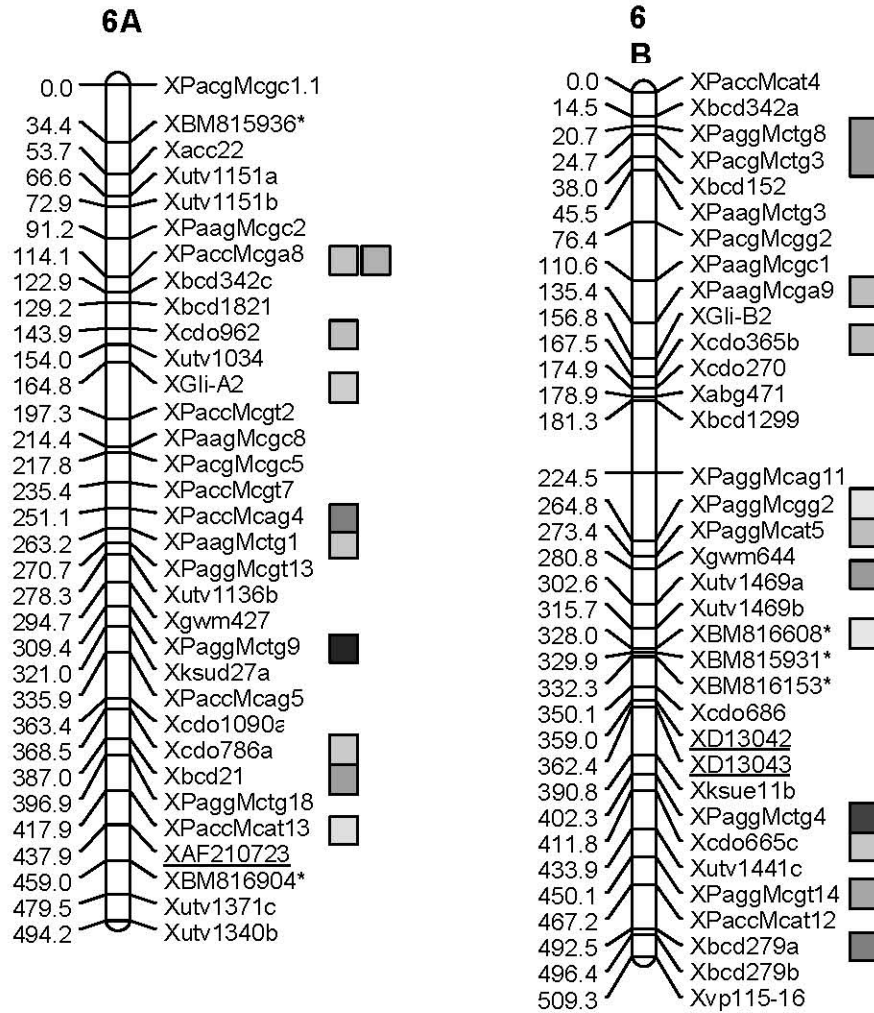


Fig. 1: Continued

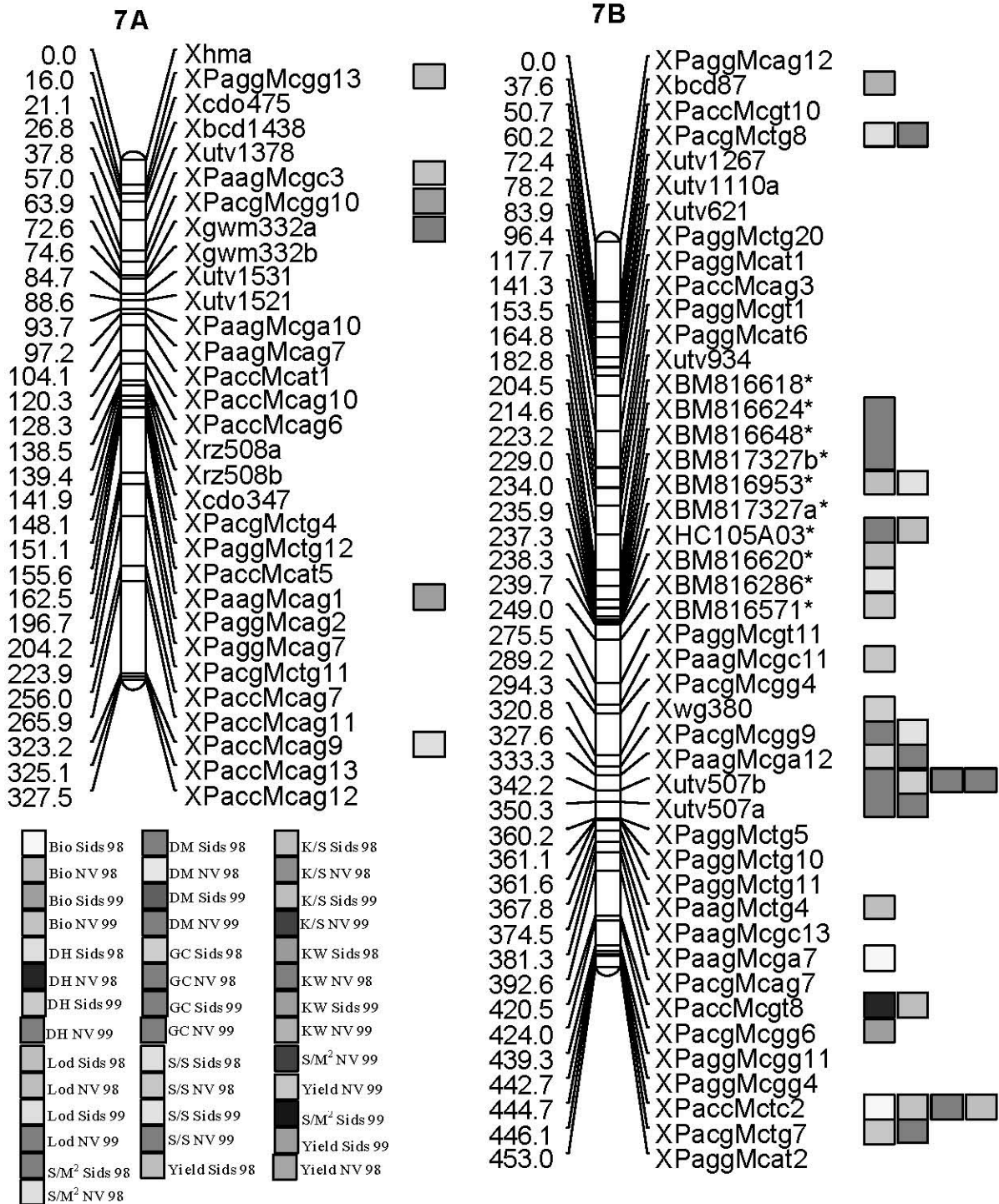


Fig. 1: Molecular linkage map of durum wheat (*T. turgidum* L. var. durum) showing positions of QTL influencing biomass (Bio), days to heading (DH), days to maturity (DM), glume color (GC), Kernel per spike (K/S), kernel weight (KW), lodging (Lod), spike per meter square (S/M), spikelet per spike (S/S) and yield. NV: New Valley/Egypt, Sids: Sids/Egypt. \* are dESTs and drought candidate genes are underlined

QTLs for glume color were consistent at the same chromosomal location (gwm136) on chromosome 1A in three environments (Sids 98, NV 98 and NV 99) and at the locus *PaggMcga12* on chromosome 7B in two environments (Sids 98 and NV 98). Both loci are comparable in the direction and magnitude of their additive effect and the amount of variance accounted for (Table 3). Another genomic region on chromosome 2B (*cdo 365a*) showed coincident for QTLs for days to heading in two environments (NV 98 and Sids 98) and two QTLs for lodging at *pacgmctg8* and *utv837* on chromosomes 7B and 2B respectively were consistent in two environments. While two QTLs for biomass at *PaggMctg19* and *PacgMctg7* on chromosomes 5B and 7B respectively were common in two environments (Sids 99 and Sids 98). One QTL for spikelets per spike at the locus *cdo1312d* was found in two environments on chromosome 4A and one QTL for yield at the locus *PaccMcat7* was detected in two environments on chromosome 5B (Fig. 1).

## DISCUSSION

The material used, a RILs population, is particularly useful for this kind of study, in that, for each genotype, an unlimited number of genetically identical individuals is available, allowing a very accurate measurement of each trait as well as a direct comparison of the data regarding different traits or different years/environments.

The map used in this study was previously constructed with data obtained from the RILs, including the data of Nachit *et al.* [13] consisted of 468 loci covering 5672.8 cM [12]. Whereas the maps developed by Blanco *et al.* [11] (for the cross *Triticum durum* X *Triticum dicoccoides* and by Nachit *et al.* [13] consisted of 259 loci covering 1352 cM and 306 loci covering 3597.8 cM respectively. The longer map used in this study is due mainly to the interspecific cross durum X durum and due to the greater number of mapped markers. The map used in this study was reliable for identifying QTLs for yield and other agronomic traits because it is more saturated and with no major gaps. Whereas, five gaps on chromosomes 1A, 2B, 3A, 6A and 7B that were reported by Nachit *et al.* [13] are eliminated in the map used in this study and 4 gaps on chromosomes 1B, 2A, 3B, 5B and 2 gaps on 3A are reduced. On the other hand, the map used in this study contains 9 genes and 37 differentially expressed sequence tags (dESTs) that were not mapped in any other durum maps, where on the B genome more dESTs were mapped than on the A genome. This suggest

that the B genome has more expressed regions than the A genome. In this study, it was interesting to find more QTLs on the B genome than on the A genome (Fig. 1). Using genetic maps with genes and dESTs hopefully will help in tagging a variety of QTL (including those derived from alien species such as barley\rye\ *etc*) for improving the efficiency of durum wheat breeding.

**QTL analysis:** Yield is a quantitatively inherited trait, controlled by several genetic loci (QTLs). Many quantitative traits can be partitioned between smaller components of a quantitative and/or qualitative nature. In this work QTL analysis for biomass, days to heading, days to maturity, glume color, Kernel per spike, kernel weight, lodging, spike per meter square, spikelet per spike and yield has been done in relation to mapped genetic markers including genes and dESTs to provide data on genome location and the relative effects of loci and alleles. A total of 175 QTLs with LOD score  $\geq 4$  have been identified for the 10 traits by composite interval mapping (CIM). CIM is a combination of simple interval mapping and multiple linear regression developed independently by Zeng [19] and Jasen [20]. This method uses other markers to control for the genetic background. Therefore, it gives powerful detection and discrimination between loci and it corrects the fact that the original interval mapping approach considers each interval independently of all other regions, even if the other QTLs are interfering with the trait under study.

QTLs for yield and other agronomic traits have been widely mapped in hexaploid wheat [21-29]. QTL for morpho-physiological traits have also been mapped in hexaploid wheat [30,31]. Some agronomic traits such as tones per hectare, protein per hectare, grain protein content (%), grain ash content (%), yellow index of semolina, days to heading, leaf rust, plant height and test weight were mapped in durum wheat [32]. Some other traits such as heading dates, spike number per plant, spike weight per plant, single spike weight, kernel number per plant, kernel number per spike, kernel number per spikelet, 100-grain weight, grain yield and spikelet number per spike were also mapped in *Triticum dicoccoides*, which is the progenitor of wheat [33]. To our knowledge, this is the first work reports the mapping of QTLs for yield component and other agronomic traits in durum wheat grown in Egyptian environment.

In this study QTLs associated with kernel weight, spike per meter square and kernel per spike were found on chromosome 3A. These results agree with that of Campbell *et al.* [25] where the same QTLs were found on



chromosome 3A of bread wheat at the same chromosomal regions. Quarrie *et al.* [21] identified QTLs for kernel weight and yield in hexaploid wheat on chromosome 7A, this result is consistent with our findings where QTLs for the same traits were identified on the same chromosome. QTLs for kernel weight, yield, spike per spikelet, heading date and kernel per spike were identified by Junhua *et al.* [33] on chromosomes 1B, 2A, 3A and 5A of a mapping population derived from a cross between *Triticum dicoccoides* and *Triticum durum*. In the present study, QTLs for days to heading and kernel per spike were identified on the same chromosomes. However, QTLs for yield and spike per spikelet were found on 1B, 2A and 5A but not on 3A. While kernel weight QTLs were mapped on 2A, 3A and 5A but not 1B. This could be due to the effect of different environment and different mapping population used in the two investigations. QTLs for lodging were mapped on chromosomes 1B, 2B and 4B. Verma *et al.* [23] found QTLs for lodging and related parameters on chromosomes 1B, 1D, 2B, 2D, 4B, 4D, 6D and 7D in bread wheat.

Multiple QTLs were found for almost all traits at different regions, the number of QTLs identified for each trait varied from 13 to 21, indicating that the genome contains multiple genes affecting different traits.

**Correlations between traits:** It has been demonstrated that correlated, or components of plant yield traits often have QTLs mapping at similar locations. This has been observed in maize [34-36], tomato [37], rice [10] and barley [9, 38]. Snape *et al.* [39] reported pleiotropic effects of plant yield and some yield components in wheat. The distinction between linkage and pleiotropy is important for breeding purposes as well as for scientific reasons. However, without fine resolution mapping or molecular cloning of QTLs, such distinction would be difficult and at best one can make inferences based on morphological and/or physiological relationships between traits under consideration.

Correlations between traits can be interpreted according to their physiological effects to determine their relevance to plant improvement under specific environment. In this work, many genomic regions were identified with significant effects on more than one trait. For example, QTLs for spike per meter square and kernel weight were mapped to the same chromosomal region. Physiologically, spike per meter square related to kernel weight and thus, an increase in spike per meter square is likely to contribute to an increase kernel weight. Therefore, the co-localization of the two QTLs is most likely due to pleiotropic effects of the same gene(s).

The same can be applied for QTLs for kernel per spike and days to maturity; biomass and lodging that are mapped in the same regions. This suggests a genetic basis for a positive correlation between these traits and this correlation is most likely due to pleiotropic effects of the same gene(s). This has been reported by Garcý'a *et al.* [40] and Reynolds *et al.* [41]. Kato *et al.* [26] identified five chromosomal regions controlling grain yield and its components QTLs on chromosome 5A and confirmed that these grain-yield QTLs were correlated with QTLs for yield components and found at the same map positions. On chromosomes 3A and 7B overlapping QTLs for days to heading, kernel per spike, kernel weight and spike per meter square biomass and lodging were found, showing that these traits are probably regulated by common factors on chromosomes 3A and 7B and these 2 chromosomes are probably an important chromosomes for yield and its components.

**Consistent QTLs in different environment:** The evaluation of mapping populations in multiple environments presents additional challenges and opportunities for QTL analysis. Paterson *et al.* [37] suggested that the studies conducted in a single environment are likely to underestimate the number of QTLs which can influence a certain trait. The positions of QTLs are presumably constant within a genome but the effects of QTLs may vary among environments due to QTL-environment interaction. These concepts were confirmed in the present study where QTLs for glume color were consistent on chromosome 1A in three environments and on chromosome 7B in two environments.

The same was observed for days to heading and biomass in two environments. Since, in several cases, the same genomic position of QTLs controlling two or more traits was revealed and this was true in different environments, environmental effects appear unlikely for these traits. It is also possible to identify environmentally sensitive QTLs, meaning that the expression of the QTLs will occur under certain environments. For such an environment-specific QTL, one would only be able to identify the QTLs at a location where these environmental conditions are satisfied.

**Candidate genes and differently expressed sequences co-segregating with traits:** The co-localization of specific genes with QTLs could be a better way to understand the molecular basis of traits and an efficient approach to identifying genes controlling yield and agronomic traits.

In this work, associations between QTLs and candidate genes were identified. Three candidate genes and 14 sequence tags differentially expressed under drought stress were associated with QTLs for traits related to yield and other agronomic traits.

**Candidate genes:** The loci *Loxmj1* and *Lox11-1* coding for lipoxygenase, co-segregated with QTL for yield and glume color respectively. Lipoxygenase is a non-heme iron-containing enzyme, which catalyzes the hydroperoxidation of fatty acids and is modulated in plants by water deficit [42]. Despite its wide distribution in the plants, the physiological role of this gene has only partly been elucidated. Lipoxygenase has been proposed to play a role in senescence, pathogen, durum wheat semolina color and wound responses and has also been implicated in the biosynthesis of ABA [43]. Also it has been reported that lipoxygenase revealed an N-terminal extension that could be a signal for chloroplast targeting [44]. Although the physiological function of lipoxygenase gene in plants is not well defined, the association of this gene with yield and glume color suggests a role for this gene in durum wheat yield and color especially that it is established that spaghetti and crumb bread color is affected by the amount of lipoxygenase. High levels of this enzyme in the milling products destroy the expected yellow color of pasta and bread products during their processing by oxidation of xanthophylls.

The gene M96856, coding for protein associated with G-Box binding complex co-segregated with a QTL for yield on chromosome 1B. The role of this gene in yield is not established; however the co-localization of this gene and QTL for yield might suggest a role in durum wheat yield.

**Differentially expressed sequences:** The location of differentially expressed sequence tags at QTLs involved in yield and agronomic traits could give some information about their role.

The loci BM816609, BM816257, BM816242, BM816608, BM816624, BM816648, BM817327b, BM816953, BM816620, BM816571, coding respectively for aluminum induced protein, actin depolymerizing factor, glutathione S-transferase, glutathione oxidase, glycine dehydrogenase, arginine 2-monooxygenase, alpha-glucanotransferase, nitrilase-like protein, glycine dehydrogenase and metallothioneine [45] co-segregated with several QTLs for yield and other agronomic traits (Table 4). The role of these ESTs has not yet been established. However, some studies reported a role for some of these genes.

For example, the locus BM816257, coding for actin depolymerizing factor (ADF), co-segregated with kernel weight on chromosome 2A. The ADF is part of the ADF/cofilin group, a family of small proteins (15-22 kD) that includes cofilin, destrin, depactin and actophorin [46, 47]. The members of this family are stimulus-responsive modulators of the cell actin cytoskeleton dynamics. They show actin monomer binding, actin-filament binding/severing and nucleotide/monomer dissociation-inhibiting activities *in vitro* [48, 49]. Using *Arabidopsis* ADF1, Carlier *et al.* [50] have suggested that one of the main functions of ADF is to increase the turnover rate of actin filaments. Several cellular processes are associated with the reorganization of the actin cytoskeleton in plants. These include cell division and differentiation, stomatal movement, gravitropic tip growth, light induced plastid migration, wound repair, response to pathogen attack, pollen development, nuclear migration, cytoplasmic streaming, secretion, cell wall biosynthesis and transmembrane signaling [51]. Actin filaments are tightly linked to the plasma membrane and believed to be involved in signal transduction events in plants [51]. Disruption or reorganization of the cytoskeleton could thus impair or modify the activity of signaling molecules associated with cytoskeletal elements. A group of small actin-binding proteins, the ADF group, has the ability to regulate actin polymerization and depolymerization. Based on the above information, the association between ADF and QTL for kernel weight suggests that this gene plays an important role in kernel weight in durum wheat.

The loci BM816242 coding glutathione S-transferase co-segregated with a QTL for yield. A gene coding for glutathione S-transferase has been used to transform tobacco [52] and *Arabidopsis* [53]. The transformed plants obtained from these studies showed sustainable yield under cold and salinity stress and resistance against aluminum toxicity and oxidative stress. It has been reported that exposure of plants to various environmental perturbations, including drought, intense light, temperature stress, the presence of metal toxicity (e.g Aluminum), can lead to the generation of Activated Oxygen Species (AOS) (reviewed in Datta [54]). These AOS cause extensive cellular damage, due to oxidative stress and inhibition of photosynthesis [55]. Plants have evolved systems to combat this oxidative stress with a battery of gene products that aid in reducing the AOS that damage membranes. Enzymes such as glutathione S-transferase are involved in such protective processes [54, 56, 57]. This information could explain the co-segregation of this gene with QTL for yield in durum wheat.

The loci BM816609 coding aluminum induced protein co-segregated with a QTL for days to heading. A gene coding for aluminum induced protein has been isolated from wheat spike infected with *Fusarium graminearum* [58]. It is reported that, one of the resistance mechanisms for fungal attack is the earliness of heading to escape the stress induced by the fungus. The co-localization of this gene with QTL for heading date might strongly support this information. However, more research is needed to identify functions for this gene and to validate its role in durum wheat.

The loci BM816571 coding metallothioneine co-segregated with a QTL for yield. A gene coding for metallothioneine has been isolated from durum wheat [59]. Metallothioneins (MTs) are small cysteine-rich polypeptides found in almost all organisms from bacteria to plants and animals. In general, they are involved in heavy-metal detoxification and metabolism of essential trace elements like copper and zinc. Structural studies have shown that in mammalian MTs metal-binding is achieved through the formation of thiol bonds with the metal ions [59]. The high metal binding capacity for different metals (Cd, Zn, Ag, Hg, Au etc.) make MTs valuable for detoxification, remediation and recycling in agricultural areas. The co-segregation of the metallothioneine gene with QTL for yield does not indicate a direct function for this gene in durum wheat yield but it may have an indirect role in improving yield through tolerance to metals stress condition in soil.

The dESTs that have unknown function and co-segregated with QTLs are BM816287a, BM816848, HC105A03 and BM816286. The association between these dESTs and QTLs for biomass, spikelet per spike and days to maturity suggests a possible role in durum wheat yield and maturity; however, more research is needed to identify functions for those dESTs and to validate their role.

QTL for kernel per spike was associated with the locus BM816848 on chromosome 4B and BM816953 and BM816620 on chromosomes 7B. These loci are sequence tags differentially expressed under drought stress [45]. Kernels per spike, has been reported by Gebeyehou *et al.* [60] and Simane *et al.* [61] as the yield component most closely associated with the yield of durum wheat genotypes in dryland conditions. Indeed, this character also relates to physiological mechanisms of resistance to drought [62]. Visual assessment of kernels per spike and a window value of earliness have been at the basis of an 'agronomic score' used successfully for estimating the response of durum wheat entries to severe drought stress in the region [63].

The observation of co-location between a QTL and a candidate gene does not provide definitive evidence for the role of the genes in trait variation. Fine mapping and analysis of gene polymorphism in coding and regulatory regions are required. Comparative mapping is another indirect but valuable method that can be used to validate the QTL association taking the advantage of possible co-location of the same QTL/candidate gene couples in different species of the same family.

**Candidates for marker-assisted selection:** The most practical application of the identified QTLs is to perform marker-assisted selection aimed at efficient pyramiding of favorable QTLs alleles to improve durum wheat yield and agronomic traits. The identification of markers linked with genes controlling economically important traits may be used for marker assisted selection in breeding programs, which would allow a more accurate and efficient selection of superior genotypes and a reduction of time and space during the early breeding program. In this work, the consistent QTLs identified in multiple environments could be useful in marker-assisted selection (MAS) program in durum wheat. Some QTLs were found to be the consistent in different environments. Therefore these QTLs may be valuable for use in MAS because they were expressed whatever the conditions and represent most of the studied environments. The inconsistency of QTL over environments may be a major limitation in utilizing QTL for MAS of genotypes for environments that differ from the environment in which the QTL were detected. However, the identification of QTLs that seem specific to one environment characterized by particular climatic conditions could also be of interest for breeders. For those QTLs, which were detected in only one environment, it is not known if the restriction of those QTLs to only one environment is related to gene expression or is indicative of sampling variation. Although the significant effect of environmental factors makes it more difficult to obtain accurate data for identifying associations between markers and QTL. Lande and Thompson [64] concluded from their studies on marker assisted selection that relative efficiency of MAS is greatest for character with a lower heritability. Even though traits with low heritability may benefit more from MAS, a moderate fraction of the additive genetic variation must be significantly associated with the marker loci for MAS to be effective.

For genetic analysis of durum wheat, the present investigation demonstrates the power of the molecular marker as a tool for locating and identifying QTLs controlling important quantitative traits. The results

obtained from this work can be useful in a breeding program aimed at improving yield and agronomic traits in durum wheat.

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