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Some Agronomic and Chemical Traits of Blue Aleurone and Purple Pericarp Wheat (*Triticum* L.)

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Abstract: Thirteen diverse anthocyanin pigmented wheat genotypes originating from different countries were investigated for agronomic and chemical traits. The results showed significant variation among wheat genotypes in yield and grain physical characteristics. Released cultivars were superior in grain yield, but other genetic resources exceeded the cultivars in regard to test weight, grain mass, or seed plumpness. In case of phytochemical content both genotypic and environmental effects were important for the observed variations. The total phenolic content varied from 120 to 177 mg ferulic acid equivalent per 100 g dry weight; total anthocyanin content from 3.4 to 75.2 ppm cyanidin glucoside equivalent; yellow pigment content from 2.6 and 7.6 ppm beta-carotene equivalent; protein content from 11.3 to 19.1%. The study demonstrated that Ethiopian wheats are a source of high levels of anthocyanins and protein content.

Key words: Anthocyanins, carotenoids, ethiopia, phytochemicals, pigments, seed colour, *Triticum.*

1. Introduction

Wheat (*Triticum* L.) is one of the most important cereals concerning cultivated area and production. Though it is a cool season crop, it grows in many different agro-climatic zones. Common wheat (*T. aestivum* L.) with the BBAADD genome has the broadest adaptation of all cereal crop species. It is the most important grain consumed directly by humans. A certain importance has also tetraploid (BBAA) durum wheat (*T. durum* Desf.), which is produced in many countries, especially in the Mediterranean region, mainly for their suitability to produce pasta. Other wheat species, e.g. diploid einkorn wheat (*T. monococcum* L.), tetraploid emmer (*T. dicoccum* Schrank *ex* Schübler) and Ethiopian wheat (*T. aethiopicum* Jakubz.), or hexaploid spelt wheat (*T. spelta* L.), are produced on a limited scale in diverse, often remote regions of the world [1].

Research studies have shown that whole-grain intake can have protective effects against cancer, coronary heart disease (CHD), diabetes and obesity [2, 3]. Whole grain foods are protective by decreasing the serum low-density lipoprotein (LDL) cholesterol and triglycerides [2, 4, 5]. CHD is the leading cause of death in most developed nations and is rapidly increasing in developing countries. Death rates from cardiovascular disease (CVD) exceed one million people in the USA per year [2].

Wheat grain is recognized as a good source of potentially health-enhancing components such as dietary fibre, phenolics, anthocyanins, tocopherols, and carotenoids [6, 7]. Phenolics are mainly concentrated in the bran tissues, i.e. the aleurone and pericarp layers [6, 8-10]. Wheat kernels contain a number of phenolic compounds such as ferulic, vanillic, gentistic, caffeic,

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salyicylic, syringic and *p*-coumaric acid [10, 11]. Blue and purple pigmented wheats contain phenolic sub-groups, i.e. anthocyanins. Other chemical compounds with potential health benefits in wheat are carotenoids. Carotenoids are responsible for the bright yellow endosperm colour of durum wheat, which is worldwide a requirement for pasta production [12]. Carotenoids are precursors of vitamin A and they are associated with reduced risk of cataract development, age-related macular degradation and antioxidant activities [13]. The main carotenoid in wheat is lutein; traces can be found of zeaxanthin and beta-carotene [14-16].

Pigmented wheat can provide a naturally coloured and/or functional food ingredient for the cereal industry. A number of varieties of tetraploid wheat originating from Abyssinia (Ethiopia) have purple coloured grains, due to the presence of anthocyanins in cells of the pericarp [17]. Körnicke [18] and Wittmack [19] were the first who described purple tetraploid wheats from Abyssinia. Later on, the purple seed colour was successfully transferred into hexaploid wheat [20]. Several reports have indicated that Ethiopian tetraploid wheats are responsible for the purple pericarp gene in common hexaploid wheat [17, 20-22]. As described by Copp [20] three wheats with purple grain colour, originally from Abyssinia, were obtained by the New Zealand Crop Research Division from the Plant Breeding Institute, Cambridge, UK, in 1930, and have been maintained at the wheat collection there. Crosses were made between all three purple wheats and several commercial hexaploid wheat varieties, but only one purple variety, *E 450*, produced viable seeds after the second backcross. After further backcrosses using a red-grained commercial variety a dark purple-grained hexaploid wheat plant in which the colour was as intensive as in the original tetraploid wheat was selected and further multiplied. This material was used to develop varieties such as *Konini* and *Charcoal* [21, 22]. The gene for blue aleurone was transferred from either *T. monococcum* or *T. boeoticum*, or *Agropyron elongatum* to common wheat [21, 23, 24]. Unlike purple wheats, blue aleurone wheats are so far not commercially exploited for speciality food products, whereas hexaploid purple wheats have been used since the 1980s in New Zealand for the production of speciality breads [25]. In 2006, the purple seeded wheat variety *Indigo* was released in Austria for the production of speciality food products.

Research on the content of phenolics of blue and purple wheat is an ongoing activity in many disciplines. Due to their importance as raw materials for functional foods and food colorants, research with the aim to identify varieties with high content of phenolics, anthocyanins and other phytonutrients is indispensable. The objectives of this study were (1) to determine the variation in agronomic traits and chemical compounds of pigmented tetraploid and hexaploid wheat genotypes, and (2) to examine the association between agronomic and chemical traits that would help provide useful information for direct or indirect selection.

2. Materials and Methods

2.1 Plant Material

Thirteen wheat genotypes which comprised four tetraploid (4 ×) Ethiopian (*T. aethiopicum*) and nine hexaploid (6 ×) common (*T. aestivum*) wheats originating from seven countries were investigated (Table 1).

2.2 Experimental Site and Trial Management

The trials were arranged in a row-column design with two replications at Raasdorf, Austria (16° 35′ E, 48° 14′ N). During the two experiment years, sowings were done on 10 October 2005 and 9 October 2006 for winter wheat, and on 3 April 2006 and on 16 March 2007 for spring wheat. The crop was grown under organic farming conditions without application of any external input. Monthly precipitation and mean temperature of the growing seasons at the study site for the two experiment years are shown in Fig. 1.

2.3 Agronomic Traits

Data were collected for heading date (DH, days after

Table 1 Description of investigated wheat genotypes.

Genotype name	Ploidy level	Growth type	Origin[1]	Grain colour[2]	Variety status
BVAL 258007	4 ×	Spring	ET	*Pp*	genetic resource
BVAL 258027	4 ×	Spring	ET	*Pp*	genetic resource
BVAL 258028	4 ×	Spring	ET	*Pp*	genetic resource
BVAL 258034	4 ×	Spring	ET	*Pp*	genetic resource
Purple	6 ×	Spring	CN	*Pp*	research germplasm
Purple Feed	6 ×	Spring	AU	*Pp*	research germplasm
Indigo	6 ×	Alternative	UK	*Pp*	released variety
Sebesta Blue 3	6 ×	Spring	US	*Ba*	research germplasm
Tschermaks Blaukorn	6 ×	Winter	AT	*Ba*	research germplasm
PWW2	6 ×	Winter	CL	*Pp*	breeding line
Amethyst	6 ×	Winter	NZ	*PP*	released variety
Capo	6 ×	Winter	AT	*R-1*	released variety
Saturnus	6 ×	Winter	AT	*R-1*	released variety

[1] AT, Austria; AU, Australia; CL, Chile; CN, Canada; ET, Ethiopia; NZ, New Zealand; UK, United Kingdom; US, United States of America; [2] *Ba*, blue aleurone; *Pp*, purple pericarp; *R-1*, red grain.

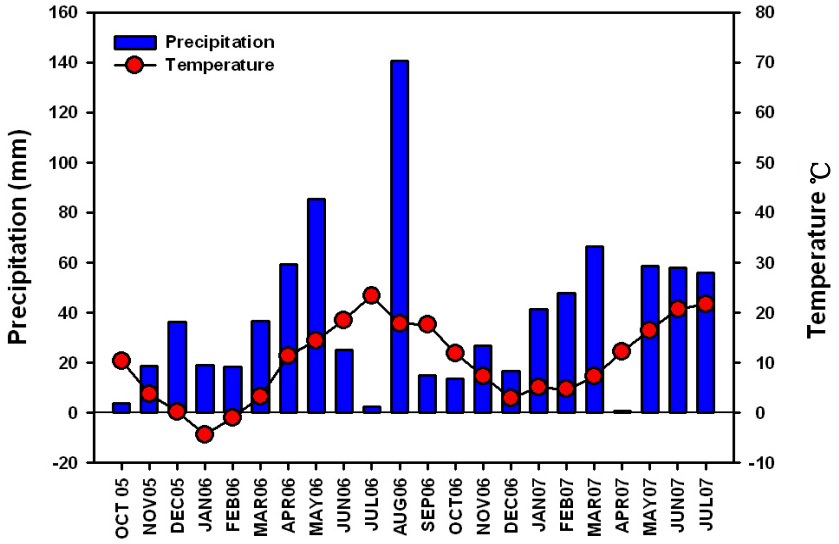

Fig. 1 Precipitation and mean temperature at the experimental site over the investigation period.

30 April), grain yield (GYLD, g m^{-2}), thousand kernel weight (TKW, g), hectolitre weight (HLW, kg hL^{-1}), and kernel plumpness (KP25, %). Plant height (PHT, cm) was recorded only in the first year. HLW was measured by a ¼ L chondrometer. Counting of seeds was done by a Contador machine (Pfeuffer GmbH, Kitzingen, Germany) and weighed on a BA2100 balance (Sartorius AG, Göttingen, Germany). KP25 was determined by sieving 100 g of grain with a Sortimat laboratory machine (Pfeuffer GmbH, Kitzingen, Germany). Grains remaining on the 2.5 mm sieve were considered as plump.

2.4 Chemical Analysis

Extraction of phenolics was carried out on 2.5 ± 0.1 g whole grain flour samples. Each sample was extracted twice with 20 mL acidified methanol (85:15 MeOH:1 M HCl) 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 ℃. Subsequently the mixture was transferred into plastic tubes and centrifuged at 4,000 rpm for 5 min. The centrifuged samples were placed into the refrigerator for another 20

min at 4 ℃ before the supernatants were filtered into 25 mL volumetric flasks fitted with funnels and folded filters ∅ 125 mm. The supernatants were filled to equal volume of 25 mL with the solvent and stored under room temperature in dark places.

The total anthocyanin content (TAC) was determined following Abdel-Aal and Hucl [26]. 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. The results were calculated using the calibration curve and expressed as mg cyanidin-3-glucoside equivalents per kg dry matter (ppm).

The total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent according to Singleton et al. [27]. The reaction mixture contained 0.1 mL acidified MeOH extract, oxidized by 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 waterbath at 50 ℃ 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 made for each extract and the results were expressed as mg ferulic acid equivalents per 100 g dry matter using the respective calibration curve.

Yellow pigment concentration (YP) was determined following the ICC Standard Method 152 [28]. 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 18-20 hrs. Afterwards the suspension was filtered into brown jars using folded filter papers with a sieve size of ∅ 110 mm. 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-carotene equivalents per 100 g dry matter (ppm).

Crude protein content was determined by the Dumas combustion method (ICC Standard Method 167 [28]) using a CN-2000 (Leco Instrumente GmbH, Mönchengladbach, Germany). In the CN analyzer the sample is combusted in an oxygen-rich environment at about 1000°C to give oxides of nitrogen which are catalytically reduced to nitrogen. Nitrogen gas is measured with a thermal conductivity detector. Total nitrogen is calculated from the detector response. The detector is calibrated with a known nitrogen standard, i.e. EDTA. The nitrogen content was transferred into protein content by multiplication with the conversion factor 5.7.

2.5 Data Analysis

MIXED model analysis of variance (ANOVA) was carried out using procedure MIXED of SAS 9.1 software with genotypes as fixed effect, year and genotype by year interaction (G × Y) as random effects. Variance components of random effects were tested by the Likelihood Ratio Test and model selection was done considering the Akaike Information Criteria (AIC). Pearson correlation analysis on genotypic best linear unbiased estimators (BLUEs) was performed using procedure CORR to investigate relationships between traits. BLUEs for the genotypes were calculated by the respective ESTIMATE statements. Procedures PRINCOMP and CLUSTER were used for principal component analysis (PCA) and subsequent hierarchical clustering of the most important principal components using Ward's minimum distance method. PCA was performed on the annual genotypic BLUEs of agronomic and quality traits. The STARS macro was used to create a star plot in order to better visualize the differences among individual wheat genotypes using

multivariate data [29-31].

3. Results

3.1 Mixed Model ANOVA and Variation of Traits

Mixed model ANOVA revealed significant differences among genotypes for all agronomic traits (Table 2). Considering random effects, the variance components for G × Y were significantly greater than that of the year effect for all traits with the exception of heading date. Hence, earliness is a stable trait influenced only by the genotype and differences between years. Similar stability showed protein content and yellow pigment concentration. The significant year effect was mainly due to differences in climatic conditions. In 2006, enough rainfall was present after sowing of spring cereals and maturity and harvest in July was characterized by warm temperatures and negligible rainfall. In 2007, sowing of spring cereals was followed by a six weeks period without rainfall resulting in reduced plant emergence and development of stands, resulting in significantly lower performance of spring wheat in regard to agronomic traits (Table 3). The best agronomic performance was recorded in both years for the hard red winter wheat (HRWW) check varieties *Saturnus* and *Capo*.

Blue and purple grained wheat samples contained higher levels of TPC and TAC compared to HRWW check varieties (Table 4). TPC ranged from about 120 to 177 mg 100 g^{-1} in both 2006 and 2007. In both years the lowest values were obtained for the HRWW check varieties. TAC ranged from 13 to 75 ppm in 2006 and from 3 to 56 ppm in 2007. As for TPC the lowest values were obtained for the HRWW check varieties, whereas the highest anthocyanin pigmentation was observed for an Ethiopian germplasm. Concerning YP, all genotypes had low values with the exception of purple pericarp breeding line *PWW2*. The lowest YP values were obtained for the HRWW varieties. All other hexaploid and tetraploid wheats exhibited YP

Table 2 Mixed model ANOVA statistic for agronomic and quality traits (F-value and probability statistic for fixed effect genotype, variance components for random effects year (Y) and genotype by year interaction (G×Y); variance components of significant random effects are printed in bold).

Trait [1]	Genotype		^2_Y	$^2_{G \times Y}$
	F-value	Pr > F		
DH	11.40	< 0.0001	**91.2**	3.5
GYLD	4.37	0.0121	156.2	**6081.7**
HLW	3.24	0.0276	**6.7**	**11.4**
TKW	7.64	0.0007	**2.8**	**3.6**
KP25	7.80	0.0005	**24.8**	**60.2**
PROT	2.73	0.0245	**4.6**	0.0
TPC	2.90	0.0388	**3.9**	**121.4**
TAC	5.96	0.0021	**100.9**	**114.6**
YP	12.27	< 0.0001	0.0	0.1

[1] abbreviations see Material and Methods.

Table 3 Best linear unbiased estimators (BLUEs) of agronomic traits of anthocyanin pigmented and check wheat varieties.

Genotype name	DH		GYLD		HLW		TKW		KP25	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
BVAL 258007	38	26	188.1	127.7	75	71	36	31	85	63
BVAL 258027	44	29	136.1	110.9	74	69	30	30	52	60
BVAL 258028	40	26	212.2	218.7	75	72	33	32	63	57
BVAL 258034	41	28	140.1	191.8	77	74	34	34	77	74
Purple	46	32	108.4	89.4	72	68	36	35	69	63
Purple Feed	40	27	277.7	268.8	81	75	42	41	92	89
Indigo	29	15	225.2	295.4	77	77	35	33	77	73
Sebesta Blue 3	37	23	232.4	191.9	75	72	30	28	29	32
Tschermaks Blaukorn	28	16	334.5	275.0	87	80	34	30	74	65
PWW2	33	19	424.0	349.1	73	68	30	27	54	42
Amethyst	33	19	186.6	267.5	68	71	28	29	53	59
Capo	28	14	614.5	529.3	86	80	39	35	88	75
Saturnus	29	15	529.9	618.5	81	84	42	39	91	81

Table 4 Best linear unbiased estimators (BLUEs) of quality traits of anthocyanin pigmented and check wheat varieties.

Genotype name	PROT		TPC		TAC		YP	
	2006	2007	2006	2007	2006	2007	2006	2007
BVAL 258007	15.7	18.7	138.7	142.9	54.5	45.3	4.4	4.5
BVAL 258027	15.0	18.0	176.6	169.5	71.9	55.8	4.8	4.7
BVAL 258028	14.8	17.7	166.6	149.8	75.2	45.6	5.0	4.8
BVAL 258034	16.1	19.1	150.4	153.3	30.6	27.0	3.9	4.0
Purple	14.1	17.1	159.6	161.6	37.1	27.2	4.8	4.8
Purple Feed	12.2	15.2	128.6	132.5	22.9	20.2	3.9	3.9
Indigo	12.8	15.7	144.1	144.3	39.1	27.6	4.6	4.7
Sebesta Blue 3	11.9	14.9	147.8	136.0	54.0	39.9	3.3	3.3
Tschermaks Blaukorn	13.2	16.2	148.2	165.1	49.7	32.6	3.5	3.4
PWW2	12.0	14.9	160.1	153.2	40.7	19.3	7.6	7.5
Amethyst	13.2	16.2	171.0	160.1	43.5	39.1	3.5	3.7
Capo	11.8	14.8	119.9	123.7	17.5	3.4	2.6	2.7
Saturnus	11.3	14.3	136.7	136.0	13.5	5.2	2.6	2.6

values between 3 and 5 ppm which is similar to YP concentration of durum wheat of lower quality. Surprisingly, the Ethiopian tetraploid wheats were not significantly higher than the hexaploid blue and purple pigmented wheats. Usually tetraploid wheat such as durum and Khorassan wheat are known for their medium to high YP, i.e. 5 to 10 ppm [32]. Due to the climatic influence PROT was generally higher in 2007. The highest PROT values were observed for the Ethiopian tetraploid wheat accessions.

3.2 Correlation Analysis

Correlations between agronomic and quality traits are given in Table 5. As expected the relationship between GYLD and PROT was significantly negative ($r = -0.53$). The negative correlation between yield and quality is well established in plant breeding. Varietal improvement is always the search for outliers of this negative correlation [33, 34]. GYLD was significantly and positively correlated to TKW and HLW. Generally, grain traits such as TKW, HLW and KP25 were significantly and positively intercorrelated. Concerning phytonutrients TPC and TAC were significantly correlated with each other. TPC was negatively correlated to GYLD and grain characters. This is most probably due to the fact that the highest yielding varieties were the adapted HRWW varieties *Capo* and *Saturnus* in which anthocyanin pigmentation is absent. A few further significant correlations were observed, however, these are most probably only artefacts, depending on the investigated plant material of this study. It is not reasonable that these correlations would be stable in any other germplasm, i.e. DH and TAC, which is due to the fact that the tetraploid Ethiopian wheats which are high in TAC are also later in maturity compared to the other genotypes.

3.3 Multivariate Analysis

Fig. 2 presents the star plot of the investigated germplasm. Using the star plot a breeder can easily identify which genotypes to use for trait improvement through crossing breeding. The larger the length of the ray, the higher will be the value for the respective trait. In this study, the star plots clearly showed that all the breeding lines and genebank accessions exhibit low grain yield and inferior grain traits, whereas their amount of phytonutrients was high.

PCA has generated four principal components (PCs) with eigenvalues > 1 which explained 49.5%, 18.7%, 11.7% and 7.0% of the total variance, respectively. Thus, 87% of the total variance were explained by the first four PCs. PC1 was largely related to TAC and TPC. With the exception of DH all other agronomic traits were negatively correlated to PC1. PC2 was

Table 5 Pearson correlation coefficients between agronomic and quality traits.

Trait	GYLD	HLW	TKW	KP25	PROT	TPC	TAC	YP
DH	-0.49 *	-0.16	0.16	0.00	-0.22	0.31	0.55 **	0.20
GYLD		0.68 ***	0.39 *	0.36	-0.53 **	-0.59 **	-0.64 ***	-0.31
HLW			0.61 ***	0.60 **	-0.47 *	-0.58 **	-0.36	-0.56 **
TKW				0.88 **	-0.31	-0.63 ***	-0.48 *	-0.42 *
KP25					-0.12	-0.50 **	-0.46 *	-0.35
PROT						0.30	0.15	0.14
TPC							0.64 ***	0.45 *
TAC								0.30

*, ** and *** significant at $P = 0.05$, 0.01 and 0.001, respectively.

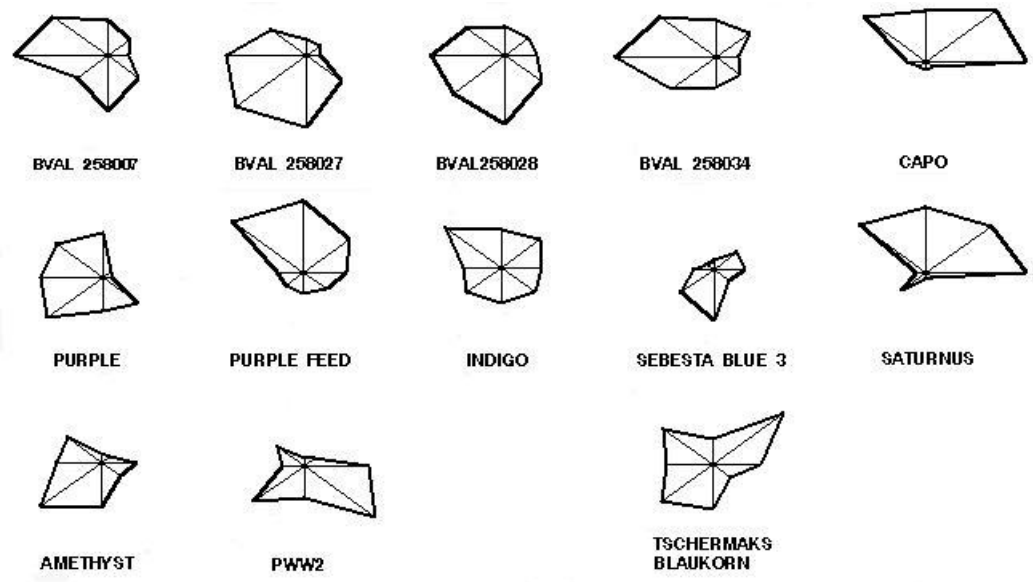

Fig. 2 Star plot of wheat genotypes based on two years' mean performance of TKW, HLW, GYLD, YP, TAC, TPC, PROT and KP25 (variables arranged clockwise from the top).

mainly a function of TKW and KP25, whereas the third PC was influenced by YP. The biplot (Fig. 3) displays the distribution of genotypes and variables according to the first two PCs. It is obvious that the Ethiopian tetraploid wheats and *Purple* form a group influenced by PROT, TPC and TAC. This group is also associated with GYLD, however, in the opposite direction which means these genotypes are characterized by low grain yields. On the opposite of the graph the high yielding HRWW check varieties are located. Between the group of tetraploid wheats and the HRWW checks the other purple and blue hexaploid wheat genotypes are located. These genotypes are somewhat grouped together. *Amethyst*, *PWW2* and *Sebesta Blue 3* form a group of genotypes from overseas, the two European sources *Indigo* and *Tschermaks Blaukorn* are located nearby, whereas *Purple Feed* takes an erratic position, which is strongly influenced by grain mass and grain grading.

Cluster analysis was run on the first four PCs and resulted in three major clusters (Fig. 4). The first cluster, Clus1, was formed by the purple wheats *Amethyst*, *Indigo* and *PWW2*, which are all descendants of the first released purple wheat *Konini*, and by the two blue wheats *Sebesta Blue 3* and *Tschermaks Blaukorn*. Clus2 was formed by the Ethiopian tetraploid wheat accessions and hexaploid *Purple*. Finally the third cluster consists of the two HRWW checks *Capo* and *Saturnus*, and *Purple Feed*. Generally, the dendrogramm from the cluster analysis corresponds well with the biplot from the PCA. It is interestingly to

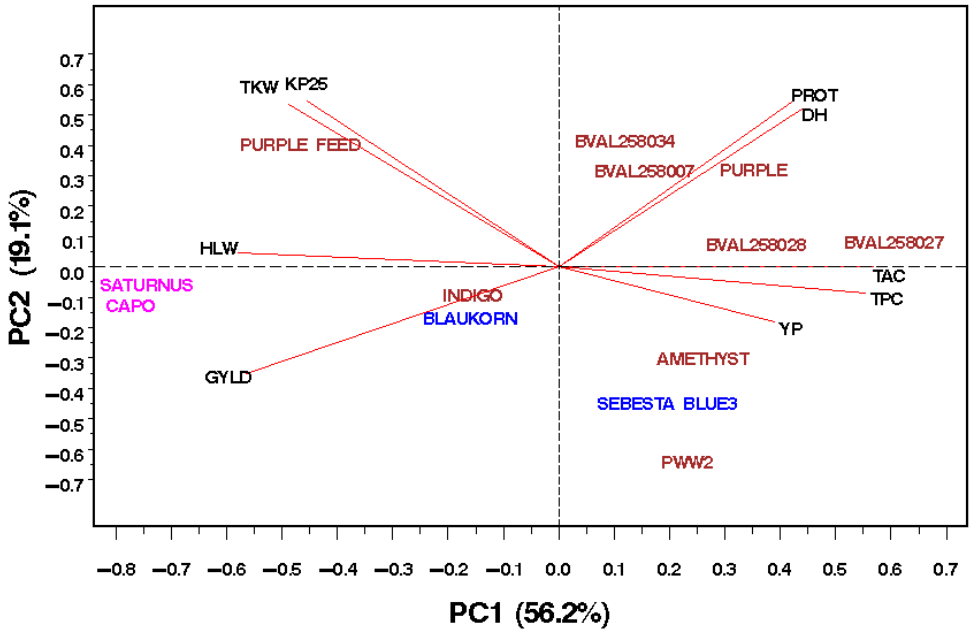

Fig. 3 Biplot of wheat genotypes and variables. The length of each variable vector is proportional to its contribution to separating the genotypes, and the direction of the vector indicates its relative contribution to PC1 and PC2.

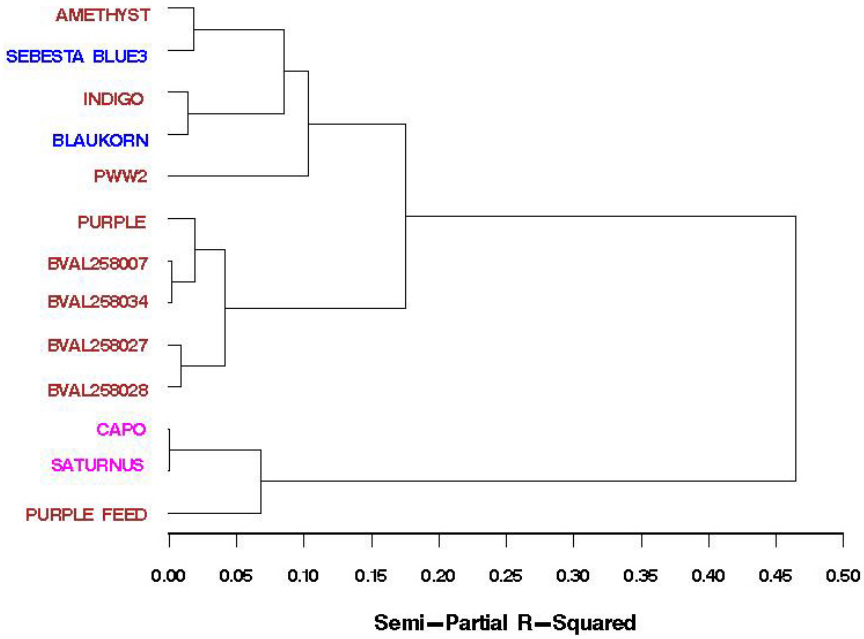

Fig. 4 Dendrogram of wheat genotypes based on similarities in agronomic and quality traits.

see that, although the purple grain character of the hexaploid wheat genotypes originates from one Ethiopian source, there exists multivariate variation between the purple hexaploid wheats. Especially *Purple* and *Purple Feed* are grouped differently from the released commercial varieties *Indigo* and *Amethyst*, and the advanced breeding line *PWW2*.

4. Discussion

In the present study, purple-pigmented Ethiopian tetraploid wheats were compared with purple, blue and red grained hexaploid wheat genotypes concerning agronomic and quality traits. The studied genotypes differed in most traits due to genotypic and environmental effects. Effect of year was more

pronounced on GYLD, TAC and PROT. Generally, it is hard to compare results from many studies since methods used for chemical analysis and used samples are diverse. In the present study, genotypic means for TPC ranged from 120 to 170 mg ferulic acid equivalents 100 g^{-1}. Li et al. [35] found 111 mg ferulic acid equivalents 100 g^{-1} in whole meal flour of Chinese 'black' grained wheat, derived from a cross of blue with purple wheat. Adom and Liu [36] compared the phenolic content of corn, oats, wheat and rice and found that the highest TPC value, i.e. 156 mg gallic acid equivalents 100 g^{-1}, was observed for corn. In their report wheat had higher TPC values (80 mg 100 g^{-1}) than oats (65 mg 100 g^{-1}) and rice (56 mg 100 g^{-1}). Yu et al. [37] found similar TPC values in three hard winter wheat varieties, ranging from 49 to 93 mg gallic acid equivalents 100 g^{-1} grain. Zhou et al. [38] examined and compared seven wheat samples from four different countries for their phytochemical compositions using HPLC and antioxidant activities, and found the TPC ranging from 220-290 mg gallic acid equivalent 100 g^{-1} bran flour. The higher TPC values in the latter study can be explained by the fact that only bran flour instead of wholegrain flour. Phenolics, however, are mainly located in the outer grain layers as was demonstrated by Adom et al. [39] who studied phytochemicals in milled fractions of five US wheat varieties and found 15-18 fold higher TPC for the bran/germ fractions compared to the endosperm fractions. In an experiment involving eleven diverse wheats, Adom et al. [7] found a free phenolic content of 120-201 µmol gallic acid equivalent 100 g^{-1} and a total phenolic content of 710-860 µmol gallic acid equivalent 100 g^{-1} in US wheat. From all hitherto published studies and our own results it becomes obvious that genetic variation in TPC is limited in wheat and also dependent on environmental conditions, e.g. drought stress.

The highest TAC values were determined in our study as expected for purple grained wheat. Contrary to our results Abdel-Aal and Hucl [6] and Abdel-Aal et al. [40] reported higher TAC values for blue pigmented wheat. Abdel-Aal and Hucl [6] analysed the anthocyanin content using HPLC and found a TAC of 152 ppm in blue wheat *Purendo*, 93 ppm in purple wheat *Konini* and 5 ppm in hard red spring wheat *Katepwa*. In a later work Abdel-Aal et al. [40] investigated the anthocyanin composition of diverse coloured cereals grain species. The blue aleurone wheat *Purendo* gave the highest TAC of 212 ppm, two purple varieties *Laval* (95 ppm) and *Konini* (38 ppm) exhibited medium to high values, whereas four white and red grained cultivars contained only 7-8 ppm. The environmental influence on TAC can be seen from the different contents the authors observed in different years for the genotypes which were present in both studies, i.e. *Purendo* and *Konini*. In our study, influence of year and genotype by year interaction on TAC was of similar magnitude.

The knowledge of the presence and magnitude of G × E is important to plant breeders concerning selection in single environments. A strong influence of environment (growing location) on TPC was reported by Beta et al. [9] for Canadian wheats. Abdel-Aal and Hucl [6] suggested that environmental effects on TAC could differ depending on the location of the respective pigments in the grain. The effect of environment could be explained in terms of the location of purple, red and blue pigments in the pericarp, testa and aleurone layers of seeds, respectively. Thus purple pigments are the most exposed to external environment whereas blue pigments the least.

In the biplot of the PCs the research germplasm *Purple* was grouped with the tetraploid Ethiopian wheats. This indicates a close relationship. In fact, the purple grain colour was originally transferred into hexaploid wheat from Ethiopian tetraploid wheat [20]. Nevertheless, enough multivariate variation, as demonstrated by PCA and cluster analysis, was found for further improvement of purple wheat. Especially interesting is the possibility to increase TAC, and most probably TPC, by combining the various allelic sources for purple pericarp and blue aleurone, if different

anthocyanins and/or phenolics would be expressed by these alleles. Li et al. [35] already reported such a 'black' grained genotype.

5. Conclusion

The standard red grained wheat cultivars had significantly lower amounts of phenolics and anthocyanins compared to blue or purple pigmented types. Hence, the use of whole grain food products processed from anthocyanin pigmented wheats can have health beneficial effects if the antioxidant activities of the respective phytochemicals remain unaffected by processing. Ethiopian purple wheats can be useful resources for improving the content of phenolics, anthocyanins and protein.

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References

[1] L.W. Briggle, B.C. Curtis, Wheat worldwide, in: Heyne E G (Ed.), Wheat and Wheat Improvement, 2nd ed., ASA-CSSA-SSSA, Madison, WI, USA, 1987, pp. 1-31.

[2] J.W. Anderson, T.J. Hanna, X. Peng, R. Kryscio, Whole grain foods and heart disease risk, J Am Coll Nutr 19 (2000) 291-299.

[3] J.M. Jones, M. Reicks, J. Adams, G. Fulcher, L. Marquart, Becoming proactive with the whole-grains message, Nutrition Today 39 (2004) 10-17.

[4] J. Dwyer, Overview: dietary approaches for reducing cardiovascular disease risks, J Nutr 125 (1995) 656-665.

[5] J. Slavin, Why whole grains are protective: biological mechanisms, Proc Nutr Soc 62 (2003) 129-134.

[6] E.S.M. Abdel-Aal, P. Hucl, Composition and stability of anthocyanins in blue grained wheat, J. Agric. Food. Chem. 51 (2003) 2174-2180.

[7] K.K. Adom, M.E. Sorrells, R.H. Liu, Phytochemical profiles and antioxidant activity of wheat varieties, J. Agric. Food Chem. 51 (2003) 7825-7834.

[8] W. Dedio, D.R. Hill, L.E. Evans, Anthocyanins in the pericarp and coleoptiles of purple wheat, Can. J. Plant Sci. 52 (1972) 977-980.

[9] T. Beta, S. Nam, J.E. Dexter, H.D. Sapirstein, Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions, Cereal Chem. 82 (2005) 390-393.

[10] S. Siebenhandl, H. Grausgruber, N. Pellegrini, D. del Rio, V. Fogliano, R. Pernice, E. Berghofer, Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley, J. Agric. Food Chem. 53 (2007) 8533-8536.

[11] M. Naczk, F. Shahidi, Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis, J Pharm. Biomed Anal. 41 (2006) 1523-1542.

[12] A. Blanco, C. De Pace, E. Porceddu, G.T. Scarascia Mugnozza, Genetics and breeding of durum wheat in Europe, in: G. Fabriani, C. Lintas (Eds.), Durum Wheat: Chemistry and Technology, Am Ass Cereal Chem, St. Paul, MN, USA, 1988, pp. 17-45.

[13] C.K.B. Ferrari, Functional foods and physical activities in health promotion of aging people, Maturitas 58 (2007) 327-339.

[14] V. Hentschel, K. Kranl, J. Hollmann, M.G. Lindhauer, V. Böhm, R. Bitsch, Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain, J. Agric. Food Chem. 50 (2002) 6663-6668.

[15] F. Leenhardt, B. Lyana, E. Rocka, A. Boussard, J. Potus, E. Chanliaud, C. Remesya, Genetic variability of carotenoid concentration, and lipoxygenase and peroxidase activities among cultivated wheat species and bread wheat varieties, Europ. J. Agron. 25 (2006) 170-176.

[16] E.S.M. Abdel-Aal, J.C. Young, I. Rabalski, P. Hucl, J. Fregeau-Reid, Identification and quantification of seed carotenoids in selected wheat species, J. Agric. Food Chem 55 (2007) 787-794.

[17] B.C. Sharman, Purple pericarp: a monofactorial dominant gene in tetraploid wheats, Nature 181 (1958) 929.

[18] F. Körnicke, Die Arten und Varietäten des Getreides, Verlag Emil Strauss, Bonn, Germany, 1885, p. 27.

[19] L. Wittmack, Violette Weizenkörner, Sitzungsber Ges Naturforsch Freunde, J.F. Starcke, Berlin, Germany, 1906, pp. 103-108.

[20] L.G.L. Copp, Purple grain in hexaploid wheat, Wheat Info Serv. 18 (1965) 19-20.

[21] A.C. Zeven, Wheats with purple and blue grains: a review, Euphytica 56 (1991) 243-258.

[22] N.F. Jensen, Registration of Charcoal wheat germplasm, Crop Sci. 17 (1977) 983.

[23] F.J. Zeller, M.C. Cermeno, T.E. Miller, Cytogenetical analysis on the distribution and origin of the alien chromosome pair conferring blue aleurone colour in

several European common wheat (*Triticum aestivum* L.) strains, Theor Appl. Genet. 81 (1991) 551-558.

[24] L.A. Morrison, R.J. Metzger, A.J. Lukaszewski, Origin of the blue-aleurone gene in Sebesta Blue wheat genetic stocks and a protocol for its use in apomixes screening, Crop Sci. 44 (2004) 2063-2067.

[25] T.N. Lindley, N.G. Larsen, Cereal processing in New Zealand: Inversion, diversification, innovation, management, in: G.M. Campbell, S.L. MacKee (Eds.), Cereals: Novel Uses and Processes, Plenum Press, New York, USA, 1997, pp. 273-279.

[26] E.S.M. Abdel-Aal, P. Hucl, A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheat, Cereal Chem. 76 (1999) 350-354.

[27] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenolics and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol 299 (1999) 152-178.

[28] ICC (International Association for Cereal Science and Technology), Standard Methods, Basic + Supplements. ICC Headquarter, Vienna, Austria, 1998.

[29] R.C. Littell, G.A. Milliken, W.W. Stroup, R.D. Wolfinger, SAS® system for mixed models, SAS Institute, Cary, NC, USA, 1996.

[30] N.W. Galwey, Introduction to Mixed Modelling, John Wiley & Sons, Ltd, Chichester, UK, 2006.

[31] M. Friendly, SAS® system for statistical graphics, SAS Institute, Cary, NC, USA, 1991.

[32] H. Grausgruber, S. Siebenhandl, F. Eticha, E. Berghofer, Carotenoids and anthocyanins in wheat (*Triticum* sp.), Vortr Pflanzenzüchtg 69 (2006) 117-125.

[33] B. Feil, The inverse yield-protein relationship in cereals: possibilities and limitations for genetically improving the grain protein yield, Trends Agron 1 (1997) 103-119.

[34] F.X. Oury, C. Godin, Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes?, Euphytica 157 (2007) 45-57.

[35] W. Li, F. Shan, S. Sun, H. Corke, T. Beta, Free radical scavenging properties and phenolic content of Chinese black-grained wheat, J. Agric. Food Chem. 53 (2005) 8533-8536.

[36] K.F. Adom, R.H. Liu, Antioxidant activity of grains, J. Agric. Food Chem. 50 (2002) 6182-6187.

[37] L. Yu, S. Haley, J. Perret, M. Harris, J. Wilson, M. Qian, Free radical scavenging properties of wheat extracts, J. Agric. Food Chem. 50 (2002) 1619-1624.

[38] K. Zhou, J. Laux, L. Yu, Comparison of Swiss red wheat grain and fractions for their antioxidant properties, J. Agric. Food Chem. 52 (2004) 1118-1123.

[39] K.K. Adom, M.E. Sorrells, R.H. Liu, Phytochemicals and antioxidant activity of milled fractions of different wheat varieties, J. Agric. Food Chem. 53 (2005) 2297-2306.

[40] E.S.M. Abdel-Aal, J.C. Young, I. Rabalski, Anthocyanin composition in black, blue, pink, purple, and red cereal grains, J. Agric. Food. Chem 54 (2006) 4696-4704.