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 transfectance 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
material resistance to penetration (8), and is closely relat-
ed to vitreousness.

Wheat hardness is affected especially by genetic fac-
tors (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 gran-
ules 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 identi-
fication of
markers for puroindoline genes (Pina and Pinb) in wheat
using polymerase chain reaction and determination of en-
dosperm 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 Kro-
měříž, 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 identi-
fication 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 fi-
nal extension step of 10 min at 72 °C. The total volume in
one reaction was 25 μL. The visualisation of PCR prod-
ucts was carried out in 1.5 % agarose gel.

A light trans-
fluc-tance meter (LTm; Brewing Research
International (BRi), Nuttfield, UK) was used for the evalu-
ation 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 geno-
type were used for one experiment. Mealy caryopses are
not transparent to light and vitreous caryopses allow

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

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.XTplus 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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Product size/bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>STS1</td>
<td>Pina-D1a</td>
<td>704</td>
</tr>
<tr>
<td>STS1</td>
<td>Pina-D1b</td>
<td>922</td>
</tr>
<tr>
<td>STS2</td>
<td>Pina-D1a</td>
<td>704</td>
</tr>
<tr>
<td>STS2</td>
<td>Pina-D1b</td>
<td>1033</td>
</tr>
<tr>
<td>STS3</td>
<td>Pina-D1a</td>
<td>744</td>
</tr>
<tr>
<td>STS3</td>
<td>Pina-D1b</td>
<td>922</td>
</tr>
<tr>
<td>STS4</td>
<td>Pina-D1a</td>
<td>744</td>
</tr>
<tr>
<td>STS4</td>
<td>Pina-D1b</td>
<td>1033</td>
</tr>
<tr>
<td>STS5</td>
<td>Pina-D1a</td>
<td>463</td>
</tr>
<tr>
<td>STS5</td>
<td>Pina-D1b</td>
<td>792</td>
</tr>
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<td>STS6</td>
<td>Pina-D1a</td>
<td>503</td>
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<td>STS6</td>
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<td>792</td>
</tr>
<tr>
<td>STS7</td>
<td>Pina-D1a</td>
<td>407</td>
</tr>
<tr>
<td>STS7</td>
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<td>736</td>
</tr>
<tr>
<td>STS8</td>
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<td>447</td>
</tr>
<tr>
<td>STS8</td>
<td>Pina-D1b</td>
<td>625</td>
</tr>
<tr>
<td>STS9</td>
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<tr>
<td>STS9</td>
<td>Pina-D1b</td>
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<tr>
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<tr>
<td>SNP A</td>
<td>Pinb-D1b</td>
<td>226</td>
</tr>
<tr>
<td>SNP G</td>
<td>Pinb-D1a</td>
<td>423</td>
</tr>
<tr>
<td>SNP A</td>
<td>Pinb-D1b</td>
<td>232</td>
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<tr>
<td>SNP T</td>
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<td>423</td>
</tr>
<tr>
<td>SNP C</td>
<td>Pinb-D1c</td>
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<tr>
<td>SNP C</td>
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<tr>
<td>SNP T</td>
<td>Pinb-D1a</td>
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<tr>
<td>SNP A</td>
<td>Pinb-D1d</td>
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<td>SNP A</td>
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<td>423</td>
</tr>
<tr>
<td>SNP T</td>
<td>Pinb-D1a</td>
<td>236</td>
</tr>
</tbody>
</table>

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=Komin, 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=48SM, 18=Skorpions, 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 

Pinb D1a

Pinb D1c

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 vegetative 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.

Non-significant differences were found for grain hardness. Starch grains in the endosperm of the grain are shown in Table 1.

The nonstandard colours of wheat caryopses 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 

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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