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Identification of Alleles of Puroindoline Genes and Their Effect on Wheat (*Triticum aestivum* L.) Grain Texture

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Summary

Grain hardness is one of the most important quality characteristics of wheat (*Triticum aestivum* L.). It is a significant property of wheat grains and relates to milling quality and end product quality. Grain hardness is caused by the presence of puroindoline genes (*Pina* and *Pinb*). A collection of 25 genotypes of wheat with unusual grain colour (blue aleurone, purple and white pericarp, yellow endosperm) was studied by polymerase chain reaction (PCR) for the diversity within *Pina* and *Pinb* (alleles: *Pina-D1a*, *Pina-D1b*, *Pinb-D1a*, *Pinb-D1b*, *Pinb-D1c* and *Pinb-D1d*). The endosperm structure was determined by a non-destructive method using light transflectance meter and grain hardness by a texture analyser. Genotype Novosibirskaya 67 and isogenic ANK lines revealed hitherto unknown alleles at the locus for the annealing of primers of *Pinb-D1*. Allele *Pinb-D1c* was found to be absent from each genotype. The mealy endosperm ranged from 0 to 100 % and grain hardness from 15.10 to 26.87 N per sample.

Key words: grain hardness, mealiness, vitreousness

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. Grain texture is a major characteristic and a determinant of end product quality, especially important in baking and noodle making (*1,2*). Flour from hard wheat is best for making bread, while flour from soft wheat is mainly used for making cakes, pastries and biscuits (*3*).

Wheat seeds contain the group of proteins called puroindolines (Pin). They belong to the broad superfamily of plant proteins consisting of a number of other cereal proteins. They are characterised by the presence of short sequences rich in the amino acid tryptophan (*4*). Grain hardness is primarily controlled by the complex hardness (*Ha*) locus, which consists of three closely-linked genes *Gsp-1*, *Pina* and *Pinb* (*5*). Wheat includes two types of protein: puroindoline a (Pina) and puroindoline b (Pinb). The milling of wheat is strongly influenced by grain hardness due to the presence or absence of the polypeptides Pina and Pinb (*6*). The key role of the *Pina* and *Pinb* genes is to determine the structure of the proteins in wheat grain and also possible antimicrobial effects (*7*).

The endosperm texture can be vitreous (steely, flinty, glassy or corneous) or mealy (starchy or chalky). Mealy endosperm contains more starch and less protein compared with vitreous endosperm. Hardness is defined as

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material resistance to penetration (*8*), and is closely related to vitreousness.

Wheat hardness is affected especially by genetic factors (*9*). Starch granules of different sizes are coated by a protein matrix created predominantly by gluten proteins. Differences in wheat hardness are due to the adhesion of storage proteins to starch granules (*10*). Cultivars with softer endosperm texture have bigger starch granules and harder wheat has smaller starch granules. Smaller granules have a larger surface available for non-covalent bonds with endosperm proteins and can be packed more effectively, which ensures harder endosperm.

The main aim of this work is the identification of markers for puroindoline genes (*Pina* and *Pinb*) in wheat using polymerase chain reaction and determination of endosperm texture of wheat.

Materials and Methods

Common wheat (*Triticum aestivum* L.) genotypes with unusual grain and endosperm colour (Table 1) from the 2014 harvest of the Agricultural Research Institute Kroměříž, Ltd., Kroměříž, Czech Republic, were investigated in the present study.

Genomic DNA was isolated from young leaf plant tissues using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). For the identification of puroindoline genes, primers for *Pina* and *Pinb* (*3*) were used. The PCR analysis for determination of puroindoline genes was performed as follows: initial denaturation for 5 min at 94 °C, then 35 cycles for 30 s at 95 °C, 30 s at 60 °C, 90 s at 72 °C and a final extension step of 10 min at 72 °C. The total volume in one reaction was 25 μL. The visualisation of PCR products was carried out in 1.5 % agarose gel.

A light transflectance meter (LTm; Brewing Research International (BRi), Nutfield, UK) was used for the evaluation of vitreousness and mealiness of caryopses (*11*). This non-destructive method is based on the quantitative measurement of laser beam propagation through a barley or wheat caryopsis. Ninety-seven caryopses of each genotype were used for one experiment. Mealy caryopses are not transparent to light and vitreous caryopses allow

Table 1. Investigated genotypes, grain colour and texture, and puroindoline alleles

Genotype	Mealiness/%	Hardness/N	Final allele	
White pericarp:				
Novosibirskaya 67 (N67)	2	1.94±0.26	n.d.	n.d.
Heroldo	34	2.74±0.41	*Pina-D1a*	*Pinb-D1d*
Purple pericarp:				
ANK-28A	10	2.63±0.37	n.d.	n.d.
ANK-28B	6	1.77±0.29	n.d.	n.d.
Abissinskaya arraseita	84	1.73±0.15	*Pina-D1a*	*Pinb-D1a*
Konini	30	1.90±0.32	*Pina-D1a*	*Pinb-D1a*
Purple	87	1.54±0.25	*Pina-D1a*	*Pinb-D1a*
Purple feed	91	1.97±0.31	*Pina-D1a*	*Pinb-D1a*
Indigo	100	1.66±0.18	*Pina-D1a*	*Pinb-D1a*
Rosso	88	2.16±0.28	*Pina-D1a*	*Pinb-D1a*
Blue aleurone:				
UC 66049	99	2.35±0.36	*Pina-D1a*	*Pinb-D1b*
Tschermaks Blaukörniger Sommerweizen	98	2.11±0.41	*Pina-D1a*	*Pinb-D1b*
Tschermaks Blaukörniger	99	1.82±0.23	*Pina-D1a*	*Pinb-D1b*
48M	97	1.79±0.24	*Pina-D1a*	*Pinb-D1a*
Skorpion (RU 440-6)	76	2.45±0.42	*Pina-D1a*	*Pinb-D1d*
RU 440-5	96	1.88±0.27	*Pina-D1a*	*Pinb-D1d*
Barevná 9	23	2.57±0.36	*Pina-D1a*	*Pinb-D1b*
Barevná 25	74	1.92±0.15	*Pina-D1a*	*Pinb-D1b*
Xiao Yian	68	1.91±0.37	*Pina-D1a*	*Pinb-D1a*
EF 02-54/9	100	2.12±0.67	*Pina-D1a*	*Pinb-D1b*
H 90-15-2	85	1.90±0.45	*Pina-D1a*	*Pinb-D1a*
Yellow endosperm:				
Citrus	0	2.10±0.25	*Pina-D1a*	*Pinb-D1b*
Luteus	50	1.77±0.26	*Pina-D1a*	*Pinb-D1b*
Bona Dea	89	2.18±0.41	*Pina-D1a*	*Pinb-D1b*
TA 4024	3	1.76±0.29	*Pina-D1a*	*Pinb-D1b*

Hardness is expressed as mean value±standard deviation, n.d.=not detected

more light to transmit. Table 1 shows the percentage of mealy caryopses. Vitreous endosperm has lower percentage of mealiness than mealy endosperm.

The hardness of wheat was determined at the University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic. The texture analysis was performed with TA.XT*plus* texture analyser (Stable Micro Systems, Godalming, Surrey, UK). The samples were examined using Exponent v. 5.0 software (Stable Micro Systems). A three-inch compression plate was installed in the 25-kg load cell of the analyser. A 5-kg weight was used to calibrate the 25-kg load cell prior to analysis and the setting was adjusted at a pretest, test and posttest speed of 1 mm/s. All samples were compressed once to 60 % of their original height using an upper fracture wedge piston. The obtained texture profiles were used to measure the instrumental hardness. The maximum positive force is the force required to penetrate the sample to the specified distance. The higher this value, the harder the sample. The maximum hardness force was measured during the first compression cycle.

Results and Discussion

The genes for *Pina-D1* and *Pinb-D1* are located on chromosome 5D and are the main determinants of grain texture in hexaploid wheat. All wheat with hard endosperm is characterised by a sequence mutation in either *Pina* or *Pinb*. The result is a change in kernel hardness from soft to hard (*6,12,13*).

We analysed nine markers for *Pina* and four combinations of markers for *Pinb* with different product sizes (Table 2). Two alleles of *Pina* for each genotype were analysed. Only one allele, *Pina-D1a*, was detected (Table 1). Three alleles of *Pinb* were found: *Pinb-D1a*, *Pinb-D1b* and *Pinb-D1d* (Table 1), while allele *Pinb-D1c* was not detected. In genotypes Novosibirskaya 67, ANK-28A and ANK-28B unknown PCR products were observed (Figs. 1 and 2). A mutation in the locus for annealing primer temperature for *Pinb-D1*, which resulted in hard texture (*2*), was detected. Mutations in the *Pina-D1* and *Pinb-D1* genes have individually been associated with grain hardness,

Table 2. Markers for identification of puroindoline *Pina* and *Pinb* genes and their product size

Marker	Allele	Product size/bp
STS1	*Pina-D1a*	704
	Pina-D1b	922
STS2	*Pina-D1a*	704
	Pina-D1b	1033
STS3	*Pina-D1a*	744
	Pina-D1b	922
STS4	*Pina-D1a*	744
	Pina-D1b	1033
STS5	*Pina-D1a*	463
	Pina-D1b	792
STS6	*Pina-D1a*	503
	Pina-D1b	792
STS7	*Pina-D1a*	407
	Pina-D1b	736
STS8	*Pina-D1a*	447
	Pina-D1b	625
STS9	*Pina-D1a*	447
	Pina-D1b	736
SNP G	*Pinb-D1a*	423
SNP A	*Pinb-D1b*	226
SNP G	*Pinb-D1a*	423
SNP A	*Pinb-D1b*	232
SNP T	*Pinb-D1a*	423
SNP C	*Pinb-D1c*	269
SNP C	*Pinb-D1c*	423
SNP T	*Pinb-D1a*	269
SNP T	*Pinb-D1a*	423
SNP A	*Pinb-D1d*	237
SNP A	*Pinb-D1d*	423
SNP T	*Pinb-D1a*	236

bp=base pair

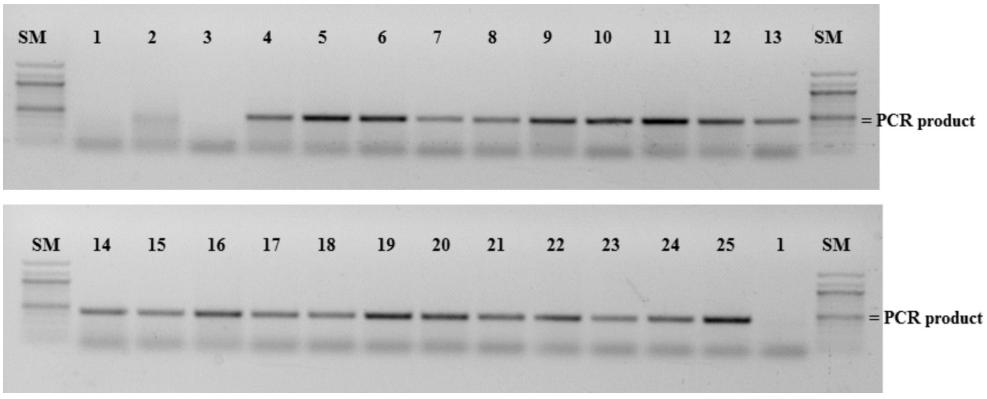

Fig. 1. PCR product of *Pina* (marker STS5) for the detection of *Pina-D1a*, product size 463 bp. SM=size marker, 1=Novosibirskaya 67, 2=ANK-28A, 3=ANK-28B, 4=Abissinskaya arraseita, 5=Konini, 6=Purple, 7=Purple feed, 8=Indigo, 9=Rosso, 10=Citrus, 11=Luteus, 12=Bona Dea, 13=TA 4024, 14=UC 66049, 15=Tschermaks Blaukörniger Sommerweizen, 16=Tschermaks Blaukörniger, 17=48M, 18=Skorpion, 19=RU 440-5, 20=Barevná 9, 21=Barevná 25, 22=Xiao Yian, 23=EF 02-54/9, 24=H 90-15-2, 25=Heroldo

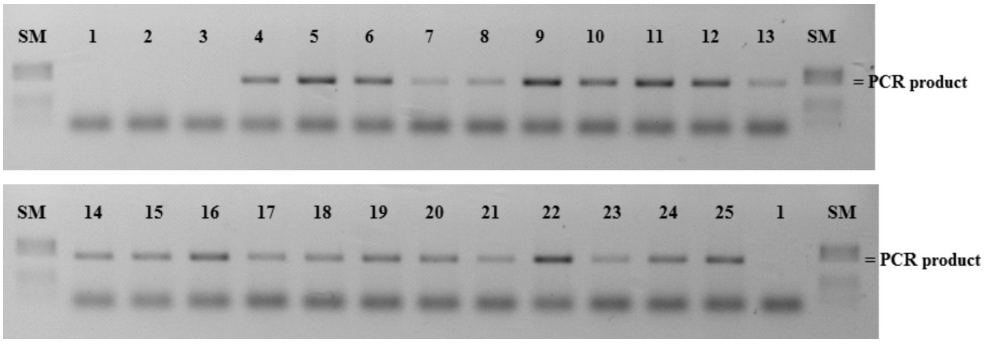

Fig. 2. PCR product of *Pinb* (marker SNP T/C) for the detection of *Pinb-D1a*, product size 423 bp. SM=size marker, 1=Novosibirskaya 67, 2=ANK-28A, 3=ANK-28B, 4=Abissinskaya arraseita, 5=Konini, 6=Purple, 7=Purple feed, 8=Indigo, 9=Rosso, 10=Citrus, 11=Luteus, 12=Bona Dea, 13=TA 4024, 14=UC 66049, 15=Tschermaks Blaukörniger Sommerweizen, 16=Tschermaks Blaukörniger, 17=48M, 18=Skorpion, 19=RU 440-5, 20=Barevná 9, 21=Barevná 25, 22=Xiao Yian, 23=EF 02-54/9, 24=H 90-15-2, 25=Heroldo

but it is not known if mutations at both loci may further increase hardness or if additional copies may reduce it (*14*).

In Novosibirskaya 67 genotype and its isogenic ANK lines with purple caryopses, alleles were not identified even when testing the primer combinations described by Gautier *et al*. (*15*). It is necessary to design primers for a sequence analysis of these genotypes, which would allow the identification of relevant alleles for *Pina* and *Pinb* loci.

The development of mealiness appears to depend on maturation. Immature grains of all wheat types are mealy. Vitreous grains are found in plants that grow and ripen quickly and mealy grains are characteristic of varieties that grow slowly and have a long maturation period. This means that vitreousness is characteristic of a short vegetation period. The mealy or vitreous character is hereditary but it is also affected by environment (*16*). The mealiness of caryopses ranged from 0 to 100 %. Eight genotypes had less than 50 % mealy caryopses. The lowest value was in Citrus genotype (0 % mealy endosperm). Mealy endosperm was observed in 17 genotypes, eight of which had 90 % or more mealy caryopses.

Mealiness is closely related to hardness. The hardness of grains is used for the evaluation of breeding material, especially for wheat and barley. Values of wheat hardness varied from 15.10 to 26.87 N. The highest and lowest values were observed in Heroldo genotype with white pericarp and Purple genotype with purple pericarp, respectively. The results for mealiness and hardness are shown in Table 1.

Nonsignificant differences were found for grain hardness. Starch grains in the endosperm of the grain are bound by different molecules of puroindoline that exhibit different grain hardness. High values are characteristic of vitreous endosperm and *vice versa* (*7*). Gaines *et al*. (*17*) found that soft wheat had a higher content of amylose bound to lipids and a lower total starch content.

The nonstandard colours of wheat caryopsis are caused by the presence of anthocyanins, and the interest in such wheat is mainly because of its positive effects on the health of consumers. The hardness of wheat endosperm is critical in determining the suitability of wheat for various end products and influences the processing and milling of wheat. The results indicate that there is no general relationship between the colour of wheat endosperm and its hardness.

Conclusion

We identified puroindoline genes in wheat and their effect on mealiness and hardness. Only *Pinb-D1c* allele was not found. Three genotypes did not have amplified PCR products, which is caused by mutation. Therefore, further studies are required for the identification of alleles (preferably DNA sequencing). The knowledge of the genetic determination of *Pina* and *Pinb* loci in wheat with nonstandard coloured caryopses can be used in breeding for marker-assisted selection of bread-making quality wheat genotypes. The grain hardness ranged from 15.10 to 26.87 N per sample. Hard wheat is considered of higher quality and suitable for bread making, while most cakes are made from soft wheat flour. Bread-making quality is essential because it determines other physical characteristics such as the volume of dough and sensory attributes. The endosperm texture of wheat showed differences among the studied genotypes in the mealiness and hardness of grains. The colour does not have any effect on grain hardness.

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