

LITERATURE REVIEW

Improving durum wheat (*Triticum turgidum* L. var *durum*) grain yellow pigment content through plant breeding

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Abstract

A. Schulthess, and A.R. Schwember. 2013. Improving durum wheat (*Triticum turgidum* L. var *durum*) grain yellow pigment content through plant breeding. Cien Inv. 40(3): 475-490. Wheat grain yellow pigment content (GYPC) is an important trait that determines pasta quality. The main objective of this review is to examine the genetics regulating GYPC to enhance it through breeding, leading to improved pasta quality. Although GYPC is a polygenic trait, its high heritability has facilitated breeding internationally. GYPC is influenced by one or two major loci with additive effects plus several minor genes, and there is evidence showing that the phytoene synthase loci *PSY1A* and *PSY1B* are strong candidate genes that regulate GYPC. Nine Chilean durum wheat (*Triticum turgidum* L. var. *durum*) genotypes showed intermediate to low levels of GYPC based upon both phenotypic and genotypic data. The next step is to improve GYPC in those materials by introgressing the high-yellowness *PSY1* allelic variants (*i.e.*, the *PSY1A_o* allele and the *PSY1B_b* allele) using plant breeding strategies such as backcrossing and marker-assisted selection.

Key words: durum wheat, grain yellow pigment content, improved color, *PSY1A*, *PSY1B*, backcrossing, marker-assisted selection.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is the only tetraploid species of wheat of commercial importance that is widely cultivated today (Blanco *et al.*, 1998; Shewry, 2009). It is used in different parts of the world for several food products, such as pasta, couscous, unleavened bread, bulgur, and mote, among others (Nachit, 1992). However, the main use of durum wheat is pasta making because of its high protein content

and vitreousness (Troccoli *et al.*, 2000; Ficco *et al.*, 2009). It has been postulated that this type of wheat was developed by artificial selection of the domesticated emmer wheat strains formerly grown in Central Europe and the Near East around 7000 B.C., which developed a naked, free-threshing form (Zohary and Hopf, 1993). In contrast to common (bread) wheat (*Triticum aestivum* L.), which after grinding produces particles of flour smaller than 212 μm (Mellado, 2007), durum wheat milling produces a coarse particle called semolina that it is used for pasta production (Sissons, 2008) and whose particle size ranges between 125 and 355 μm (Mellado, 2007). Furthermore, its intense

semolina yellow color (Kneipp, 2008), high protein content, gluten content, and strength make durum wheat ideal for pasta making purposes (Harlan, 1995).

In general, there is a positive correlation between high semolina yellowness and pasta quality (Borrelli *et al.*, 2003). Pasta color is highly associated with the consumer's choice, and the competition in the pasta market has made this trait even more important (Dexter and Marchylo, 2001), especially after the legal ban of the use of artificial coloring in pasta production in certain countries in Europe (Hare, 2006). Pasta color essentially depends on the combination of semolina yellowness and brownness (Porceddu, 1995). The desirable yellowness of pasta and semolina comes mostly from carotenoid pigments (Borrelli *et al.*, 2008), mainly trans-lutein at the semolina level (Hentschel *et al.*, 2002; Ramachandran *et al.*, 2010), and their oxidative degradation (Borrelli *et al.*, 2008). Two groups of objective methods are used for the evaluation of the yellow color of pasta, flour or semolina from wheat. The first group of methods is based upon the extraction of pigments using *n*-butanol followed by the estimation of their concentrations using spectrophotometry techniques such as the standard 14-50 method (AACC, 1995) or the ICC 152 method (Hentschel *et al.*, 2002). The second method relies on a reflectance colorimeter for recording the b^* value (Digesù *et al.*, 2009), which denotes yellowness when positive (Feillet *et al.*, 2000).

The main objective of this review is to examine the genetics regulating the endosperm yellowness with the aim of improving grain yellow pigment content (GYPC) and pasta quality through durum wheat breeding.

Desirable traits and Chilean institutions involved in durum wheat breeding

The superior cultivars of durum wheat encompass traits such as high semolina yellowness, high

grain protein content, pasta firmness, and minimal cooking loss (Troccoli *et al.*, 2000; Sissons *et al.*, 2005). The yellow color is also desired for the yellow alkaline noodles that are made from common wheat (Mares and Campbell, 2001) and consumed in Japan and southeastern Asia, in which GYPC contributes significantly to their color (Fu, 2008). In this sense, wheat lines for these products need higher GYPC (Kruger *et al.*, 1992). Conversely, lower GYPC values are preferred for Chinese-style foods such as steamed bread and Chinese noodles made from common wheat (He *et al.*, 2004; Fu, 2008) because these products require a bright white to creamy flour color (Mares and Campbell, 2001).

In Chile, the INIA (Instituto de Investigaciones Agropecuarias) is the institution that has carried out most of the durum wheat breeding activities nationally, and it has launched fifteen cultivars of durum wheat between 1956 and 2002, covering approximately 90% of the national crop surface (Matus, 2007). To avoid using artificial coloring, improving the content and the quality of GYPC is an essential and central topic related to future plant breeding programs of durum wheat that would benefit both the consumers and the national pasta industry (Schulthess, 2013). One of the INIA main objectives in the medium and long term is to improve the semolina yellowness of durum wheat from 20 to 26 on the Minolta colorimetric scale (Matus, 2007). The second durum wheat breeding program, in order of importance, is associated with the Pontificia Universidad Católica de Chile (PUC). This program was active between 1972 and 2008 and then was resumed in 2010. It has launched over ten cultivars of durum wheat, common wheat and triticale, based primarily on materials introduced from Mexico belonging to the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). This program has relied on hybridizations and selections that have been made in Chile, and Ambra UC and Brescia UC are durum wheat cultivars that continue to be used by growers (Schwember, 2012).

There have been no breeding efforts for improving the semolina color in Chile until recently, which is evidenced by the medium to low GYPC levels of nine Chilean genotypes (eight cultivars plus one advanced breeding line) compared to the values of other durum and common wheat materials bred in North America and Australia (Schulthess, 2013) (Figure 1).

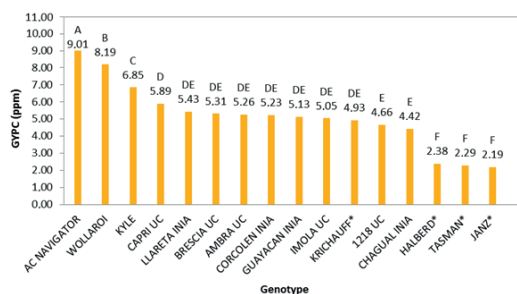


Figure 1. Grain yellow pigment content (GYPC) (ppm) values of the eight main durum wheat Chilean cultivars, Chagual INIA, Corcolén INIA, Guayacán INIA, Llareta INIA, Ambra UC, Brescia UC, Capri UC, and Imola UC and the advanced breeding line 1218 UC showed intermediate to low levels of semolina yellowness relative to other elite international genotypes (AC Navigator, Wollaroi and Kyle) cultivated under the same environmental conditions. GYPC was determined by spectrophotometry as described by Zhang and Dubcovsky (2008), and three replicates per genotype were averaged. Genotypes that share letters are not significantly different from each other with 95% confidence based on the Student-Newman-Keuls (SNK) test. Asterisks indicate common wheat cultivars (Schulthess, 2013).

Carotenoids and the genetic control of endosperm yellowness

The carotenoid pigment family comprises more than 750 members that are present in plants, bacteria and fungi and constitutes the second most abundant class of naturally occurring pigments (Britton, 1998; DellaPenna and Pogson, 2006). All carotenoids are derived from phytoene, and most of them are C₄₀ polyenes. They play a crucial role in photosynthesis, being required for the correct assembly of the photosystems and light-harvesting complexes, and as photoprotective compounds by limiting oxidative damage (Demmig-Adams and Adams, 1996; Yamamoto *et al.*, 1999; Cuttriss *et al.*, 2006). In addition to their photosynthetic functions, carotenoids are

involved in plant reproduction through their color and natural aromas attracting birds and insects. They are also essential components of the human diet because β -carotene is a precursor to vitamin A (Yeum and Russell, 2002), and lutein and zeaxanthin have been associated with the prevention of age-related macular degeneration and cataracts (Landrum and Bone, 2004). Vitamin A deficiency, a major problem in parts of the developing world, can result in permanent blindness and increased susceptibility to infectious diseases (West and Darnton-Hill, 2001).

There has been an increasing focus to study carotenoids in non-green tissues, particularly in grains produced by staple food crops such as cereals (Howitt and Pogson, 2006). The majority of the research has been conducted in maize, as the grains contain high levels of carotenoids, and mutants are readily available (Howitt *et al.*, 2009). Research has focused on increasing the carotenoid content of maize or changing the relative proportion of various carotenoids within the grain through conventional breeding (Harjes *et al.*, 2008). As a result, a number of biosynthetic genes involved in carotenogenesis have been characterized (Buckner *et al.*, 1990; Hable *et al.*, 1998; Matthews *et al.*, 2003; Singh *et al.*, 2003; Gallagher *et al.*, 2004; Li *et al.*, 2007), and some of them are linked to quantitative trait loci (QTL) for carotenoid content (Wong *et al.*, 2004; Chander *et al.*, 2008; Harjes *et al.*, 2008). Rice, which does not contain any carotenoids in the endosperm, has been genetically engineered to accumulate β -carotene in this seed tissue (Ye *et al.*, 2000; Paine *et al.*, 2005). In contrast, research into carotenoid biosynthesis and content in wheat endosperm is not as advanced. This reflects the complexity of wheat due to its polyploid nature, causing it to possess various homeoforms of every gene. Nonetheless, wheat is an interesting model, where an almost white endosperm still has variation in color, for which there are identified QTLs (Howitt *et al.*, 2009). The color of the wheat endosperm is the major determinant of flour color and is primarily influenced by its

carotenoid content (Mares and Campbell, 2001), which requires a complex metabolic pathway for synthesis, involving at least ten different enzymes (Hirschberg, 2001) (Figure 2).

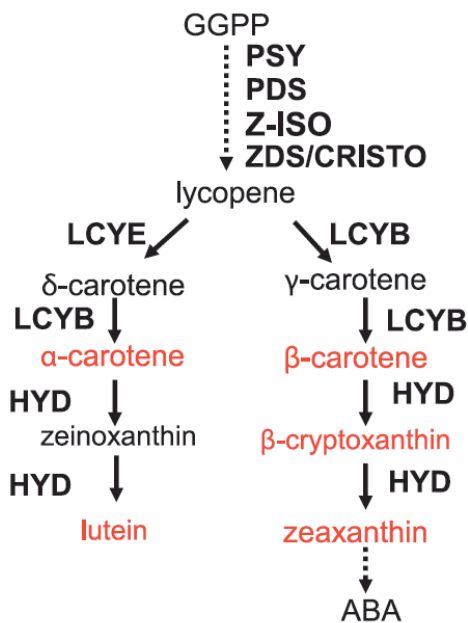


Figure 2. Summary of the synthesis of various carotenoid compounds and the enzymes involved in the pathway (Harjes *et al.*, 2008). Abbreviations: GGPP: geranylgeranyl pyrophosphate; PSY: phytoene synthase; PDS: phytoene desaturase; Z-ISO: zeta-carotene isomerase; ZDS: zeta-carotene desaturase, CRISTO: carotenoid isomerase; LCYE: lycopene epsilon cyclase; LCYB: lycopene beta cyclase; HYD: carotene hydroxylase; ABA: abscisic acid.

The main carotenoid pigment of wheat grains is lutein (Kaneko *et al.*, 1995; Panfili *et al.*, 2004), a compound that contributes to the organoleptic quality of pasta (yellowness) (Hentschel *et al.*, 2002). Lutein has been used as supplement in the treatment of eye diseases and to protect visual function since the 1950s, although its nutritional function is unknown. The nutritional function of a compound refers to its essentiality in the diet and thus its capacity to prevent deficiency states. As there is no clinical condition reported in humans specifically associated with lutein deficiency, this carotenoid pigment cannot satisfy the definition of a nutrient (Granado *et al.*, 2003). However, yellowness is a desirable trait for

pasta and therefore an important target in durum wheat breeding programs. Conversely, white flour varieties are usually selected in common wheat breeding programs because yellow pigments are considered a detrimental quality for breadmaking. Consequently, both durum and common wheat breeders can benefit from a better understanding of the genetic factors controlling GYPC. Such knowledge will also be used in future attempts to engineer the nutritionally important carotenoid pathway in cereals (Zhang and Dubcovsky, 2008).

The yellow pigmentation of the endosperm is mainly controlled by additive gene effects and has high heritability in durum (Elouafi *et al.*, 2001) and common wheat (Mares and Campbell, 2001). Multiple QTLs linked to endosperm yellowness have been reported in the literature in the last years (Table 1). Genetic studies on GYPC or endosperm yellowness have been somewhat constrained because some of the first QTLs associated with grain pigment were identified in crosses, including wild tetraploid parental lines (Joppa *et al.*, 1997; Gonzalez-Hernandez *et al.*, 2004), and therefore, they were thought to have limited application to modern durum wheat germplasm. However, many studies have been conducted to localize for genetic factors associated with endosperm yellowness variation in wheat (Table 1), and the results indicate that the trait is influenced by one or two major loci plus several minor genes (He *et al.*, 2009b). Additionally, non-additive genetic effects such as epistatic interactions have also been reported for this trait in durum wheat (Zhang *et al.*, 2008; Roncallo *et al.*, 2012). Elouafi *et al.* (2001) and Pozniak *et al.* (2007) identified a region towards the end of chromosome 7B of durum wheat with major QTLs linked to GYPC. Zhang and Dubcovsky (2008) also reported a QTL associated with GYPC in this region linked to a SSR marker, *Xgwm146*, in agreement with the result reported by Pozniak *et al.* (2007), suggesting that the three QTLs identified in different durum wheat populations were the same locus. Similarly, GYPC-linked QTLs near the end of chromosome 7A have also been

Table 1. Chromosome locations and reported references related to quantitative trait loci (QTL) regulating wheat endosperm yellowness (*Triticum* sp.) identified by linkage mapping or association mapping (Schulthess, 2013).

Chromosome	References
1A	Patil <i>et al.</i> , 2008 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang <i>et al.</i> , 2009 ¹ ; Sadeque and Turner, 2010 ¹
1B	He <i>et al.</i> , 2008 ¹ ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang <i>et al.</i> , 2008 ² ; Zhang <i>et al.</i> , 2009 ¹ ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ²
1D	-
2A	Pozniak <i>et al.</i> , 2007 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Blanco <i>et al.</i> , 2011 ² ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ²
2B	Reimer <i>et al.</i> , 2008 ^{2,3} ; Sadeque and Turner, 2010 ¹
2D	Mares and Campbell, 2001 ¹
3A	Parker <i>et al.</i> , 1998 ¹ ; Mares and Campbell, 2001 ¹ ; Reimer <i>et al.</i> , 2008 ^{2,3}
3B	Mares and Campbell, 2001 ¹ ; Patil <i>et al.</i> , 2008 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Howitt <i>et al.</i> , 2009 ¹ ; Sadeque and Turner, 2010 ¹ ; Blanco <i>et al.</i> , 2011 ²
3D	-
4A	Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang <i>et al.</i> , 2008 ² ; Zhang <i>et al.</i> , 2009 ¹ ; Roncallo <i>et al.</i> , 2012 ²
4B	Mares and Campbell, 2001 ¹ ; Pozniak <i>et al.</i> , 2007 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang <i>et al.</i> , 2008 ² ; Sadeque and Turner, 2010 ¹
4D	-
5A	Reimer <i>et al.</i> , 2008 ^{2,3} ; Blanco <i>et al.</i> , 2011 ² ; Roncallo <i>et al.</i> , 2012 ²
5B	Mares and Campbell, 2001 ¹ ; Patil <i>et al.</i> , 2008 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Sadeque and Turner, 2010 ¹ ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ²
5D	Mares and Campbell, 2001 ¹
6A	Mares and Campbell, 2001 ¹ ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang <i>et al.</i> , 2008 ² ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ²
6B	Pozniak <i>et al.</i> , 2007 ² ; Reimer <i>et al.</i> , 2008 ^{2,3}
6D	-
7A	Parker <i>et al.</i> , 1998 ¹ ; Elouafi <i>et al.</i> , 2001 ² ; Mares and Campbell, 2001 ¹ ; He <i>et al.</i> , 2008 ¹ ; Patil <i>et al.</i> , 2008 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang and Dubcovsky, 2008 ² ; Zhang <i>et al.</i> , 2008 ² ; Howitt <i>et al.</i> , 2009 ¹ ; Singh <i>et al.</i> , 2009 ² ; Zhang <i>et al.</i> , 2009 ¹ ; Blanco <i>et al.</i> , 2011 ² ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ² ; Ravel <i>et al.</i> , 2013 ¹
7B	Elouafi <i>et al.</i> , 2001 ² ; Mares and Campbell, 2001 ¹ ; Kuchel <i>et al.</i> , 2006 ¹ ; Pozniak <i>et al.</i> , 2007 ² ; Patil <i>et al.</i> , 2008 ² ; Reimer <i>et al.</i> , 2008 ^{2,3} ; Zhang and Dubcovsky, 2008 ² ; Zhang <i>et al.</i> , 2008 ² ; Pozniak <i>et al.</i> , 2012 ^{2,3} ; Roncallo <i>et al.</i> , 2012 ² ; Ravel <i>et al.</i> , 2013 ¹
7D	-

¹ Use of hexaploid genetic background (*Triticum aestivum* x *Triticum aestivum*).

² Use of tetraploid genetic background (*Triticum turgidum* spp. *durum* x *Triticum turgidum* spp. *durum* or x *Triticum dicoccoides*).

³ Marker-phenotype associations identified by association mapping (otherwise by linkage mapping).

identified in durum wheat (Elouafi *et al.*, 2001; Patil *et al.*, 2008), which gives the chance for a homeologous copy of the QTL on chromosome 7B to exist. In addition, major QTLs on chromosomes 7A and 7B were detected in common wheat (Parker *et al.*, 1998; Ma *et al.*, 1999; Mares and Campbell, 2001; Kuchel *et al.*, 2006; He *et al.*, 2008; Zhang *et al.*, 2009), demonstrating the importance of these two chromosomes for the endosperm yellowness.

Phytoene synthase: the main candidate gene for endosperm yellowness

The enzyme phytoene synthase (PSY) catalyzes the dimerization of two molecules of geranylgeranyl pyrophosphate into phytoene (Dogbo *et al.*, 1988) and is considered the rate-limiting enzyme in the accumulation of carotenoids in seeds (Lindgren *et al.*, 2003) (Figure 2). The *PSY* gene underwent duplication during the evolution of Angiosperm

plants, resulting in two paralogous genes (*PSY1* and *PSY2*) in dicotyledonous species, whereas monocotyledonous species have one additional paralogous copy, resulting in the genes *PSY1*, *PSY2*, and *PSY3* in the genomes of the grass family (Dibari *et al.*, 2012). The duplication of the *PSY* gene in grasses could provide finer control of carotenoid biosynthesis under different regulation scenarios. For instance, a specific mechanism could be provided to alter gene expression in the seeds without causing deleterious effects on the photosynthetic organs (Li *et al.*, 2008). In maize, some studies showed that only *PSY1* exhibited strong association with endosperm yellowness (Palaisa *et al.*, 2003) or carotenoid content (Gallagher *et al.*, 2004; Li *et al.*, 2008). In agreement with this result, the homeologous copies of *PSY1* were mapped within the GYPC-linked QTLs positioned on the chromosomes 7A (Singh *et al.*, 2009) and 7B (Pozniak *et al.*, 2007; Zhang and Dubcovsky, 2008) in durum wheat, suggesting that the genes responsible for those QTLs were the wheat *PSY1* orthologues.

The *PSY1* genes contain six exons and five introns in common wheat, durum wheat and related species (Wang *et al.*, 2009), maize, rice and other grasses (genera *Sorghum*, *Tripsacum*, *Zea* and *Coix*) (Fu *et al.*, 2009). The lengths of the exons 2, 3, 4 and 5 are perfectly conserved among the grass species, contrary to the introns, based upon sequencing the *PSY1* gene in several grasses (He *et al.*, 2008; Fu *et al.*, 2009; He *et al.*, 2009a; Singh *et al.*, 2009; Wang *et al.*, 2009). Because there is polyploidy in wheat, hexaploid (common) wheat possesses three genomes (A, B and D), while tetraploid durum wheat has two genomes (A and B), and thus three and two *PSY1* homeoforms, respectively, are present in these species: *PSY1A*, *PSY1B* and *PSY1D* (Howitt *et al.*, 2009).

The magnitude of the influence of the *PSY1* loci on the endosperm yellowness of wheat is erratic, and this result shows how the specific experimental context of each study affects this trait (Schulthess, 2013). Thus, the percentages of explained vari-

ability of endosperm yellowness for *PSY1A* have been low (< 10%) (Ravel *et al.*, 2013), medium (10–30%) (He *et al.*, 2008; Zhang *et al.*, 2009; Ravel *et al.*, 2013), high (30–50%) (Zhang *et al.*, 2009), and very high (> 50%) (Howitt *et al.*, 2009) in common wheat. In parallel, the corresponding *PSY1A* values of the explained variation of this trait have been medium (10–30%), high (30–50%) and very high (> 50%) (Blanco *et al.*, 2011) in durum wheat. In the case of *PSY1B*, the percentages of explained variability of endosperm yellowness reported have been very high (> 50%) in common wheat (Ravel *et al.*, 2013), and low (< 10%) to medium (10–30%) (Zhang *et al.*, 2008; Roncallo *et al.*, 2012) in durum wheat. The effect of the *PSY1D* locus on the yellowness of the endosperm has been less well studied, but recent evidence shows that it also significantly influences this trait (Ravel *et al.*, 2013). However, the percentages of endosperm yellowness or GYPC variability explained by the *PSY1* loci are not reported in several studies (Pozniak *et al.*, 2007; Reimer *et al.*, 2008; Zhang and Dubcovsky, 2008; He *et al.*, 2009a; He *et al.* 2009b; Singh *et al.*, 2009). Therefore, those values previously mentioned are only a partial sample of all cases in which the homeologous *PSY1* loci have been studied so far (Schulthess, 2013).

With regard to the allelic variants of the *PSY1* loci, the Komugi genetic resources database of wheat (Komugi, 2012) shows that eighteen, fifteen and thirteen allelic variants have been reported to date for *PSY1A*, *PSY1B* and *PSY1D*, respectively. In addition, phenotypic-genotypic association studies have investigated the effects of some of the *PSY1* allelic variants on the endosperm yellowness using different genetic backgrounds of common and durum wheat (Table 2). One ongoing study at the PUC in collaboration with INIA-Quilamapu is currently characterizing the semolina yellowness of a durum wheat population and using molecular markers to identify *PSY1A* (Singh *et al.*, 2009) and *PSY1B* (Zhang and Dubcovsky, 2008) allelic variants of those materials. Regarding *PSY1A*, the three allelic variants are

PSYIAa (1776 bp, low semolina yellowness), *PSYIAI* (1089 bp, intermediate yellowness), and *PSYIAo* (897 bp, high yellowness) (Singh *et al.*, 2009) (Figure 3). Concerning *PSYIB*, the two allelic variants correspond to *PSYIBa* (220 bp, lower semolina yellowness) and *PSYIBb* (200 bp, higher yellowness) (Reimer *et al.* 2008; Zhang and Dubcovsky, 2008) (Figure 4). In this work, the nine Chilean durum wheat genotypes studied exhibited the same PCR banding pattern with both the *PSYIAI* and *PSYIBa* alleles (Figure 3 and 4), which are associated with intermediate (Singh *et al.*, 2009) and low levels of GYPC (Reimer *et al.*, 2008; Zhang and Dubcovsky, 2008), respectively, in agreement with the phenotypic results previously reported (Figure 1).

Table 2. Marker-phenotype association studies reporting polymorphisms within the *PSYI* loci affecting endosperm yellowness of different wheat genetic backgrounds (ppm: yellow pigment content; b*: colorimetric value; Δb*: colorimetric value adjusted to the value of a control genotype) (Schulthess, 2013).

Locus	Alleles or 'haplotypes'	Genetic background	Phenotypic effect	Reference
PSYIA	a, b	217 cultivars and advanced lines of Chinese wheat	a: 1,82 ppm A b: 1,30 ppm B	He <i>et al.</i> (2008)
	a, b, c	342 spring wheat lines CIMMYT	a: 2,56 ppm A b: 2,12 ppm B c: rare allele	He <i>et al.</i> (2009a)
	e, p, q, r, s	Common wheat: Sunco, Janz, Glenlea, Tasman, Cranbrook, Chara, Halberd, Schomburgk, Krichauff	e: white p and q: pale yellow r: yellow s: very yellow	Howitt <i>et al.</i> (2009)
	'p', 'jt', 'e', 'r', 'ak', 'c'	372 accessions of common wheat from diverse origins (INRA core collection)	'p': 11,12 b* A 'jt': 11,04 b* AB 'e': 10,89 b* AB 'r': 10,76 b* AB 'ak': 10,56 b* B 'c': rare allele	Ravel <i>et al.</i> (2013)
	a, l, o	Collection of 93 durum wheat genotypes from diverse origins	o: 2,2 ppm higher than the l allele on average l: at least 2,4 ppm higher than the a allele on average a: at least 4,6 ppm lower than the o allele on average	Singh <i>et al.</i> (2009)
PSYIB	a, b, c, d	217 lines of Chinese winter wheat	c: 2,01 ppm A a: 1,71 ppm B b: 1,40 ppm C d: rare allele	He <i>et al.</i> (2009a)
	a, b, d, e	342 spring wheat lines CIMMYT	No significant differences	He <i>et al.</i> (2009a)
	f, g	100 durum wheat lines CIMMYT	f: 1,83 Δb* A g: 0,59 Δb* B	He <i>et al.</i> (2009b)
	'cm', 'b', 'a', 'd'	372 accessions of common wheat from diverse origins (INRA core collection)	'cm': 11,16 b* A 'b': 10,91 b* B 'a': 10,56 b* B 'd': 10,15 b* C	Ravel <i>et al.</i> (2013)
	a, b	Collection of 93 durum wheat genotypes from diverse origins	b: 8,96 ± 0,39 ppm a: 7,59 ± 0,19 ppm	Reimer <i>et al.</i> (2008)
PSYID	'g', 'a'	372 accessions of common wheat from diverse origins (INRA core collection)	'g': 11,37 b* A 'a': 10,64 b* B	Ravel <i>et al.</i> (2013)

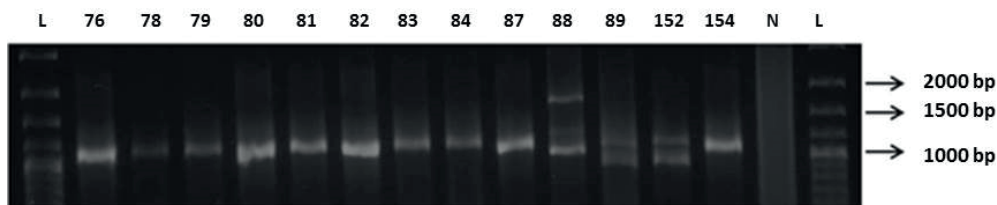


Figure 3. Allelic characterization of the *PSYIA* gene using the co-dominant marker *Psyl-A1_STS* (Singh *et al.*, 2009) on nine Chilean genotypes (eight cultivars plus one advanced breeding line) of durum wheat. All these genotypes exhibited the *PSYIAI* allelic variant, previously associated with intermediate GYPC (Singh *et al.*, 2009). Lane letters: L: GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific), N: negative control. Each lane number corresponds to a genotype: UC 1113 (88) carries the *PSYIAa* allele (1776 bp, low semolina yellowness). Wollaroi (89) and Commander (152) are positive controls for the *PSYIAo* allele (897 bp, high yellowness), and Strongfield (154) is the positive control for the *PSYIAI* allele (1089 bp, intermediate yellowness) (Singh *et al.*, 2009). The Chilean materials are the advanced breeding line 1218 UC (76) and the cultivars Ambra UC (78), Brescia UC (79), Capri UC (80), Chagual INIA (81), Corcolén INIA (82), Guayacán INIA (83), Imola UC (84) and Llareta INIA (87). The double banding pattern in the genotypes carrying alleles other than *PSYIAI* is the result of a non-specific PCR product previously described by Singh *et al.* (2009) (Schwember, unpublished results).

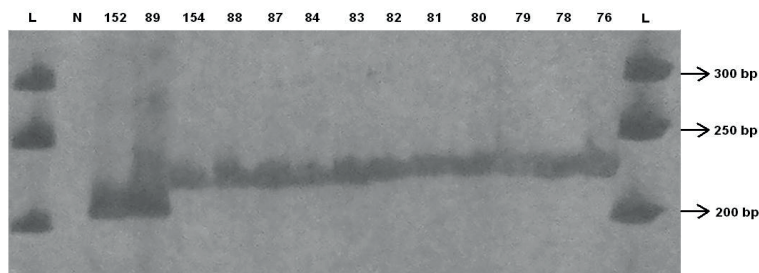


Figure 4. Allelic characterization of the *PSYIB* gene using the co-dominant marker developed by Zhang and Dubcovsky (2008) on nine Chilean genotypes, which showed exclusively the *PSYIBa* allele (220 bp). Lane letters: L: O'GeneRuler 50 bp DNA Ladder (Thermo Scientific), N: negative control. Each lane number corresponds to a genotype: Commander (152) and Wollaroi (89) are positive controls for the *PSYIBb* allele (200 bp, high levels of GYPC) (Reimer *et al.*, 2008), and Strongfield (154) (Reimer *et al.*, 2008) and UC 1113 (88) (Zhang and Dubcovsky, 2008) are positive controls for the *PSYIBa* allele (220 bp, lower levels of GYPC). The Chilean materials are Llareta INIA (87), Imola UC (84), Guayacán INIA (83), Corcolén INIA (82), Chagual INIA (81), Capri UC (80), Brescia UC (79), Ambra UC (78) and 1218 UC (76) (Schwember, unpublished results).

Other genes potentially involved in determining endosperm yellowness

Marker-phenotype associations of the *PSYI* loci with endosperm yellowness are far from being perfect, sometimes resulting in unexpected values for the trait. In this regard, some studies (He *et al.*, 2008, He *et al.*, 2009a, He *et al.*, 2009b) exhibited wide phenotypic variability between genotypes sharing a particular allelic variant of the *PSYI* loci, which possibly reflects the polygenic nature of the endosperm yellowness observed in previous studies (Table 1). In addition, not only are there studies where the *PSYI* loci explain only a small proportion of the endosperm yel-

lowness variability (Zhang *et al.*, 2008; Roncallo *et al.*, 2012; Ravel *et al.*, 2013), but there are also some extreme cases of no association due to the scarcity or the absence of polymorphisms in the regions studied within the sequence of *PSYIA* (Pozniak *et al.*, 2007, Patil *et al.*, 2008; Zhang and Dubcovsky, 2008, He *et al.*, 2009b), *PSYIB* (Singh *et al.*, 2009) or *PSYID* (Wang *et al.*, 2009; Ravel *et al.*, 2013). Additionally, in other studies (see as examples Pozniak *et al.*, 2007 and He *et al.*, 2009a) the variation within these loci simply had no significant association with the endosperm yellowness. Moreover, Zhang and Dubcovsky (2008) raised the possibility that a second gene (or another regulatory element) is involved in control-

ling wheat endosperm yellowness and maps very close to *PSYI* on the long arm of chromosome 7. There is some evidence reported to support this idea. First, the peak of a QTL associated with GYPC identified in a population of 93 recombinant inbred lines (RILs) from the cross Kofa x UC1113 does not exactly match the *PSYIB* locus but rather matches the closely linked proximal marker *Xbarc340-7B* (Zhang and Dubcovsky, 2008). Second, Roncallo *et al.* (2012), using the same mapping population but grown in different environments in Argentina, encountered an additional QTL linked to semolina yellowness (other than the *PSYIB* QTL), which was located in the distal region of chromosome 7B. Third, Singh *et al.* (2009) and Blanco *et al.* (2011) reported additional QTLs influencing semolina yellowness that were positioned 23 and 26 cM from *PSYIA*, respectively. Fourth, Zhang and Dubcovsky (2008) and Roncallo *et al.* (2012) found a QTL associated with semolina yellowness in the long arm of chromosome 7A, but the *PSYIA* region showed no polymorphisms between the parents of the RIL population. However, further studies are required to elucidate which specific gene (or regulatory element) is involved in GYPC in the vicinity of *PSYI*.

Not all the genes or the regulatory factors that affect endosperm yellowness remain anonymous. For example, another durum wheat endosperm yellowness candidate gene is *Lpx-A3*, which maps to chromosome 4A and encodes a variant of lipoxygenase that has been hypothesized to be active during early developmental stages of the wheat grains (Carrera *et al.*, 2007). Howitt *et al.* (2009) reported that ϵ -*LCY*, which encodes a lycopene epsilon cyclase enzyme (LCYE) (Figure 2), co-localizes with a QTL positioned on chromosome 3B that influences endosperm yellowness in a double haploid (DH) population derived from the common wheat cross Sunco x Tasman. Additionally, one QTL on the long arm of chromosome 2D associated with common wheat GYPC co-localizes with the *TaZds-D1* locus, which encodes a zeta-carotene desaturase

enzyme (ZDS) (Figure 2) (Zhang *et al.*, 2011). More recently, two genes (each with three homeologs), *HYD1* and *HYD2* (Figure 2), which encode for beta-hydroxylase enzymes in wheat, were cloned and characterized. Although the developing grains showed a decreasing trend in carotenoid accumulation, the expression of *HYD1*, particularly *HYD-B1*, reached its highest levels at the last stage of tetraploid and hexaploid grain development, suggesting that carotenoids (at least xanthophylls) were still actively synthesized in mature grains. This result challenges the common perception that carotenoids are simply turned over during wheat grain development after their initial biosynthesis at the early grain development stages (Qin *et al.*, 2012).

Despite the identification of alternative genes, several studies on candidate genes associated with endosperm yellowness agree on the important effect of the homeologous *PSYI* loci on GYPC (Table 2), and they will consequently remain the principal candidate genes regulating this trait, especially in durum wheat.

Transfer of QTLs or genes linked to high GYPC

One effective breeding method for transferring or introgressing a GYPC-related QTL (or gene) in self-pollinated crops (*i.e.*, wheat) is backcrossing (Figure 5). This method consists of transferring a specific trait governed by one or a few genes into an otherwise superior cultivar and fully recovering the recurrent parent genotype, but including the additional gene(s) controlling the desirable trait provided by the donor (non-recurrent) parent (Briggs and Knowles, 1967). The main advantages of backcrossing are: (1) there is a high degree of genetic control during the process; thus the outcome is predictable and repeatable, (2) the previous selection gains are not lost because it is a stepwise improvement, and (3) there is no need for extensive field trials or note taking (Briggs and Knowles, 1967; Fehr, 1987).

Conversely, the main disadvantage of backcrossing is that it does not permit the achievement of unusual combinations of genes from two or more cultivars because the effectiveness of selection decreases with an increasing number of genes under transfer (Fehr, 1987).

In marker-assisted selection (MAS), a marker (morphological, biochemical or based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants linked to the trait(s) of interest. Consequently, this method relies on genotypic rather than phenotypic selec-

tion (Dubcovsky, 2004). MAS normally greatly increases the efficiency and effectiveness in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or QTLs of interest have been identified, breeders typically use specific DNA markers as a diagnostic tool to identify plants carrying the alleles of interest of these genes or QTLs (Michelmore 1995; Ribaut *et al.*, 1997), such as the molecular markers previously mentioned for *PSYI* (Zhang and Dubcovsky, 2008; Singh *et al.*, 2009). The advantages of MAS based on biochemical and/or DNA/RNA variation include: (1) time

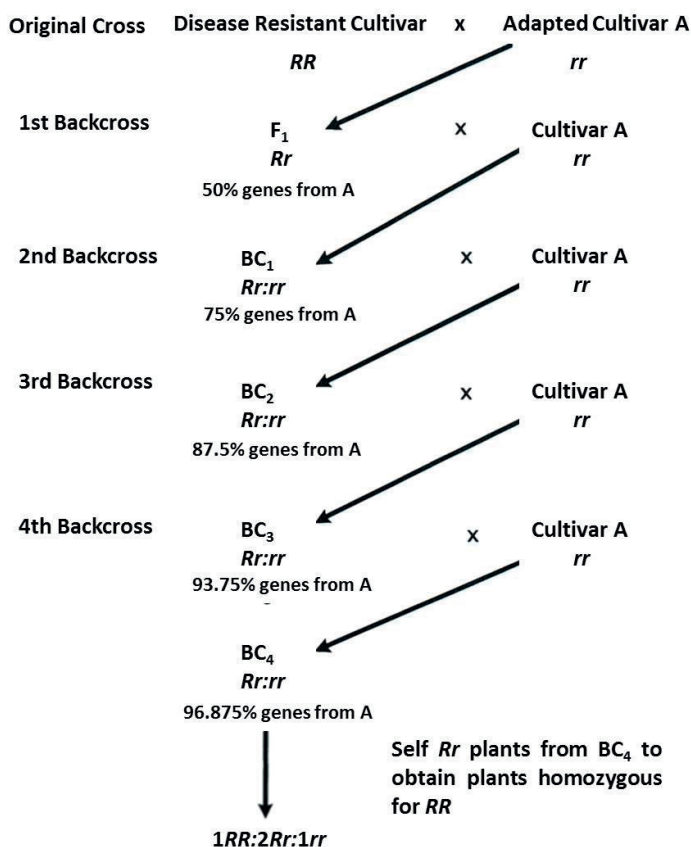


Figure 5. The backcross breeding method illustrated for a dominant allele for disease resistance (R) from a resistant cultivar (donor or non-recurrent parent) that is transferred to a susceptible, adapted cultivar A (recurrent parent). The R allele can be analogous to a high-yellowness *PSYI* allele (the *PSYIAo* allele and the *PSYIBb* allele) that is introgressed into a cultivar (recurrent parent) with very good agronomic and industrial characteristics, except for high GYPC (individuals carrying the *PSYIAI* allele and the *PSYIBa* allele). Marker-assisted selection can be performed from BC_1 onwards (Sleper and Poehlman, 2006).

savings from the substitution of complex field experiments with molecular tests, (2) elimination of unreliable phenotypic evaluation associated with environmental variation present in field trials, (3) selection of genotypes at the seedling stage, (4) gene 'pyramiding' or combining multiple genes at the same time, (5) avoiding the transfer of undesirable deleterious genes, (6) selection for traits with low heritability, and (7) testing for specific traits where phenotypic evaluation is not possible (*i.e.*, quarantine restrictions may prevent the use of exotic pathogens for screening) (Collard *et al.*, 2005).

Conclusions

Pasta color is partly determined by the intrinsic characteristics of the semolina from which it is made. In this sense, the yellow component of semolina is highly associated with GYPC, which mainly corresponds to carotenoids (especially in the form of trans-lutein). Pasta yellow color is also associated with the degradation of these pigments during grain milling and the pasta production process.

This review shows that the GYPC of nine Chilean genotypes of durum wheat is insufficient with respect to the values present in a group of international elite cultivars. The use of artificial coloring in pasta production is banned in several countries, which is an important limitation for local pasta production and potential exports. In agreement with the GYPC levels of the nine Chilean durum wheat genotypes studied, these materials carried the *PSYIAI* allele and the *PSYIBa* allele, which are associated with intermediate and low levels of GYPC, respectively. In summary, this evidence justifies ongoing and future breeding work in Chile for this particular

trait; for example, work is currently being carried out by the PUC and INIA-Quilamapu to improve the grain quality of durum wheat. This project consists of characterizing the semolina yellowness of a durum wheat base population of 124 genotypes mainly from the CIMMYT and the INIA breeding programs. This work has also focused on the genotypic characterization of the *PSYIA* and *PSYIB* loci, which are appropriate candidate genes associated with GYPC based on the existing literature. One objective of the genotypic characterization is to identify the desired haplotype for its potential introduction into the national durum wheat breeding programs. This includes identifying individuals that contain the *PSYIAo* and *PSYIBb* alleles because these alleles were associated with high GYPC values in previous studies. Subsequently, these individuals will be used as donor parents, and the alleles conferring high GYPC will be introgressed into the Chilean durum wheat cultivars using backcrossing and marker-assisted selection to efficiently accelerate the improvement of semolina yellowness of those materials. Finally, other candidate genes or markers related to GYPC could be studied in the future, and their association with endosperm yellowness could be sought in the base population to obtain better estimates of the phenotypic values already characterized and to improve the semolina yellowness predictions for plant material not yet evaluated.

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Resumen

A. Schulthess y A.R. Schwember. 2013. Mejora del contenido de pigmentos amarillos del grano de trigo candeal (*Triticum turgidum* L. var *durum*) a través de fitomejoramiento. Cien Inv. Agr. 40(3): 475-490. El contenido de pigmentos amarillos del grano de trigo (GYPC) es un rasgo de calidad importante que determina la calidad de la pasta. El objetivo principal de esta revisión de literatura es examinar la regulación genética de la amarillez endospermática para mejorar el GYPC a través del fitomejoramiento, lo que finalmente se traduciría en una mejor calidad de la pasta. Aunque el GYPC es un rasgo poligénico, es un carácter altamente heredable, aspecto que ha facilitado el trabajo de mejoramiento a nivel internacional. El GYPC está controlado por uno o dos loci principales de efectos aditivos, además de varios genes menores, y existe evidencia que demuestra que los loci de la fitoeno sintasa *PSY1A* y *PSY1B* son los principales genes candidatos que regulan el GYPC. Nueve genotipos chilenos de trigo candeal (*Triticum turgidum* L. var. *durum*) mostraron niveles intermedio a bajos de GYPC basado en datos fenotípicos y genotípicos. El siguiente paso consistirá en incrementar el GYPC de estos materiales, introgressando las variantes alélicas de *PSY1* de alta amarillez (es decir, el alelo *PSY1A_o* y el alelo *PSY1B_b*) a través de estrategias de fitomejoramiento como el retrocruzamiento y la selección asistida por marcadores moleculares.

Palabras clave: Color mejorado, contenido de pigmentos amarillos del grano, *PSY1A*, *PSY1B*, retrocruzamiento, selección asistida por marcadores, trigo candeal.

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