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Multivariate analysis of agronomic and quality traits of hull-less spring barley (*Hordeum vulgare* L.)

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A study was conducted to characterize a world collection of hull-less barley using multivariate traits. Significant variations were observed among genotypes in grain yield and grain physical characteristics. Genotypic as well as environmental effects were important for the variations occurred in chemical contents. Total phenolic content ranged from 166.0 to 295.0 mg/100 g ferulic acid equivalent. The total anthocyanin ranged from 3.0 to 284.5 ppm cyanidin glucoside equivalent. Yellow pigment content ranged from 3.9 to 8.7 ppm and protein content from 12.3 to 17.3%. Beta-glucan ranged from 3.5 - 7.4% for barley genotypes. Purple pigmented barley found to have high total anthocyanin content whereas the black pigmented barleys were superior in their total phenolics and yellow pigments. Waxy barleys contained higher levels of beta-glucan.

Key words: *Hordeum*, hull-less, barley, pigments, Ethiopia.

INTRODUCTION

Cultivated barleys can be classified according to caryopsis form in hulled (syn. covered) and hull-less (syn. naked) types. In the hulled form, the lemma and palea are fused to the pericarp, whereas in the hull-less forms, the chaff can be easily separated from the grain by threshing. Thus, for food uses hulled barley requires extensive processing, e.g. pearling, before use and hull-less barley is preferred over hulled barley for food production (Bhatty 1999). The hull-less trait is controlled by a single recessive gene (*nud,* nudum), located on the long arm of chromosome 7H (Lundqvist et al., 1997). The expression of *nud* precludes permanent adhesion of hulls to the kernel (Xue et al., 1997). No other member of *Triticeae* shows such a hull-caryopsis adhesion than hulled barley (Taketa et al., 2004, 2008). Cultivation of hull-less barley is as old as that of hulled barley but it is less common worldwide than that of hulled barley (Atanassov et al., 2001; Pandey et al., 2006). Compared to hulled barley, hull-less barley has higher contents of protein and limiting amino acids, lysine and threonine (Baidoo and Liu, 1998; Bhatty, 1999). Moreover, it has lower levels of fibre components but contains considerably higher levels of beta-glucan (Xue et al., 1997; Baidoo and Liu, 1998). However, hull-less barley is also characterised by low grain yield, small grain size and superior growth requirements. This might explain why hull-less barley have been grown unsuccessfully in many regions of Ethiopia (Asfaw, 1989; Assefa and Labuschagne, 2004). Therefore, improvement in grain yield and related traits and grain quality for different end uses is essential in hull-less barley cultivar development. Barley kernel colour shows a great diversity, from white-yellowish to blue, purple and black. Seed colour depends on different pigments in different seed layers. Recently interest in some of these pigments, such as anthocyanins, increased due to their possible health promoting effects (Abdel-Aal and Hucl, 2003; Abdel-Aal et al., 2006; Hu et al., 2007). Moreover, grain colour can be used as a marker to detect genes conferring resistance to certain diseases if linkage and co-segregation with the respective genes exists (Bonman et al., 2005). Blue seed colour is due to anthocyanins in the

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aleurone layer of the kernel (Wang et al., 1993). The genetics of blue colour was described by Finch and Simpson (1978). Aleurone colour is the result of five complementary factors, the non-blue aleurone xenia alleles (*blx*) 1 to 5. White aleurone results if the alleles are present in their re-cessive forms (Lundqvist et al., 1997). Colour intensity can be influenced by environmental factors and modifying genes. Information on the distribution of blue barleys in the world is rare. But they occur in different barley growing regions in Ethiopia (Negassa, 1985). Purple pigmentation of the lemma, palea and pericarp is controlled by two dominant complementary genes (*Pre1* and *Pre2*). Purple or red colouration of the hull and pericarp develops during the soft dough stage of grain fill and fades as the grain matures (Lundqvist et al., 1997). The pigmentation is due to anthocyanins (Woodward and Thieret, 1953). Black lemma and pericarp is controlled by the *Blp* locus at the long arm of chromosome 1H (Lundqvist et al., 1997). The black pigmentation is considered to be due to melanin-like pigments (Buckley, 1930; Woodward, 1941). The black colour develops be-fore maturation of the spike. Pigmented organs may in-clude all parts of the spike, awns, the upper portion of the stem, and upper leaves. The intensity of pigmentation is relatively stable over environments.

Multivariate analysis refers to all statistical methods that simultaneously analyse multiple measurements on each individual or object under investigation. More explicitly, any simultaneous analysis of more than two variables can be considered as multivariate analysis (Hair et al., 1998). Multivariate data analysis facilitates a graphic display of the underlying latent factors and interface between individual samples and variables (Nielsen and Munck, 2003). Principal component analysis (PCA) has been widely used in plant sciences for reduction of variables and grouping of genotypes. Kamara et al. (2003) used PCA to identify traits of maize (*Zea mays* L.) that accounted for most of the variance in the data. Granati et al. (2003) used PCA to investigate the re-lationship among *Lathyrus* accessions. Žáková and Benková (2006) identified traits that were the main sources of variation of genetic diversity among 106 Slovakian barley accessions. Cartea et al. (2002) and Salihu et al. (2006) used PCA and cluster analysis to group kale populations and winter wheat genotypes, respectively. In the current study, a set of data comprising agronomic and quality traits of a world collection of 81 hull-less barley genotypes were subjected to multivariate data analysis, namely, PCA, cluster analysis and CDA.

The main objectives of the study were to (1) characterize and classify diverse hull-less barley genotypes based on their overall similarity in agronomic and qualitative data and (2) identify the genotypes that best combine both agronomic and quality characters for the future use in hull-less barley breeding.

MATERIALS AND METHODS

Plant materials

A broad range of hull-less spring barley genotypes comprising breeding lines, landraces and cultivated varieties were investigated. Descriptions of the investigated germplasm in regard to name, gene bank accession code, donor institution and country of origin is given in Table 1.

Experimental site and trial management

The field trials were planted in row-column designs with two replicates at Raasdorf, Austria (16°35'E, 48°14'N) in spring 2006 and 2007. The entries were grown under organic farming conditions without application of external inputs. Sowings were done on April 3 in 2006 and on March 16 in 2007.

Agronomic traits

Data were collected for heading date (DH, days after April 30), grain yield (GYLD, g m^{-2}), thousand kernel weight (TKW, g), hectolitre/test weight (HLW, kg hL^{-1}), and kernel plumpness (KP25, %). HLW was measured by a ¼ l chondrometer (Institut für Laborbedarf, Wien, Austria). TKW was determined by using a Contador seed counter (Pfeuffer GmbH, Kitzingen, Germany). KP25 was determined by sieving 100 g grain with a Sortimat laboratory machine (Pfeuffer GmbH, Kitzingen, Germany); the percentage of grains with a width >2.5 mm was recorded.

Chemical analysis

Extraction of phenolics

Each grain sample was extracted twice and subsequently the dry matter content was determined. The solvent used for extraction was acidified methanol (85:15 MeOH: 1 m HCl). Grain samples of 2.5 ± 0.1 g were extracted with 20 ml solvent in 50 ml Erlenmeyer flasks. The mixtures were homogenized at ambient condition using a magnetic stirrer for 20 min and then stored in a refrigerator for 20 min at 4°C. Subsequently the mixture was transferred into plastic tubes and centrifuged at 4000 rpm for 5 min. The centrifuged samples were placed into the refrigerator for another 20 min at 4°C before the supernatants were filtered into 25 ml volumetric flasks fitted with funnels and folded filters Ø 125 µm. The supernatants were filled to equal volume of 25 ml with the solvent and stored under room temperature in dark places.

Anthocyanins

The total anthocyanin content (TAC) was determined following Abdel-Aal and Hucl (1999). The acidified MeOH extracts were filled in cuvettes of 1 cm thickness and measured at 525 nm in a type U-1100 spectrophotometer (Hitachi, Tokyo, Japan). The reading was first adjusted to zero with an empty microcuvette and afterwards by a cuvette with acidified MeOH solely. According to the calibration curve the results were calculated into mg cyanidin-3-glucoside equivalents per kg dry matter and/or parts per million (ppm).

Total phenolics

The total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent according

Table 1. Description of the investigated hull-less spring barley genotypes.

No.	Genotype	Origin[1]	Donor	Head	Grain
				Rows	Colour[2]
1	00/900/19/3/1	DE	SZ Ackermann, Irlbach, DE	2	
2	00/900/19/3/7	DE	SZ Ackermann, Irlbach, DE	2	
3	00/900/19/3/12	DE	SZ Ackermann, Irlbach, DE	2	
4	00/900/19/3/13	DE	SZ Ackermann, Irlbach, DE	2	
5	00/900/19/6/4	DE	SZ Ackermann, Irlbach, DE	2	*Blp*
6	00/900/19/6/8	DE	SZ Ackermann, Irlbach, DE	2	*Blp*
7	00/900/19/6/11	DE	SZ Ackermann, Irlbach, DE	2	*Blp*
8	A 032	GB	BGC, Okayama, JP	6	*Pre*
9	A 330	GT	BGC, Okayama, JP	2	*Blx*
10	BVAL 358117	ET	AGES, Linz, AT	2, 6	*Blp*
11	BVAL 358163	ET	AGES, Linz, AT	2, 6	*Blp*
12	C 051	CN	BGC, Okayama, JP	6	*Blp*
13	C 359	CN	BGC, Okayama, JP	6	*Pre*
14	C 651	CN	BGC, Okayama, JP	6	*Blx*
15	C 661	CN	BGC, Okayama, JP	6	*Blp*
16	CDC Candle	CA	VUKROM, Kromeriz, CZ	2	
17	Digersano	IT	ISC, Fiorenzuola d'Arda, IT	2	
18	Dometzkoer Paradies (U 347)	CZ	BGC, Okayama, JP	2	
19	E 048	ET	BGC, Okayama, JP	2	
20	E 056	ET	BGC, Okayama, JP	2	*Blx*
21	E 339	ET	BGC, Okayama, JP	2	*Blx*
22	E 359	ET	BGC, Okayama, JP	6	*Blx*
23	E 360	ET	BGC, Okayama, JP	2	*Blp*
24	E 515	ET	BGC, Okayama, JP	2	*Blx*
25	E 550	ET	BGC, Okayama, JP	6	*Blx*
26	E 552	ET	BGC, Okayama, JP	6	*Pre*
27	E 604	ET	BGC, Okayama, JP	2	*Blp*
28	E 632	ET	BGC, Okayama, JP	6	*Blx*
29	E 639	ET	BGC, Okayama, JP	2	*Blx*
30	E 649	ET	BGC, Okayama, JP	6	*Blx*
31	GE 037	AT	Arche Noah, Schiltern, AT	6	
32	GE 040 sel BA	AT	BOKU-DAPP, Vienna, AT	6	*Blx*
33	GE 090	AT	Arche Noah, Schiltern, AT	2	
34	HB 803	CA	VUKROM, Kromeriz, CZ	2	
35	HOR 345	DE	IPK, Gatersleben, DE	2	
36	HOR 346	DE	IPK, Gatersleben, DE	2	
37	HOR 816	DE	IPK, Gatersleben, DE	2	
38	HOR 2172	DE	IPK, Gatersleben, DE	2	
39	HOR 2199	DE	IPK, Gatersleben, DE	6	*Pre*
40	HOR 2593	DE	IPK, Gatersleben, DE	6	*Pre*
41	HOR 3647	DE	IPK, Gatersleben, DE	2	
42	HOR 3710	DE	IPK, Gatersleben, DE	6	*Pre*
43	HOR 3727	DE	IPK, Gatersleben, DE	6	*Pre*
44	HOR 3756	DE	IPK, Gatersleben, DE	2	
45	HOR 3803	DE	IPK, Gatersleben, DE	2	
46	HOR 4024	IN	IPK, Gatersleben, DE	2	*Pre*
47	HOR 4076	NP	IPK, Gatersleben, DE	6	*Pre*
48	HOR 4769	UN	IPK, Gatersleben, DE	6	*Pre*
49	HOR 4940	US	IPK, Gatersleben, DE	6	*Pre*
50	HOR 10955	NP	IPK, Gatersleben, DE	6	

Table 1. Continued.

51	HOR 11402	CN	IPK, Gatersleben, DE	6	*Pre*
52	HORA (BVAL 350010)	DE	AGES, Linz, AT	2	
53	I 002	IN	BGC, Okayama, JP	2	
54	I 026	IR	BGC, Okayama, JP	2	
55	I 311	IN	BGC, Okayama, JP	6	*Blx*
56	I 329	IN	BGC, Okayama, JP	6	*Blx*
57	ICARDA Black Naked	SY	SL, Reichersberg, AT	6	*Blp*
58	J 203	JP	BGC, Okayama, JP	2	
59	KM 1910B	CZ	VUKROM, Kromeriz, CZ	2	
60	KM 2074	CZ	VUKROM, Kromeriz, CZ	2	
61	KM 2384	CZ	VUKROM, Kromeriz, CZ	2	
62	Lawina	DE	GF Darzau, DE	2	
63	Merlin	CA	VUKROM, Kromeriz, CZ	2	
64	Mihori Hadaka 3 (J 373)	JP	BGC, Okayama, JP	6	
65	Murasaki Hadaka (J 307)	JP	BGC, Okayama, JP	2	*Blp*
66	N 023	NP	BGC, Okayama, JP	6	*Blp*
67	N 040	NP	BGC, Okayama, JP	6	*Blx*
68	N 308	NP	BGC, Okayama, JP	6	*Blx*
69	N 623	NP	BGC, Okayama, JP	6	*Blx*
70	N 624	NP	BGC, Okayama, JP	6	
71	Nackta (HOR 6936)	DE	IPK, Gatersleben, DE	2	
72	Namoi	AU	DPS, Aidelaide Univ., AU	2	
73	Priora	IT	ISC, Fiorenzuola d'Arda, IT	2	
74	Rimpaus Nackte (HOR 1629)	DE	IPK, Gatersleben, DE	2	
75	Rondo	IT	ISC, Fiorenzuola d'Arda, IT	6	
76	SNG 04	AT	BOKU-DAPP, Vienna, AT	2	*Blp*
77	T 045	TR	BGC, Okayama, JP	2	
78	T 247	TR	BGC, Okayama, JP	2	
79	Taiga (BVAL 358017)	DE	AGES, Linz, AT	2	
80	Torrens	AU	DPS, Aidelaide Univ., AU	2	
81	U 047	CZ	BGC, Okayama, JP	2	
82	U 363	SU	BGC, Okayama, JP	2	
83	U 647	CZ	BGC, Okayama, JP	2	
84	U 687	SU	BGC, Okayama, JP	2	
85	Wanubet	US	VUKROM, Kromeriz, CZ	2	
86	Washonubet	US	VUKROM, Kromeriz, CZ	2	

[1]ISO 3166 country codes: AU, Australia; AT, Austria; CA, Canada; CN, China; CZ, Czech Republic; DE, Germany; ET, Ethiopia; GB, United Kingdom; GT, Guatemala; IN, India; IR, Iran; IT, Italy; JP, Japan; MN, Mongolia; NP, Nepal; NI, Nicaragua; PK, Pakistan; SE, Sweden; UN, unknown/Germany; US, United States of America; SU, Soviet Union; SY, Syria.
[2]Grain colour is white/yellow unless indicated: *Blp*, black pericarp; *Blx*, blue aleurone; *Pre*, purple/red pericarp.

to Singleton et al. (1999). The reaction mixture contained 0.1 ml acidified MeOH extract, 0.5 ml Folin-Ciocalteu reagent (1: 10 Folin-Ciocalteu: H_2O) and 0.8 ml 7.5% Na_2CO_3. The latter was added 2 min after the extract and the Folin-Ciocalteu reagent were mixed. The blank sample was prepared simultaneously with 0.1 ml H_2O instead of extract. The mixture was heated in a water bath at 50°C for 5 min and cooled to ambient temperature before measuring the absorbance at 760 nm in a type U-1100 spectrophotometer (Hitachi, Tokyo, Japan). Two readings were recorded for each extract and the results were expressed as mg ferulic acid equivalents per 100 g dry matter (dm) according to the calibration curve.

Yellow pigments

Yellow pigment concentration (YP) was determined following ICC Standard Method 152. In brief, 2 ± 0.1 g of wholemeal flour was dispersed in 20 ml of distilled water-saturated n-butanol (1:6 v/v H_2O: butanol) in Erlenmeyer flasks. The suspension was well mixed and subsequently the flasks were stored overnight under room temperature and in dark for 18 to 20 h. Afterwards the suspension was filtered into brown jars using folded filter papers with a sieve size of Ø 110 µm. The extracts were measured at 440 nm wavelength in a type U-1500 spectrophotometer (Hitachi, Tokyo, Japan) against the standard solvent. Results were expressed

according to the calibration curve as mg beta-carotene equivalents per 100 g dry matter (dm) and/or as parts per million (ppm).

Beta-Glucan

Beta-Glucan content (GLUC) was determined enzymatically using Megazyme kits (ICC Standard Method 166; Megazyme Int. Ireland Ltd., Wicklow, Ireland) and Near Infrared Spectroscopy (NIRS) using a Matrix-I FT-NIR Spectrometer (Bruker Optik GmbH, Ettlingen, Germany). For the enzymatic determination of beta-glucan, 100 ± 0.1 mg milled sample was suspended and hydrated in a sodium phosphate buffer solution of pH 6.5 and incubated with purified lichenase enzyme (specific, endol-(1→3), (1→4)-beta-D-glucan 4-glucanohydrolase, EC 3.2.1.73).

The glucose produced was assayed using glucose/peroxidise (GOPOD) reagent. The beta-glucan contents were determined in two to four replicated measurements. In 2007 determination of beta-glucan was carried out by NIRS on wholemeal grain flour in two replicates per sample. NIRS spectra were transferred into predicted beta-glucan contents using the calibration developed by Schmidt (2007). Both results of the two methods were combined and the results were reported as % on dry weight basis.

Protein content

Protein content (PROT) was determined by the Dumas (combustion) method (ICC Standard Method 167) using a CN-2000 analyzer (Leco Instrumente GmbH, Mönchengladbach, Germany). The nitrogen detected after combustion of each sample was transferred into protein by multiplying with a conversion factor 5.7. That is, protein content (%) = N × 5.7. Duplicate analysis was done for each grain sample and means were used for further statistical analysis.

Data analysis

Principal component analysis

PCA was performed using procedure PRINCOMP (SAS 1999a). Combined analysis of variance (ANOVA) has shown that there was significant genotype by environment interaction (G×E) effects. Therefore, annual means for the single traits were considered for PCA. PCA relies upon the Eigen vector decomposition of the covariance or correlation matrix (Granati et al., 2003). In the present study the correlation matrix was used for PCA. Any trait that does not have a significant correlation with PC scores is considered unimportant in classifying genotypes (Kamara et al., 2003). Correlation analysis was performed using procedure CORR (SAS, 1999b) to determine relationships between PC scores and the original data as well as between agronomic and quality traits.

Cluster analysis

Genotype scores (Eigen vectors) of the seven PCs which had Eigen values greater than unity were subjected to hierarchical cluster analysis using procedure CLUSTER and Ward's minimum variance method as a clustering algorithm (SAS, 1999c). Ward's minimum method is a hierarchical clustering procedure in which similarity used to join clusters is calculated as the sum of squares between the two clusters summed over all variables (Hair et al., 1998). It minimizes the within cluster sums of squares across all partitions. ANOVA and Tukey-Kramer mean comparison test were performed between clusters (groupings) by the procedure GLM (SAS, 1999d).

Canonical discriminant analysis

CDA was carried out using procedure CANDISC (SAS, 1999c) using grain colour for defining groups of genotypes. The first two canonical variables were plotted by procedure GPLOT (SAS, 1990; Friendly, 1991) to show the distribution of genotypes on two dimensional planes.

RESULTS

Significant variations were observed among genotypes in grain yield and grain physical characteristics (data not shown). Between the two experimental years, DH was highly correlated (r=0.69, P<0.0001). A significant correlation between the two contrasting years was also observed for TKW (r=0.70, P<0.0001), KP25 (r=0.57, P<0.0001) and HLW (r=0.56, P<0.0001), demonstrating that these traits are mainly qualitatively inherited and, therefore, relatively stable across environments. Plant height varied from 30 - 133 cm in 2006 indicating that there is enough variation for selecting short stalked genotypes with improved lodging resistance. Due to the drought stress in 2007, higher values of GYLD were generally obtained in 2006. The significant G×E interaction is underlined by a non significant correlation of the yield data of both test years (r=0.03, P=0.75).

Summary of data for the chemical content analysis was presented in Table 2. Genotypic as well as environmental effects were important for the variations occurred in chemical contents. High variation in TPC was observed among genotypes ranging from 155 to 291 mg 100 g^{-1} in 2006 and from 143 to 350 mg 100 g^{-1} in 2007. Genotypic values were higher in 2007 and most probably associated with drought stress. Extremely high variation was observed among genotypes for TAC. The range was slightly higher in 2006 (2 - 342 ppm) than in 2007 (3 - 304 ppm). Considerable variation was also observed for YP. The highest value obtained in 2006 was 8.2 ppm; in 2007 it was 10 ppm. PROT varied from 10.7 to 16.7% in 2006 and from 11.1 to 18.6% in 2007. GLUC was stable across the years, ranging from 3.3 to 7.4% in 2006 and from 3.6 to 7.4% in 2007.

Principal component analysis

Seven principal components (PC) had Eigen values >1 and accounted for 72.7% of the total variance in the data (Table 3). The proportions of the total variance attributable to the first three PC were 18.6, 14.4 and 11.0%. The importance of traits to the different PC can be seen from the corresponding Eigen vectors which are presented in Table 4. The results showed that hectolitre weight (HLW) and total anthocyanin content (TAC) had the highest loadings in PC1 indicating their significant importance for this component. On the other hand, other traits are less important to PC1. Thousand kernel weight (TKW), grain grading (KP25) and beta-glucan content

Table 2. Chemical composition of hull-less spring barley.

Genotype	2006					2007				
	TPC	TAC	YP	PROT	GLUC	TPC	TAC	YP	PROT	GLUC
00/900/19/3/1	–	–	–	14.1	3.4	–	–	–	15.4	4.2
00/900/19/3/7	205	5	5.3	12.3	3.7	268	10	4.5	14.0	5.9
00/900/19/3/12	–	–	–	14.6	3.3	–	–	–	14.2	4.2
00/900/19/3/13	–	–	–	14.2	3.5	–	–	–	14.8	4.3
00/900/19/6/4	236	5	7.1	12.5	3.3	222	6	7.8	14.5	3.6
00/900/19/6/8	239	8	5.3	12.9	4.1	238	6	7.6	15.3	3.6
00/900/19/6/11	260	6	6.2	13.5	3.5	220	6	7.8	14.1	3.6
A 032	205	75	4.7	13.1	5.9	299	86	5.8	15.8	4.5
A 330	209	11	4.4	15.8	5.5	148	10	4.4	17.3	5.5
BVAL 358117	225	14	7.9	14.4	4.9	279	13	9.5	17.2	4.7
BVAL 358163	228	23	6.6	14.8	5.6	249	24	8.4	15.8	5.2
C 051	254	74	5.6	12.7	5.0	207	42	6.8	15.6	5.1
C 359	219	46	4.6	13.6	5.6	264	50	5.6	14.0	4.3
C 651	186	22	5.8	13.6	5.0	272	54	6.2	15.8	4.8
C 661	190	37	5.9	12.4	5.8	247	24	7.1	15.0	5.6
CDC CANDLE	210	5	6.6	13.1	6.3	174	5	6.1	13.7	6.8
DIGERSANO	256	12	3.5	12.9	3.8	275	8	5.2	15.2	4.8
DOMETZKOER PARADIES	254	11	5.5	13.9	5.9	266	5	5.9	14.3	6.8
E 048	171	5	5.4	13.9	5.0	179	5	5.4	16.2	5.2
E 056	260	21	5.5	14.2	5.7	248	17	5.7	17.4	6.2
E 339	210	6	5.6	14.5	6.0	265	6	6.0	17.5	5.3
E 359	205	27	5.1	13.0	4.8	149	22	4.6	12.0	4.8
E 360	181	12	5.6	13.8	4.6	271	16	6.8	16.8	4.6
E 515	199	25	3.8	13.9	5.5	279	28	6.2	15.4	6.0
E 550	207	20	5.1	15.3	5.4	250	20	5.5	16.8	6.3
E 552	200	26	6.2	14.3	4.5	178	57	5.8	15.0	5.5
E 604	214	39	4.4	15.8	5.6	350	27	8.2	16.9	4.5
E 632	186	28	5.8	13.5	4.9	162	17	6.2	16.0	5.4
E 639	191	20	5.7	12.9	5.4	178	8	5.8	16.5	4.8
E 649	267	31	6.7	14.9	4.6	251	18	6.0	15.9	4.5
GE 037	207	19	4.8	14.6	4.6	233	13	5.7	14.6	4.9
GE 040 sel BA	224	27	4.3	14.2	4.2	319	25	4.8	15.8	5.2
GE 090	225	7	5.2	14.9	5.0	180	4	5.4	13.9	5.1
HB 803	259	32	5.6	14.6	7.2	294	13	6.0	15.6	5.9
HOR 345	232	6	6.5	14.3	6.0	158	6	5.0	15.5	5.9
HOR 346	219	5	5.2	14.5	5.7	157	5	4.4	15.6	5.6
HOR 816	219	12	7.1	15.1	5.5	239	6	5.9	14.3	5.7
HOR 2172	179	6	6.3	14.5	5.9	254	7	6.8	15.4	6.4
HOR 2199	285	135	5.1	14.1	5.5	305	115	6.4	16.7	5.4
HOR 2593	266	301	6.4	13.4	3.6	259	123	6.7	17.4	5.4
HOR 3647	191	11	7.2	15.0	5.7	260	6	5.6	15.1	5.1
HOR 3710	291	117	4.6	13.5	5.3	276	113	7.1	15.3	4.7
HOR 3727	252	342	4.9	12.6	4.9	228	172	6.9	14.2	4.8
HOR 3756	199	12	4.6	15.0	5.5	257	6	5.3	15.4	5.2
HOR 3803	200	7	6.4	14.3	3.6	167	6	5.9	15.0	5.4
HOR 4024	237	265	3.6	11.1	5.5	321	304	4.8	13.5	5.1
HOR 4076	192	24	5.4	13.8	4.9	143	15	5.6	15.7	4.9
HOR 4769	289	130	5.4	13.2	5.0	258	73	5.9	15.3	4.7
HOR 4940	222	278	6.0	12.9	4.5	205	132	6.4	17.2	5.5
HOR 10955	187	8	4.9	14.9	5.4	228	4	4.9	13.4	5.5

Table 2. Continued.

HOR 11402	260	249	5.1	13.5	4.5	214	121	6.2	17.7	7.0
HORA	274	4	7.2	12.4	4.3	256	5	5.5	15.5	4.1
I 002	255	25	6.6	13.8	4.5	148	6	4.5	15.4	6.0
I 026	204	12	6.7	13.7	5.8	245	7	4.3	13.9	5.2
I 311	212	28	4.3	13.1	5.3	261	24	5.5	15.8	5.3
I 329	207	28	5.1	15.0	4.9	172	14	5.4	14.8	5.3
ICARDA BLACK NAKED	268	20	5.5	14.4	4.1	285	12	10.0	15.0	4.7
J 203	203	4	3.4	13.5	5.2	260	4	4.8	15.6	5.2
KM 1910B	155	6	6.6	11.3	4.2	261	5	5.4	14.9	4.6
KM 2074	205	6	7.4	10.7	3.8	246	8	7.3	15.8	4.4
KM 2384	203	6	5.7	11.2	4.3	227	6	5.1	16.2	3.9
LAWINA	255	5	4.6	13.4	4.6	274	4	5.5	16.5	4.7
MERLIN	223	5	7.9	14.3	5.4	221	3	6.6	15.0	5.6
MIHORI HADAKA 3	178	5	4.9	14.3	4.9	210	4	4.7	15.6	5.0
MURASAKI HADAKA	228	26	6.9	14.7	5.0	341	26	9.1	17.7	5.2
N 023	256	30	5.7	13.5	6.0	173	22	6.2	13.6	5.1
N 040	190	21	3.4	13.9	5.5	236	16	4.3	13.8	4.8
N 308	164	20	4.5	13.3	5.0	224	20	5.0	12.4	5.0
N 623	183	22	4.4	13.0	4.9	149	13	5.2	16.6	4.6
N 624	195	23	5.0	12.7	4.7	286	22	6.9	15.3	5.2
NACKTA	280	13	5.0	13.3	3.7	271	5	5.2	15.9	4.5
NAMOI	224	4	6.5	13.4	4.4	189	3	5.8	14.6	5.4
PRIORA	200	4	4.5	14.8	5.1	199	3	4.6	15.5	5.0
RIMPAUS NACKTE	194	4	5.7	14.0	5.1	263	7	6.8	15.7	4.0
RONDO	216	13	4.0	15.2	4.8	215	9	5.3	15.6	4.5
SNG 04	289	25	5.4	15.0	5.7	245	31	7.8	11.1	4.5
T 045	206	9	4.0	13.4	4.4	240	6	5.3	13.9	4.6
T 247	188	9	5.5	13.4	4.8	157	6	5.4	14.3	4.2
TAIGA	240	11	5.4	13.9	4.1	277	8	5.6	14.3	5.1
TORRENS	252	11	5.7	13.1	4.3	274	6	6.1	14.1	4.6
U 047	215	9	5.5	16.0	6.4	253	8	6.0	18.6	4.9
U 363	207	12	6.8	14.0	5.4	233	7	5.9	15.6	6.1
U 647	291	6	4.5	16.7	5.0	248	5	5.6	16.1	5.1
U 687	192	7	6.5	14.4	5.5	157	4	5.4	16.0	5.7
WANUBET	218	2	6.5	13.6	7.4	257	4	6.4	14.4	7.4
WASHONUBET	208	8	8.2	13.8	6.8	270	8	5.7	14.4	6.8
CHECK (LAWINA+TAIGA)	248	8	5.0	13.8	4.3	276	6	5.6	15.0	5.0
Mean	221	37	5.5	13.8	4.9	236	27	6.0	15.3	5.1
Minimum value	155	2	3.4	10.7	3.3	143	3	4.3	11.1	3.6
Maximum value	291	342	8.2	16.7	7.4	350	304	10.0	18.6	7.4
CV	15	188	19.2	7.9	17.4	20	173	19.2	8.3	14.8

(GLUC) are the main traits of PC2. For PC3 traits such as TKW, KP25 and TAC were the most important, whereas PROT and GLUC were mainly contributing to PC4 and yellow pigment content (YP) affected PC5. Multiple traits contributed to the sixth PC in varying proportions in different seasons. The seventh PC was derived mainly from the variance due to differences in GLUC. In general, it is possible to see some traits that inconsistently contributed to the total variance in different seasons.

Correlation analysis was performed between the original data and the PC scores to determine the contribution of each trait to the total variance (Table 5). PC1 showed a significant correlation with HLW and TAC. The second principal component PC2 was positively associated with TKW, KP25 and GLUC and negatively with TPC and TAC. PC3 had a significant correlation with traits such as TKW, KP25 and TAC. In the fifth PC the most important traits which showed significant correlation

Table 3. Principal component analysis of 81 hull-less spring barley genotypes: Eigen values and percent variation accounted by the first seven principal components.

Principal component	Eigen value	Variance (%)	Cumulative variance (%)
PC1	3.71	18.6	18.6
PC2	2.89	14.4	33.0
PC3	2.20	11.0	44.0
PC4	1.78	8.9	52.9
PC5	1.60	8.0	60.9
PC6	1.38	6.9	67.8
PC7	1.03	5.2	73.0

Table 4. Eigen vectors (loadings) of the first seven principal components.

Trait × Year[1]	Eigen vector						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DH 06	-0.37	0.02	0.23	-0.06	0.01	-0.01	0.04
DH 07	-0.38	-0.10	0.26	-0.10	-0.03	0.04	0.05
GYLD 06	0.17	-0.07	0.13	-0.28	0.11	0.33	0.23
GYLD 07	0.11	0.13	-0.37	-0.05	0.45	0.13	-0.01
HLW 06	0.31	-0.03	0.13	-0.10	-0.33	0.22	0.26
HLW 07	0.43	0.07	-0.15	0.01	0.04	0.05	-0.05
TKW 06	0.05	0.38	0.34	0.04	0.02	0.16	-0.24
TKW 07	-0.12	0.39	0.37	-0.07	0.12	0.07	-0.06
KP25 06	0.31	0.17	0.26	-0.08	0.22	-0.01	-0.05
KP25 07	0.12	0.35	0.29	-0.11	0.20	-0.18	0.11
PROT 06	-0.10	0.17	-0.11	0.39	-0.19	0.37	-0.42
PROT 07	-0.12	-0.13	0.27	0.26	-0.28	0.11	0.19
TPC 06	0.01	-0.25	0.15	0.17	0.32	0.09	-0.52
TPC 07	0.08	-0.23	0.14	0.10	0.18	0.54	0.27
TAC 06	0.24	-0.29	0.29	0.24	0.04	-0.32	-0.09
TAC 07	0.30	-0.28	0.26	0.19	0.04	-0.23	-0.01
YP 06	-0.24	0.05	-0.05	0.17	0.42	-0.24	0.32
YP 07	-0.14	-0.27	0.05	0.17	0.39	0.23	0.10
GLUC 06	0.08	0.28	-0.04	0.44	0.02	0.13	0.25
GLUC 07	0.04	0.21	-0.06	0.52	-0.05	-0.15	0.26

[1]Abbreviations for traits followed by year: GYLD: grain yield; HLW: hectolitre/test weight; KP25: percentage kernel plumpness >2.5 mm; TKW: thousand kernel weight; GLUC, beta-glucan content; PROT, protein content; TAC, total anthocyanin content; TPC, total phenolics content; YP, yellow pigment content.

with the scores were KP25, TPC and YP. For the last two PC many traits have shown different correlation coefficients depending on the season.

Cluster analysis

Based on cluster analysis, the 81 hull-less spring barley genotypes were separated into seven major groups which each have two or more subgroups. Figure 1 illustrates the seven clusters formed by hierarchical clustering. Table 6 summarizes the number of genotypes in each cluster and their proportion in terms of their countries of origin. ANOVA revealed significant differences between clusters for all traits. Means of clusters and Tukey-Kramer mean comparisons are presented in Table 7.

The first cluster (Clus1) is composed by the largest number of genotypes (n=25). Yellow grained genotypes comprised 80% and blue aleurone types 16% of the group. One black grained genotype was also included in the group. The yellow grained genotypes originated mainly from Western Europe, e.g. Germany and Italy, whereas the pigmented forms originate almost all from

Table 5. Pearson correlation coefficients of significant (P<0.05) correlations between trait × year means and loadings of the first seven principal components.

Trait × Year[1]	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DH 06	-0.72		0.34				
DH 07	-0.74		0.39				
GYLD 06	0.32			-0.38		0.39	0.23
GYLD 07		0.23	-0.55		0.56		
HLW 06	0.59				-0.41	0.26	0.26
HLW 07	0.83		-0.22				
TKW 06		0.64	0.51				-0.25
TKW 07	-0.23	0.65	0.54				
KP25 06		0.29	0.38		0.27		
KP25 07		0.59	0.43		0.25		
PROT 06		0.29		0.52	-0.24	0.44	-0.43
PROT 07	-0.24		0.40	0.35	-0.35		
TPC 06		-0.42	0.23	0.23	0.40		-0.53
TPC 07		-0.40			0.23	0.64	
TAC 06	0.47	-0.49	0.42	0.32		-0.38	
TAC 07	0.58	-0.48	0.39	0.25		-0.27	
YP 06	-0.47			0.22	0.53	-0.28	0.32
YP 07	-0.26	-0.46		0.23	0.50	0.27	
GLUC 06		0.47		0.59			0.26
GLUC 07		0.36		0.69			0.26

[1]Abbreviations for traits followed by year: GYLD: grain yield; HLW: hectolitre/test weight; KP25: percentage kernel plumpness >2.5 mm; TKW: thousand kernel weight; GLUC, beta-glucan content; PROT, protein content; TAC, total anthocyanin content; TPC, total phenolics content; YP, yellow pigment content

Ethiopia. The group is mainly characterized by high values for HLW, TKW and PROT (Table 7).

The second cluster (Clus2) is a small group of only three varieties and is tightly linked to Clus1. This group consists of 'waxy' starch cultivars *Wanubet* and *Washonubet* from USA and *CDC Candle* from Canada. This cluster is especially distinct to the other ones concerning GLUC and YP. For these traits the three varieties exhibit the highest contents over all environments. It is worth mentioning that the other two 'waxy' cultivars, that is, *Merlin* and *HB 803*, were grouped in the neighbouring Clus1.

The third cluster (Clus3) comprises the German variety *Hora* together with Czech and German breeding lines. The latter genotypes show black grain pigmentation. This group of genotypes are late in heading (DH) and exhibited the lowest mean values for GLUC. Together Clus1, 2 and 3 form a major cluster of mainly yellow grained barley of European and/or American origin.

Contrary, the next three clusters (Clus4, 5 and 6) form a major group of accessions originating from diverse countries, amongst others from centres of barley domestication and/or diversification. Clus4 is heterogeneous in regard to grain pigmentation and origin, however, includes the German and Italian cultivars *Lawina* and *Nackta*, and *Digersano*, respectively. This is interesting since all other standard European varieties and/or breeding lines are concentrated in the first major group formed by Clus1, 2 and 3. Genotypes of Clus4 are characterized by high values of HLW, medium to high GYLD and TPC, and low content of GLUC. For all other traits the genotypes show average performance. Clus4 forms another bigger group together with Clus5. In the latter cluster mainly pigmented Asian and Ethiopian barley accessions are present. Like Clus4 this group is characterized by intermediate values for agronomic and quality traits. Concerning KP25 Clus5 shows significantly lower values of plump kernels. From a breeding perspective, one of the most interesting groups is Clus6. This cluster contains yellow, blue and black pigmented genotypes from Ethiopia, Nepal, Japan and Australia. The accessions are characterized by early heading, medium to high GYLD, high HLW and KP25. Unlike in other clusters the agronomic traits have contributed to a greater extent for the classification of this cluster.

The last and very distinct cluster, Clus7, consists of only purple pigmented barely accessions. Concerning their origin it must be supposed that all accessions originate from the Himalaya region. Germany and the USA represent only countries which carried out collection missions to the Himalaya and stored these genotypes in their national gene banks. Clus7 genotypes are

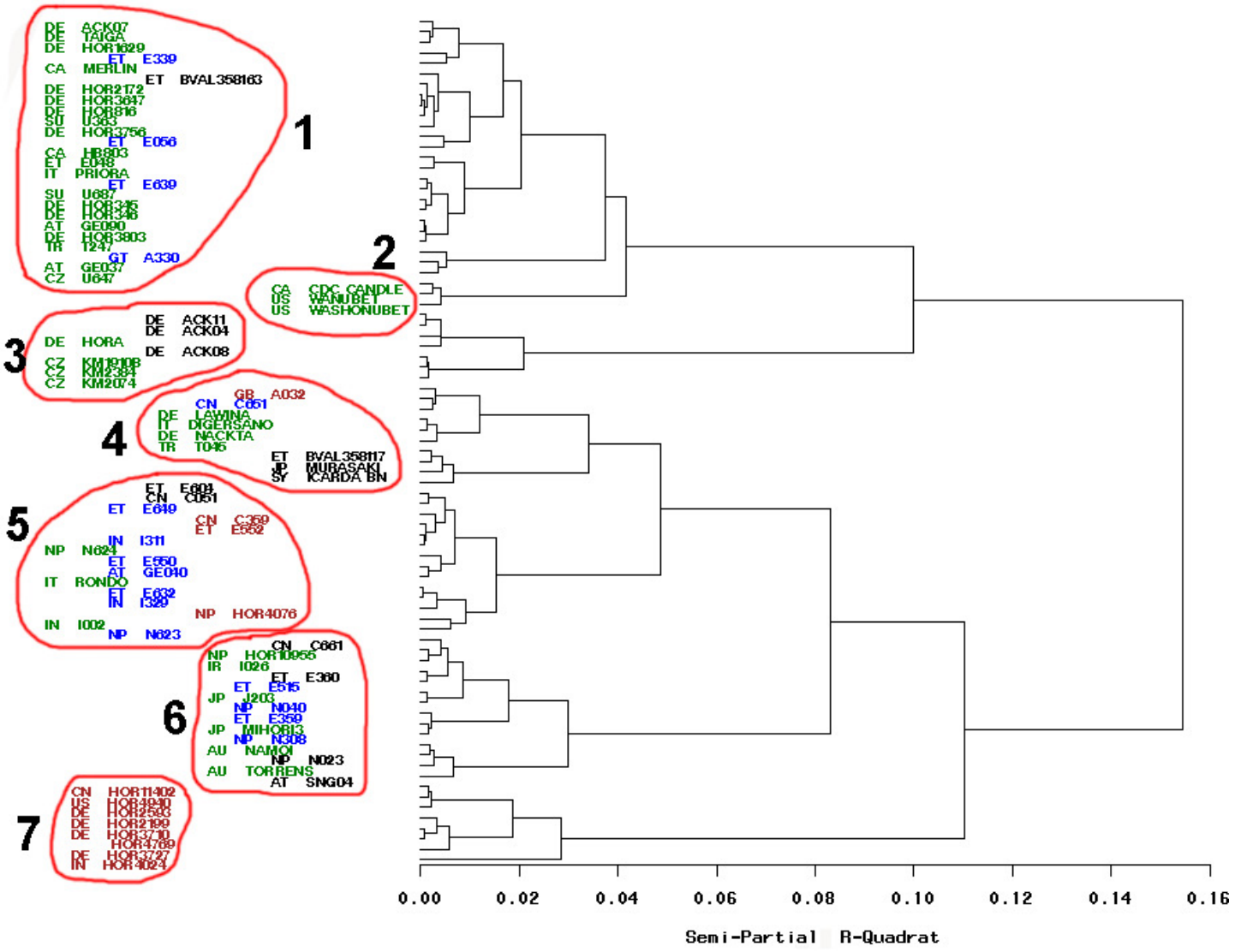

Figure 1. Dendrogram of the cluster analysis based on the genotypic scores of the first seven principal components. Colours of genotype names indicate their grain colour (green, white/yellow; blue, blue aleurone; red, purple pericarp; black, black pericarp). ISO3166 country codes are indicated before the genotype name (for abbreviation of country codes and genotype names see Table 9.2). Breeding lines of Saatzucht Ackermann are abbreviated as follows: ACK07, 00/900/19/3/7; ACK11, 00/900/19/6/11; ACK04, 00/900/19/6/4; ACK08, 00/900/19/6/8.

Table 6. The number and proportion of genotypes in each cluster with respect to their countries of origin.

Origin[1]	Clus1		Clus2		Clus3		Clus4		Clus5		Clus6		Clus7	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
AT	2	50							1	25	1	25		
AU											2	100		
CA	2	67	1	33										
CN							1	20	2	40	1	20	1	20
CZ	1	25			3	75								
DE	10	50			4	20	2	10					4	20
ET	5	36					2	14	4	29	3	21		
GB							1	100						
GT	1	100												
IN									3	75			1	25

Table 6. Contd.

IR											1	100		
IT	1	50					1	50						
JP							1	33			2	67		
NP									4	50	4	50		
SU	2	100												
TR	1	50					1	50						
UN							1	50					1	50
US			2	67									1	33
Total	25	31	3	4	7	9	10	12	14	17	14	17	8	10

[1] ISO3166 country codes: AT, Austria; AU, Australia; CA, Canada; CN, China; CZ, Czech Republic; DE, Germany; ET, Ethiopia; GB, United Kingdom; GT, Guatemala; IN, India; IR, Iran; IT, Italy; JP, Japan; NP, Nepal; SU, Soviet Union; UN, unknown; US, United States of America.

Table 7. Means of agronomic and quality traits of the seven clusters. Means with the same letter are not significantly different from each other at P=0.05.

Cluster	DH06[1]	DH07	GYLD06	GYLD07	HLW06	HLW07	TKW06	TKW07	$KP_{25}06$	$KP_{25}07$
1	42 abc	25 ab	221 b	201 b	74 a	77 ab	40 a	39 a	28 abc	32 a
2	46 ab	26 ab	224 ab	323 a	67 b	73 bc	32 b	32 cd	19 c	14 ab
3	47 a	32 a	276 ab	182 b	71 ab	71 c	32 b	36 ab	18 c	23 ab
4	40 bc	26 ab	357 a	209 b	77 a	77 ab	33 b	32 bc	24 bc	13 ab
5	36 cd	20 bc	205 b	192 b	75 a	77 ab	30 b	27 d	16 c	10 b
6	34 d	17 c	315 ab	258 ab	75 a	80 a	36 ab	33 bc	48 a	32 a
7	36 cd	22 bc	264 ab	185 b	76 a	80 a	34 ab	32 cd	41 ab	25 ab

Cluster	YP06	YP07	PROT06	PROT07	TPC06	TPC07	TAC06	TAC07	GLUC06	GLUC07
1	5.8 ab	5.7 a	14.4 a	15.5 ab	214 b	220 b	11 b	8 b	5.3 b	5.4 b
2	7.1 a	6.1 a	13.5 ab	14.2 b	212 b	234 ab	5 b	6 b	6.8 a	7.0 a
3	6.5 ab	6.6 a	12.1 c	15.2 ab	225 ab	239 ab	6 b	6 b	3.9 c	4.0 c
4	5.2 b	7.0 a	13.9 ab	16.0 a	232 ab	289 a	23 b	24 b	4.7 bc	4.7 bc
5	5.2 b	5.7 a	13.9 ab	15.6 ab	215 b	215 b	30 b	24 b	4.9 bc	5.1 b
6	5.1 b	5.6 a	13.7 ab	14.1 b	209 b	231 ab	17 b	15 b	5.2 b	5.1 b
7	5.1 b	6.3 a	13.0 bc	15.9 a	263 a	258 ab	227 a	144 a	4.9 bc	5.3 b

[1] For abbreviations followed by years see descriptions under Tables 4 and 5.

characterized by the highest content of TAC and TPC. High values were also observed for HLW and KP25, whereas the yield level is only moderate to low. Correlations between agronomic and quality traits were found to be in most cases non-significant or not consistent across the environments. The only relationship showing significant negative correlations in both years was between PROT and GYLD (r = -0.23*; r = -0.47**).

Figure 2 displays the relationships between selected traits and beta-glucan content within the seven clusters. It is obvious that with the exception of Clus3 in each cluster genotypes with GLUC >5% and high levels of TAC, TPC, YP or PROT are available. These genotypes could be used in a first step in a breeding programme for 'healthy' hull-less barley varieties by pyramiding the respective traits. Concerning GYLD major improvements are necessary to reach yields comparable to spring malting barley varieties adapted to the growing conditions of eastern Austria which range from 50 to 60 dt ha^{-1}.

Canonical discriminant analysis

CDA using grain colour as classifying variable resulted in three significant discriminant functions (Table 8). Since the first two functions explained together 88% of the multivariate variation only these two functions were plotted in Figure 3. High scores for the first canonical function were associated with TAC as the most important parameter for differentiating the genotypes. In addition to TAC, TPC and TKW contributed to the separation of genotypes with the first function (Can1). Concerning the second canonical function (Can2) YP, GLUC and HLW were the most important traits for differentiation between

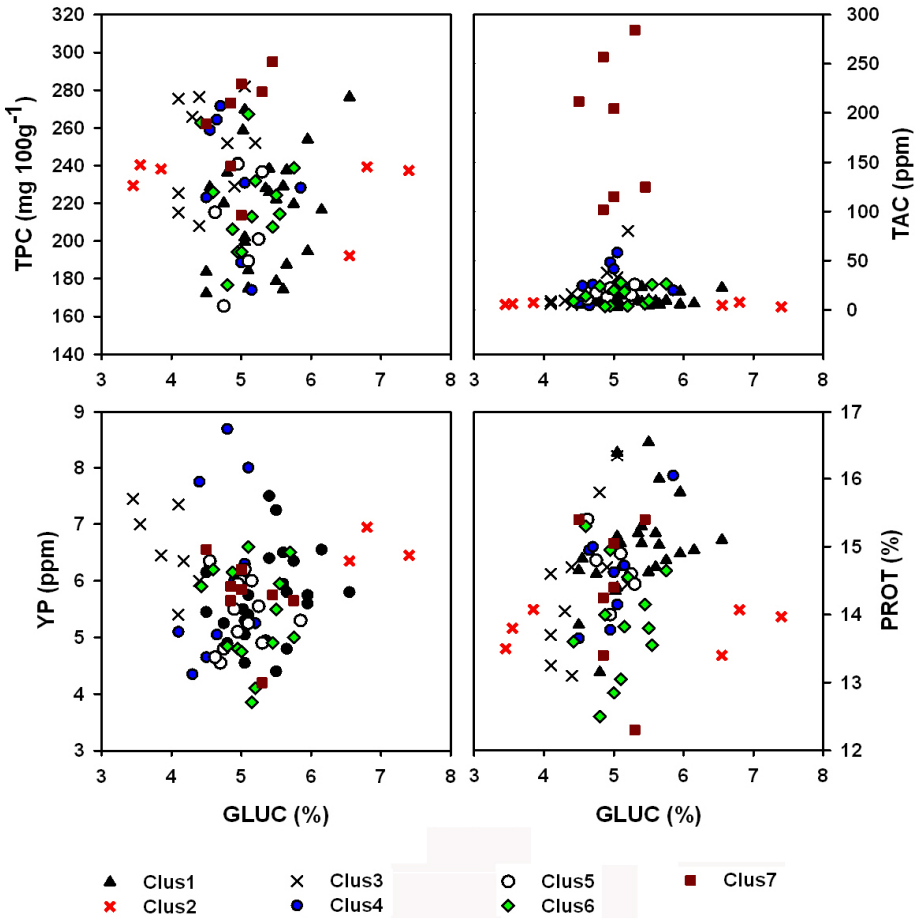

Figure 2. Scatter plot of quality traits (PROT, protein content; TPC, total phenolic content; TAC, total anthocyanin content; YP, yellow pigment content) within clusters in relation to beta-glucan content (GLUC).

Table 8. Canonical discriminant analysis of hullless spring barley.

CAN	Canonical correlation	Eigen value	Proportion	Likelihood ratio (Wilks' lamda)	Pr>F
1	0.899	4.19	0.58	0.033	<.0001
2	0.826	2.15	0.30	0.172	<.0001
3	0.676	0.85	0.12	0.542	0.0014

groups. From Figure 3, it is obvious that along Can1 only three groups of hull-less barley can be differentiated clearly: yellow (*blx*), blue (*Blx*) and black (*Blp*), and purple (*Pre*) seeded genotypes. Blue aleurone and black pericarp types are not significantly separated by Can1, however, a clear separation of these two groups is possible by Can2.

DISCUSSION

Effective application of multivariate analysis on agronomic and quality characters can result in meaningful grouping of genotypes. On the basis of genetic diversity in regard to important agronomic and quality traits, the investigated hull-less spring barley germplasm was grouped into seven groups by PCA and following cluster analysis. This procedure revealed that European and American varieties and breeding lines differentiated more or less significantly from African and Asian material. Exceptions were the Italian varieties *Digersano* and *Rondo*, the German varieties *Nackta* and *Lawina* and the Australian varieties *Torrens* and *Namoi*. In the latter cases the early heading and maturity date of

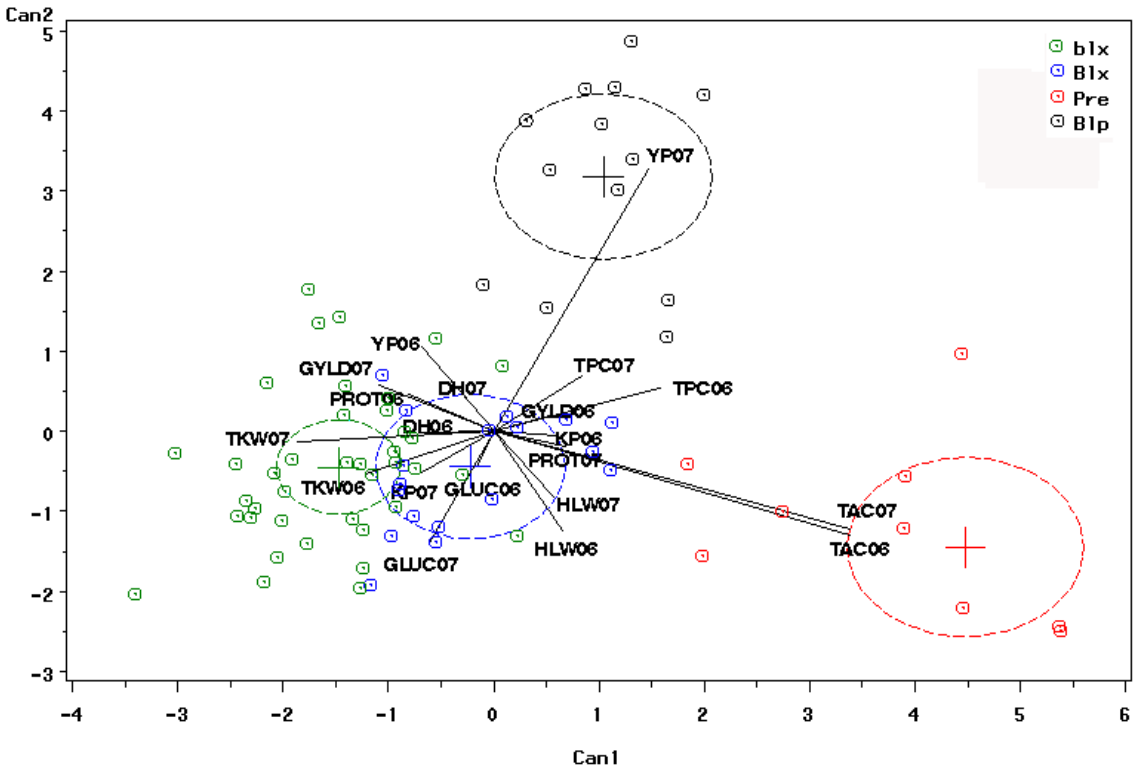

Figure 3. Distribution of hullless spring barley genotypes according to canonical discriminant analysis. The length of each variable (for abbreviations see footnote of first chapter page) vector is proportional to its contribution to separating the grain colour classes (*blx*: non blue aleurone; *Blx*: blue aleurone; *Blp*: black pericarp; *Pre*: purple/red pericarp), and the direction of the vector indicates its relative contribution to the Can1 and Can2 linear combinations. Single genotypes are marked as dots. Mean values of kernel colour groups are indicated by the plus symbol, and their 95% confidence interval by dashed circles.

the Australian barleys are more similar to the Ethiopian and Asian genotypes. The phenotype of *Rondo*, 6-row *uzu*-dwarf barley, resembles also more some of the Japanese genotypes. The narrow genetic background of waxy barley is demonstrated by the fact that three of these genotypes formed a small distinct cluster, while the other two genotypes were located in the neighbouring cluster. An efficient separation using different multivariate analysis techniques was demonstrated for the purple pericarp accessions. Both PCA combined with cluster analysis and CDA clearly separated the purple pigmented genotypes. Recently PCA has been used by various authors for the reduction of multivariate data into a few artificial varieties which can be further used for classifying material. This approach is especially valuable for the screening of a large number of genetic resources by a large number of descriptor variables (Cartea et al., 2002; Granati et al., 2003; Kamara et al., 2003; Salihu et al., 2006).

Canonical discriminant analysis revealed that TAC was the best variable for distinguishing purple pericarp, blue aleurone and non blue aleurone types of hull-less barley. Zeven (1991) reported that in wheat purple and blue colour types are caused by anthocyanins located in the pericarp or aleurone, respectively. Contrary to our results Abdel-Aal et al. (2006) indicated that blue aleurone wheat had significantly higher TAC than purple types. However, Kim et al. (2007) found non-significant differences between TAC levels of blue (337.6 ± 152.4 µg g^{-1}) and purple (312.7 ± 244.3 µg g^{-1}), but significantly lower TAC values for black (84.5 ± 37.2 µg g^{-1}) genotypes of Korean hull-less barley. Abdel-Aal et al. (2006) also found that blue barley *Tankard* had a TAC value of 35 µg g^{-1} which is similar to our results for some of the blue types. Significantly higher amounts of TAC were reported for black rice and purple corn (Abdel-Aal et al. 2006). Hence, various TAC values were reported depending on genotypes and crop species suggesting the requirement for further studies by including more genotypes and species.

Black pericarp barley was efficiently separated by the other grain colour classes by YP. Hitherto, no references are available that black barley generally contains higher amounts of yellow pigments. Since both methods for the determination of TAC and YP are simple spectrophotometrical methods, it is necessary as a

following step to quantify and identify the exact anthocyanins and carotenoids of blue aleurone and black pericarp barley by e.g. HPLC. The correlation analysis between agronomic and quality traits was found to be non-significant between almost all the traits. The absence of correlation and the negative correlation among agronomic and quality traits could be a big challenge and/or impediment in the future to develop hull-less barley cultivars that are endowed with quality traits like phenolics and soluble fibre (beta-glucan). Our results showed that there were no correlation of GLUC with GYLD, HLW, TKW and KP25. However, Hang et al. (2007) reported that GLUC was found to be positively correlated with KP25, but showed a weak negative correlation with GYLD in 27 barley genotypes. Similar to our results, Peterson et al. (1995) reported that the correlations between GLUC and GYLD, HLW, and TKW were generally nonexistent or inconsistent across years or nurseries in oat.

The knowledge of the phenolic metabolic pathways has now opened the possibility of directly developing new varieties with specifically modified phenolic profiles. In particular, the use of molecular techniques to 'fine tune' the control of phenolic metabolism to up-regulate desirable metabolic routes or to down-regulate undesirable ones is now a very real possibility (Parr and Bolwell 2000). Based on the present results it was recommended to make crosses among genotypes in Clus1, Clus5, Clus6 and Clus7 in breeding programmes. Classifying genotypes according to their agronomic and quality traits with sophisticated multivariate techniques can reduce the cost of time and money in crop improvement. However, stability analysis of different traits on the already established groups of the current study requires further investigations based on sufficient data that cover different years and experimental locations.

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