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Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat (*Triticum turgidum* L. var. durum)

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The objective of this study is to identify QTLs linked to yellow pigment content in durum wheat. A durum-dicoccoides genetic linkage map was constructed using 124 microsatellites, 149 amplified fragment length polymorphism (AFLPs), and six seed storage proteins (SSP) in a population of 114 recombinant inbred lines (F8). The population has been obtained from a cross between a durum cultivar Omrabi5 and *Triticum dicoccoides*600545 and backcrossed to Omrabi5. The map consists of 14-durum chromosomes plus an unknown group; and shows a good synteny to the previously published wheat maps. Yellow pigment was measured in the population in three different locations during 3 seasons. Analysis of QTLs was based on simple and simplified composite interval mapping (SIM and sCIM). Three QTLs for yellow pigment were detected on the chromosomal group 7 (7AL and 7BL telomeres) explaining 62% of the total variation. On 7BL, a major microsatellite (*Xgwm344*) explained by itself 53%, whereas on 7AL, the other two QTLs have contributed 13 and 6%. All determined QTLs showed a strong genetic effect and a weak QTL × E effect. The QTLs effect was consistent across all environments and showed a large effect. Consequently, promising QTLs will be used in the marker assisted breeding program to enhance the selection efficiency for yellow pigment.

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Durum wheat (*Triticum turgidum* L. var. durum) is mainly produced and consumed in the Mediterranean region; it is used to produce several specific endproducts. The wheat grain quality traits are considered to be inherited as quantitative traits as it is known to be controlled by a group of genes and being very affected by environmental variations (KUSPIRA and UNRAN 1957; DIEHL et al. 1978; NACHIT et al. 1995). The quality cannot be expressed in terms of a single property, but depends on several milling, chemical, baking, processing, and physical dough characteristics; each one of them is important in the production of each end-product. The flour and semolina color is considered important in the assessment of durum quality.

Durum has normally an amber vitreous kernel that produces yellow semolina. Due to its importance, many studies were conducted to define the biochemical pathways and genetic control of this trait (SAX 1923; TSEN and HLYNKA 1963; DAHLE 1965; MOSS 1967; LEPAGE and SIMS 1968; LAIGNELET 1983; NACHIT 1990; HATCHER and KRUGER 1993; 1997; PARKER et al. 1998; BORRELLI et al. 1999). The semolina color is the result of the natural carotenoid pigments present in the seeds (CUBADDA 1988) and of their residual contents after the storage of the grain (DAHLE 1965) or semolina and after milling (BORRELLI et al. 1999), of their oxidative degradation by lipoxygenase during processing (IRVINE and WINKLER 1950; MCDONALD 1979; BORRELLI et al. 1999), and of processing conditions (TROCCOLI et al. 2000). The carotenoid pigments are classified into carotenes, unsaturated hydrocarbons, and xanthophylls. Xanthophylls are the most abundant and possess one or more oxygen-bearing functional groups. They are including many types; e.g. triticoxanthin, taraxanthin, flavoxanthin, and canthaxanthin (LAIGNELET 1983). Using chromatography, it was reported that the pigments in durum variety are composed of 84.8% free lutein (xanthophylls), 9.8% lutein monoester, and 5.3% lutein diester (LEPAGE and SIMS 1968). Carotenoides are antioxidant compounds that reduce the oxidative damage to biological membranes by scavenging peroxyradicals. They are mainly located in the outer layers of the kernel, while the embryo, bran, and endosperm contain smaller amounts (TROCCOLI et al. 2000). In durum, the yellow pigment is highly heritable, the heritability values range from 0.90 to 0.97, and is controlled by additive gene effects (NACHIT et al. 1995). Some studies suggest that the major genes are probably on chromosomes 2A and 2B (JOPPA and WILLIAMS 1988), whereas, more recently PARKER et al. (1998) reported a major locus on 7A in bread wheat explaining 60% of the

genetic variation. Nevertheless, a high level of carotenoid pigments in semolina does not guarantee high color pasta, as the color is also affected by the level of lipoxygenase (LOX) activity (MCDONALD 1979) and polyphenol oxidase activity (DEXTER et al. 1984).

Positioning QTLs on genetic maps is a powerful technique to portion quantitative traits on Mendelian genes, especially that more genetic maps are available now (PATERSON et al. 1991). These QTLs can assist the marker-assisted selection and enhance the efficiency of plant breeding (PATERSON et al. 1991). During the last years, several QTLs linked with important traits have been identified in many crops. In wheat, QTLs related to amylose content, disease resistance, and grain yield have been identified (ARAKI et al. 1999; WALDRON et al. 1999; KATO et al. 2000). In this context, we used a mapping population showing a high polymorphism and a significant variation for yellow pigment. The objective of this study was to identify molecular markers linked to yellow pigment content in durum.

MATERIAL AND METHODS

Plant material

The durum cultivar Omrabi5 is a cross between Haurani × Jori-C69, bred for Mediterranean dryland conditions by CIMMYT/ICARDA durum program (Nachit pers. com.). Omrabi5 is released in Turkey, Algeria, Iran, and Iraq for commercial production in dry areas. It combines drought tolerance with high yield and yield stability. *Triticum dicoccoides* accession number: 600545 was collected in Jordan, 25 km west of Amman on the Amman-Dead See highway; it shows resistance to yellow rust and tolerance to drought.

A cross was made between Omrabi5 and the *T. dicoccoides*600545. The F1-cross was backcrossed to the maternal parent Omrabi5 (pedigree ICDMN-91XMP (Nachit pers. com). The population was advanced to F6 generation with single seed descent method (SSD) and up to the F8 generation with bulk (114 RILs). The genetic linkage map of this population was constructed using microsatellites, AFLPs, and SSP markers. Its length was 2288.8 cM, with an average distance between markers of 8.2 cM (publication under preparation).

Sites description and experimental design

The mapping population was grown in three locations for the 4 consecutive seasons from 1996 to 2000. First in Tel-hadya, the main research station of ICARDA, where a double gradient screening technique was applied to generate contrasting environments (Early Planting, EP; Rainfed, Rf; Irrigated, Ir; Late Planting, LP; Summer Planting, Sum; Sowing after hay legume crop, Inc). This station is characterized by wet and cold winters and warm and dry summers. Secondly in Breda station, characterized by harsh continental climatic conditions, and finally in Terbol station, characterized with cold winters, favorable seasons, and high soil fertility.

The field design used was the augmented design (FEDERER 1956; PETERSON 1985). Thus the 114 RILs were divided over 6 blocks; in each block 19 RILs were included with 5 commercial durum genotypes as checks (Omrabi5, Haurani, Korifla, Cham1, and Gidara2). The trial was sown either in 2 rows of 2.5m long spaced by 30 cm; in 8 rows of 2.5 m long spaced by 22.5 cm or in 6 rows of 2.5 m long spaced by 30 cm.

Yellow Pigment Assessment

Data for yellow pigment content were analyzed for 16 environments (Table 1). Yellow pigment content of the 114 RILs was determined on 8 g of semolina extracted overnight with 40 ml of water-saturated n-butyl alcohol. After filtration of the extract through a Whatman No. 1, a light transmission is determined in a spectrophotometer at 440 nanometers (nm). The determined values were estimated according to the concentration scale based on β-carotene (AACC 1976).

QTL analysis

The QTL analysis was performed with the software package Multiple Quantitative Trait Loci (MQTL) (TINKER and MATHER 1995) and the sCIM was applied based on few background markers (ELOUAFI 2001).

RESULTS AND DISCUSSION

Quantitative trait

The summary of yellow pigment data over the 16 studied environments is presented in Table 1. The recombinant inbred lines RILs mean over the 16 environments was 5.6 ppm, ranging from 3.1 to 8.6 ppm, whereas the mean of the two parents: Omrabi5 and *T. dicoccoides*600545 were 6.6 and 5.3 ppm, respectively. Studying the distribution in each environment, a negative transgressive inheritance was detected in some stressed environments such as Breda98, Breda00, Early Planting98, and Rainfed98, whereas the average frequency distribution for the RILs illustrates transgressive inheritances in both positive and negative directions (Fig. 1). The yellow pigment mid-parent value is slightly higher than the

Table 1. *Yellow pigment mean, minimum, maximum, heritability, variance, and CV in Omrabi5/T. dicoccoides600545//Omrabi5 population and the parents (P1, P2)*

Trait-year-site	RILs mean	RILs min	RILs max	P_1	P_2	h^2	Variance (σ^2)	Gen. CV (100)
YP98Br	4.9	3.1	7.7	5.7	5.2	0.58	1.2	22.4
YP98EP	5.5	3.3	8.4	6.3	5.8	0.48	1.4	21.5
YP98LP	5.9	3.2	9.1	7.0	5.7	0.98	2.1	24.5
YP98Rf	5.3	2.9	8.4	6.0	5.5	0.97	1.4	22.3
YP99Br	5.9	3.6	8.7	6.6	5.5	0.87	1.7	22.8
YP99Inc	5.9	3.2	9.2	7.3	5.9	0.98	2.4	26.2
YP99Kf	5.6	3.1	8.9	7.2	5.8	0.76	1.8	23.9
YP99LP	5.3	2.8	8.0	6.9	5.5	0.99	1.5	23.1
YP99Rf	5.6	3.1	8.8	6.7	4.9	0.96	2.3	27.0
YP99Tr	5.2	2.9	8.7	6.9	4.8	0.95	2.1	27.8
YP00Tr	5.5	2.9	9.3	6.6	5.0	0.97	2.2	26.9
YP00Rf	5.4	3.2	8.0	6.4	5.6	0.96	1.7	24.1
YP00Br	6.2	4.1	8.8	6.9	6.1	0.91	1.3	18.3
YP00EP	5.3	3.1	7.9	7.1	5.5	0.99	1.8	25.3
YP00Sum	6.2	2.1	8.8	6.4	4.2	0.85	1.7	21.0
YP00Ir	5.1	2.8	8.9	5.9	3.6	-	2.5	31.0
Mean	5.6	3.1	8.6	6.6	5.3	0.88	1.8	24.3

mean of the RILs. The values of the parents differed by one standard deviation ($\sigma = 1.3$), and there were transgressive RILs segregates that differed from both parents by two standard deviations. The broad sense heritability estimated for the 16 environments, showed values varying from 0.48 to 0.99 with a mean of 0.87. Actually, most of the environments showed a heritability higher than 0.8 except for 98Br and 98Ep, where relatively low heritabilities were shown (Table 1). This could be explained by early and late dry conditions experienced during 1997/98 seasons. These high heritability values indicate the strong genotypic effect on yellow pigment content and confirm earlier published studies on carotenoid content heritability in durum wheat (JOPPA and WILLIAMS 1988; PARKER et al. 1998; NACHIT et al. 1995; BORRELLI et al. 1999) and in *Tritordeums* (ALVAREZ et al. 1998). Further, the average genetic coefficient of variability for the RILs was high (24.2%), varying from 18.3% (00Br) to 31.0% (00EP, 99LP).

QTL determination

In the simple interval mapping (SIM) analysis for yellow pigment content, the test statistic exceeded the significance threshold in 2 regions, first on chromosome 7A, and secondly on chromosome 7B (Fig. 2). Following the same strategy as the one developed for Jennah Khetifa × Cham 1 population (NACHIT et al. 2001), for gluten strength QTLs (ELOUAFI et al. 2000), the significant peaks for the test statistic for SIM were chosen as background markers. The resulting sCIM scan confirmed the major peak on the telomeric region of 7BL with both its strong main genetic effect and small interaction effect. This QTL was at 0 cM from the microsatellite *Xgwm344*. Further; sCIM analysis annulated the peak detected by SIM close to *Xgwm63e* on the telomeric region of 7AL, but it did detect a new QTL peak close to the centromeric region in an area where SIM showed a small non-significant peak (Fig. 2). This new QTL corresponded to the AFLP marker *XMcaaEacg198*. The inability of sCIM to detect the QTL detected by SIM could be explained by the fact that sCIM eliminates sampling variance that contributed to type-I error for SIM (TINKER et al. 1996). On the other hand, sCIM detection of a new QTL could be explained by its ability to detect QTL with small effects, as it accounts for the genetic component of the background variance, and its ability to show regions

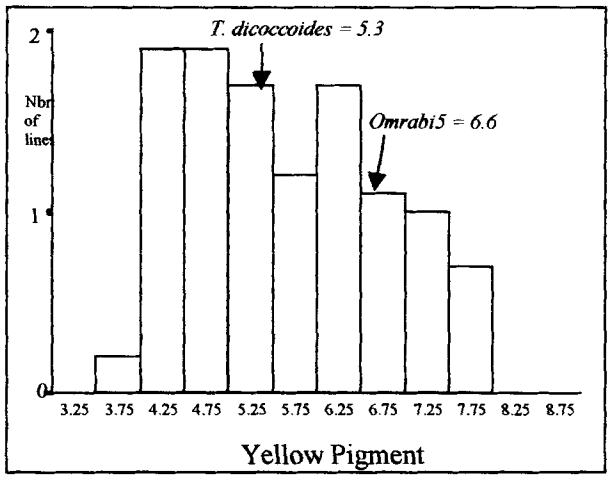

Fig. 1. Distribution frequency for average yellow pigment content in Omrabi5/*T. dicoccoides*600545//Omrabi5 (16 environments).

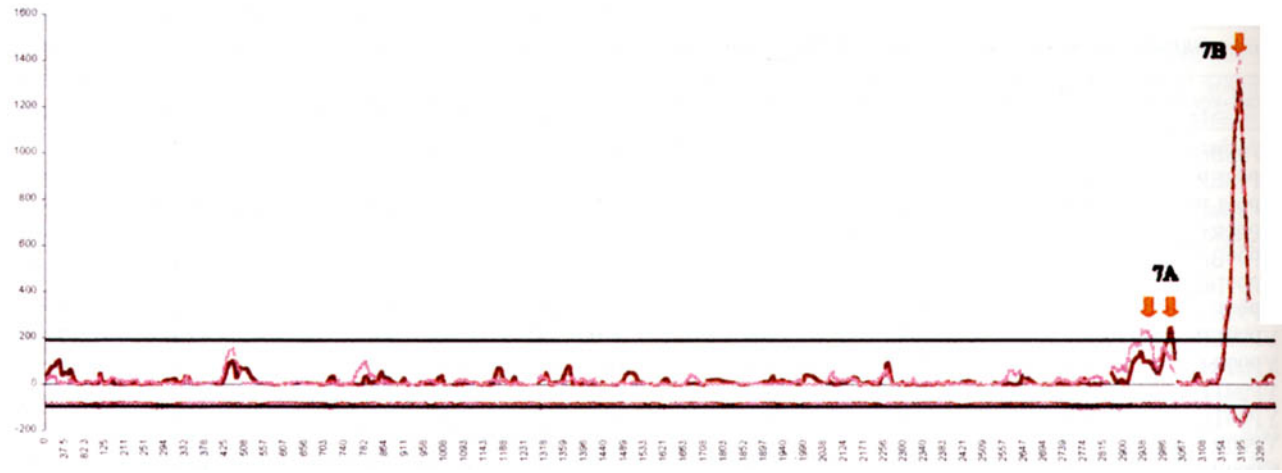

Fig. 2. Yellow pigment scans of a test statistic for SIM (solid lines) and sCIM (broken lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 durum chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of detected QTLs.

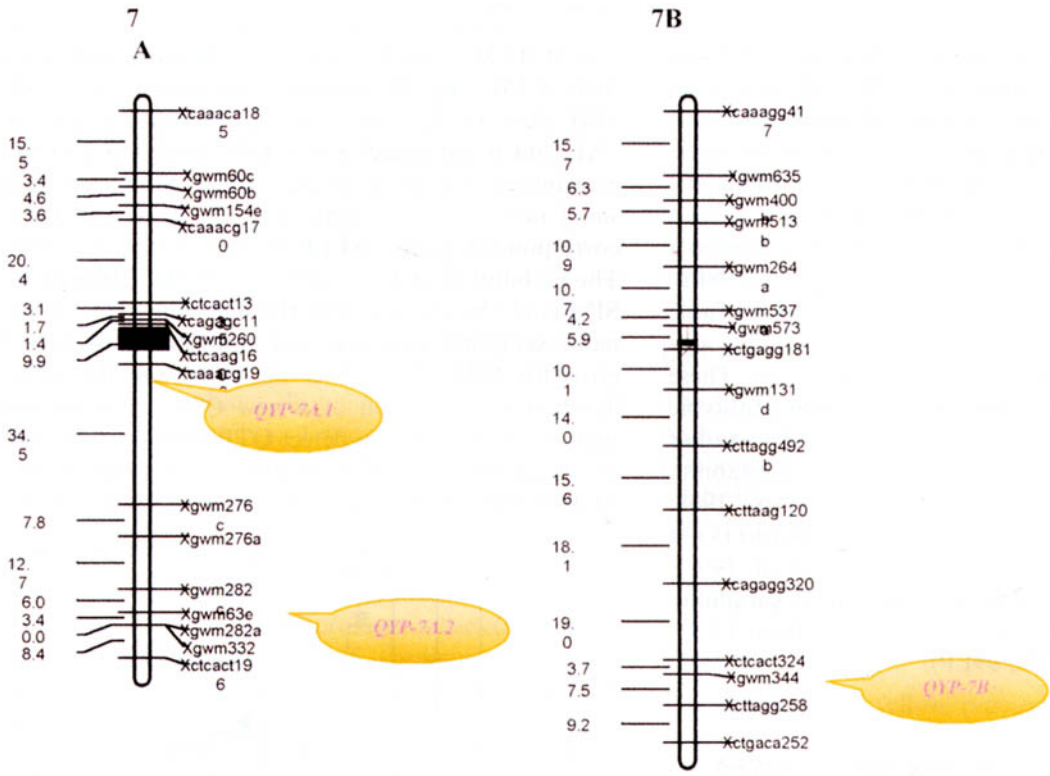

Fig. 3. Estimated yellow pigment QTLs relative magnitude, chromosomal location and effect/nature in different environments. (M = main effect; I = QTL × E effect)

where there is evidence for multiple linked QTLs (TINKER et al. 1996).

In general, the scan showed that there is strong genotypic effect on the yellow pigment trait. The two peaks on 7A presented only a main effect and the major peak on chromosome 7B a strong main effect and a weak QTL × E interaction effect. These results corroborate earlier findings showing that yellow pigment trait is mainly affected by genotypic effect in an additive manner (JOPPA and WILLIAMS 1988; PARKER et al. 1998; NACHIT et al. 1995; BORRELLI et al. 1999). Furthermore, The peak on 7B was very high suggesting a major QTL on this region (Fig. 2).

Thus, all yellow pigment QTLs in Omrabi5/*T. Dicoccoides*600545//Omrabi5 population were detected on the homoeologous regions of chromosomal group 7 (Fig. 3). A major QTL on 7BL and 2 minor QTLs on 7AL. These findings are in agreement with PARKER et al. (1998) work on bread wheat, which reported a major QTL on chromosome 7AL. This bread wheat QTL appears to be the homoeologous locus of *Xgwm344* major QTL detected in this population on 7BL. Moreover, the chromosomal group localization is also in agreement with the findings in tritordeums, where it was reported that carotene content is controlled by the chromosome $7H^{ch}$ of *Hordeum chilense* (ALVAREZ et al. 1998). Nevertheless, earlier studies suggested rather QTLs on chromosomes 2A and 2B in durum wheat (JOPPA and WILLIAMS 1988) and chromosome 4B close to the LOX gene, in Jennah Khetifa × Cham1 durum population (Nachit, pers. com.).

The three detected QTLs explained 62% of the total yellow pigment variation (Table 2). The major QTL at zero cM of *Xgwm344* on 7BL showed a significant effect by explaining 53% of the total variation, out of which 52% is a genetic variation. The QTL detected on bread wheat on 7AL, was reported to explain 60% of the total flour color genetic variation (PARKER et al. 1998). The other two QTLs on 7AL, *XMcaaEacg198* and *Xgwm63e*, have explained 6 and 13% of the total variation, respectively. This is a good example of the complementarity between SIM and sCIM analysis, as the QTL on *Xgwm63e* was detected only by SIM, whereas *XMcaaEacg198* by sCIM analysis.

All the determined yellow pigment QTLs showed that the Omrabi5 alleles had a significant positive effect (Fig. 4), indicating that the improved durum variety Omrabi5 alleles increased the yellow pigment content. This positive contribution was indicated for all detected QTLs and was consistent across sites and years with minor magnitude changes. Therefore, they can be considered as candidate QTLs for marker assisted breeding, in order to enhance selection efficiency for yellow pigment. In fact, yellow pigment still showed some transgressive segregation that may be accounted for other QTLs and were not detected in this study. For sure the chemical yellow pigment assessment is relatively an easy protocol, but it is time consuming and need at least 8 grams to be performed, meaning about 300 grains, which is not available till quite advanced generations. Therefore, finding a putative QTL that could replace this chemical protocol will be of great help to the breeding programs, though the practical

Table 2. *Yellow pigment QTLs and their contributions*

Chr. localization	QTL marker	CM	V_g/V_{ph}	V_g+V_{QTLxE}/V_{ph}
7BL	*Xgwm344*	0	52	53
7AL	*Xgwm63e*	0	13	13
7A	*XMcaaEacg198*	5	6	6
Total explanation			60%	62%

V_g = genetic variance; V_{QTLxE} = QTL × E variance; V_{ph} = phenotypic variance.

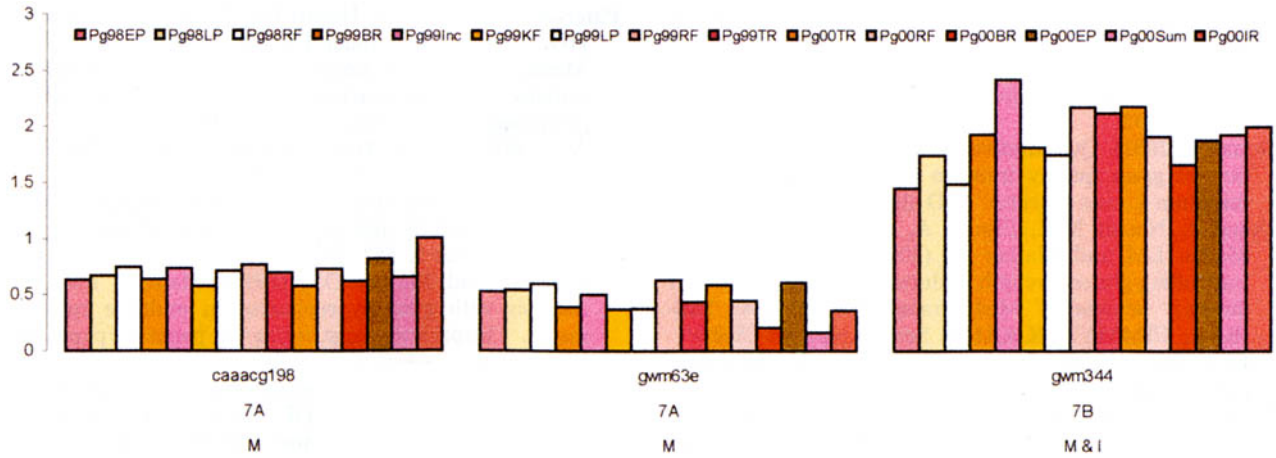

Fig. 4. Positioning of yellow pigment detected QTLs on Omrabi5/*T. dicoccoides*600545//Omrabi5 genetic linkage map.

utility of marker-assisted selection for quantitative traits remains speculative. Nevertheless, detecting a major yellow pigment QTL may lead to the isolation of the gene responsible for the accumulation of carotenoids and may highlight its pathway.

Furthermore, this population can also be used to determine other QTLs of interest for stress resistance, as the parents harbor contrasting resistance genes for biotic and abiotic stresses.

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