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Durum wheat (*Triticum turgidum L. var. durum*) a suitable vehicle for Biofortification strategies to increase yellow pigment content in staple cereal based food: A review

KEYWORDS: Durum wheat, GYPC, QTL, PSY, MAS.

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ABSTRACT *In effort to reduce micronutrient malnutrition in human populations, Biofortification is the best sustainable and cost effective way, nowadays various efforts to biofortify various staple crops are under progress. Durum wheat (Triticum turgidum L. var. durum) because of its quality and high nutritional value can serve as a suitable vehicle for biofortification of cereal based food to ensure nutritional security to humanity. Grain yellow pigment content (GYPC) is an important trait that determines pasta and other product quality. The main objective of this review is to examine the genetics regulating GYPC and to provide a comprehensive literature for wheat breeders to increase Pro vitamin A (β-carotene) for consumers. Although GYPC is a polygenic trait, its high heritability has facilitated breeding internationally. GYPC is influenced by two major loci with additive effects plus several minor genes, and this study provide evidence showing that the Phytoene synthase loci PSY1A and PSY1B are strong candidate genes that regulate GYPC, using functional markers available for these putative QTL it is easy to validate the transfer of important Psy loci for development of superior wheat varieties for GYPC.*

Introduction

Wheat is the major staple food crop in many parts of the world, contributing 28% of the world edible dry matter production and up to 60% of daily energy intake in several developing countries (Cakmak 2008; Wang et al. 2011). Durum wheat ***(Triticum turgidum L. var. durum)*** is the second most important wheat species grown in various part of world with global production estimates of 35 million tonnes (Gillen 2013. world Durum outlook CWB Market Research). Durum wheat cultivation offers many advantages like field tolerance to loose smut and Karnal bunt diseases, lodging resistance, termite resistance, higher abiotic stress tolerance etc. Durum is nutritionally superior to Bread wheat as they contain comparatively higher protein, Carotenoids and minerals like Iron, Zinc (Dick and Matsuo 1988) etc. Because of its widespread geographic distribution, acceptance, stability and versatility; wheat and its products are suitable vehicle for delivering micronutrients to mankind. Yellow pigment content of durum wheat is one of the important characteristics in determining pasta and other end products quality (Troccoli et al. 2000; Zhang et al. 2008), GYPC is also very important as per human health prospective because of its antioxidant properties and being precursor for 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). The color of the wheat endosperm is the major determinant of flour color and is primarily influenced by its Total Carotenoid Content (TCC) (Mares and Campbell 2001). 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). Synthesis of these Carotenoids requires a complex metabolic pathway for synthesis, involving at least ten different enzymes (Hirschberg 2001). IARI RS Indore is conducting research on GYPC of Durum wheat from several years, so based on our study other existing literature we can say that there is wide genetic diversity in durum wheat for Carotenoid (GYPC) content which is suitable for breeding program to develop more superior varieties for GYPC. There is a rising demand for fortified foods to fight malnutrition and Biofortification is the best sustainable and cost effective way to fulfill this demand. Durum wheat can serve as a suitable vehicle for Biofortification of cereal based food to ensure nutritional security to mankind and can be used for nutritive food preparations, because of its comparatively high and stable Carotenoid & Protein content and high levels of micronutrients.

Durum wheat Quality

The quality cannot be expressed in terms of a single property, but depends on several characteristics; each one of them is important in the production of each end-product. Durum wheat is superior in quality than bread wheat as it contains comparatively high protein (13-14%), high total Carotenoid content (6-8 ppm) and high Iron (35-40 ppm) zinc (45-50ppm) content. It is also at par in yield attributes to bread wheat and good for various processed end products like pasta. Durum wheat (*Triticum durum*) is preferred for the production of pasta or macaroni products, mainly because of its elevated level of yellow pigments and appropriate protein and gluten characteristics (Hoseney 1994; Bushuk 1998; Troccoli et al. 2000). In addition to color and protein characteristics, kernel size and vitreousness are also important in durum wheat quality, as they are strongly related to semolina yield, bright yellow appearance of semolina and cooking properties of pasta products (Hoseney 1994; Bushuk 1998; Troccoli et al. 2000; Dziki and Laskowski 2005). Protein contents of the Durum genotypes ranging from 10.7% to 16.8% with a mean of 13.1%. SDS sedimentation volumes of the Durum genotypes ranging from 20.5 to 38.0 mL (mean: 25.7 mL) and grain yellow pigment contents of the genotypes ranged from 3.2 to 8.3 mg/ kg with a mean of 5.53 mg/ kg.

Grain yellow pigment assay

Yellow pigment content

Yellow pigment content was determined on 8 g of flour extracted overnight with 40 ml of water-saturated n-butyl alcohol. After filtration of the extract through a Whatman

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No.1, a light transmission is determined in a spectrophotometer at 440 nanometers (nm) (Elouafi et al. 2001; Santra et al. 2003). The determined values were estimated according to the concentration scale based on β-carotene (AACC 1976). YP content was expressed as mg/kg using a correction coefficient 30.1 (AACC 1995).

Endosperm colour

Yellow index (YI) was determined using the reflectance colorimeter (Blanco et al. 2011) Chroma Meter CR-410 (Minolta) equipped with a pulsed xenon arc lamp. Absolute measurement in L*, a*, b* (CIE, 1986) coordinates in the Munsell colour system were taken using D65 lightning. Samples to be analyzed were placed into a granular material support. The b* value was used in subsequent analysis since it represents the variation in yellow intensity. It is the accurate and rapid method of screening large number of samples for endosperm yellowness which is positively & significantly correlated with total Carotenoid content. Hunter color parameters could also be used for measurement of endosperm yellowness (Sanchez et al. 2013).

Individual Carotenoid components

Carotenoid extraction was performed on 2 g of ground endosperm according to (Adom et al. 2003; Konopka et al. 2005) with some modifications reported by (Digesu et al. 2009). A sample volume of 20 µL was injected to an Agilent Technologies 1100 HPLC system equipped with an automatic sampler and a diode array detector (DAD) (Antonio Blanco et al. 2011). Separation was done on an YMC C30 column (250 X 4.6 mm i.d., particle size = 5 µm). Spectrophotometric detection was achieved by means of a diode array detector in the range 400-600 nm. Peaks were detected at 450 nm. Carotenoids were identified through their characteristic spectra and comparison of their retention times with commercial standards (Lutein, Zeaxanthin, β-cryptoxanthin, a-carotene and β-carotene) obtained from LGC Promochem. Carotenoid concentrations were calculated using a linear regression (concentration versus area) of the five-point standard curve.

2.0. Putative QTL for Grain yellow pigment content

The wheat grain 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 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; Parker et al. 1998; Borrelli et al. 1999; Santra et al. 2005; Patil et al. 2008; He et al. 2008). 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). The yellow colour of semolina and pasta is mainly due to Carotenoid accumulation in pericarp and endosperm. Yellow pigment concentration (GYPC) in durum wheat is a quantitative trait controlled by a complex genetic system and influenced by environmental factors. GYPC concentration has high heritability across different environment (Santra et al. 2005).Heritability estimates for GYPC in durum wheat range from 0.90 to 0.97, and controlled by additive gene effects (Nachit et al. 1995; Clarke et al. 2006; Elouafi et al. 2001). Significant marker trait association for GYPC were detected on all chromosome of the durum wheat through association mapping (Reimer et al. 2008) indicating the complexity of trait. Though the major QTL are reported on 7AL explaining 60 % of the genetic variations (Parker et al.1998; Howitt et al. 2009; Mares and Campbell 2001; Patil et al. 2008; Zhang and Dubcovsky 2008; Zhang et al. 2008-09) and 7BL explaining 53% of the genetic variation by(Elouafi et al. 2001; Kuchel et al. 2006; Pozniak et al. 2007; Zhang et al. 2008 and Zhang and Dubcovsky 2008). Minor QTL for GYPC were detected on chromosomes 3A (Parker et al. 1998), 4A and 5A (Hessler et al. 2002), 2A, 4B and 6B (Pozniak et al. 2007), 4B and 6B (Zhang et al. 2008), 1A, 3B and 5B (Patil et al. 2008), 3B and 5B (Howitt et al. 2009), 1A, 1B, 3B and 4A (Zhang et al. 2009).

Table 1: Marker-phenotype association studies reporting polymorphisms within the PSY1 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 et al. 2013).

Locus	Alleles	Genetic background	Phenotypic effect	References
Psy-A1	a, b	Chinese wheat	a: 1.82 ppm b: 1.30 ppm	He et al. (2008)
	a, b, c	spring wheat lines CIMMYT	a: 2.56 ppm b: 2.12 ppm c: rare allele	He et al. (2009)
	e, p, q, r, s	Common wheat	e: white p and q: pale yellow r: yellow s: very yellow	Howitt et al.(2009)
	'p', 'jt', 'e', 'r', 'ak', 'c'	Common wheat from diverse origins (INRA core colle tion)	'p': 11.12 b* 'jt': 11.04 b* 'e': 10.89 b* 'r': 10.76 b* 'ak': 10.56 b* 'c': rare allele	Ravel et al. (2013)
	a, l, o	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 et al. (2009)

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Psy-B1	a, b, c, d	Chinese winter wheat	c: 2.01 ppm a: 1.71 ppm b: 1.40 ppm d: rare allele	He et al. (2009)
	a, b, d, e	spring wheat lines CIMMYT	No significant differences	He et al. (2009)
	f, g	durum wheat lines CIMMYT	f: 1.83 Δb* g: 0.59 Δb*	He et al. (2009)
	'cm', 'b', 'a', 'd'	Common wheat from diverse origins (INRA core collection)	'cm': 11.16 b* 'b': 10.91 b* 'a': 10.56 b* 'd': 10.15 b*	Ravel et al. (2013)
	a, b	Durum wheat genotypes from diverse origins	b: 8.96 ppm a: 7.59 ppm	Reimer et al.(2008)
Psy-D1	'g', 'a'	Common wheat from diverse origins (INRA core collection)	'g': 11.37 b* 'a': 10.64 b*	Ravel et al. (2013)

3.0. MAS & Functional marker for Yellow pigment content

In plants, steps involved in Carotenoid biosynthesis are well reviewed (Moise et al. 2005; Romer and Fraser 2005) and phytoene synthase (psy) was found to be the key enzyme to form phytoene by condensing two molecules of geranylgeranyl pyrophosphate at the beginning of the pathway. Characterization of Psy genes and the development of functional markers are very important for accurate discrimination of contrasting alleles in marker assisted selection in wheat breeding (Bagge et al. 2007; Tommasini et al. 2006; Yang et al. 2004). Functional markers developed from polymorphic trait sites within genes that causally affect phenotypic variation are ideal tools for marker assisted selection. Several molecular markers were identified on chromosomes sets A & B. The major QTL QYp.macs-7A was located on chromosome 7AL (Patil et al. 2008) and gene sequence of phytoene synthase locus psy1-2, on chromosome 7AL, was used to design the primers specific for psy1-2; the markers Xscar807 and Xscar3362 were found to be tightly linked to the major QTL QYp.macs-7A. Carotenoid biosynthetic pathway, shows high association with the yellow pigment (YP) content in wheat grain (He et al.2008). Using an RIL population from cross PH82-2/Neixing 188, and a set of Chinese Spring nullisomic–tetrasomic lines and ditelosomic line 7AS a Psy gene (*Psy-A1*) located on chromosome 7A was characterized by in silico cloning and experimental validation. The cloned *Psy-A1* comprises six exons and five introns, 4,175 bp in total, and an ORF of 1,284 bp. A co-dominant marker, *YP7A* was developed based on polymorphisms of two haplotypes of *Psy-A1*, yielding 194 and 231-bp fragments in cultivars with high and low YP content, respectively (He et al.2008) and two allelic variants, Psy-A1a and Psy-A1b, were detected in Chinese winter wheat cultivars. A co-dominant functional marker, YP7A, was developed and validated. Subsequently, Zhang et al. (2009) demonstrated a significant influence of Psy-A1 on flour YP content. A similar study conducted on the Chinese winter wheat (He et al. 2009) cultivars revealed four allelic variants of this gene on chromosome 7B (Psy-B1), designated as Psy-B1a, Psy-B1b, Psy-B1c and Psy-B1d. The frequencies of these four alleles were 39.6, 43.8, 15.7 and 0.9%, respectively. A co-dominant marker YP7B-1 based on a 5-bp InDel of polyC in the fifth intron of Psy-B1 amplified a 151-bp PCR fragment in accessions with the medium YP content allele Psy-B1a, and a 156-bp fragment in lower YP content accessions with Psy-B1b. Two dominant markers YP7B-2 (428 bp) and YP7B-3 (884 bp) were designed for accessions with Psy-B1c and Psy-B1d, respectively. Allele Psy-B1c was associated with high YP content. Although, markers for many economically important traits have been identified in wheat, very few of them have been validated to find out their usefulness in different genetic backgrounds (Sharp et al. 2001; Prasad et al. 2003; Torada et al. 2005).

Table 2: Important functional markers for major QTL of grain Carotenoid content in wheat:

Marker	Sequence (5'-3')	Phenotypic variance (%)	specific Allele/ PCR Product size (bp)	Reference
scar807-F	GAGAGAGTCTTATCTGATGTACCG	26.8	psy1–2 (967&465bp)	Patil et al. 2008
scar807-R	GAGAGAGTGGAATCACTTTGTGAG			
scar3362-F	TTGGCTTATTCCAATGCACA	16.8	psy1–2 (255&247bp)	Patil et al. 2008
scar3362-R	TGTAAGGGCAACTCCCACAT			
F	GGACCTTGCTGATGACCGAG	20 -28	Psy-A1a(194bp) Psy-A1b(231bp)	He et al. 2008
YP7A-R	TGACGGTCTGAAGTGAGAATGA			
YP7A-2 F	GCCAGCCCTTCAAGGACATG	48-51	Psy-A1a, Psy-A1b1 (686bp) Psy-A1c (1001bp)	He et al. 2009
YP7A-2 R	CAGATGTCGCCACACTGCCA			
YP7B-1 F	GCCACAACTTGAATGTGAAAC	50.6	Psy-B1a(151bp), Psy-B1b (156bp)	He et al. 2009
YP7B-1 R	ACTTCTTCCATTTGAACCCC			
YP7B-2 F	GCCACCCACTGATTACCACTA	29.2	Psy-B1c (428 bp)	He et al. 2009
YP7B-2 R	CCAAGGTGAGGGTCTTCAAC			

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YP7B-3 F	GAGTAAGCCACCCACTGATT	19.6	Psy-B1d (884 bp)	He et al. 2009
YP7B-3 R	TCGCTGAGGAATGTACTGAC			
YP7B-4 F	AGGTACCAGCCAGCCCATA	Rare allele	Psy-B1e(717bp)	He et al. 2009
YP7B-4 R	CTCGTCAAAGTTCGTGTACC			

4.0. Conclusion

Being superior in quality and agronomic aspects Durum wheat is the suitable source to deliver the important micronutrient to human population. GYPC is one of the very important Quality parameter for its commercial value internationally, this is also required for pasta natural color, this yellowness is because of various Carotenoid present in durum wheat which are also the source of pro Vitamin A(β- carotene) .This study describe that GYPC is complex quantitative trait, regulated by additive gene effects, providing information on major genetic loci for this trait and the genotypic interrogation of the *PSY1A* and *PSY1B* 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 international durum wheat breeding programs. This includes identifying individuals that contain the desirable *PSY1A* and *PSY1B* alleles because these alleles were associated with high GYPC. Subsequently, these individuals will be used as donor parents, and facilitate backcrossing and marker-assisted selection to efficiently accelerate the improvement of semolina yellowness made from durum wheat. 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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