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# Characterization of Phytoene synthase 2 (*Psy2*) genes in wheat

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**Abstract.** Phytoene synthase (*Psy*) is a key enzyme responsible in plant metabolism of carotenoids. *Psy* genes are important for their contribution to flour color and nutritional aspects in human diet since they are precursor of vitamin A. In the grass family, *PSY* are nuclear enzymes encoded by a small gene family consisting of three genes: *Psy1*, *Psy2*, and *Psy3* localized on group 7 and 5 chromosomes.

The goal of our study was to characterize *Psy2* gene sequences and to verify its assessment with quantitative trait loci (QTL) involved in carotenoid expression in durum wheat. In the work we described the isolation of the *Psy2* sequences on A and B genomes in durum wheat cvs. Latino and Primadur characterized by a different carotenoid content. *Psy-A2* (2,593 bp) and *Psy-B2* (2,646 bp) were comprised of 6 exons separated by 5 introns. Alignment with *Brachypodium* and rice genomes confirmed the intron/exon structure. The study localized *Psy2* genes on chromosomes 5B and revealed the absence of linkage with QTLs for carotenoid content.

**Keywords.** Durum wheat – Phytoene synthase gene – Carotenoid.

## Caractérisation des gènes de la phytoène synthase 2 (*PSY2*) du blé

**Résumé.** La phytoène synthase (*Psy*) est une enzyme clé responsable du métabolisme des caroténoïdes chez les plantes. Les gènes *Psy* sont importants pour leur contribution à la couleur de la farine et aux aspects nutritionnels dans l'alimentation humaine, car ils sont précurseurs de la vitamine A. Dans la famille des graminées, les *PSY* sont des enzymes nucléaires codées par une petite famille de gènes composée de trois membres : *Psy1*, *Psy2*, et *Psy3*, localisés sur les groupes chromosomiques 7 et 5.

Le but de notre étude était de caractériser les séquences du gène *Psy2* et de vérifier son évaluation avec des locus de caractères quantitatifs (QTL) impliqués dans l'expression des caroténoïdes chez le blé dur. Dans ce travail, nous avons décrit l'isolement des séquences *Psy2* dans les génomes A et B du blé dur cvs. Latino et Primadur, caractérisés par une différente teneur en caroténoïdes. Les *Psy-A2* (2593 pb) et *Psy-B2* (2646 pb) sont constitués de 6 exons séparés par 5 introns. L'alignement avec les génomes de *Brachypodium* et du riz a confirmé la structure intron/exon. L'étude a localisé les gènes *Psy2* sur le chromosome 5B et a révélé l'absence d'association avec les QTL pour la teneur en caroténoïdes.

**Mots-clés.** Blé dur – Gène de la phytoène synthase – Caroténoïdes.

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## I – Introduction

Yellow pigment content (YPC) represents one of the major criteria in the assessment of durum wheat semolina quality. It is important in determining the commercial and nutritional quality of end-products such as pasta. Semolina colour is influenced by several factors, including the carotenoid pigments accumulation in grain (Panfili *et al.*, 2004), the oxidative degradation processes and the transformation events of end-products (Borrelli *et al.*, 1999).

The carotenoid biosynthesis involves several enzymatic steps, among which the step catalyzed by phytoene synthase (*PSY*) is assumed to be the rate-limiting one (Hirschberg, 2001). Phytoene synthase encodes for an enzyme responsible of the first step of C40 phytoene compound formation condensing two geranylgeranyl diphosphate molecules (GGDP).

In the grass species, *Psy* genes are classified into three paralogous sub-families: *Psy1*, *Psy2*, and *Psy3* (Li *et al.*, 2008). The three *Psy* genes were characterized in rice (Gallagher *et al.*, 2004; Welsh *et al.*, 2008), maize (Li *et al.*, 2008), sorghum (Fernandez *et al.*, 2008) and recently in wheat (Pozniak *et al.*, 2007; Dibari *et al.*, 2012).

In wheat the YPC is under complex genetic control and several QTLs have been located on chromosomes 3A (Parker *et al.*, 1998), 3B (Patil *et al.*, 2008), 5A (Hessler *et al.*, 2002), 7A and 7B (Crawford *et al.*, 2011, 2013). However, the group 7 chromosomes appeared to contain genes critical for the carotenoid expression (Zhang *et al.*, 2008; Blanco *et al.*, 2011). Indeed *Psy1* locus was located on the long arm of group 7 chromosomes where a major QTL for YP was detected (Pozniak *et al.*, 2007; Zhang and Dubcovsky, 2008; Blanco *et al.*, 2011). The role and function of *Psy1* have been largely investigated since it was correlated with accumulation of endosperm carotenoids.

Partial sequences of *Psy2* from several durum wheat varieties are available, but there are no information about its function. Cenci *et al.* (2004) and Pozniak *et al.* (2007) located this locus on the short arm of group 5 chromosomes with no clear association to carotenoid content. On the same chromosome group (arm 5L), *Psy3* have been recently mapped and characterized. Dibari *et al.* (2012) showed their expression in roots during stress conditions (drought and salt stress) underlining the *Psy3* roles in the downstream carotenoid and abscisic acid (ABA) accumulation.

In the present work, we focused our attention on *Psy2* gene with the objectives: (a) to isolate and characterize the complete genomic sequences of this gene in the A and B genomes of wheat; (b) to develop and map functional markers for *Psy2*; (c) to assess the linkage between *Psy2* gene and QTLs for carotenoid pigment content.

## II – Material and methods

A set of 121 F<sub>2</sub>:F<sub>3</sub> families derived from crossing two durum wheat cultivars, Latino and Primadur (characterized by low and high values of carotenoid content), were used for *Psy2* mapping. Nulli-tetrasomic, di-telosomic, and deletions lines (NTs) of *Triticum aestivum* cv. Chinese Spring (Sears 1954; Sears and Sears 1978; Endo and Gill 1996) were used for the physical location of *Psy2* amplicons on chromosome bins. Genomic DNA was isolated from young leaves using the protocol published by Dvorak *et al.* (1998).

The identification of *Psy2* genes in *Brachypodium* (Bradi4g01100) and *Oryza sativa* (Os12g43130) genomes was carried out searching in BLASTn (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Phytozome database (<http://www.phytozome.net/>). The physical mapping and the functional annotation in genomes of closed related species to wheat genome were obtained using the COGE website (<http://genomeevolution.org/CoGe/CoGeBlast.pl>) (Lyons *et al.*, 2008). An expectation value (E) of e<sup>-10</sup> was used as significant threshold. All detected sequences were aligned and analysed using ClustalW tools (<http://www.ebi.ac.uk>).

To isolate the phytoene synthase genes in wheat, we used partial cDNA sequences from durum wheat (DQ642445, DQ642446, DQ642441 and DQ642442) identifying *Psy-A2* and *Psy-B2*, respectively (Pozniak *et al.*, 2007). The reconstruction of the complete gene sequences in wheat ran out with the cereals databases (<http://www.cerealsdb.uk.net/>).

A set of primer pairs was designed using Primer3 (<http://frodo.wi.mit.edu/primer3/>) to cover the entire gene sequence in the genome A and B of the hexaploid cv. Chinese Spring. Primer design was focused mostly on the nucleotide regions characterized by polymorphisms between the homoeologous wheat genes. The primer sequences used for the physical mapping are: for (5'-CCTCTCTGACACGGCGTCA-3') and rev (5'-AGGTCATATACCTCGATTTCCAA-3') primers for A genome, for (5'-TTGGAATCGAGGTATATGACCT-3') and rev (5'-ACTGGACGAACTGG

CACAG-3') primers for B genome. Single PCR fragments were directly purified with EuroGold Cycle Pure Kit and sequenced following the manufacturer's instructions (<http://www.bmr-genomics.it/>).

In order to investigate the role of *Psy2* genes in cv. Chinese Spring and to have a preliminary correlation between YPC and gene transcript, an analysis *in silico* was carried out against a wheat 61K microarray platform (Dash *et al.*, 2012). The Plant Expression Database (PLEXdb) Blast (E value <-10) allowed to identify the corresponding probe set on wheat GeneChip.

### III – Results and Discussion

*Psy* genes play a key role in the biosynthesis of carotenoids components, important for their impact on flour color and on human diet for nutritional aspects. The syntenic relationships within monocots (rice, maize, *Brachypodium*, sorghum and *Triticeae*) are recently reassessed and allow the development of tools to identify precisely chromosome-to-chromosome orthologous relationships (Colasuonno *et al.*, 2013).

The analysis, based on BLASTn and Phytozome databases, allowed the isolation of the *Psy2* gene in *Brachypodium* and rice genomes. In particular, in *Brachypodium* genome the *Psy2* gene, located on chromosome 4 (locus name: Bradi4g01100) (Fig.1a), consisted of 2,560 bp length, mRNA sequence of 1,185 bp length and protein of 394 amino acids. In rice, the *Psy2* gene was mapped on chromosome 12 (Os12g43130) and had a genomic sequence of 2,791 bp, a mRNA of 1,197 nucleotides and a protein of 398 amino acids. In both species, the gene had 5 introns and 6 exons structure. The syntenic analysis allowed the isolation of a partial wheat gene sequences. The 5' UTR region of *Psy2* genes showed high GC content regions. The complete gene sequences were obtained using primer pairs derived from the contig reconstruction sequences by Cereals db website.

The *Psy-A2* and *Psy-B2* genomic structures and sequences were obtained for the cv. Chinese Spring and confirmed in the two durum wheat cultivars (Latino and Primadur). The *Psy-A2* genomic sequence was 2,592 bp long with 50.2% GC content. The predicted gene sequence counted out a region 811 bp long of 5' untranslated sequence and 1,049 bp long of 3' untranslated sequence. The mRNA had a 1,193 bp length translating for 397 amino acids. The *Psy-B2* genomic sequence had a length of 2,645 bp (50.5% GC content) and a mRNA region of 1,193 bp. The 5' and 3' untranslated sequences was of 778 bp and 747 bp, respectively. The predicted protein length consisted of 397 amino acids. In both genes, the intron borders matched the canonical plant intron borders (GT..AG) for all 5 introns. Consensus exon/introns boundaries were predicted using Softberry program (Fig.1). The two homoeologous wheat genes shared the same number of introns/exons with no differences in the open reading frame (ORF), an identity of 93% and 98% between genomic and mRNA sequences. In comparison with model species, *Psy-A2* gene shared a sequence identity of 88% with *Brachypodium* and rice, whereas *Psy-B2* gene presented a sequence identity of 92% with *Brachypodium* and of 95% with rice (Fig. 1).

The *Psy2* genes were physically mapped on wheat group 5 chromosomes using NT lines. They were localized on chromosome bin 5AS3-0.75-0.98 and 5BS6-0.18-1.00, respectively. The Latino x Primadur linkage map reported by Blanco *et al.* (2011) and Colasuonno *et al.* (2013) was used for the *Psy2* genetic mapping. The *Psy-A2* and *Psy-B2* functional markers designed on the basis of *Psy2* sequences produced polymorphic fragments only for the B genome. In details *Psy-B2* marker produced polymorphic fragments of 491 bp in the cv. Latino and 483 and 519 bp in the cv. Primadur. The segregation analysis in the Latino x Primadur population integrated the *Psy-B2* marker into a linkage group of 112 cM including 37 markers (15 SSRs, 3 EST-SSRs and 19 DArTs).

**Figure 1. *Psy2* gene characterization. a) Schematic representation of physical position of *Psy2* on wheat chromosomes was showed. The physical mapping was referred to cv. *Chinese Spring*. b) Comparison of *Psy2* gene structure between *Oryza sativa*, *Brachypodium* and *Triticum aestivum* was presented basing on coloured boxes highlighting conserved exons. Introns and exons sizes were reported as well as the total gene (in brackets). cDNA and genomic sequence lengths were reported next to colour boxes.**

The segregant population Latino × Primadur was evaluated for carotenoid content in four field trials in southern Italy as reported by Blanco *et al.* (2011). The ICIM (Inclusive Composite Interval Mapping) analysis detected the QTL on chromosome arm 5BS, flanked by *Xwmc73* and *wPt-3661*, closest to *Xgwm274* marker. The analysis revealed a different localization of the detected QTL-5B and *Psy-B2* gene, indicating the absence of the gene association to carotenoid content.

To further investigate the role of *Psy2* genes during plant development, a comparative matching analysis was done using the wheat 61k microarray platform at Plant Expression Database (PLEXdb). The BLAST search identified the probe Ta.18880.1.S1\_at that had a high sequence identity with *Psy2*. The probe expression showed a constant pattern during wheat development suggesting its involvement in several biological mechanisms rather than in the endosperm development phase as the *Psy1*. This could justify an alternative *Psy2* role as detected in rice by Welsh *et al.* (2008). Indeed they detected PSY2 transcript levels increments in response to illumination during greening.

The present analysis identifies suitable *Psy2* markers and assesses the absence of linkage between the gene and QTLs for YPC, as confirmed by the 35 cM of distance between them in the linkage group map. This suggests a different role of *Psy2* in carotenoid expression, compared the other PSY gene family members (PSY1 and PSY3) involved directly in the control of the agronomic trait and resistance of stress, respectively.

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