MAPPING OF PHYTOENE SYNTHASE (PSY2) GENES ON GROUP 5 CHROMOSOMES OF DURUM WHEAT

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Introduction

Yellow pigment content is one of the criterion in the assessment of semolina quality of durum wheat and is of particular importance in determining the commercial and nutritional quality of end-products such as pasta. A yellow to amber colour is generally preferred by consumers rather than a brown or cream one. Semolina colour is the result of carotenoid pigment content of the grain and of their residual content after the storage of the grain or semolina, of the carotenoid oxidative degradation by lipoxigenase during processing, and of processing conditions. The biosynthetic pathway of carotenoids involves more than ten enzymatic steps, among which the step catalyzed by phytoene synthase (Psy) is assumed to be rate-limiting in the carotenoid biosynthesis. Duplicated Psy genes (*Psy1* and *Psy2*) in 12 species (including common wheat) from eight subfamilies of the grass family were identified by Gallagher et al (2004), and *Psy1,* but not *Psy2*, exhibited a strong association with yellow pigment content of endosperm. A recently identified *Psy3* gene in maize was found to be highly expressed in embryo and roots particularly in response to abiotic stress. Characterization of Psy genes and the development of functional markers are of importance for marker-assisted selection in wheat breeding.

Genetic mapping

A genetic linkage map of the Latino x Primadur F_3 population was constructed using the Kosambi mapping function within the software JoinMap 4. A total of 102 SSRs and 241 DArT markers were assigned to 26 linkage groups, with the number of loci per group ranging from 4 to 43. Linkage groups were assigned chromosome names by comparing markers on the generated map to previously published durum maps and the hexaploid wheat SSR consensus map (Somers et al 2004). The final map consisted of 443 markers; however, some chromosome arms were not represented.
Nine primer sets were developed based on the *Psy2* sequences from durum wheat (GenBank accession DQ642445 and DQ642442) to amplify the *Psy-A2* and *Psy-B2* genes in the Latino and Primadur cultivars of durum wheat. Because of the high similarity of *Psy1* and *Psy2* genes, the nulli-tetrasomic lines of Chinese Spring were used to differentiate A and B genome derived *Psy2* sequences. The primer sets amplifying fragments assigned to 5A and 5B chromosomes were subsequently used for mapping the *Psy-A2* and *Psy-B2* genes. Physical mapping of the *Psy2* loci, performed using ditelosomic and deletion lines of Chinese Spring, assigned *Psy-A2* and *Psy-B2* to 5AS3-0.75-0.98 and 5BS6-0.81-1.00 bins, respectively. Genetic mapping of *Psy-B2* using the polymorphic primers PSY_AB2 (F-CAAATCCAGCGCGCCAGGGT; R-ACAGCGACAGCGATGCCAGG) confirmed that *Psy-B2* was located on 5BS6-0.81-1.00 bin with other known SSR markers (Fig. 1).

Field trait analysis

Grain carotenoid content was evaluated in the segregant population Latino x Primadur in two replicated trials conducted at two locations in southern Italy in 2006. Pigment content was measured using a modified version of the AACC 14-50 procedure. The analysis of variance revealed highly significant differences among F_3 progenies for YP in each of the two environments. Large segregation was observed in each trial, with the phenotypic values of F_3 progenies being normally distributed. Trasgressive segregation was observed in each environment, with trasgressive segregates higher than the high parent (Primadur), indicating that positive alleles were present in both parents (Table 1). Estimates of heritability were found to be superior to 0.90 in both environments.

Table 1. Mean, range, genetic variance (s^2_G) and heritability (h^2_B) of pigment content (µg/g) in the segregant population of 121 F_3 progenies derived from the cross Latino x Primadur evaluated in two environments.

Parental lines or F_3 progenies	Environments	
	Valenzano 2006	Gaudiano 2006
Latino	5.21	5.09
Primadur	9.63	10.61
F_3 progenies	7.64	7.61
Range	(5.25-10.94)	(4.75-11.49)
C.V. (%)	3.37	4.22
s^2_G	1.51	2.07
h^2_B	0.96	0.95

Objectives

❖ Genetic and deletion mapping of *Psy2* genes in a segregant population of F_3 progenies derived from crossing the durum wheat cultivars Latino and Primadur characterised by low and high values of yellow pigment content, respectively.

❖ Detection of quantitative trait loci (QTLs) and assessment of the possible association of *Psy2* genes with carotenoid content.

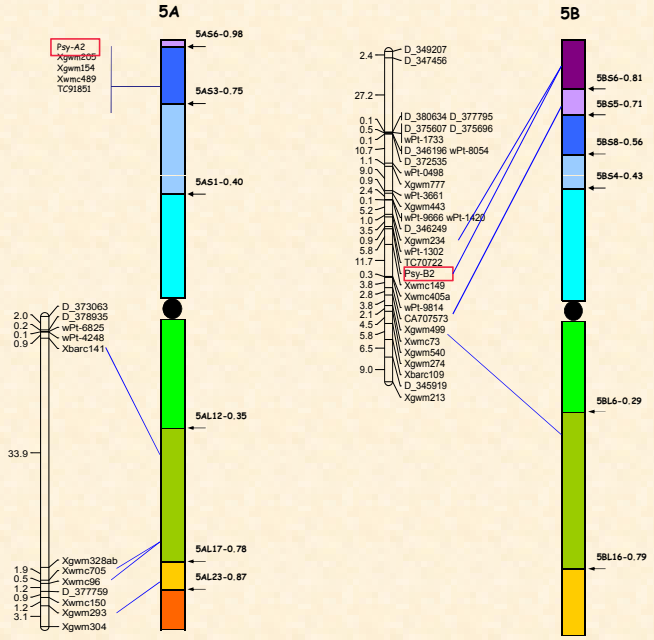

Fig. 1 Genetic and deletion mapping of *Psy-A2* and *Psy-B2* genes on chromosome 5A and 5B

QTL detection

The Simple Interval Mapping (SIM) method was employed for QTL mapping using QGene 4.0. Putative QTLs for carotenoid content in individual environments are listed in Table 2. SIM identified three QTLs on chromosome arms 3BL, 5AL and 7AL, accounting for a large proportion of the total phenotypic variation. The amount of phenotypic variation explained by individual QTLs (R^2) ranged from 12.2 to 50.2%. The major QTL with an additive effect of 1.21-1.51 ppm was detected on chromosome arm 7AL, most likely corresponding to the *Psy-A1* gene. The QTL on 5AL was also consistent in both environments, with additive effect of 0.75-0.76 ppm and explaining 13.0-18.7% of phenotypic variation. The minor QTL on 3BL was significant only in a single environment. *Psy-B2* was not associated with carotenoid content

Table 2. Simple interval mapping results for carotenoid content in F_3 progenies derived from the cross Latino x Primadur

Chromosome arm	Closest marker	Valenzano 2006			Gaudiano 2006		
		LOD	Effect	R^2	LOD	Effect	R^2
3B	Xgwm299L	ns			3.4*	0.66	12.2
5AL	Xwmc150	5.4***	-0.76	18.7	3.6*	-0.75	13.0
7AL	Xcfa2240	15.6***	-1.21	44.8	19.0***	-1.51	50.2

LOD = Logarithm-of-odd; Effect = Additive effect with positive and negative values indicating contribution of parent Latino and Primadur, respectively; R^2 = Phenotypic variation explained by QTL (%)

*, **, *** LOD score detected by permutation test significant at the P≥0.05, 0.01, 0.001 levels, respectively; ns = LOD score not significant.

Conclusion

❖ *Psy-A2* and *Psy-B2* genes were located on the short arms of 5A and 5B chromosomes (bins 5AS3-0.75-0.98 and 5BS6-0.81-1.00), respectively.

❖ The genetically mapped *Psy-B2* was not associated with phenotypic variation for grain carotenoid content