CHARACTERISTIC OF GRAIN PHYSICAL TRAITS OF SPRING BARLEY

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Abstract
The objective of this study was to examine the range of variation and correlation relationships of some grain physical traits of different barley types. Field experiments were carried out at the State Stende Cereal Breeding Institute from 2004 to 2006. Grain samples of 52 spring barley (Hordeum vulgare L.) genotypes were analysed for 1000 grain weight, test weight, relative hardness index and hull content. The mean value of 1000 grain weight and test weight for two-row barley was significantly higher than for six-row barley. Test weight for hulless barley was significantly higher than for covered ones (mean values - 774.6 g l⁻¹ and 669.8 g l⁻¹ respectively). The hull content was higher for six-row barley type (10.4%) than for two-row (8.6%) barley. There was not significant difference in relative hardness index between different types of barley. The coefficient of variation for grain hardness ranged from 13.7% for six-row barley to 18.7% for hulless barley. Among varieties bred in Latvia relative hardness index ranged from 47.1 for two-row variety 'Sencis' to 80.4 for only six-row variety 'Druvis'. Significant (p<0.05) positive correlation was detected between grain hardness index and β-glucans for covered two-row head types of barley (r = 0.418 > r = 0.396).

Key words: barley genotypes, 1000 grain weight, test weight, grain hardness, hull content, variation.

Introduction
Barley is the primary livestock feed grain in the areas where it is grown. The nutrient composition of barley is variable. This variation may be caused by geographic location, year of production and variety. Grain quality is important consideration in all cereal crop improvement. Breeders must use the available genetic variability in the development of new varieties. Barley quality complex consists of wide range physical and chemical criteria.

Barley is one of only four commercial cereal species that retain a hull after harvest. The hull amount is approximately 13% of grain weight, but can range between 7 to 25% depending upon type, growing environment and grain size (Evers et al., 1999). The hull plays an important role before and after harvest. During the later stages of grain ripening, the hull has been considered to have a role in grain dormancy and therefore preharvest sprouting resistance (Benech-Arnold et al., 1999). During harvest, the hull acts to protect the germ during the abrasive threshing process in the harvester. Post harvest, the hull plays a role in processing for the malting, brewing and feed industries. The intensive livestock industry also benefits from using a hulled barley grain. The hull aids in holding crushed or pressed grains together. A thinner hull is desirable, as thicker hulls will result in higher levels of deleterious compounds, in particular lignin (Fox et al., 2006b), which have been shown to have a negative impact on feed performance in livestock (Kaiser, 1999).

Kernel size and shape contain information relevant to the end use quality characteristics of cereals in general. The grain weight of barley, usually expressed as 1000 grain weight, is one of the most important yield components. It is influenced by both genetic and environmental factors (Fox et al., 2006a). Most two-row varieties have larger grain than six-row varieties (Fregeau-Reid et al., 2001). Grain weight is the latest yield component of barley that compensates for earlier stresses when favourable conditions prevail during the grain filling period (Evers and Millar, 2002).

Test weight is actually a measure of the density of weight per unit of volume of a grain. Test weight is important because of direct relationship this factor has between energy content and feeding value of grain. Grains of high test weight have a high percentage of large, plump grains. Plumper barley is generally higher in starch and with a lesser proportion of hull and consequently lower in fiber. The higher test weights had higher feeding value than lower test weight grains due to higher starch content in the grain (Chirstison and Bell, 1975). The seed coat of hulless barley is loosely attached and easily removed during harvesting, resulting in the feed grain with a test weight and physical appearance similar to wheat (Evers et al., 1999).

Barley varieties differ in traits related to grain texture (Beecher et al., 2002). Grain hardness is a product of the complex interaction between compositional and structural endosperm components, including starch, protein and β-glucan (Fox, 2003). Hardness may contribute significantly to barley quality. Malting barley varieties generally are classified as soft grain whereas
non-malting or feed varieties were classified as hard grain (Alison et al., 1976). J. Bowman et al. (2001) demonstrated that the higher kernel hardness and slower dry matter digestibility is associated with higher feed quality. This research described that the grain hardness variation exists among barley genotypes. Therefore substantial advances in the selection for both malting and feed quality could partially depend on the grain textural differences inherent in the barley grain. According to B. Osborne et al. (2005) results the hardness index was shown to be influenced by genetic and environmental effect. Depending upon the method used differing techniques to measure of barley grain hardness. These methods are particle size method, milling energy and Single Kernel Characterisation System (SKCS) (Fox et al., 2003). SKCS was developed to determine wheat hardness. B. Osborne et al., (2005) demonstrated application of this method in the measurement of barley grain hardness. This instrument enables measurements of the mechanical properties of the botanical layers of grain (Osborne et al., 2007).

As the evaluation of barley quality is usually expensive and time consuming process the needs for screening methods in barley breeding in order to facilitate higher sample throughput. The rapid analytical methods for grain quality analysis also for feed grain analysis should reflect both the composition and nutritional value of the grain. Physical measurements can be advantageous commercially because they are usually more rapid and less expensive than chemical assays. Therefore the evaluation methods of the grain physical traits could be used as potential early screening tools in barley breeding also for characterisation of grain nutritional quality.

The objective of this study was to establish the variation of some grain physical traits (1000 grain weight, test weight, grain hardness, hull content) of different types of spring barley selected on the basis of their different characteristics – two-row and six-row, covered and hulless types. The correlation relationships between grain hardness and other grain quality traits will be found.

**Materials and Methods**

There were chosen 52 barley genotypes that represented a broad range of germplasm (two-row, six-row, covered, and hulless) of different origin (Table 1). Thirty-eight genotypes of covered spring barley, from which 27 with two-row and 10 with six-row ear types, and 15 hulless genotypes were used in this study. Only two-row hulless genotypes were included in this investigation.

**Table 1**

<table>
<thead>
<tr>
<th>Barley type</th>
<th>n</th>
<th>Genotype, origin country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-row, covered</td>
<td>27</td>
<td>Ansis, Abava, Sencis, Kristaps, Rasa, Linga, Idumeja, Balga, Ruja, Gate, Malva, Klinta (Latvia); Hanka, Annabell, Danuta, Justina, Polygena (Germany); Austrian early, Landsorte Aus Tirol (Austria); Primus II (Sweden); Lysimax (Denmark); Hatvani 45/25 (Hungary); Cork, Century (Great Britain); Lechtaler (Portugal); Grimmet (Australia); 379 (Chile)</td>
</tr>
<tr>
<td>Six-row, covered</td>
<td>10</td>
<td>Druvis (Latvia); Colsess IV, July (Denmark); B90A, RNB-367 (Nepal); Zoapila, Puebla (Mexico); IV/192 (Macedonia); Valluno (Bolivia); Chosen (North Korea)</td>
</tr>
<tr>
<td>Two-row, hulless</td>
<td>15</td>
<td>L 302 (Latvia); KM 2084 (the Czech Republic); SW 1291 (Sweden); McGwire, Gainer, Candle (Canada); X-4 (Lithuania); 10250 (Russia); Orzo Nudo di Altamura (Italy); 2474, Chho 7799 (Guatemala); C.P.I. 22817 (Russia); Sumire Mochi (Japan), Merlin, Wanubet (USA)</td>
</tr>
</tbody>
</table>

The genotypes were grown at the State Stende Cereal Breeding Institute from 2004 to 2006. The soil at the site was sod-podzolic sandy loam, humus content – 12-15 mg kg\(^{-1}\), soil pH – 6.0-6.7, precrop – potato, available for plants P – 88-94 mg kg\(^{-1}\), and K – 103-122 mg kg\(^{-1}\). Plot size was 2 m\(^2\), 2 replicates, seed rate - 400 germinable seeds per m\(^2\). The plots were fertilized with N60 P15 K40 kg ha\(^{-1}\).

1000 grain weight (TGW) (LV ST ZM 43-95) and test weight (TW) (LVS ISO 7971-2) were analyzed. Crude protein (CP) content (N x 6.25) was determined by Kjeldahl method (LVS 277), starch content (ST) (ISO 10520). Content of β-glucans (BG) was analyzed enzymatically following the barley grains procedures of the commercial kits from Megazyme (Megazyme International Ireland Ltd.) (McCleary and Glennie-Holmes, 1985).

For hull content (HC) determination duplicate sample of 50 grains from each variety was weighted. The sample was dipped into 10 ml of 3% solution of sodium hydroxide (NaOH) and kept at room temperature for 1 h and 15 min. Then the samples were washed in cold water and hulls removed by tweezers. The removed hulls were dried for
1 h at 130 °C. The dried hulls were then weighted and
the difference in weight from initial and final weights was
calculated taking into account that due to interaction with
sodium hydroxide solution hulls lost 1/12 part of weight.

A Single Kernel Characterisation System (SKCS) 4100
(Perten instrument, USA) was used for assesment of
barley single kernel hardness. Kernel hardness were
recorded as the average of 300 grains. Since no apparent
hardness standard has been established for barley, the
hardness is reported as a relative hardness indekss (RHI).
For this hardness method, the hardness range from soft to
hard corresponds with low to high values. Samples with
RHI<30 are characterized as soft, 30<RHIL60 as semi-soft,
and samples with RHI>60 as hard (Nielsen, 2003).

ANOVA procedures were used for statistical data
analysis. Significance level was determined at p<=0.05
between groups of two-row and six-row, two-row covered
and two-row hulless genotypes. The value of genotypic
variability for traits was determined and expressed by
coefficient of variation of traits. Pearson correlation
coefficients between two year phenotypic means were
calculated. Correlation coefficients for variables of
different barley head types were labeled as follows - for
covered - \( r_{2\text{-row},C} \) and for hulless - \( r_{2\text{-row},H} \)

**Results and Discussions**

The genotypic variability of spring barley grain
physical traits depends from both the barley type and
evaluated trait. The coefficient of variation of traits was
from 2.0 to 18.9% (Table 2).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mean value 2</th>
<th>Standard deviation</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Coefficient of variation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 grain weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-row, covered</td>
<td>45.7a</td>
<td>2.99</td>
<td>39.6</td>
<td>50.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Six-row, covered</td>
<td>40.2b</td>
<td>4.83</td>
<td>29.6</td>
<td>44.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Two-row, hulless</td>
<td>43.1ab</td>
<td>5.51</td>
<td>31.2</td>
<td>49.6</td>
<td>12.8</td>
</tr>
</tbody>
</table>

|                   | Test weight, g l^{-1}                                    |
| Two-row, covered  | 682.4a         | 20.4               | 635.7         | 712.2         | 2.9                          |
| Six-row, covered  | 635.8b         | 19.1               | 601.8         | 661.8         | 3.0                          |
| Two-row, hulless  | 774.6c         | 15.7               | 738.0         | 798.3         | 2.0                          |

|                   | Relative hardness index                                   |
| Two-row, covered  | 64.4a          | 9.48               | 43.5          | 80.8          | 14.7                         |
| Six-row, covered  | 67.1a          | 9.22               | 54.6          | 80.4          | 13.7                         |
| Two-row, hulless  | 64.8a          | 12.2               | 40.3          | 84.9          | 18.9                         |

|                   | Hull content, %                                          |
| Two-row           | 8.6a           | 0.89               | 7.0           | 10.6          | 10.4                         |
| Six-row           | 10.4b          | 0.71               | 9.6           | 11.7          | 6.8                          |

\( 1 \) mean of 2004-2005 for relative hardness index.

\( 2 \) means in each comparison between pairs of barley types followed by different letters are significantly different
at the p<=0.05 level.
The greatest value of feed grain from barley improvement, besides increasing yield, is that of increasing the available energy content. For pigs there is a much clearer and distinct relationship between available energy and hull (fibre) content (Darlington et al., 1996). Lowering of the relatively indigestible hull content and/or lignin content of the hull in barley would increase available energy also for ruminants (Zinn et al., 1996). According to the results of this study the higher hull content was for six-row barley type (10.4%) than for two-row (8.6%) ones that was confirmed also in other studies (Evers et al., 1999; Kong et al., 1995). Since two-row barley produces larger kernels with higher test weight and 1000 grain weight, and with lower hull content than six-row barley, it is very likely that two-row barley produces in general better quality feed than six-row barley.

Rather high genotypic variability was stated between genotypes also for 1000 grain weight of six-row (12.0%) and hulless barley types (12.8%) as well as in hull content for two-row barley (10.4%).

Figure 1 represents data of relative grain hardness index for 13 varieties bred in Latvia. There were found high variability between varieties in this grain quality indice. Hardness index ranged from 47.1 for two-row variety ‘Sencis’ to 80.4 for an only six-row variety ‘Druvis’. According to results varieties ‘Sencis’ and ‘Klinta’ are defined as semi-soft varieties therefore they are more corresponding to malt barley requirements. Other barley varieties that characterized with hard endosperm could be more suitable for feed application as they should provide the slower dry matter digestibility.

Correlation relationships were calculated between relative grain hardness index and other grain physical chemical components of barley grain – starch, crude protein and β-glucan (Table 3).

**Table 3**

<table>
<thead>
<tr>
<th>Grain quality indice</th>
<th>Two-row, covered</th>
<th>Six-row, covered</th>
<th>Two-row, hulless</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_{0.05} = 0.396 )</td>
<td>( r_{0.05} = 0.707 )</td>
<td>( r_{0.05} = 0.514 )</td>
</tr>
<tr>
<td>1000 grain weight</td>
<td>0.031</td>
<td>-0.432</td>
<td>0.212</td>
</tr>
<tr>
<td>Test weight</td>
<td>-0.027</td>
<td>0.289</td>
<td>-0.435</td>
</tr>
<tr>
<td>Crude protein</td>
<td>-0.255</td>
<td>-0.043</td>
<td>0.089</td>
</tr>
<tr>
<td>Starch content</td>
<td>0.177</td>
<td>-0.036</td>
<td>-0.213</td>
</tr>
<tr>
<td>β-glucans</td>
<td>0.418*</td>
<td>0.426</td>
<td>0.503</td>
</tr>
</tbody>
</table>

*significant at 95% probability level.
The significant positive correlation was detected between grain hardness and β-glucans for covered two-row head types of barley ($r_{2\text{-row, }c}=0.418 \text{> } r_{2\text{5; }c=0.396}$) (Table 3). Also for six-row head type and two-row hulless barley this relationship was positive ($r_{6\text{-row, }c}=0.426$; $r_{2\text{-row, }H}=0.503$) even if unsignificant at 95% probability level. The similar result was obtained also in another studies (Henry and Cove, 1990; Chandra et al., 1999). As 70% of β-glucans are found in the endosperm cell walls their thickness might be able to contribute to grain hardness (Nielsen, 2003). Also the genetic mapping identified a number of common regions on chromosomes 4 that have been associated with barley grain hardness and content of β-glucans (Darlington et al., 2001). In the other studies differences were found regarding to correlation relationships between grain hardness and grain protein. There were studies where grain protein was positively correlated with hardness (Henry and Cove, 1990; Chandra et al., 1999). In this study no significant correlation was found between SKCS hardness and protein concentration that coincided also with B. Beecher et al. (2002) study. C. Brennan et al. (1996) found that strong starch-protein binding (high grain hardness) is related to poor malting barleys, and good malting barleys have a weak association between starch granules and protein matrix (low grain hardness). This association is independent of the nitrogen level in the grain. SKCS instrument showed potential as early screening tool in barley breeding for grain nutritional quality in breeding for different end uses.

Conclusions
1. The genotypic variability of spring barley grain physical traits was depending from both the barley type and evaluated trait. The coefficient of variation of traits was from 2.0 to 18.9%.
2. The mean value of 1000 grain weight and test weight for two-row barley was significantly higher than for six-row barley. Test weight for hulless barley was significantly higher than for covered ones. Higher genotypic variability was stated in 1000 grain weight for six-row (12.0%) and hulless barley types (12.8%).
3. The higher hull content was for six-row barley type than for two-row ones. Rather high genotypic variability was stated in hull content for two-row barley (10.4%).
4. There was not significant difference in relative hardness index between different types of barley. The genotypic variability for this trait was high for all types of barley. The coefficient of variation ranged from 13.7% for six-row barley to 18.7% for hulless barley.
5. The significant positive correlation was detected between grain hardness and β-glucans for covered two-row head types of barley. Also for six-row head type and two-row hulless barley this relationship was positive, only unsignificant.
6. The potential exist to exploit the variation in grain relative hardness for the development of the improved barley germplasm specifically designed for different end uses. SKCS instrument showed potential as early screening tool in barley breeding for grain nutritional quality.

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References


