Genetic Variability for Coloured Caryopses in Common Wheat Varieties Determined by Microsatellite Markers

Milena MUSILOVÁ, Václav TROJAN, Tomáš VYHNÁNEK and Ladislav HAVEL

Department of Plant Biology, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic

Abstract

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.

Products made from wheat are the most important components of the human diet, and could also become a source of functional foods and feed ingredients, e.g. minerals, vitamins and/or phytochemicals. The caryopses of certain wheat genotypes contain antioxidants, i.e. anthocyanins or carotenoids, which cause purple, blue or yellow coloration. The first step before the introduction of these traits into individual wheat cultivars is the characterization of relationships and the possibility of new gene combinations. In this study, relationships among 24 genotypes with different types of caryopsis colour were investigated by means of microsatellite markers. Using 44 SSR (Simple Sequence Repeat) markers it was possible to detect a total of 184 alleles; on average, approximately 4 alleles were detected at a microsatellite locus. Using a set of 5 SSR markers (*Xgwm636*, *Xbarc077*, *Xwmc262*, *Xgwm397* and *Xwmc219*) with PIC (polymorphic information content) values higher than 0.70, it was possible to differentiate among all the genotypes analysed. A dendrogram was created on the basis of all SSR markers, and showed that the genotypes were divided into two groups. Three, and one genotype with purple and blue caryopsis, respectively, belonged to one cluster, while the remaining twenty formed the second, greater cluster, which was subdivided into 2 sub-clusters: one of them involved genotypes with blue caryopses, and the other those with yellow and red caryopses. The genotype of tall wheatgrass (*Thinopyrum ponticum*), as a possible donor of genes responsible for blue caryopses, was also classified. These results can be used in wheat breeding programmes aimed at the selection of functional foodstuffs.

Keywords: aleurone layer; endosperm; grain; pericarp; SSR markers; *Triticum aestivum*; *Thinopyrum ponticum*

Products made of wheat represent the most important components of the human diet, and could become a source of functional foods and feed ingredients. At present, wheat breeding is orientating towards certain nutritional aspects, e.g. contents of minerals, vitamins and/or phytochemicals. One possibility to reach this goal is to increase the content of antioxidants in the caryopsis and, thus, also in flour. The effect of antioxidants on human health is very well documented (Pham-Huy *et al.* 2008). There are wheat genotypes that contain antioxidants, e.g. anthocyanins in the purple pericarp, blue aleurone layer and/or carotenoids in the yellow endosperm. Modern varieties of hexaploid wheat have red caryopses and their colour is controlled by one to three dominant genes (*R-A1*, *R-B1*, and *R-D1*), which are localized on chromosomes 3A, 3B and 3D. The red colour is caused by the deposition of catechin-tannin derivatives in the pericarp. White caryopses develop when their genotype contains recessive alleles at these three genes (Miyamoto & Everson 1958). The deposition of the anthocyanin called cyanidin-3-glycoside predominates in the pericarp of purple

caryopses. Purple pericarp was transferred into today's wheat from tetraploid wheats originating from Ethiopia, Somalia and Yemen. In this case, four genes determining the purple colour of the wheat pericarp were mapped to different donors, viz. *Pp1* (on chromosome 7B) and *Pp2* (on chromosome 6A) (Arbuzova & Maystrenko 2000). However, Piech and Evans (1979) localized these genes on chromosome 7A while Dobrovolskaya *et al.* (2006) renamed the gene *Pp2* to *Pp3a* and detected *Pp3b* on chromosome 2A.

The presence of delphinidin-3-glycoside in the aleurone layer is typical for blue caryopses (Abdel-Aal & Hucl 2003), and is controlled either by a co-dominant gene *Ba1* (on chromosome 4B) or by the gene *Ba2* (on chromosome 4A), which was transferred to the wheat genome through a disomic substitution from a yet unknown species, but probably from tall wheatgrass (Zeven 1991). Yellow colour is determined by carotenoids that are deposited in the endosperm (Howitt *et al.* 2009). Genes *Psy1* and *Psy2* are responsible for the production of yellow pigments. They are located on chromosomes of homoeologous groups 7 and 5 (Pozniak *et al.* 2007).

Anthocyanin-coloured caryopses can be used as functional foodstuffs (e.g. when manufacturing whole-grain products) or as functional food colourants (e.g. caryopses fractions with a high content of anthocyanin) (Abdel-Aal *et al.* 2006). Before the beginning of a breeding programme, selection of wheats with coloured caryopses, and their development as a functional food requires a detailed description of all available genotypes. To reach this goal, it is possible to use morphological markers, protein markers, and DNA markers (Zhang *et al.* 2007). DNA markers such as microsatellites, SSR (Simple Sequence Repeat) markers, are especially suitable for this purpose, above all due to their high degree of polymorphism and co-dominant inheritance (Yi *et al.* 2008; Chandra *et al.* 2010).

The aim of this study was to detect genetic variability amongst wheat genotypes with non-standard coloured caryopses that are available in the Czech Republic, and to compare them with selected genotypes that show the standard (i.e. red) colour.

In future, it will be necessary to study these genotypes in wheat breeding programmes focused on the development of new wheat cultivars that could represent a source of functional foods. Genotypes with purple pericarp, blue aleurone layer, yellow endosperm, white pericarp, and red pericarp were analyzed.

The genotype of tall wheatgrass (*Thinopyrum ponticum*/Podp./Z.-W. Liu & R.-C. Wang), which is a probable source of a blue colour of aleurone layer, was analysed as well (Jan *et al.* 1981).

MATERIAL AND METHODS

Genotypes of spring and winter wheat (*Triticum aestivum* L.), with a standard caryopsis colour (i.e. with red pericarp) and those showing a non-standard colour were used as the experimental material (Table 1). Wheat genotypes were provided by the Agricultural Research Institute (ARI) Kromeriz, Ltd., and the tall wheatgrass genotype was obtained from the Gene Bank of the Crop Research Institute in Praha-Ruzyně. These 25 genotypes were subjected to an analysis of genetic variability using 44 SSR markers located on chromosomes close to genes determining different pigmentation characteristics of the caryopsis. These were obtained from the database GrainGenes (2010) and from the literature dealing with wheat and triticale variation (Röder *et al.* 1998; Sourdille *et al.* 2001; Somers *et al.* 2004, Dobrovolskaya *et al.* 2006; Kuelung *et al.* 2006). Genomic DNA, isolated by means of a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) from mixed samples of 3–5 cultivated plants from the one leaf stage (i.e. 5–7 days old), was used for the molecular analysis. The reaction mixture of a total volume of 25 µl contained: 30 ηg DNA template, 0.5 U *Taq* polymerase (Promega, Madison, USA), 1× corresponding buffer, 7.5µM of each primer and 100µM of each dNTP. The temperature and the time profiles of the reaction were as follows: 1 cycle (93°C – 120 s) and 30 cycles (93°C – 60 s, 54°C – 120 s, 72°C – 120 s). Samples were separated electrophoretically (300 V) in 8% non-denatured polyacrylamide (PAA) gel containing the TBE (Tris-Borate-EDTA) buffer. For product visualization, the method of staining with silver nitrate (0.2%) was used. Results of molecular analysis were evaluated using the binary matrix where values of 1 and 0 indicated either presence or absence of the product, respectively. Subsequently, the results were analysed by means of the statistical software FreeTree, version 9.1 (Hampl *et al.* 2001) using the UPGMA (Unweight Pair Group Method with Arithmetic Mean) clustering method, and the Jaccard similarity coefficient (Jaccard 1908). The graphical

Table 1. Characteristics of analysed genotypes

Name	Caryopsis colour	Type	Donor country
Novosibirskaya 67	white	spring	RUS
ANK-1A, ANK-1B, ANK-1C, ANK-1D, ANK-1E	red	spring	RUS
ANK-28A, ANK-28B	purple	spring	RUS
Abyssinskaya Arraseita		spring	CZE
Konini		spring	NZL
Purple		spring	USA
Purple feed		spring	USA
Indigo		winter	ENG
UC66049	blue	spring	USA
Tschermaks Blaukörniger Sommerweizen		spring	GER
Tschermaks Blaukörniger		spring	JAP
48M		winter	POL
RU 440-5, RU 440-6 (Skorpion*), Barevna 9, Barevna 25		winter	CZE
Citrus, Luteus	yellow	winter	GER
Bona Dea		winter	SVK
Thinopyrum ponticum	donor of blue colour	winter	TUR

CZE – Czech Republic; ENG – England; GER – Germany; JAP – Japan; NZL – New Zealand; POL – Poland; RUS – Russia; SVK – Slovakia; TUR – Turkey; USA – United States; *Martinek *et al.* (2013)

representation of the matrix was performed using the software TreeView, version 1.6 (Page 1996). For every SSR marker, statistical values of DI (diversity index), PI (probabilities of identity) and PIC (polymorphic information content) were calculated (Russell *et al.* 1997).

RESULTS AND DISCUSSION

SSR markers for the A- and B-genomes enabled the detection of 184 alleles, so there was an average number of four alleles per microsatellite locus.

A similar number of alleles (i.e. 5.1 per locus) was also detected by MacCaferri *et al.* (2010) who analysed a greater number (189) of genotypes of durum wheat (*Triticum durum* Desf.), and also used a higher number of microsatellites (altogether 90 SSR markers). The highest number, 9 alleles, was detected at microsatellites *Xgwm397* and *Xwmc219*. The highest variability in the size of amplicons was found using primers for *Xbarc176* and *Xbarc077*, and the size of amplified products ranged from 90 to 260 bp.

Statistical values calculated from these data represented an average degree of variability in the collection of wheat genotypes under study (Table 2). Average values of DI (0.40), PI (0.43), and PIC (0.38) reflected the existence of a uniform spectrum of 6 SSR markers (U values in Table 2), the presence of near-isogenic lines (ANK-1A–28B), and the existence of genotypes with a very similar genetic basis (Tschermaks Blaukörniger Sommerweizen and Tschermaks Blaukörniger; RU 440-5 and RU 440-6).

The letter P indicated a microsatellite marker with the highest degree of polymorphism and with values of DI ranging from 0.71 to 0.79; its values of PI and PIC ranged from 0.02 to 0.06 and from 0.70 to 0.79, respectively (Table 2). A combination of these five most polymorphic SSR markers enabled the reliable identification of all genotypes, and for that reason could be used as a microsatellite panel to enable an easier identification of parent combinations during the process of hybridisation. Null alleles (0 in Table 2) were detected at 13 SSR markers. Kuelung *et al.* (2006) also mentioned presence of null alleles.

The similarity dendrogram was constructed on the basis of the statistical data. It was found that there were two clusters of genotypes (Figure 1), and that the genotype of *Thinopyrum ponticum*,

which is a donor of blue colour, existed outside of these two clusters. The smaller cluster (Cluster II.) involved three genotypes with purple caryopses (Abyssinskaya Arraseita, Purple, and Purple Feed) and one with a blue colour. This fact indicates that Cluster II was significantly different from the remaining 20 genotypes that belonged to clusters I.A, I.B, and also from the genotype of tall wheatgrass.

The large cluster was further divided into 2 subclusters. The smaller one (subcluster I.B) consisted only of blue genotypes, and in case of two of them, a close relationship was observed. The relationship between genotypes Tschermaks Blaukörniger Sommerweizen and Tschermaks Blaukörniger was subsequently confirmed. These two lines are probably genetically identical. They were added into the collection of ARI Kromeriz, Ltd. just from different sources (P. Martinek 2012, ARI Kromeriz, personal communication). A statistically significant relationship of genotypes Citrus and Luteus (subcluster I.A) was evidently pre-determined by the existence of common ancestors in their pedigree. Both these genotypes were selected by Jahn in Deesbach, Germany, and their pedigree was as follows: Citrus (Sunnan/Monopol//Stamm GI 912) and Luteus (Sunnan/Monopol//Giessener Stamm) (V. Horáková 2012, Central Institute for Supervising and Testing in Agriculture Brno, personal communication).

Tall wheatgrass (a probable donor of the blue caryopses colour) ranked outside these two clusters in the overall dendrogram of relationships (Figure 1). This was confirmed also by the fact that this wild species did not share homology in all wheat chromosomes monitored. Relationships among coloured lines were investigated in a dendrogram involving only lines with blue aleurone (Figure 2). The dendrogram was constructed from variation at 19 SSR markers. These SSR markers are located on chromosomes 4A and 4B while *Ba1* and *Ba2* genes control blue aleurone (Qualset *et al.* 2005).

The similarity of the tall wheatgrass genotype to others with blue caryopses was shown by a statistically highly significant value after bootstrapping. In the context of a more specific dendrogram, genotypes with blue caryopses (aleurone layer) were divided into 2 clusters (A and B). We expected a higher degree of similarity of tall wheatgrass with

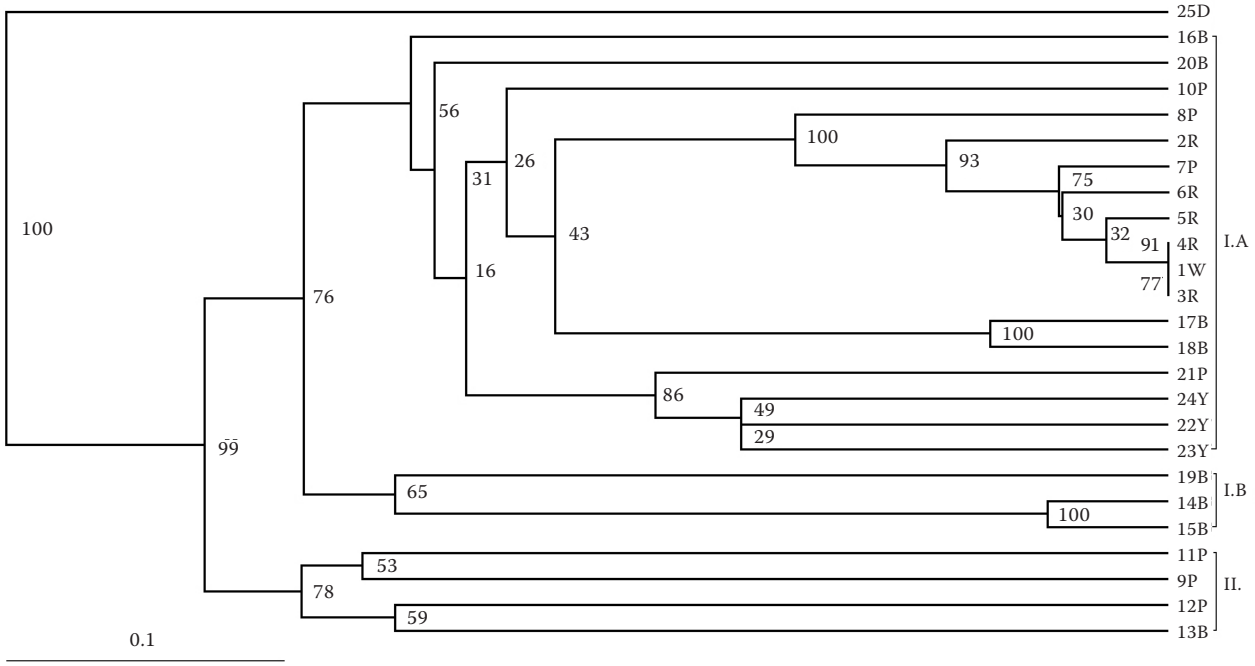

Figure 1. Dendrogram showing variability and clustering of the 25 genotypes included in this study
1 – Novosibirskaya 67; 2 – ANK-1A; 3 – ANK-1B; 4 – ANK-1C; 5 – ANK-1D; 6 – ANK-1E; 7 – ANK-28A; 8 – ANK-28B; 9 – Abyssinskaya Arraseita; 10 – Konini; 11 – Purple; 12 – Purple feed; 13 – Indigo; 14 – UC66049; 15 – Tschermaks Blaukörniger Sommerweizen; 16 – Tschermaks Blaukörniger; 17 – 48M; 18 – RU 440-5; 19 – RU 440-6; 20 – Barevna 9; 21 – Barevna 25; 22 – Citrus; 23 – Luteus; 24 – Bona Dea; 25 – *Thinopyrum ponticum*; D – donor; B – blue aleurone; P – purple pericarp; R – red pericarp; W – white pericarp; Y – yellow endosperm

Table 2. Characterization of wheat SSR (Simple Sequence Repeat) markers and their statistical analysis

SSR marker	Localization	Number of alleles	DI	PI	PIC	Note
Xgwm122	2A	7	0.52	0.23	0.51	
Xgwm294	2A	4	0.65	0.16	0.60	
Xgwm445	2A	6	0.60	0.17	0.58	
Xgwm328	2A	1	0.00	1.00	0.00	U
Xgwm356	2A	1	0.00	1.00	0.00	U
Xgwm666b	2A	3	0.28	0.54	0.26	
Xgwm636	2A	6	0.71	0.05	0.70	P
Xwmc428	3A	5	0.54	0.24	0.51	
Xcfa2134	3A	2	0.08	0.86	0.07	
Xgwm674	3A	3	0.28	0.54	0.26	
Xgwm155	3A	6	0.51	0.24	0.50	
Xgwm480	3A	5	0.44	0.27	0.44	0
Xbarc077	3B	6	0.72	0.05	0.72	P, 0
Xgwm181	3B	5	0.69	0.06	0.68	
Xgwm340	3B	4	0.60	0.09	0.60	0
Xgwm4a	3B,4A	3	0.39	0.35	0.38	
Xgwm314	3D	2	0.08	0.86	0.07	
Xgwm664	3D	2	0.08	0.86	0.07	
Xgwm456	3D	2	0.21	0.64	0.19	
Xbarc170	4A	7	0.63	0.10	0.63	0
Xwmc173	4A	4	0.45	0.32	0.42	0
Xgwm601	4A	2	0.48	0.39	0.36	
Xbarc206	4A	3	0.22	0.60	0.22	0
Xbarc138	4A	3	0.28	0.54	0.26	
Xwmc262	4A	8	0.72	0.06	0.72	P, 0
Xgwm397	4A	9	0.78	0.03	0.78	P
Xwmc232	4A	2	0.27	0.57	0.23	0
Xgwm160	4A	6	0.46	0.28	0.46	
Xwmc219	4A	9	0.79	0.02	0.79	P, 0
Xgwm251	4B	6	0.69	0.13	0.65	0
Xgwm6	4B	2	0.15	0.74	0.14	
Xgwm513	4B	1	0.00	1.00	0.00	U
Xgwm192	4B	5	0.51	0.22	0.51	
Xgwm149	4B	1	0.00	1.00	0.00	U
Xwmc47	4B	4	0.34	0.41	0.34	
Xbarc163	4B	8	0.52	0.23	0.52	0
Xbarc195	6A	4	0.51	0.30	0.44	
Xbarc003	6A	6	0.64	0.09	0.64	0
Xgwm427	6A	2	0.08	0.86	0.07	
Xgwm570	6A,6B	1	0.00	1.00	0.00	U
Xgwm60	7A	1	0.00	1.00	0.00	U
Xgwm861	7A,7B	6	0.67	0.10	0.65	
Xbarc176	7B	7	0.65	0.11	0.65	
Xgwm276	7B	3	0.28	0.54	0.26	0
Mean			0.40	0.43	0.38	

DI – diversity index; PI – probabilities of identity; PIC – polymorphic information content; P – SSR marker with the highest level of polymorphism; U – uniform SSR marker; 0 – null allele

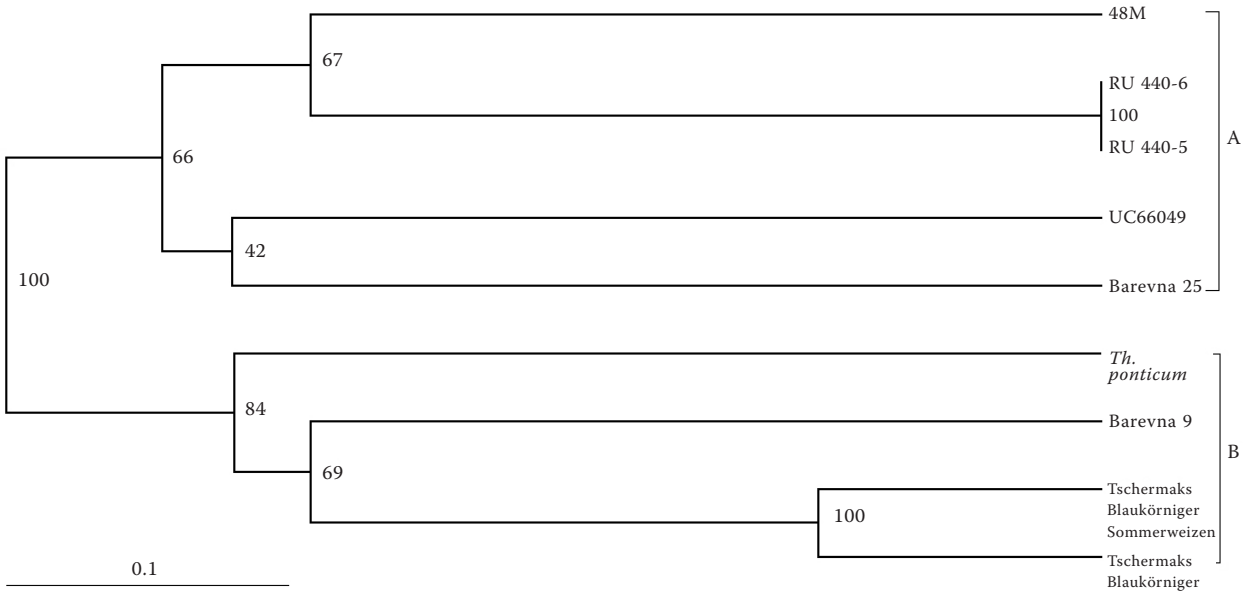

Figure 2. Dendrogram showing variability and clustering of the genotypes with blue aleurone and *Thinopyrum ponticum*
A, B – clusters

the genotype UC66049. Morrison *et al.* (2004) mentioned the existence of lines BS-1, BS-2, and BS-3 (Blue Sebesta) with blue caryopses which had chromosomal segments transferred from a genotype of tall wheatgrass which seemed to have a similar origin as UC66049. Here the analysed genotype of tall wheatgrass, which is freely accessible in Gene Bank CRI Prague-Ruzyně, had the code ECN 01C3200011. Morrison *et al.* (2004) did not specify the exact characteristics of this genetic resource; it was not possible to include a clearly defined set of genetic resources so that the comparison with our results was not possible.

This study indicates that the most closely related genotypes with blue aleurone were Tschermaks Blaukörniger, Tschermaks Blaukörniger Sommerweizen, RU 440-5 and RU 440-6 (now new cultivar Skorpion). Near isogenic lines ANK with red and purple pericarp showed a high level of similarity with Novosibirskaya 67 (white pericarp). Our results also confirmed the close relationship between two genotypes with yellow endosperm (Citrus and Luteus).

These results can be used for monitoring of hybridisation in wheat breeding programmes and also for the characterisation of donors of non-standard caryopses colour.

Acknowledgements. The authors gratefully acknowledge the support of the Czech Scientific Foundation, Grant GACR 204/09/H002, P. Martinek from Agricultural Research Institute Kromeriz, Ltd. Czech Republic, the Gene Bank of the Crop Research Institute Praha-Ruzyně for providing of the experimental material, H. Buerstmayr from the Department for Agrobiotechnology, BOKU Wien, and H. Grausgruber from the Department of Crop Sciences, University of Natural Resources and Life Sciences Tulln, Austria for their critical readings of the manuscript.

References

Abdel-Aal El-S.M., Hucl P. (2003): Composition and stability of anthocyanins in blue-grained wheat. Journal of Agricultural and Food Chemistry, **51**: 2174–2180.

Abdel-Aal El-S.M., Young J.C., Rabalski I. (2006): Anthocyanin composition in black, blue, pink, purple, and red cereal grains. Journal of Agricultural and Food Chemistry, **54**: 4696–4704.

Arbuzova V.S., Maystrenko O.I. (2000): Chromosomal location of genes for purple grain colour introgressed in common wheat. Cereal Research Communication, **28**: 235–237.

Chandra R., Gupta S., Ahmad A., Iqbal M., Prasad M. (2010): Variability in Indian bread wheat (*Triticum aestivum* L.) varieties differing in nitrogen efficiency as assessed by microsatellite markers. Protoplasma, **242**: 55–67.

Dobrovolskaya O., Arbuzova V.S., Lohwasser U., Röder M.S., Börner A. (2006): Microsatellite mapping

of complementary genes for purple grain in bread wheat (*Triticum aestivum* L.). Euphytica, **150**: 355–363.

GrainGenes (2010): A Database for *Triticeae* and *Avena*. Available at http://www.wheat.pw.usda.gov/GG2/index.shtml (accessed February 2010).

Hampl V., Pavlíček A., Flegr J. (2001): Construction and bootstrap analysis of DNA fingerpriting-based phylogenetic trees with a freeware program FreeTree: Application to Trichomonad parasites. International Journal of Systematic and Evolutionary Microbiology, **51**: 731–735.

Howitt C.A., Cavanagh C.R., Bowerman A.F., Cazzonelli Ch., Rampling L., Mimica J.L., Pogson B.J. (2009): Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes are associated with lutein accumulation in wheat endosperm. Functional and Integrative Genomics, **9**: 363–376.

Jaccard P. (1908): Nouvelles recherches sur la distribution florale. Bulletin del la Societé Vaudoise des Scientes Naturelles, **44**: 223–270.

Jan C.C., Dvořák J., Qualset C.O., Soliman K.M. (1981): Selection and identification of a spontaneous alien chromosome-translocation in wheat. Genetics, **98**: 389–398.

Kuelung C., Baezinger P.S., Kachman S.D., Dweikat I. (2006): Evaluating the genetic variability of triticale with wheat and rye SSR markers. Crop Science, **46**: 1692–1700.

MacCaferri M., Sanguineti M.C., Demontis A., El-Ahmend A., Del Moral L.C., Maalouf F., Nachit M., Nserallah N., Ouabbou H., Rhouma S., Royo C., Villegas D., Tuberosa R. (2010): Association mapping in durum wheat grown across a broad range of water regimes. Journal of Experimental Botany, **10**: 1–30.

Martinek P., Škorpík M., Chrpová J., Fučík P., Schweiger J. (2013): Development of the new wheat variety Skorpion with blue grain. Czech Journal of Genetics and Plant Breeding, **49**: 90–94.

Miyamoto T., Everson E.H. (1958): Biochemical and physiological studies of wheat seed pigmentation. Agronomy Journal, **50**: 733–734.

Morrison L.A., Metzger R.J., Lukaszewski A.J. (2004): Origin of the blue-aleurone gene in Sebesta blue wheat genetic stocks and a protocol for its use in apomixis screening. Crop Science, **44**: 2063–2067.

Page R.D.M. (1996): TreeView: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences, **12**: 357–358.

Piech J., Evans L.E. (1979): Monosomic analysis of purple grain colour in hexaploid wheat. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, **82**: 212–217.

Pham-Huy L.A., He H., Pham-Huyc C. (2008): Free radicals, antioxidants in disease and health. International Journal of Biomedical Science, **4**: 89–96.

Pozniak C.J., Knox R.E., Clarke F.R., Clarke J.M. (2007): Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. Theoretical and Applied Genetics, **114**: 525–537.

Qualset C.O., Soliman K.M., Jan C.C., Dvořák J., McGuire P.E., Vogt H.E. (2005): Registration of UC66049 *Triticum aestivum* blue aleurone genetic stock. Crop Science, **45**: 432.

Röder M.S., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganal M.W. (1998): A microsatellite map of wheat. Genetics, **149**: 2007–2023.

Russell J., Fuller J., Young G., Thomas B., Taramino G., Macaulay M., Waugh R., Powell W. (1997): Discriminating between barley genotypes using microsatellite markers. Genome, **40**: 442–450.

Somers D.J., Isaac P., Edwards K. (2004): A high-density wheat microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, **109**: 1105–1114.

Sourdille P., Tavaud M., Charmet G., Bernard M. (2001): Transferability of wheat microsatellites to diploid *Triticeae* species carrying the A, B and D genome. Theoretical and Applied Genetics, **103**: 346–352.

Yi Y.J., Liu H.Y., Huang X.Q., An L.Z., Wang F., Wang X.L. (2008): Development of molecular markers linked to the wheat powdery mildew resistance gene *Pm4b* and marker validation for molecular breeding. Plant Breeding, **127**: 116–120.

Zhang L., Li H., Wangh H., Li L. (2007): Genetic diversification of the Chinese wheat landrace Mazhamai as revealed by morphological characteristics, seed storage proteins, and microsatellite markers. Canadian Journal of Plant Science, **87**: 763–771.

Zeven A.C. (1991): Wheats with purple and blue grains: A review. Euphytica, **56**: 243–258.

Received for publication August 9, 2012
Accepted after corrections August 20, 2013

Corresponding author:

Prof. RNDr. Ladislav Havel, CSc., Mendelova univerzita v Brně, Agronomická fakulta, Zemědělská 1, 613 00 Brno, Česká republika
e-mail: ladislav.havel@mendelu.cz