Lehr- und Forschungszentrum für Landwirtschaft
Raumberg-Gumpenstein

64. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2013, 75 – 78
ISBN: 978-3-902849-00-7

Use of wheat gene resources with different grain colour in breeding

Petr Martinek[1*], Ondřej Jirsa[1], Kateřina Vaculová[1], Jana Chrpová[2], Nobuyoshi Watanabe[3],
Veronika Burešová[4], David Kopecký[4], Klára Štiasna[5], Tomáš Vyhnánek[5] and Václav Trojan[5]

Abstract

The interest in wheat genetic resources with different grain colour has recently increased. Differences in grain colour are caused by the presence of polyphenols, tannins, anthocyanins and carotenoids. Resources of uncommon grain colour have blue aleurone (*Ba* genes), purple pericarp (*Pp* genes) and yellow endosperm (*Psy* genes), which are determined by the presence of anthocyanins and carotenoids, respectively. These substances have antioxidant activity and are useful for the production of functional foods with positive effects on consumers' health. In 2011, the winter wheat variety Skorpion with blue aleurone, which had been bred in the Czech Republic, was registered in Austria. The used donor of blue aleurone comes from the heritage of Erich von Tschermak-Seysenegg (1871-1962). The breeding program at Agrotest Fyto, Ltd., Kroměříž, is focused on the development of winter wheat genotypes with dark grain combining different genes for grain colour and simultaneously agronomic important traits (yield, resistance to stress) and good baking quality. In different kernel tissues there are different amounts of coloured substances that affect their content in bran and flour and, thus, their content in bakery and biscuit products.

Keywords

Antioxidants, blue aleurone, purple pericarp, *Triticum aestivum*, yellow endosperm

Introduction

Various types of plants synthesize a number of substances from the group of flavonoids that cause the characteristic colour of tissues in response to the respective environments. The occurrence of these substances is associated with adaptive role to stressful environmental factors (KHLESTKINA 2013, ZEVEN 1991). Also, the grain colour of cereals may be different. For the vast majority of current wheat varieties the grain colour is red, less often it is white. However, there are genetic resources of wheat with a grain colour significantly differing from current varieties due to the presence of coloured pigments (e. g. carotenoids, flavonoids, anthocyanins, some phenolic compounds). Like many types of vegetables and fruits, these substances are characterized by antioxidant properties and have an irreplaceable role in a healthy diet for people. Generally, antioxidants are considered essential for humans to prevent inflammation, diabetes, cancer, oxidative stress and ocular diseases (LAMY et al. 2006). Antioxidants in cereals could be used for the production of functional foods and positively affect consumers' health. The use of existing wheat genetic resources with different genes for coloured substances and understanding the pathway of their biosynthesis could be employed for the breeding of varieties accumulating a higher number of relevant genes, which would allow to increase the content of health-promoting substances. The successful application of varieties with coloured grains into practice will depend on the level of yield and agronomic properties comparable to commercially used varieties. The current donors of genes for blue and purple grain, and yellow flour have usually lower yield compared to standard varieties. Therefore, wheat breeding programmes should focus on eliminating this deficiency.

It will be also necessary to know the extent of natural degradation of dyes during thermal processing of the wheat grain when during Maillard reaction chemical changes occur. These new compounds can have a differerent influence on health compared to the original compounds. Production and processing technology will have to be adapted in order to conserve and use best these natural substances. An alternative technology could be extrusion or expansion (puffing) where the exposure of the raw material to high temperatures is reduced.

Wheat grain colours and their genes

Red and white grain

The red colour of grain occurs in most common European wheat varieties. It is controlled by one to three dominant alleles *R-A1* (on chromosome 3AL), *R-B1* (3BL) and *R-D1* (3DL). Contrary, white grain colour is determined by the recessive alleles, i.e. *r-A1*, *r-B1* and *r-D1*. The pigment is composed of catechin and tannin derivatives generated in the process of biosynthesis of flavonoids (HIMI and NODA 2005). The red colour of the grain is associated with a higher content of bitter phenolic components, lower activity

---

[1] Agrotest Fyto, Ltd., Havlíčkova 2787, 767 01 KROMĚŘÍŽ, Czech Republic
[2] Crop Research Institute, Drnovská 507/73, 161 06 PRAGUE, Czech Republic
[3] College of Agriculture, Ibaraki University, 3-21-1 Chuo, Ami, Inashiki, IBARAKI 300-0393, Japan
[4] Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Šlechtitelů 31, 78371 OLOMOUC, Czech Republic
[5] Mendel University in Brno, Zemědělská 1, 613 00 BRNO, Czech Republic
[*] Corresponding author: Petr MARTINEK, martinek.petr@vukrom.cz

of hydrolytic enzymes, and better resistance to sprouting. Phenolic acids are low molecular weight compounds with antioxidant activities and can be oxidised in the grain by polyphenol oxidase to darker colour compounds (tannins and lignin). They inhibit lipoxygenase and are uncompetitive inhibitors with protective effects against diseases (LACHMAN et al. 2003). White grains have low amounts of polyphenol oxidase, the absence of bitter substances makes the product naturally sweeter, which can be important in confectionery. Wheats with white grains are more susceptible to sprouting, the milling allows higher yield of flour and, thus, the flour can contain more fibre, minerals and proteins.

Purple pericarp

The purple grain colour is caused by genes for purple pericarp *Pp*, which were transferred to common wheat from tetraploid wheat *Triticum turgidum* L. subsp. *abyssinicum* Vavilov, coming from the Abyssinian region in Ethiopia. It is characterized by the presence of anthocyanins in the surface layer (pericarp) of the grain. According to ABDEL-AAL and HUCL (2003) and KNIEVEL et al. (2009) cyanidin 3-glucoside, cyanidin 3-rutinoside, and succinyl glucoside are most represented in purple grains. The mean content of total anthocyanins was 104 $mg{\cdot}kg^{-1}$ in wholemeal flour and 251 $mg{\cdot}kg^{-1}$ in the bran (ABDEL-AAL and HUCL 1999). So far several genes for purple pericarp were identified. Using monosomic analysis two genes, i.e. *Pp1* and *Pp2*, on chromosomes 7B and 7A, respectively, were detected in variety 'Purple Feed', whereas variety 'Purple' contained the genes *Pp1* and *Pp3* (ARBUZOVA and MAYSTRENKO 2000). Later it was found that *Pp3* is composed of two alleles that have been named *Pp3a* and *Pp3b* (DOBROVOLSKAYA et al. 2006). Both are located in the centromeric region of chromosome 2A. For genes *Pp1* and *Pp3* complementary effect was described. Feeding hens with purple wheat resulted in increased weight of meat (+6.2%) and a higher number of eggs (+3.4%), whereas no effect was found on the colour of yolk (RÜCKSCHLOSS et al. 2010).

Blue aleurone

Blue grain colour is determined by genes for blue aleurone *Ba*. QUALSET et al. (2005) reported that blue aleurone in spring wheat 'UC66049' is controlled by the codominantly acting gene *Ba1*. This gene has been transferred to wheat by an entire chromosome arm from *Thinopyrum ponticum* Podp. which was incorporated into chromosome 4B (4BS-$4el_2$). Dr. Emil Šebesta from USDA-ARS at Oklahoma State University developed during 1958-1988 the blue grained lines 'SB-1', 'SB-2', and 'SB-3' ('Sebesta Blue') with chromosomal segments from *Th. ponticum* (MORRISON et al. 2004). This material seems to have the similar origin as 'UC66049'. Gene *Ba2* has been transferred to wheat from *T. monococcum* ssp. *aegilopoides* (syn. *T. boeoticum*) as disomic substitution of 4A ($4A^mL$) (DUBCOVSKY et al. 1996, SINGH et al. 2007). It is assumed *Ba2* is present in 'Thatcher Blue', originating from the John Innes Centre (Watanabe, personal commun.). Deviations in the inheritance demonstrate that *Ba1* and *Ba2* are distinct genes (METTIN et al. 1991). According to the 'Catalogue of Genetic Symbols for Wheat', there is also weaker gene expression 'half-blue' in addition to the above two genes, which occurred in a sample of *T. boeoticum*. Another possible gene for blue aleurone could be on chromosome 4D, which has been substituted by chromosome pair of *Agropyron elongatum* in the variety 'Xiao Yian' from China (ZELLER et al. 1991). Genbank accession TRI2401 (*T. aestivum* var. *tschermakianum* Mansf.; IPK Gatersleben, Germany) with the name 'Tschermaks Blaukörniger Sommerweizen' is reported to have a different origin and gene (ZEVEN 1991). The results based on the FISH and GISH analysis show that the transferred chromosomal segments of *Th. ponticum* in blue grained wheats have different length, position and we can divide the material according to the length, position and number of transferred segments into six different groups (BUREŠOVÁ et al. 2013). This demonstrates a great genetic diversity among individual blue grained gene sources. It is also necessary to consider that the donors *Agropyron elongatum* and *Th. ponticum* are some of the many synonyms of the highly heterogeneous decaploid group with chromosomal constitution 2n = 10x = 70 (StStEeEbEx).

Blue grain differs from purple grain by the composition and presence of individual anthocyanins (ABDEL-AAL and HUCL 2003), which can be seen clearly on cross sections of kernels. For blue aleurone wheat the dominant anthocyanins were delphinidin 3-glucoside and delphinidin 3-rutinoside, whereas cyanidin 3-glucoside and cyanidin 3-rutinoside were, unlike in purple grains, present only in smaller quantities (KNIEVEL et al. 2009). Wholemeal flour contained 157 $mg{\cdot}kg^{-1}$ and bran 458 $mg{\cdot}kg^{-1}$ anthocyanins (ABDEL-AAL and HUCL 1999). Generally, the content of total anthocyanins seems to be higher in blue grained wheat compared to purple wheat (SYED JAAFAR et al. 2013).

In the Czech Republic, Miroslav Škorpík from the Crop Research Institute Prague-Ruzyně was interested in blue grained wheat for a long time. After World War II, he received donor material coming from the heritage of Erich von Tschermak-Seysenegg (one of the rediscoverers of Mendel's laws in 1900) (ŠKORPÍK et al. 1983). We assume it was a material similar to 'Tschermaks Blaukörniger Sommerweizen', which is preserved in the genebank of IPK Gatersleben. The initial material was gradually significantly improved by crossing with conventional wheat varieties. The activities led to the development of winter wheat variety 'Skorpion', which was registered in Austria in 2011. In 2012 it was enrolled in the European Catalogue of Varieties. 'Skorpion' has blue grain, but in comparison with common winter wheat varieties it has lower grain yield, medium winter hardiness and lower resistance to Fusarium head blight (FHB) (MARTINEK et al. 2013).

Yellow endosperm

The yellow endosperm colour is determined by two loci *Psy1* and *Psy2*, located on homoeologous chromosome groups 7 and 5 (POZNIAK et al. 2007). They affect the biosynthetic pathway of carotenoids, in particular phytoen synthase enzyme. The most investigated loci are *Psy1-A1* (7AL), *Psy1-B1* (7BL), *Psy1-D1* (7DL), *Psy2-A1* (5A) and *Psy2-B1* (5B) (HOWITT et al. 2009, Catalogue of Genetic Symbols for Wheat). The content of the yellow pigment is currently the most studied in *T. durum* (ZHANG and DUBCOVSKY 2008, HE et al. 2008) due to a need of yellow products in the pasta production industry. In the Czech Republic, winter wheat 'Citrus' and spring wheat 'Luteus' from Germany containing the carotenoids lutein and zeaxanthin were registered in 2011. The yellow substances favourably affect the colour of egg yolk in feeding trials with poultry.

Breeding for high anthocyanin content

The aim is to develop breeding lines of wheat with a high content of anthocyanins, good baking quality and satisfactory yield levels. In the available wheat genetic resources with uncommon grain colour the genetic similarity was evaluated using SSR markers (MUSILOVA et al. 2013). This information is used together with the assessment of the length of chromosomal segments in blue grained wheats (BUREŠOVÁ et al. 2013) to select parental combinations for crossing. At Agrotest Fyto, Ltd., we have now advanced lines with dark purple or blue grain colour which were selected on visual assessment. In some cases a combination of both colours, which is characterized by a dark purple with a violet shade, was observed. The problem is still the significant yield penalty, especially in the lines with blue grains. We assume that the low yield is conditioned by the negative influence of genes linked to the gene for blue aleurone on the chromosome segment from the wild species. It would be desirable to disrupt these linkages by evoking recombination. Most contemporary breeding lines with blue grain are characterized by a low resistance to FHB and snow mould, and frequent drying of grain. Lines with purple pericarp often exhibit small grains, whereas their resistance to FHB is usually good. Back-crosses are used for the transmission of non-traditional colour into the genetic background of common wheat varieties. It will be important to understand the biosynthetic pathways of anthocyanins in grain and regulatory mechanisms of their expression in different tissues of the grain. It would be useful to find such genetic mechanisms that enable gene expression also in the grain endosperm through unlocking regulatory genes or responsible transcription factors. Currently, the first steps have been made to clarify the biosynthetic pathway of enzyme chalcone synthase and the corresponding gene in wheat has been sequenced (TROJAN et al. 2013). New candidate sequence for other genes of the biosynthetic pathway of anthocyanins, e. g. dihydroflavonol 4-reductase, chalcon isomerase, including the first data on their expression during caryopsis maturation have been described.

Acknowledgements

The work was supported by project No. QJ1210257 financed by the Ministry of Agriculture of the Czech Republic and IGA FA MENDELU No. IP 12/2013.

References

ABDEL-AAL ESM, HUCL P, 1999: A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem 76, 350-354.

ABDEL-AAL ESM, HUCL P, 2003: Composition and stability of anthocyanins in blue-grained wheat. J Agric Food Chem 51, 2174-2180.

ARBUZOVA VS, MAYSTRENKO OI, 2000: Chromosomal location of genes for purple grain colour introgressed in common wheat. Cereal Res Commun 28, 235-237.

BUREŠOVÁ V, KOPECKÝ D, ŠAFÁŘ J, VYHNÁNEK T, MARTINEK P, DOLEŽEL J, 2013: Genomic constitution of cereals with blue aleurone trait. In: CHVÁTALOVÁ K (Ed), Olomouc Biotech 2013 - Plant Biotechnology: Green for Good II, BIO-TECHNO session: Industry meets academia, Book of abstracts, p 15. Centre of the Region Haná for the Biotechnological and Agricultural Research, Olomouc, Czech Republic.

DOBROVOLSKAYA O, ARBUZOVA VS, LOHWASSER U, RÖDER MS, BÖRNER A, 2006: Microsatellite mapping of complementary genes for purple grain colour in bread wheat (*Triticum aestivum* L.). Euphytica 150, 355-363.

DUBCOVSKY J, LUO MC, ZHONG GY, BRANSTEITTER R, DESAI A, KILIAN A, KLEINHOFS A, DVOŘÁK J, 1996: Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143, 983-999.

HE XY, ZHANG YL, HE ZH, WU YP, XIAO YG, MA CX, XIAL XC, 2008: Characterization of phytoene synthase 1 gene (*Psy1*) located on common wheat chromosome 7A and development of a functional marker. Theor Appl Genet 116, 213-221.

HIMI E, NODA K, 2005: Red grain colour gene (*R*) of wheat is a Myb-type transcription factor. Euphytica 143, 239-242.

HOWITT CA, CAVANAGH CR, BOWERMAN AF, CAZZONELLI C, RAMPLING L, MIMICA JL, POGSON BJ, 2009: Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes are associated with lutein accumulation in wheat endosperm. Funct Integr Genomics 9, 363-376.

KHLESTKINA EK, 2013: The adaptive role of flavonoids: Emphasis on cereals (review). Cereal Res Commun 41, 185-198.

KNIEVEL DC, ABDEL-AAL ESM, RABALSKI I, NAKAMURA T, HUCL P, 2009: Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.). J Cereal Sci 50, 113-120.

LACHMAN J, DUDJAK J, ORSÁK M, PIVEC V, 2003: Effect of accelerated ageing on the content and composition of polyphenolic complex of wheat (*Triticum aestivum* L.) grains. Plant Soil Env 49, 1-7.

LAMY S, BLANCHETTE M, MICHAUD-LEVESQUE J, LAFLEUR R, DUROCHER Y, MOGHRABI A, BARRETTE S, GINGRAS D, BELIVEAU R, 2006: Delphinidin, a dietary anthocyanidin, inhibits vascular endothelial growth factor receptor-2 phosphorylation. Carcinogenesis 27, 989-996.

MARTINEK P, ŠKORPÍK M, CHRPOVÁ J, FUČÍK P, SCHWEIGER J, 2013: Development of the new winter wheat variety Skorpion with blue grain. Czech J Genet Plant Breed 49, 90-94.

METTIN D, SCHLEGEL G, LEHMANN C, 1991: Instability of the blue grain colour in a strain of *Triticum aestivum* L. Genome 34, 745-750.

MORRISON LA, METZGER RJ, LUKASZEWSKI AJ, 2004: Origin of the blue-aleurone gene in Sebesta Blue wheat genetic stocks and a protocol for its use in apomixis screening. Crop Sci 44, 2063-2067.

MUSILOVÁ M, TROJAN V, VYHNÁNEK T, HAVEL L, 2013: Genetic variability for coloured caryopses in common wheat varieties determined by microsatellite markers. Czech J Genet Plant Breed 49, 116-122.

POZNIAK CJ, KNOX RE, CLARKE FR, CLARKE JM, 2007: Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. Theor Appl Genet 114, 525-537.

QUALSET CO, SOLIMAN KM, JAN CC, DVORAK J, MCGUIRE PE, VOGT HE, 2005: Registration of UC66049 *Triticum aestivum* blue aleurone genetic stock. Crop Sci 45, 432.

RÜCKSCHLOSS Ľ, MATÚŠKOVÁ K, HANKOVÁ A, JANČÍK D, 2010: Influence of winter wheat with purple colour of the corn on laying hens' efficiency and eggs quality. Potravinárstvo 4 (Special Issue), 231-235 (In Slovak, English abstract).

SINGH K, GHAI M, GARG M, CHHUNEJA P, KAUR P, SCHNURBUSCH T, KELLER B, DHALIWAL HS, 2007: An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum*×*T. monococcum* RIL population. Theor Appl Genet 115, 301-312.

ŠKORPÍK M, ROD J, ŠÍP V, SEHNALOVÁ J, KOŠNER J, 1983: Coloured wheat from the effects of E. Tschermak. Acta Agron Acad Sci Hung 2, 147-157.

SYED JAAFAR SN, BARON J, SIEBENHANDL-EHN S, ROSENAU T, BÖHMDORFER S, GRAUSGRUBER H, 2013: Increased anthocyanin content in purple pericarp×blue aleurone wheat crosses. Plant Breed 132, 546-552.

TROJAN V, MUSILOVÁ M, VYHNÁNEK T, KLEJDUS B, HANÁČEK P, HAVEL L, 2013: Chalcone synthase expression and pigments deposition in wheat with purple and blue colored caryopsis. J Cereal Sci 59, 48-55.

ZELLER EJ, CERMEÑO MC, MILLER TE, 1991: Cytological analysis on the distribution and origin of the alien chromosome pair conferring blue aleurone color in several European common wheat (*Triticum aestivum* L.) strains. Theor Appl Genet 81, 551-558.

ZEVEN AC, 1991: Wheats with purple and blue grains: a review. Euphytica 56, 243-258.

ZHANG W, DUBCOVSKY J, 2008: Association between allelic variation at the phytoene synthase 1 gene and yellow pigment content in the wheat grain. Theor Appl Genet 116, 635-645.