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Mediterranean durum wheat landraces as a source of variability for quality improvement

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Abstract. A collection of 154 durum wheat landraces from 20 Mediterranean countries and 18 modern varieties was used to examine the existing variability for the main quality traits and to identify potential quality-enhancing genotypes for use in breeding programs. Field experiments were conducted during 3 years under rainfed conditions in north-eastern Spain. Based on yield and quality attr butes, landraces were clustered according to their region of origin in eastern Mediterranean, western Mediterranean, and North-Ba kan Peninsula. Landraces from the eastern Mediterranean countries had the highest global quality and the widest variability for quality traits, but were characterized by relatively small grains. Landraces from the western Mediterranean countries had the heavier grains, while landraces from the North-Balkan Peninsula had low quality and small quality variability. Modern varieties showed the highest global quality, but they had the lowest grain protein content and phenotypic variability. The assessment of the allelic composition at five glutenin loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, and *Glu-B2*) allowed identifying 114 alleles/banding patterns in the collection. Three rare banding patterns, found on a few number of landraces, affected significantly gluten strength. Landraces with improved quality traits, particularly gluten strength and grain weight were identified as potential donors for quality improvement.

Keywords. Protein content – Gluten strength – Kernel weight – HMW-GS – LMW-GS.

Variétés locales de blé dur de la Méditerranée comme source de variabilité pour l'amélioration de la qualité

Résumé. *Une collection de 154 variétés locales de blé dur provenant de 20 pays méditerranéens et 18 variétés modernes a été utilisée pour examiner la variabilité existante pour les principaux caractères de qualité et pour identifier des potentiels génotypes améliorateurs de qualité à intégrer dans les programmes de sélection. Des expériences de terrain ont été menées en sec pendant 3 ans dans le nord-est de l'Espagne. Sur la base du rendement et des attributs de qualité, les variétés locales ont été regroupées en fonction de leur région d'origine dans l'est de la Méditerranée, dans l'ouest de la Méditerranée et dans le nord de la péninsule balkanique. Les cultivars traditionnels provenant des pays de la Méditerranée orientale se caractérisent par la qualité globale la plus élevée et la plus large variabilité des caractères de qualité, mais ils présentent des grains petits. Les cultivars traditionnels provenant des pays de la Méditerranée occidentale ont les grains les plus lourds, tandis que les variétés locales du nord de la péninsule balkanique ont une faible qualité et une faible variabilité de la qualité. Les variétés modernes ont affiché la qualité globale la plus élevée, mais elles ont la plus faible teneur en protéines du grain et la plus faible variabilité phénotypique. L'évaluation de la composition allélique dans cinq loci de la gluténine (Glu-A1, Glu-B1, Glu-A3, Glu-B3 et Glu-B2) a permis d'identifier 114 allèles/profils de bandes dans la collection. Trois profils de bandes rares, observés sur un petit nombre de variétés locales, influent de manière significative sur la force du gluten. Les variétés locales avec des caractères de qualité améliorés, en particulier la force du gluten et le poids du grain, ont été identifiées comme donneurs potentiels pour l'amélioration de la qualité.*

Mots-clés. *Teneur en protéines – Force du gluten – Poids du grain – HMW-GS, LMW-GS.*

I – Introduction

The Durum wheat (*Triticum turgidum* L. var. *durum*) is a major crop in the Mediterranean Basin, a region close to the Fertile Crescent, where wheat was domesticated about 10,000 years BP. During the migration process East ward into the Mediterranean Basin, both natural and human

selection resulted in the establishment of landraces adapted to a diversity of agro-ecological zones and to local consumer preferences. From the early 1970s landraces were displaced by improved semi-dwarf cultivars which resulted in a decrease of diversity and the near-extinction of on-farm native genetic variability (Skovmand *et al*., 2005). Landraces, with their broad diversity within the species, are highly valuable sources for widening the genetic variability for numerous traits when made available to breeding programs. In particular, durum wheat landraces and old varieties from the Mediterranean region seem to retain a high level of polymorphism and large genetic diversity for grain and end-product quality (Aguiriano *et al*., 2008; Moragues *et al*., 2006).

The overall quality of durum wheat grain may be evaluated through the quality index (QI) established in 2003 by an EU Commission Regulation. The QI is based on protein content, gluten strength, yellow color index and test weight (or thousand kernel weight), and is expressed as a percentage with reference to specific check varieties. Gluten strength, one of the main factors influencing grain quality, strongly depends on the composition of storage proteins, among which glutenins are the most influential. The high molecular weight-glutenin subunits (HMW-GS) are encoded by the complex at the *Glu-1* loci (*Glu-A1* and *Glu-B1*), whereas the low molecular weight-glutenin subunits (LMW-GS) are encoded by genes at *Glu-A3*, *Glu-B3* and *Glu-B2* (Shewry *et al*., 1992; Vázquez *et al*., 1996).

This study was conducted with the aim of evaluating and characterizing the grain quality of a collection of Mediterranean landraces and a set of representative modern varieties −with special emphasis on banding patterns related to allelic variability for HMW-GS and LMW-GS−in order to detect the presence of variants of potential interest for breeding purposes. The geographic structure existing in the region was also assessed on the basis of yield and quality traits.

II – Material and methods

A collection 154 durum landraces from 20 Mediterranean countries and a set of 18 representative modern cultivars (see Nazco *et al*., 2012 for a complete description of genotypes), was tested during three years in Lleida (north-eastern Spain) in non-replicated experiments with three replicated checks. Plots were mechanically harvested, yield was expressed at 12% moisture level, and a sample of grain for each plot was used for quality determinations. Protein content (%) was determined by a near infrared spectroscope and gluten strength was assessed by the SDS (sodium dodecyl sulphate) sedimentation test (ml). Yellow color index was determined by means of a reflectance colorimeter. These three quality traits plus thousand kernel weight (TKW, g) were used to calculate the EU quality index (QI) relative to cv. 'Simeto', 'Gallareta' and 'Vitron' that were used as reference checks. The quotient between gluten strength and protein content was the sedimentation index (SI, ml/protein unit); test weight (TW, kg/hl) was determined by the Dickey-John equipment. The best linear unbiased predictors (BLUPs) were estimated for yield and quality data by Restricted Maximum Likelihood (REML). High- and low molecular weight glutenin subunit compositions at *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-B2* were assessed by electrophoretic analysis (1D SDS-PAGE).

III – Results and discussion

1. Variability and geographic structure

A large genotypic effect was observed for QI and gluten strength, but a high environmental influence was obtained for protein content as reported by Rharrabti *et al.* (2001, 2003). Landraces showed much larger variability than modern cultivars for the quality traits evaluated (Fig. 1), with

the widest variability recorded in landraces from the eastern Mediterranean Basin (Nazco *et al.,* 2012).

The first two PC axes of the Principal Component Analysis (PCA), conducted with yield and quality data, grouped the germplasm under study in four clusters, corresponding to (1) modern cultivars, (2) landraces from the Eastern Mediterranean Basin, (3) landraces from the western Mediterranean Basin, and (4) landraces from the north-Balkan countries (see Nazco *et al*., 2012). Landraces from the eastern Mediterranean Basin had the best overall quality among the set of landraces, on the basis of their high gluten strength and yellow index. Landraces from the north Balkan countries had high grain weight and low overall quality and gluten strength, while landraces from the western Mediterranean countries had intermediate properties between both groups (Fig. 2). Modern varieties showed the best average grain yield, gluten strength, yellow index, SI and overall quality. However, their protein content was lower than that of landraces (Fig. 2) as reported by previous studies (De Vita *et al*., 2007; Royo *et al*., 2007). Nevertheless, landraces were identified that could be used in breeding programs with the least negative effect on overall quality.

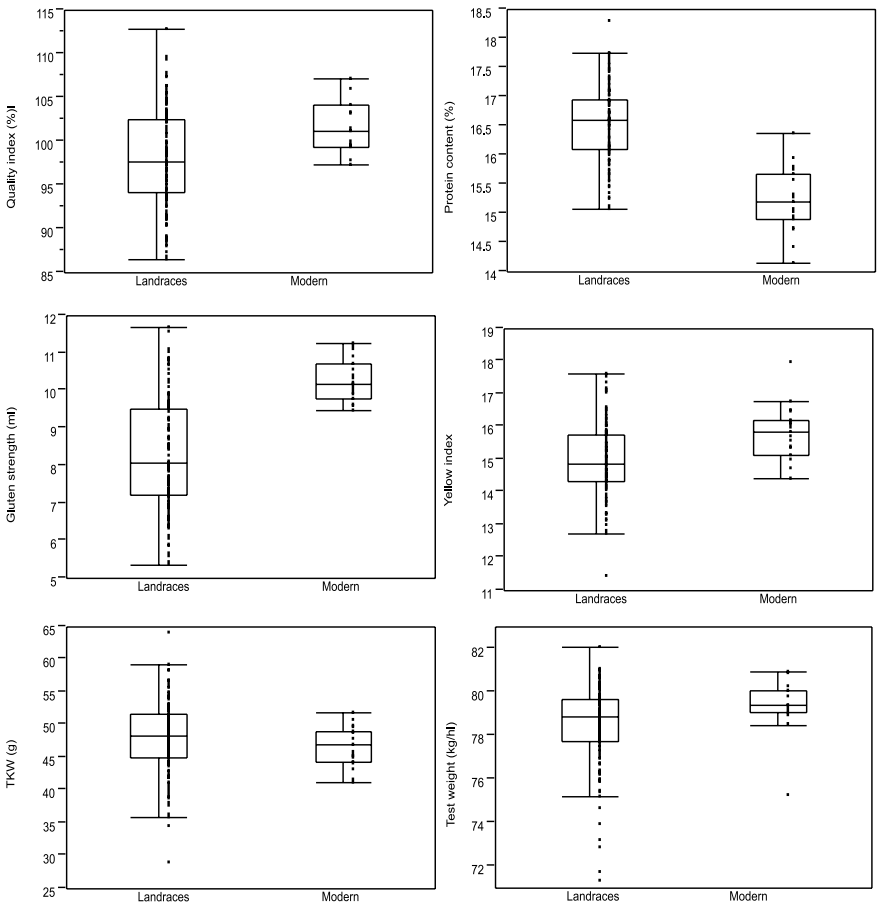

Figure 1. Box plots showing the variability existing within landraces and modern cultivars for grain quality traits. Data are 3-years adjusted means of samples from field experiments conducted in north-eastern Spain.

be associated with their frequency. They were banding pattern 2+4+14+15+18 (present in the Egyptian landrace PI-366109) and 2+4+15+18+19 (Fig.3), present in two landraces: 'Trigo Glutinoso' from France and 'Lobeiro de Grao Escuro' from Portugal (Nazco *et al*., 2013).The two alleles reported at *Glu-B2* (*Glu-B2b* or null and *Glu-B2a* or band 12) were found in both sets of germplasm at high frequencies and with a significant effect on the gluten strength of the landraces. However, while band 12 had a positive effect on gluten strength, the effect of the null allele was detrimental (Fig 3).

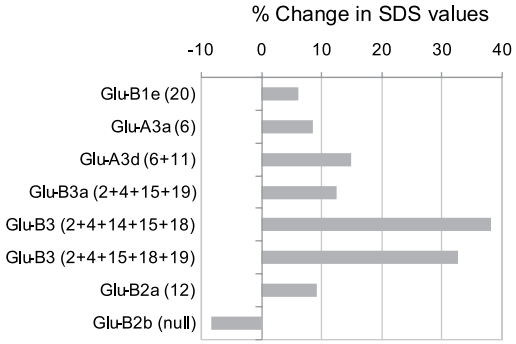

Figure 3. Alleles/banding patterns with significant effect (*P*<0.05) on the SDS-sedimentation volume of the landraces carrying them. Prepared from data shown in Nazco *et al*., 2013.

3. Landraces useful to enhance grain quality in breeding programs

At country level the greatest QI was recorded in Cypriot landraces, which also showed the best gluten strength and SI values (Nazco *et al.*, 2012). For individual genotypes, the best QI (113% relative to the average of the three check cultivars) was recorded in the Egyptian landrace identified in the USDA Germplasm Bank as PI-366109. This landrace and also 'Lobeiro de Grao Oscuro' from Portugal were among the 10th percentile for QI, protein content and gluten strength simultaneously.

The Spanish landrace 'Raspinegro de Alcalá' was among the best for QI, gluten strength, yellow index and SI simultaneously. The Israeli landrace 'Hati' was among the best entries for yellow index and TW simultaneously. The Spanish landrace 'Enano de Andújar' had the heaviest grains with a TKW surpassing a 23.5% that of Simeto, the modern cultivar with the heaviest grains. Among the modern set, the Italian cv. 'Svevo' and the U.S. desert durum cv.'Ocotillo' reached the greatest overall quality standards. Many new/un-reported banding patterns were identified and some apparently influencing the expression of gluten strength. These will need to be transferred to modern backgrounds and subsequently evaluated for their potential strength-enhancing effects or their capacity to produce gluten qualities that may be useful in the production of specific types of products.

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Purple grain colour genes in wheat

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Abstract. Purple colour of wheat grain is caused by accumulation of anthocyanins in the pericarp. Potential health benefits and adaptability may be the reason for a renewed interest in wheat with high anthocyanin content. Genetic bases underlying purple grain character as well as some practical aspects are reviewed in this paper. Two complementary dominant genes control purple pericarp coloration in both durum (*Triticum durum* Desf., *2n* = *4x* = 28) and bread (*T. aestivum* L., *2n* = *6x* = 42) wheat. In durum wheat, these genes are mapped to be on chromosomes 2A (*Pp3*) and 7B (*Pp-B1*), whereas in bread wheat they are located on chromosomes 2A (*Pp3*) and 7D (*Pp-D1*). Functional alleles of at least one of the two complementary genes determining purple pericarp exist in *Ae. speltoides*, *Ae. tauschii* and *T. timopheevii* – the species, in which purple-grained plants have never been descr bed. The wheat *Pp-1* and *Pp3* genes regulate transcription of the anthocyanin biosynthesis structural genes and may encode transcription regulatory factors that belong to the MYB- and MYC-l ke superfamilies, respectively. Their orthologues were cloned in maize, rice and barley and can be used for homology-based cloning of wheat *Pp-1* and *Pp3*. Usefulness of microsatellite markers closely linked to the *Pp* genes for marker-assisted selection has been demonstrated.

Keywords. Purple grain – Durum wheat – Bread wheat – Gene – Mapping – Orthologous genes – Marker-assisted selection.

Les gènes de la couleur violette du grain chez le blé

Résumé. *La couleur violette du grain de blé est déterminée par l'accumulation des anthocyanes dans le péricarpe. Les bienfaits potentiels pour la santé et l'adaptabilité peuvent être la raison d'un regain d'intérêt pour le blé ayant une teneur en anthocyanes élevée. Dans ce travail, nous allons examiner les bases génétiques du caractère grain violet ainsi que certains aspects pratiques. Deux gènes complémentaires dominants contrôlent la coloration violette du péricarpe chez le blé dur (Triticum durum Desf., 2n = 4x = 28) et chez le blé tendre (T. aestivum L., 2n = 6x = 42). Chez le blé dur, ces gènes sont cartographiés sur les chromosomes 2A (Pp3) et 7B (Pp-B1), alors que chez le blé tendre, ils sont situés sur les chromosomes 2A (Pp3) et 7D (Pp-D1). Les allèles fonctionnels d'au moins un des deux gènes complémentaires déterminant le péricarpe violet sont présents sur Ae. speltoides, Ae. tauschii et T. timopheevii - les espèces, pour lesquelles les plantes à grain violet n'ont jamais été décrites. Les gènes du blé Pp-1 et Pp3 régulent la transcription des gènes de structure de la biosynthèse des anthocyanines et peuvent coder pour des facteurs de transcription régulateurs qui appartiennent à la superfamille de type MYB et MYC, respectivement. Leurs orthologues ont été clonés chez le maïs, le riz et l'orge et peuvent être utilisés pour le clonage basé sur l'homologie du Pp-1 et PP3 du blé. L'utilité des marqueurs microsatellites étroitement liés aux gènes Pp pour la sélection assistée par marqueurs a été démontrée.*

Mots-clés. *Grain violet – Blé dur – Blé tendre – Gènes – Cartographie – Gènes orthologues – Sélection assistée par marqueurs.*

I – Introduction

Wheat grain may have white, red, blue or purple colour. Red colour is caused by proanthocyanidins in the seed coat, blue colour is due to production of anthocyanins in the aleurone layer, whereas purple colour is caused by accumulation of anthocyanins in the pericarp. Anthocyanins are flavonoid pigments well-known for their free radical scavenging capacity (Kähkönen and Heinonen, 2003). Besides antioxidant activity, other properties of anthocyanins have been described, such as estrogenic activity, enzyme inhibition, anti-inflammatory activity, capillary permeability and

fragility decrease, membrane strengthening, protection from DNA cleavage, *etc.* (Lila, 2004). The potential nutritional and health benefits may not be the only reason for a renewed interest to wheat with high anthocyanin content. Comparative analysis of wheat near-isogenic lines differing by anthocyanin content in the coleoptile and pericarp (Tereshchenko *et al*., 2013) showed higher drought tolerance of intensely colored seedlings (Tereshchenko *et al*., 2012a). Furthermore, the purple-grained NILs had better viability after artificial ageing compared to the recurrent parent lacking anthocyanins (Gordeeva and Khlestkina, 2013a). This is in agreement with finding of Debeaujon *et al*. (2000) in Arabidopsis: mutants affected in testa pigmentation showed a reduced germination capacity after long storage.

II – Inheritance of the purple grain character and mapping *Pp* genes using molecular markers

Investigation of the genetic basis of the purple pericarp trait has been performed in both durum (*Triticum durum* Desf., *2n* = *4x* = 28) and bread (*T. aestivum* L., *2n* = *6x* = 42) wheat. Sharman (1958) described dominant monogenic inheritance of purple pericarp trait in tetraploid wheat. Bolton (1970) reported dominant digenic inheritance with possible complementary effect in hexaploid wheat. Two dominant complementary genes for purple grain were localized by Piech and Evans (1979) on chromosomes 3A and 7B of bread wheat. Arbuzova *et al.* (1998) localized one of the two complementary *Pp* genes on chromosome 7B (*Pp1*) and suggested another two loci on chromosomes 6A (*Pp2*) or 2A (*Pp3*) of bread wheat. However, certain number of *Pp* genes and their precise chromosome location remained unclear until the molecular marker era.

In durum wheat cross 'TRI 15744' (purple-grained) × 'TRI 2719' (white-grained), the segregation ratio for grain colour was consistent with 9:7, suggesting the presence of two dominant complementary *Pp* genes in the purple-grained accession 'TRI 15744' (Khlestkina *et al.*, 2010a). One of these genes, *Pp1*, forms a cluster with dominant genes *Pc*, *Plb*, *Pls* (Khlestkina *et al.*, 2010a) and *Rc* (Tereshchenko *et al.*, 2012b) determining anthocyanin coloration of culm, leaf blades, leaf sheaths and coleoptile, respectively. This cluster has been mapped to be located on the short arm of chromosome 7B between the microsatellite loci *Xgwm0951* and *Xgwm0573* (Khlestkina *et al.*, 2010a). The complementary *Pp* gene has been mapped to the long arm of chromosome 2A between the loci *Xgwm0328* and *Xgwm0817* close to the centromere. This position is highly comparable with localization of the *Pp3* gene in bread wheat (Dobrovolskaya *et al.*, 2006), thus the *Pp* gene of durum wheat mapped to chromosome 2A has been designated *Pp3*. This gene is closely linked to the dominant *Pg* gene determining purple glume in durum wheat. Unlike purple pericarp, purple glume is a monogenically inherited trait (Khlestkina *et al.*, 2010a). Dobrovolskaya *et al.* (2006) has shown the bread wheat *Pp2* gene (suggested on chromosome 6A by Arbuzova *et al*., 1998) to be an allelic variant of *Pp3* on chromosome 2A.

In bread wheat, the gene complementary to *Pp3*, inherited from either 'Purple' or 'Purple Feed', has been mapped to the short arm of chromosome 7D (Tereshchenko *et al.*, 2012b). This gene forms a cluster with *Pan* (Laikova *et al.*, 2005), *Pc*, *Plb*, *Pls* and *Rc* genes (Tereshchenko *et al.*, 2012b), determining anthocyanin coloration of anthers, culm, leaf blades, leaf sheaths and coleoptile, respectively. This cluster has been mapped between the microsatellite loci *Xgwm0044* and *Xgwm0111* on chromosome 7DS. The bread wheat *Pp* gene on 7DS is homoeoallelic to the durum *Pp1* gene on 7BS, therefore these loci have been designated *Pp-D1* (on 7DS of bread wheat) and *Pp-B1* (on 7BS of durum wheat), respectively (Tereshchenko *et al.*, 2012b). Localization of one of the complementary *Pp* genes in the D-genome of bread wheat doesn't support the former view that the purple pericarp trait of bread wheat has been transferred from purple-grained Ethiopian tetraploid wheats (Copp, 1965; Bolton, 1970). At least one of the two complementary genes in bread wheat was inherited from *Aegilops tauschii* (Tereshchenko *et al.*, 2012b).

Bread wheat 'Novosibirskaya 67' carries the dominant *Pp* gene allelic to the *Pp-D1* locus of 'Purple' or 'Purple Feed', but has a recessive allele at the *Pp3* locus, therefore anthocyanins are not synthesized in the pericarp of this cultivar (Dobrovolskaya *et al.*, 2006). However, introgression of tetraploid timopheevii wheat chromosome segment (2G) into 'Novosibirskaya 67' chromosome 2B (Leonova *et al.*, 2002) causes production of anthocyanin pigment in pericarp (Gordeeva *et al*., unpublished). Furthermore, bread wheat introgression line 'PC' having *Pp-S1* gene inherited from *Aegilops speltoides* and introgressions of timopheevii wheat to chromosomes 2A and 2B has purple pericarp too (Tereshchenko *et al.*, 2012c). Thus, in tetraploid timopheevii wheat, the gene either allelic or homoeoallelic to *Pp3* may exist. Overall, functional alleles of at least one of the two complementary genes determining purple pericarp still exist in *Ae. speltoides*, *Ae. tauschii* and *T. timopheevii* – the species, in which purple-grained stocks have never been described (Tereshchenko *et al.*, 2012b, 2012c).

III – Orthologues and future prospects for homology-based cloning

In order to understand a nature of the two complementary factors encoded by the wheat *Pp-1* and *Pp3* loci, we compared map positions of these genes (Dobrovolskaya *et al.*, 2006; Khlestkina *et al.*, 2010a; Tereshchenko *et al.*, 2012b) with locations of well-studied rice and maize purple seed colour genes (Table 1; Fig. 1).

The most probable orthologues of the wheat *Pp3* locus are rice *Pb*/*Ra* and maize *Lc*/*R* genes determining pericarp colour and encoding MYC-like regulatory factor of anthocyanin biosynthesis (Dooner and Kermicle, 1976; Ludwig *et al.*, 1989; Hu *et al.*, 1996; Wang and Shu, 2007). The *Pb*/*Ra* gene is located on rice chromosome 4, which has a colinearity with Triticeae chromosome 2 (Stein *et al.*, 2007). The *Lc*/*R* gene has been mapped to maize chromosome 10, which has colinearity with rice chromosome 4 (Ahn and Tanksley, 1993). Recently, Cockram *et al.* (2010) isolated a candidate gene for the barley *ant2* (*anthocyaninless 2*) locus. *Ant2* is located on chromosome 2H (Lundqvist *et al.*, 1996), and its candidate gene encodes a transcriptional factor, which also belongs to the bHLH (MYC) family (Cockram *et al.*, 2010). In rye, the gene determining pericarp colour was localized in chromosomes 2R (de Vries and Sybenga, 1984) which has colinearity with *Triticum* chromosome 2 (Devos *et al*., 1993).

A putative orthologue of the wheat *Pp-1* gene is the maize *C1* gene (*colorless 1*) encoding MYB-like regulatory factors affecting transcription of the structural anthocyanin biosynthesis genes (Cone *et al*., 1986, 1993). Li *et al*. (1999) used the *C1* sequence as a probe to locate homologous sequences in wheat. They identified loci (designated *Mpc1*) in those regions of wheat homoeologous group 7 chromosomes (Li *et al*., 1999), that correspond to map positions of the genes *Pp-B1* and *Pp-D1* (Tereshchenko *et al.*, 2012b). In rice, the gene *Pa* (*purple apiculus*) has been mapped on chromosome 6, which has colinearity with Triticeae chromosome 7 (Stein *et al.*, 2007). In barley, the *ant1* (*anthocyaninless 1*) locus mapped to the short arm of chromosome 7H (Lundqvist *et al*., 1996) can be an orthologue of wheat *Pp-B1* and *Pp-D1* (Table 1).

Thus, the wheat *Pp-1* and *Pp3* genes may encode transcription regulatory factors that belong to the MYB- and MYC-like superfamilies, respectively. Tereshchenko *etal*. (2013) used wheat near-isogenic lines differing by allelic state of the *Pp-1* and *Pp3* genes and compared transcriptional activity of the anthocyanin biosynthesis structural genes (*Chs* encoding chalcone synthase, *Chi* – chalcone-flavanone isomerase, *F3h* – flavanone 3-hydroxylase, *Dfr* – dihydroflavonol-4-reductase, and *Ans* – anthocyanidin synthase) in the pericarp. It was shown that dominant *Pp-D1* and *Pp3* alleles activate *F3h* expression in pericarp and induced more intensive transcription of the other structural genes (*Chs*, *Chi*, *Dfr*, and *Ans*). This is in accordance with a suggestion that the *Pp-1* and *Pp3* genes are transcriptional regulators in the anthocyanin biosynthesis network.

Homology-based cloning of these genes can be performed using known sequences of the maize, rice and barley genes encoding the MYB- and MYC-like anthocyanin biosynthesis regulatory factors.

Table 1. Regulatory genes of the anthocyanin biosynthesis in barley, rice and maize, having orthologous map positions compared to wheat *Pp* genes, determining purple pericarp colour.

	MYB-like regulatory factor			MYC-like regulatory factor		
	gene designation	chromosome location	reference	gene designation	chromosome location	reference
Bread- wheat	*Pp-D1*	7D	Tereshchenko *et al*., 2012b	*Pp3*	2A	Dobrovolskaya *et al*., 2006
Durum- wheat	*Pp-B1*	7B	Khlestkina *et al*., 2010	*Pp3*	2A	Khlestkina *et al*., 2010
barley	*Ant1*	7H	Lundqvist *et al*., 1996	*Ant2*	2H	Lundqvist *et al*., 1996
rice	*Pa*	6	Liu *et al*., 2012	*Pb*/*Ra*	4	Wang and Shu, 2007
maize	*C1*	9	Cone *et al*., 1993	*Lc*/*R*	10	Dooner and Kermicle, 1976

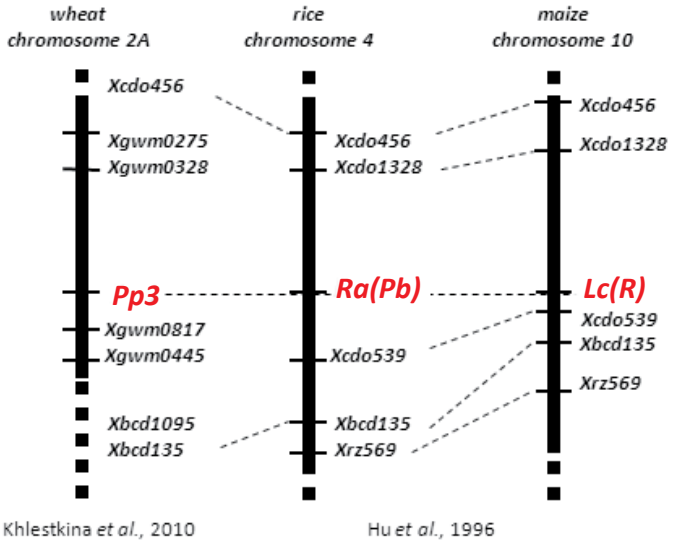

Figure 1. Comparative map positions of durum wheat *Pp3* gene determining purple pericarp colour and genes for purple seed in rice and maize.

IV – Marker-assisted selection in basic and applied research

In order to better understand the mechanisms of anthocyanin biosynthesis regulation in wheat pericarp and to determine particular role of each of the two complementary *Pp* genes, isogenic lines with different combinations of the *Pp* alleles were needed. To obtain a set of such lines we performed marker-assisted backcrossing of the near-isogenic line 'i:S29Pp1Pp2^{PF}' of bread wheat 'Saratovskaya 29' (Gordeeva and Khlestkina, 2013b). This line carries two introgressions from bread wheat 'Purple Feed' at the 'Saratovskaya 29' background (Arbuzova *et al*., 1998). The introgressions extend from microsatellite locus *Xgwm0558* on chromosome 2AS to *Xgwm0294* on 2AL and from *Xgwm0044* to *Xgwm0111* on 7DS (Tereshchenko *et al*., 2012b). These two

donor's segments carry dominant alleles of the genes *Pp3* (on chromosome 2A) and *Pp-D1* (on chromosome 7D), thus conferring intensive purple pericarp colour in 'i:S29Pp1Pp2^{PF}'. Donor's segment on chromosome 7D also carries dominant alleles at the *Rc-D1*, *Pc-D1*, *Pan-D1*, *Pls-D1*, *Plb-D1* loci, determining intensive anthocyanin pigmentation of the coleoptile, culm, anthers, leaf sheath and leaf blade, respectively (Tereshchenko *et al.*, 2012b). 'Saratovskaya 29' has no anthocyanin pigment in the pericarp, but has light pigmentation on the coleoptile, culm, leaf sheath and leaf blade, controlled by the dominant genes *Rc-A1*, *Pc-A1*, *Pls-A1* and *Plb-A1*, respectively (Khlestkina *et al.*, 2010b). We crossed 'i:S29Pp1Pp2^{PF}' with 'Saratovskaya 29' and used combined foreground and background marker-assisted selection approach to get homozygous F_2 lines with different combinations of *Pp* alleles (Gordeeva and Khlestkina, 2013b).

To choose the line with dominant *Pp-D1* and recessive *Pp3*, foreground (using chromosome 7D markers) and background (2A) selection was performed. In the selected line the recipient's chromosome 2A has been completely recovered, whereas chromosome 7D still carries the donor's segment. This line has lost anthocyanin pigment in the pericarp but retained intensive coloration of other organs (Gordeeva and Khlestkina, 2013b). Overall, wheat *Pp-1* gene is tightly linked with genes determining anthocyanin pigmentation of coleoptile, stem, leaves and anthers. This relationship was observed in durum wheat for *Pp-B1* (Khlestkina *et al.*, 2010a), in bread wheat for *Pp-D1* (Tereshchenko *et al.*, 2012b). In addition to material described in Khlestkina *et al.* (2010a) and Tereshchenko *et al.* (2012b), we studied coleoptile coloration of purple-grained wheats 'Konini', 'ANK-28A' and 'ANK-28B' and observed intensive anthocyanin pigmentation in each of them. However, this relationship can be a specific feature of wheat. For just a few of the purple-grained barley accessions maintained in IPK-Genbank have colored coleoptile.

To choose the line with dominant *Pp3* and recessive *Pp-D1*, foreground (using chromosome 2A markers) and background (7D) selection was carried out. In the selected line the recipient's chromosome 7D has been completely recovered, whereas chromosome 2A still carries the donor's segment. This line has light anthocyanin pigmentation of coleoptile, culm, leaf sheath and leaf blade similar to the recurrent parent 'Saratovskaya 29'. However, pericarp pigmentation is light purple, that is distinct from both parents, 'i:S29Pp1Pp2^{PF}' with intense anthocyanin pigmentation and 'Saratovskaya 29' without anthocyanins in pericarp (Gordeeva and Khlestkina, 2013b). This finding suggests interaction between dominant *Pp3* inherited from 'i:S29Pp1Pp2^{PF}' with another *Pp* gene, most likely *Pp-1* (*Pp-A1*) within a cluster of anthocyanin regulatory genes on chromosome 7A (Khlestkina *et al.*, 2010b). Further marker-assisted split of complementary *Pp* genes (*Pp3* and *Pp-A1*) into different near-isogenic lines is in progress.

The microsatellite markers successfully used for marker-assisted backcrossing of 'Saratovskaya 29' NILs, can be recommended for marker-assisted breeding of bread and durum wheat for purple grain colour. We propose that breeding material having red coleoptile is needed addition of *Pp3* dominant allele only in order to obtain purple-grained phenotype, whereas plants with green coleoptiles require introduction of both complementary genes *Pp3* and *Pp-1*.

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