

RELATIONSHIPS BETWEEN GRAIN QUALITY TRAITS IN COVERED AND HULLESS SPRING BARLEY

Mara BLEIDERE

State Stende Cereal breeding institute

LV-3258, p. Dižstende, Talsi region, Latvia

E-mail: maara.bleidere@stendeselekcija.lv

Abstract

The study was conducted from 2004 to 2006 at the State Stende Cereal breeding Institute in Latvia. Correlation and principal component analysis (PCA) of barley grain quality traits (crude protein, starch, crude fat, β -glucan, crude fibre, crude ash, phosphorus, 1000 grain weight, test weight) was carried out for 27 two-row covered and 15 hulless spring barley genotypes. PCA for both types of barley generated three significant principal components. The correlation estimates and the effect of traits on variation of principal components varied depending on barley hull type. The obtained results showed the possibility of selecting independently or jointly for two or more traits.

Key words: spring barley, grain quality, correlation analysis, principal component analysis.

Introduction

It is estimated that about 85 % of the world's barley production is destined for feeding animals. The rest is used for malt production, seed production and food consumption as well as for production of starch either for food use or for the chemical industry /Fischbeck, 2002/. In Latvia spring barley (*Hordeum vulgare* L.) is the second most important crop after wheat. It is also mainly used for animal feed.

The single recessive gene *nud*, located on chromosome 7HL, controls the hulless cariopsis character. Hulless barley is distributed widely in the world, but there is higher preference for this type of barley in East Asian countries. However hulless barley was grown also in northern Europe in ancient time /Pourkheirandish, Komadsuda, 2007/. The hulless gene has multiple effects on many traits of barley, like yield reduction, lower plant density, shorter plant height, lower seed weight, higher volume weight /Choo et al., 2001/. However from nutritional quality view the hulless barley has many advantages. Therefore ten years ago also Latvian barley breeders started the investigation of this type of barley /Legzdina, 2001/.

Nowadays plant breeders have developed new barley genotypes that could be useful for new products and to improve traditional applications. Therefore the grain quality of barley is of great importance for its use in any application. The barley varieties suitable for human consumption with high soluble fibre and nutritive contents are desirable. These varieties could contain high contents of β -glucans, soluble non-starch

polysaccharides, protein, and lower starch content. The hulless barley is more desirable for this purpose /Holtejolen et al., 2006/.

The key analysis of animal feeds is a proximate analysis that includes the following grain chemical components such as starch, crude protein, crude fat, crude ash and crude fibre. Animal feeders value barley mainly as an energy source. They prefer barley with thin hulls (low fibre) and high starch levels (high digestible energy levels). Barley protein is also economically significant due to high level of lysine, which is important for growing pigs and poultry. As the protein level in the animal's diet increases, the value of extra protein in the barley also increases. The presence of β -glucan in barley is also essential. The levels of β -glucan in barley have anti-nutritive effects when fed to young pigs and poultry and this is more of a quality issue with hulless barley /Hickling, 1999/.

Parental genotypes that are used in breeding programs are usually selected on the basis of their phenotypic values for important traits. A successful breeding program requires a high degree of genetic and phenotypic diversity among the progeny. This can be achieved by using unrelated and diverse parents at both phenotypic and genetic levels /Falconer, Mackay, 1996/.

It is also useful to determine the degree of linear interrelationships (correlations) between individual traits in the breeding material to ensure whether the studied traits are affected simultaneously by genes and /or environmental conditions in the gene pool and the target environment. The genetic background of any pair of characters, also grain quality characters, is unlikely to be under totally separate control, mainly due to linkage or pleiotropy /Falconer, Mackay, 1996/. Knowledge about relationships among grain quality components is of great importance because it is likely to facilitate breeders to choose the most efficient selection criteria. This information is useful in predicting how a breeding gain in one trait will affect also a grain in the other traits included in the breeding program. In many cases the reported correlations between grain quality traits are highly dependent on the environment and on the genetic material /Fregeau-Reid et al., 2001; Nielsen, Munck, 2003; Holtejolen et al., 2006; Zakova, 2006/. Many genetic loci influencing key quality attributes have been identified by gene mapping. Biochemical studies and information about relationships between quality attributes are important to advance understanding of the molecular basis of barley quality /Fox et al., 2003/.

In the classical quality evaluation, each parameter from raw barley is carefully controlled to be within the limits of the specification. However, the results of these different analyses are not independent. In fact, they form characteristic relationships. The principal component analysis (PCA) was applied to the pooled data to establish the relationships among the characteristics of the barley and to detect the significant factors responsible for variation and to be utilized also in a characterization of barley grain quality /Nielsen, Munck, 2003/. Therefore also Zakova (2006) considered PCA as powerful technique for data reduction which removes interrelationships among components. The best breeding results in terms of grain quality could be expected when genotypes of the two opposite components are crossed.

The main objective of this research was to study the relationships between grain chemical and physical characteristics using correlation and principal component analysis.

Materials and Methods

42 two-row barley genotypes were chosen that represented a broad range of germplasm of different origin. 27 genotypes of covered spring barley and 15 hulless genotypes were used in this study (Table 1).

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 – potatoes, available for plants P – 88–94 mg kg⁻¹ and K – 103–122 mg kg⁻¹. Plot size was 2 m², 2 replicates, seed rate – 400 seeds per m². The plots were fertilized with N60 P15 K40 kg ha⁻¹.

Prior to analysis, representative grain sample from both replications was ground in a Perten cyclone mill to pass a 0.8 mm screen. Crude protein content (N x 6.25) was determined by Kjeldahl method (LVS 277). β -glucan content (BG) was analyzed enzymatically following the barley grain procedures of the commercial kits from Megazyme (Megazyme International Ireland Ltd.). Starch content (ST) (ISO 10520), crude fibre content (CFB) (ISO 5498), crude fat content (CF) (ISO 6492), crude ash content (CA) (LVS 276:2000) and phosphorus (P) (ISO 6492) content were determined. All chemical analyses were reported on a dry matter basis. Dry matter content of the ground grains was determined by oven-drying at 130C° for 2 h. 1000 grain weight (TGW) and test weight (TW) were analyzed by conventional procedures.

Table 1. Spring barley genotypes used in the study
1 lentelė. Vasarinių miežių genotipai, naudoti tyrime

Barley type <i>Miežių tipas</i>	n	Genotype, country of origin <i>Genotipas, kilmės šalis</i>
Covered <i>Lukštėtieji</i>	27	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, Pongo, (Sweden); Lysimax (Denmark); Hatvani 45/25 (Hungary); Cork, Century (Great Britain); Lechtaler (Portugal); Grimmert (Australia); 379 (Chile).
Hulless <i>Belukščiai</i>	15	L 302 (Latvia); KM 2084 (the Czech Republic); SW 1291 (Sweden); McGwire, Gainer, Candle (Canada); X-4 (Lithuania); Orzo Nudo di Altamura (Italy); 2474, Clho 7799 (Guatemala); C.P.I. 22817 (Russia); Sumire Mochi (Japan), Merlin, Wanubet (USA).

The data were analyzed using correlation analysis and principal component analysis (PCA) (Zakova, 2006). When a significant F-value ($P < 0.05$) was found, the means was separated using Fisher's least significant difference values. The phenotypic correlation was calculated as the Pearson correlation coefficient between across year phenotypic means of the both traits and calculated by Excel for Windows. Correlation coefficients for covered barley variables were labelled as r_C , but for hulless – r_H . PCA was performed by using SPSS 10.0 for Windows. To overcome the effects of differences in scale, means of each trait were standardized before analysis. In PCA, principal components with eigenvalues greater than one were considered as significant. The traits which correlation or contribution in principal component (PC) was more than 0.5 showed significant genetic variance.

Results and Discussion

The basic statistics of grain quality traits for covered and hulless spring barley genotypes are represented in Table 2. This study included samples covering a wide range of variation in barley quality traits. Covered barley genotypes were characterized by lower mean value of crude protein, starch, crude fat, β -glucan, phosphorus content, test weight and higher crude fibre, crude ash, 1000 grain weight compared with hulless ones. These differences for all of the traits except for starch were significant (Table 2).

Table 2. Basic statistics of grain quality traits for covered and hulless spring barley (2004-2006)

2 lentelė. Lukštėtųjų ir belukščių vasarinių miežių grūdų kokybės pagrindiniai rodikliai

Trait <i>Požymis</i>	Covered / <i>Lukštėti</i>			Hulless / <i>Belukščiai</i>			<i>t</i>
	Mean <i>Vidurkis</i>	Min value <i>Min. vertė</i>	Max value <i>Maks. vertė</i>	Mean <i>Vidurkis</i>	Min value <i>Min. vertė</i>	Max value <i>Maks. vertė</i>	
Crude protein, g kg ⁻¹ <i>Žali baltymai g kg⁻¹</i>	128.9	109.9	170.7	149.4	118.4	177.0	*
Starch, g kg ⁻¹ <i>Krakmolas g kg⁻¹</i>	620.1	581.6	650.0	630.1	585.5	680.7	ns
Crude fat, g kg ⁻¹ <i>Žali riebalai g kg⁻¹</i>	23.2	19.7	29.4	25.4	17.3	28.6	*
β -glucan, g kg ⁻¹	38.6	34.1	43.9	50.4	37.0	66.5	*
Crude fibre, g kg ⁻¹ <i>Žalia ląsteliena g kg⁻¹</i>	46.0	34.9	53.1	23.8	21.2	30.7	*
Crude ash, g kg ⁻¹ <i>Žali pelenai g kg⁻¹</i>	22.9	21.0	25.4	21.2	18.8	23.9	*
Phosphorus, g kg ⁻¹ <i>Fosforas g kg⁻¹</i>	3.9	3.2	4.7	4.7	4.1	5.2	*
1000 kernel weight, g <i>1000-čio grūdų masė g</i>	45.7	39.6	50.0	43.1	31.2	49.6	*
Test weight, g l ⁻¹ <i>Natūrinis svoris g l⁻¹</i>	682.4	635.7	712.2	767.9	665.5	798.3	*

* Difference between means significant at 0.05 probability level; ns – not significant ($P > 0.05$)

* *Skirtumas tarp vidurkių patikimas 0,05 tikimybės lygiu, ns – nepatikimas*

Selection for grain quality using conventional breeding methods could be confused by the interrelations among quality traits. The correlations that were found in this study could be useful for understanding the relationship between grain quality components and for optimizing selection criteria. The numerous analyses made on these samples provided a good opportunity to study relationships between different chemical and physical variables. The phenotypic correlation coefficients among the various traits measured in this study are shown in Table 3. Taking in to account the significant differences in grain quality traits between covered and hulless barley the correlation coefficients were calculated separately for these both types.

Table 3. Phenotypic correlation coefficients between covered (below diagonal) and hulless (above diagonal) spring barley grain quality traits

3 lentelė. Fenotipiniai koreliacijos koeficientai tarp lukštėtųjų ir belukščių vasarinių miežių grūdų kokybės rodiklių

	CP	ST	CF	BG	CA	CFB	P	TGW	TW
CP		-0.942*	-0.163	-0.012	0.733*	0.644*	0.824*	0.386	-0.252
ST	-0.764*		0.043	-0.092	-0.643*	-0.568*	-0.802*	-0.448	0.474
CF	0.395	-0.568*		0.710*	-0.348	-0.341	0.006	-0.630*	-0.308
BG	0.128	-0.029	-0.261		0.126	-0.453	0.341	-0.131	-0.308
CA	0.452*	-0.671*	0.363	0.226		0.489	0.822*	0.613*	0.131
CFB	0.222	-0.511*	0.149	-0.026	0.470*		0.349	0.227	0.254
P	0.293	-0.503*	0.401*	-0.375	0.397*	0.174		0.404	-0.224
TGW	0.151	0.112	-0.090	0.130	-0.357	-0.345	-0.085		-0.188
TW	-0.184	0.339	-0.125	-0.068	-0.223	-0.432*	0.069	0.278	

* Significant at 0.05 probability level ($r_{0.05/hulled} = 0.380$, $n = 27$; $r_{0.05/hulless} = 0.482$, $n = 15$)

* Patikima 0,05 tikimybės lygiu ($r_{0.05/lukštėti} = 0,380$, $n = 27$; $r_{0.05/belukščiai} = 0,482$, $n = 15$)

The correlation analysis showed that there were numerous significant correlations among variables of covered and hulless barley used in this study. Crude protein content had significant ($P < 0.05$) negative correlation with starch content for both covered and hulless barley genotypes ($r_C = -0.764$; $r_H = -0.942$). Other studies also indicated the same relationships between these main constituents of grain endosperm /Bowman et al., 2001; Zakova, 2006/. Starch content was significantly negatively correlated with crude fibre ($r_C = -0.511$, $r_H = -0.568$), crude ash ($r_C = -0.671$; $r_H = -0.643$) and phosphorus ($r_C = -0.503$; $r_H = -0.802$). Only covered varieties that were characterized with heightened starch content had reduced not only crude fibre, crude ash, phosphorus content, but also crude fat content in the grain dry matter. It happened because of significant negative correlation between starch and crude fat variables for this type of barley ($r_C = -0.568$).

The results showed that with the increase of crude fibre content, crude ash content in the grains increase as well. It confirmed the positive correlation between these two variables for both types of barley ($r_C = 0.470$; $r_H = 0.489$). There was found a significant positive correlation for covered and hulless genotypes between crude ash and phosphorus ($r_C = 0.397$; $r_H = 0.822$) indicating that with increasing ash content in the grain dry matter accordingly increases phosphorus content. Also, in the study of Andersson et. al. (1999) constituents found mainly in the hull were positively correlated with each other.

Analysis also showed the differences in correlation patterns between the traits of covered and hulless genotypes. There was obtained the significant and positive correlation between crude fat and β -glucan only for hulless barley ($r_H = 0.710$). It also indicated that it is possible to perform simultaneously breeding of hulless barley varieties with high β -glucan and crude fat content. That is an important process in order to improve the nutritional quality of barley in the food application. There was also found

a significant positive correlation between endosperm cell wall constituents, such as crude fat and β -glucan in the different studies /Welch, 1987; Andersson et al., 1999/. For covered barley variables of those traits this relationship was negative and not significant. The results presented here showed that there were no other significant correlations between β -glucan and grain physical traits and other chemical constituents for both types of barley. Also Engstrom et al. (1992) and Fregeau-Reid et al. (2001) established that crude protein content for covered barley negatively correlated with β -glucan. Lack of this correlation enabled the breeding of the varieties with high crude protein and low β -glucan content in the grain that would be more suitable in feed application for hog and poultry /Hickling, 1999/. Nevertheless, Holtejolen et al. (2006) studying interrelationships between different quality parameters in barley varieties of different origin found a strong positive correlation between protein content and β -glucan. It means that interrelationship between β -glucan and protein depends on genetic diversity of barley population used in the study.

The correlation between test weight and crude fibre was significantly negative ($r_c = -0.432$) only for covered barley. It means that the higher test weight for covered barley, the lower crude fiber content that would subsequently improve digestibility of barley. The price of feed barley has traditionally been based on test weight indicating a greater percentage of starch or energy in the grains with higher test weight /Mathison et al., 1991/. In this study the correlation between test weight and starch content was positive for both types of barley ($r_c = 0.339$; $r_H = 0.474$). Nevertheless this relationship has to be considered and verified using greater number of samples. Test weight could be used as simple selection criteria for increasing energy and digestibility of barley grain.

For hulless barley significant negative correlation was found between 1000 grain weight and crude fat ($r_H = -0.630$), and significant positive correlation between 1000 grain weight and crude ash ($r_H = 0.613$). There were no significant correlations between test weight and chemical composition variables for hulless barley genotypes (Table 3).

The analysis of the contribution of each trait to the principal components showed valuable information about the importance of each in the dataset (Table 4). PCA for both types of barley generated three significant principal components (PCs). It accounted for 70.42 % of the entire variability among the covered genotypes and 84.56 % entire variability among the hulless genotypes for all the traits investigated (Table 4). There were differences found between covered and hulless genotypes regarding the effect of traits to variation of principal components.

The first principal component (PC1) accounted for 37.5 % of variance for covered genotypes, whereas PC2 and PC3 contributed 17.29 % and 15.55 %, respectively of total variation (Table 4). The traits, which correlated more positively with PC1, were crude protein (0.880), crude fat (0.596), crude ash (0.664) and phosphorus content (0.507) while starch content contributed negatively to the first component (-0.899). This study indicated that the populations of covered barley with greater PC1 values were characterized with high protein, and formed by having more crude fat, crude ash and phosphorus content, but having lesser starch content. The maximum genetic variance to PC2 of covered barley was contributed by crude fibre (-0.730), 1000 grain weight (0.794) and test weight (0.676). The results indicated that barley genotypes with plumper grains were characterized with lower crude fibre. The third principal component PC3

was strongly associated with β -glucan (0.882). It meant that for covered barley genotypes with different β -glucan content an insignificant genetic variance for other grain quality traits was observed.

Table 4. Principal Component Analysis for grain quality traits of covered and hulless spring barley genotypes (2004–2006)

4 lentelė. Lukštėtųjų ir belukščių grūdų kokybės rodiklių pagrindinių komponentų analizė

Barley type / <i>Miežių tipas</i>	Covered / <i>Lukštėti</i>			Hulless / <i>Belukščiai</i>		
	Principal component (PC) / <i>Pagrindinis komponentas</i>					
Parameter / <i>Parametras</i>	1	2	3	1	2	3
Eigenvalue <i>Eigen vertė</i>	3.38	1.55	1.39	4.18	2.31	1.11
Percentage variance, % <i>Variantiškumo %</i>	37.58	17.29	15.55	46.53	25.68	12.34
Cumulative variance, % <i>Kumuliacinis variantiškumas %</i>	37.57	54.86	70.42	46.53	72.22	84.56
Character / <i>Požymis</i>	Eigenvector / <i>Eigen vektorius</i>					
Crude protein <i>Žali baltymai</i>	0.880	0.047	0.115	0.953	-0.084	0.004
Starch <i>Krakmolas</i>	-0.899	0.301	0.104	-0.923	0.064	0.207
Crude fat <i>Žali riebalai</i>	0.596	-0.058	-0.472	-0.074	0.928	-0.272
β-glucan	0.164	-0.001	0.882	0.149	0.647	-0.569
Crude fibre <i>Žalia ląsteliena</i>	0.313	-0.730	-0.018	0.648	-0.191	0.610
Crude ash <i>Žali pelenai</i>	0.664	-0.484	0.017	0.842	-0.251	0.043
Phosphorus <i>Fosforas</i>	0.507	-0.006	-0.663	0.910	0.061	-0.203
1000 grain weight <i>1000-čio grūdų masė</i>	0.163	0.794	0.252	0.416	-0.775	-0.329
Test weight <i>Natūrinis svoris</i>	-0.161	0.676	-0.198	-0.174	-0.049	0.830

For hulless barley genotypes the PC1 accounted for 46.53 % of variance. This portion of variation was mainly due to variation in crude protein (0.953), starch (-0.923), crude fibre (0.648) and its components crude ash (0.842) and phosphorus (0.910). It was not confirmed with the study of Andersson et al. (1999) where the major trend found was that hull content and endosperm composition varied independently of each other and included the different principal components. The same as for covered barley, also for hulless barley genotypes starch content contributes negatively for PC1. The traits, which more contributed correlated more positively with PC2 of hulless barley, were β -glucan content (0.647) and crude fat content (0.928). The results of PC2 showed that the hulless populations with high values of crude fat content have also heightened β -glucan content

in the grain. 1000 grain weight for hulless barley correlated negatively with PC2 (-0.775). It indicated that hulless barley genotypes that are characterized by lower 1000 grain weight have heightened β -glucan and crude fat content.

The third principal component PC3 was strongly and positively associated with test weight (0.830) and crude fibre (0.610), but negatively with β -glucan content (-0.569). These results indicated that hulless genotypes with heightened test weight characterized with higher crude fiber content and contained less β -glucan content in the grain. This relationship between test weight and crude fibre was an opposite pattern for covered barley genotypes.

Conclusions

Obtained results provided barley breeders with useful information if the genetic material would be group according to hull type.

1. Increase of starch content reduced the amount of crude protein, crude fibre, crude ash, phosphorus content in the grain for both the covered and hulless barley genotypes.

2. Relationships between grain quality traits, such as 1000 grain weight, volume weight, crude fibre, crude fat, β -glucan, were different and depended on variation in genetic material based on barley hull type.

3. Lack of correlation between crude protein and β -glucan enabled to perform a separate selection for both these traits.

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LUKŠTĖTŲJŲ IR BELUKŠČIŲ MIEŽIŲ GRŪDŲ KOKYBĖS RODIKLIŲ TARPUSAVIO KORELIACIJA

M. Bleidere

Santrauka

Bandymai buvo daryti nuo 2004 iki 2006 metų Latvijos valstybiniame Stendės javų selekcijos institute. Ištirta 27-ių dvieilių lukštėtųjų ir 15-kos belukščių vasarinių miežių genotipų grūdų kokybė: žalių baltymų, krakmolo, žalių riebalų, β-gliukano, žalios ląstelienos, žalių pelenų, fosforo kiekis bei 1000-čio grūdų masė ir natūrinis svoris. Atlikta kokybės rodiklių tarpusavio koreliacijos ir pagrindinių kokybės komponentų (PC) identifikavimo statistinė analizė. PC analizė parodė esant po tris esminius kokybės komponentus kiekvieno tipo miežių grupėse. Pagrindinių komponentų variacija priklausė nuo miežių tipo. Gauti rezultatai rodo, kad galima selekcionuoti tiek atsižvelgiant į atskirus kokybės rodiklius, tiek įvertinant dviejų ar daugiau rodiklių tarpusavio priklausomumą.

Reikšminiai žodžiai: vasariniai miežiai, grūdų kokybė, koreliacinė analizė, pagrindinių komponentų analizė.