

Nutritional Analyses and Their Inheritance Properties in Colored Wheat Seed lines from Different Origins Using Near-Infrared Spectroscopy

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ABSTRACT

Black, purple and blue wheat could provide a potential replacement of synthetic color with a nutritional ingredient for the cereal industry. Near Infrared reflectance spectroscopy (NIRS) has been widely used for the analysis of seed color and quality traits of intact seeds from cereals and oilseeds plants. The objective of this work was to study nutritional properties and their inheritance in winter and spring colored wheat from different origins by NIRS. Hybrid seeds from 'Black wheat76' crossed with 'Purendo 38', 'Konini' and 'Lo2147-3-4' were all black in color. The gene(s) responsible for Chinese winter wheat seed color was shown to have a dominant effect in nature as the reflectance spectra of the hybrid seed was found to be same as Chinese parental lines. Black colored wheat contained higher protein levels than the blue and purple colored wheat lines from both Canadian and Chinese origins. Highest protein percentage was observed in the hybrid between 'Black wheat76' and 'Purendo 38', whereas purple colored seed 'Konini' and black colored seed 'Lo2147-3-4' showed a high level of starch content. A strong positive correlation was found between protein and crude fibre percentage, while a high negative correlation was found between protein and moisture content, as well as protein and starch content. All hybrid plants had high protein content, high crude fibre and high minerals content but lower starch and moisture content than their respective parental lines. Further quantification of individual anthocyanins in black, purple and blue color wheat with high nutrient content would facilitate the use of these grains as natural colorants and functional food ingredients in the future.

Keywords: Black wheat76, blue grain, purple grain, black grain, NIR

INTRODUCTION

The use of wheat (*Triticum aestivum* L.) as natural dyes is gaining increased attention from both producers and consumers. These colored grains are crushed and used as natural dye in multigrain breads and soy sauce (Yang *et al.* 2001). Black, purple and blue are more uncommon pigmentation than the red and white pigmentation in wheat seeds. Flavonoids, anthocyanidins, anthocyanins and tannins are important biochemicals for expression of these colors in wheat seed (Yang *et al.* 2003). Since the 1930's the blue character in wheat seed was introduced from *Thinopyrum ponticum* (Host.) Barkworth & D.R. Dewey (syn. *Agropyron elongatum* (Host) Beauvoir: syn. *Elytrigia elongata* (Host) Nevski; 2n = 70) which have been the most important perennial *Triticeae* species for wheat improvement. A substitution line blue grained wheat Blue 58, was developed through wide hybridization between *Th. ponticum* and common wheat *T. aestivum* in China. This trait of the blue seed in common wheat, which is caused by blue pigments in the triploid aleurone layer, was introgressed from *Th. ponticum* by addition or substitution of the 4Ag chromosome (Tschermak 1938; Li *et al.* 1983; Zhang *et al.* 1992). The Chinese black colored 'Black wheat76' (Chinese name: 'Heilixiao-mai 76') was developed from existing blue line ('No. 1 Lan' or 'Blue1'), *T. durum* and a purple line (Sun *et al.* 1999).

Similarly development of synthetic purple seed wheat lines derived from *T. durum* has a long history in North America, Europe and Asia (Zeven 1991). As pointed out by Gilchrist and Sorrells (1982), the purple pigment is located in the pericarp, which follows a maternal inheritance pattern.

Sometimes these colors might be deep purple to brownish purple or almost black. Deep purple and almost black grain color was found in *T. durum* 'Tukur Sinde' (black wheat) and 'Charcoal' wheat varieties, respectively (Zeven 1991). Among the different blue seed wheat lines, a composite spring type blue-aleuroned, awnless wheat 'Purendo-38' was developed in Northern America (Abdel-Aal and Hucl 1999) and the stability of the blue-aleurone trait in 'Purendo-38' was tested over six generations (Matus-Cádiz *et al.* 2004). The blue-grained trait is a dominant gene marker that has been applied within gene flow studies (Hucl and Matus-Cádiz 2001; Matus-Cádiz *et al.* 2004). The color of wheat grain was used as a genetic marker for male sterile plant development (Ji and Deng 1985; Tian and Liu 2001; Zheng *et al.* 2006), natural crossing (Bolton 1968), confirming haploid plants derived from anther culture (Jan *et al.* 1981), gamete transformation (Li *et al.* 2004a; Morrison *et al.* 2004) and pigment characterization (Zeven 1991). The quality of this seed was tested in China (Bai *et al.* 2000) and is now used as raw food materials for value added products (Li *et al.* 2004b).

Previously color was measured visually in wheat but this measurement depends on light, background color, texture and observer's experience. Visual measurements are most effective when they are based on standard samples (van Deynze and Pauls 1994), but consistency of the results for color may vary from lab to lab. Instrumental methods were developed to measure the intensity of the color. Reflectance spectroscopy accurately measures the amount of light reflected by samples at different wavelengths from the visible to infra-red spectrum. Therefore, Near Infrared Ref-

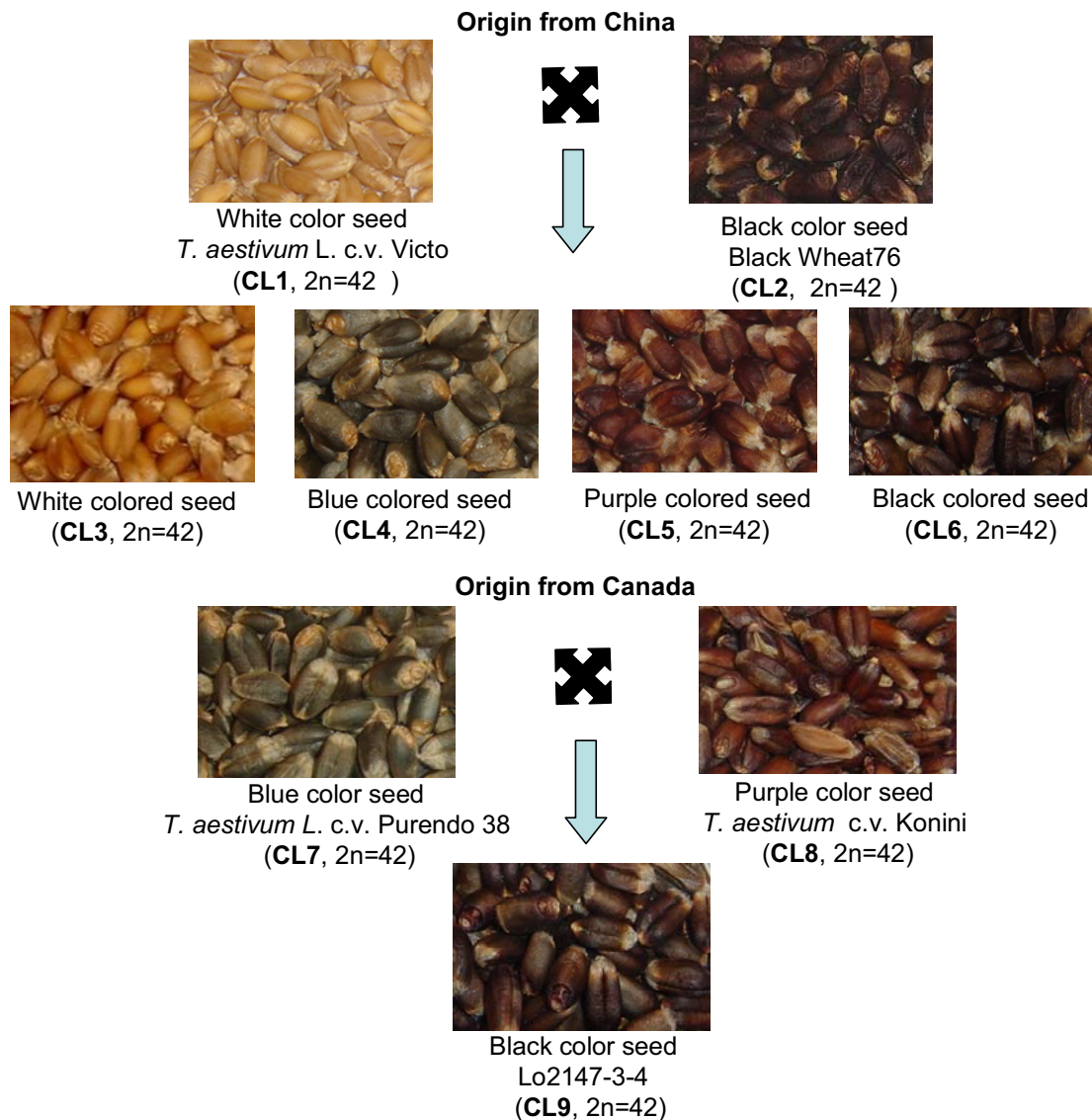


Fig. 1 Pedigree of the wheat lines used in this study.

lectance Spectroscopy (NIRS) has been widely used for the analyses of seed color, quality traits of intact seeds from cereals and oilseeds plants (Velasco *et al.* 1999). The use of these devices have permitted the development of bulked single-seed calibration equations for the analysis of protein content in wheat (Abe *et al.* 1995), oil and protein content in corn (*Zea mays* L.) and soybean (*Glycine max* L.) (Orman and Schumann 1992; Abe *et al.* 1995; Dyer and Feng 1995) and oil content in *Brassica* sp. (Velasco and Becker 1998; Velasco *et al.* 1999). Very limited number of research has been recently undertaken in this direction. NIRS has been used to analyse bulk samples of intact seeds for quality measurement in white wheat seeds. Developing new wheat cultivars with different color and good nutritive value is a new trend for the food industry.

The objective of this work was to study the genetic variation of nutritional properties for colored wheat seeds between two different genetic backgrounds using NIRS method and estimate the nutritive values in these black, purple and blue colored seeds.

MATERIALS AND METHODS

Plant material

The first black grain wheat 'Black wheat76' (2n=42, CL2) was developed by Sun *et al.* (1999) using distance hybridization and chromosome engineering. Four distinct colored hybrid lines (white,

blue, purple and black) were later developed by crossing with white grained *T. aestivum* cv. Victo (2n=42, CL1) and 'Black wheat76' in China. Grain samples of these winter Chinese white grained wheat (2n=42, CL3), blue grained wheat (2n=42, CL4), purple grained wheat (2n=42, CL5) and black grained wheat (2n=42, CL6) were collected from China.

Another black color substitution line 'Lo2147-3-4' (*T. aestivum*, 2n=42, CL9) was developed from crosses between spring type blue-grained wheat (*T. aestivum* cv. Purendo 38, 2n=42, CL7) and purple grained wheat (*T. aestivum* cv. Konini, 2n=42, CL8) (Fig. 1) in Canada. Seeds of blue-grained wheat ('Purendo 38'), purple grained wheat ('Konini') and black grained wheat ('Lo2147-3-4') were obtained from Canada and used for the study of genetic and nutrition of winter and spring colored wheat from different origins. Blue, purple and black grained wheat plants were selected from the most promising homozygous lines of their respective cultivar based on grain color, quality, and agronomic traits. Chinese winter wheat plants were crossed with Canadian spring wheat plants to develop six hybrid lines at Lethbridge Research Center, AAFC, Canada. Three hybrids were developed from crosses with 'Black wheat76' and three Canadian colored spring wheats separately (CL2 × CL7, CL2 × CL8, and CL2 × CL9) in 2007. Another three hybrids were developed by crossing with the same colored Chinese winter wheat and Canadian spring wheat (CL4 × CL7, CL5 × CL8, and CL6 × CL9). In all the cases Chinese winter wheat was used as the maternal parent.

All these lines were grown in greenhouse with 10 replications in the spring season. Winter wheat seeds were vernalised for one

month at 1°C prior to sowing of spring wheat. Conventional techniques were used for emasculation and crossing. Only lines with well formed and mature seed samples were used for this analysis. F₁ seeds and all parental lines were harvested and evaluated for their color and nutritive values. Ten F₁ plants from each cross were grown in the greenhouse. Cross compatibility related parameters like number of florets pollinated, number of seed obtained, seed set percentage, 100-grain weight, number of spikes per plant, length of spike and presence of awn were also measured for all parental lines as well as their hybrids.

NIRS analyses

Foss Model 6500 Feed and Forage Analyzer (Foss NIRSystems Inc. Silver Spring, MD, USA. Model 6500) was used for NIRS. The spectra were collected using ISIScan™. Three replications were taken to draw mean spectral data and calculated mean nutritional data for each line. The NIRS instrument determined chemical and physical composition of the nutrient material by measuring log (1/R) values, where R stands for reflectance (Blanco and Villarroya 2002). The essential information from spectra was extracted using chemometric techniques such as multivariate analysis by WinISI software (WinISI™ II Calibration development software, Infracore International). A relationship between reference data and log (1/R) values of a set of samples of known composition was already established by regressing reference data to the spectral data (log 1/R) by wheat calibration, which was also purchased from 'Foss' (Foss NIRSystems Inc. Silver Spring, MD, USA). Starch, protein, crude fibre, calcium, phosphorus, potassium, and sulphur in whole wheat seed, were determined by approved methods using spectral data, wheat calibration and WinISI software. All these predicted nutritive values were calculated at 13.5% moisture basis of wheat calibration.

Statistical analyses

Statistical analyses such as mean, variance, LSD test from mean values were performed using SAS software (SAS Institute 2003). Correlation was also calculated between two nutritional traits. All graphs were plotted using Microsoft Excel software.

RESULTS AND DISCUSSION

Plant secondary metabolites are a source of many natural products with diverse applications, including pharmaceuticals, food colors, dyes, fragrances, attraction of pollinating insects and protection against pests and pathogens (Memelink *et al.* 2001; Verhoeven *et al.* 2002). Therefore, it is very important to identify the genetic nature of black, purple and blue grain pigments in colored wheat. Light reflectance by colored wheat seeds changed as the wavelength of the illuminating light was increased from 400 to 2498 nm in the scanning spectrophotometer. The increase in reflectance began at violet (400–450 nm) to blue spectrum (450–500 nm) and an increasing tendency was also observed in yellow (570–590 nm) to red spectrum (610–750 nm) in this study. From these spectral data (Fig. 2), it was observed that all genotypes had distinct color intensity and reflectance spectra. Highest log (1/R) value was obtained for hybrids between CL2 and CL8 within a range of 400 to 500 nm wavelength. This indicated that this hybrid was darker than the parental line CL2. F₁ seeds from the CL2 crossed with CL7, CL8 and CL9 were all black. The spectral data (log 1/R) from NIRS results in visual range also showed that all the hybrids crossed with CL2 (*viz.* CL2 × CL7, CL2 × CL8, and CL2 × CL9) were similar or close to the CL2 spectra within the range of wavelength between 400–750 nm (Fig. 2). From all the spectral data it was clear that the CL2 seed color gene(s) was dominant in nature. A positive correlation (0.78) between the degree of red pigmentation and number of dominant R alleles were obtained by Wang *et al.* (1999) using NIRS method. Many scientists have also discussed the inheritance of the blue aleurone trait in wheat seed (Zeven 1991; Hucl and Matus-Cádiz 2001; Metzger and Sebesta 2004). Hurd (1959) suggested that two complemen-

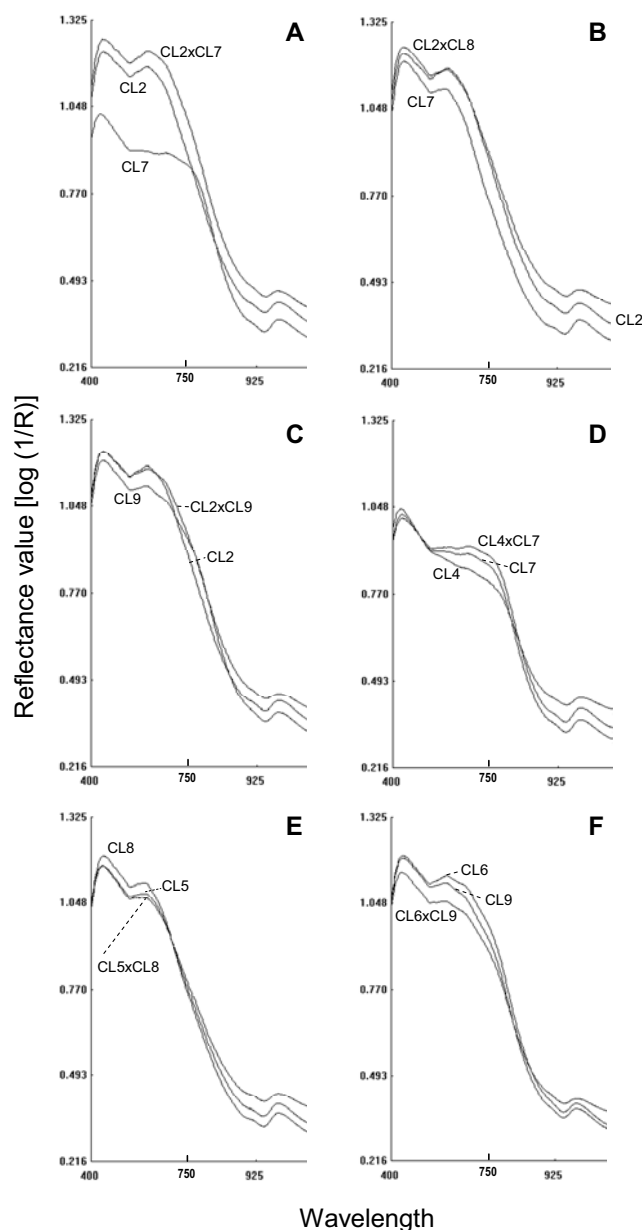


Fig. 2 Reflectance spectra for black, purple and blue colored wheat seeds using NIRS in different light wavelength. (A) Reflectance spectra of CL2, CL7 and hybrid CL2 × CL7; (B) Reflectance spectra of CL2, CL8 and hybrid CL2 × CL8; (C) Reflectance spectra of CL2, CL9 and hybrid CL2 × CL9; (D) Reflectance spectra of CL4, CL7 and hybrid CL4 × CL7; (E) Reflectance spectra of CL5, CL8 and hybrid CL5 × CL8; (F) Reflectance spectra of CL6, CL9 and hybrid CL6 × CL9.

tary dominant genes conditioned the blue grain color. According to results of test crosses, a dominant gene designated *Ba* was believed to control the expression of blue pigment (Keppenne and Baenziger 1990). The heredity of black grained wheat, CL2 was derived from blue grained (Bule1), *T. durum* and *Elymus dasystachys* Trin. Whereas, blue grained wheat (Blue1) was selected cytogenetically from crossing between common wheat and *Agropyron* (Sun *et al.* 1999). The black grain color in CL2 was controlled by two pairs of incompletely dominant genes (Sun *et al.* 2003). Blue grained wheat derived from common wheat and *Th. ponticum* has been studied; it was shown that this gene clearly exhibited a dose effect, which was revealed by the intensity of the seed color correlating with the 4Ag chromosome dosage in the endosperm cells (Li *et al.* 1986). Dominance of purple alleles was found in F₁ plants by Sharman (1958) in *T. durum* wheat crossing. Two genes controlling purple pericarp were found by Capron (1918) in a cross between *T. polonicum* × *T. elboni*. In this study, 61, 49 and

Table 1 List of parameters studied with relation to wheat pollination and seed settings.

Seed code	Color of seeds	Presence of awn	100 grain weight (g)	Nº of florets pollinated	Nº of seeds obtained	% of seed set	Nº of spikes/plant	Spike length (cm)
Colored parental lines								
CL1	White	Yes	3.54	457	390	85.19	9.6	6.93
CL2	Black	No	4.72	496	424	85.43	8.9	9.45
CL3	White	No	4.34	524	446	84.86	6.8	7.72
CL4	Blue	No	5.08	558	486	87.08	8.1	7.58
CL5	Purple	No	4.52	558	510	91.24	8.1	8.15
CL6	Black	No	4.98	515	412	79.92	6.5	7.44
CL7	Blue	No	4.20	531	454	85.49	11.9	11.1
CL8	Purple	Yes	4.74	380	321	83.46	11.8	7.44
CL9	Black	Yes	3.70	594	458	76.85	12.0	12.17
Colored hybrid lines								
CL2 X CL7	Black	No	4.02	272	160	60.69	6.5	10.85
CL2 X CL8	Black	No	4.06	260	132	48.53	6.8	8.85
CL2 X CL9	Black	No	3.62	207	153	72.85	10.3	12.16
CL4 X CL7	Blue	No	4.00	304	173	56.94	11.3	10.51
CL5 X CL8	Purple	No	3.60	220	173	79.09	9.8	10.21
CL6 X CL9	Light blue	No	3.08	241	118	49.35	11.0	11.89

Table 2 Predicted wheat seed nutritional properties by Near-Infrared Spectroscopy.

Parental lines and hybrids	Seed code	Starch	Protein	Moisture	Crude fibre	Calcium	Phosphorus	Potassium	Sulphur
Mean values in percentage									
Origin from China									
Victo	CL1	52.2	14.7	11.2	5.8	0.09	0.31*	0.36**	0.14
Black Wheat76	CL2	50.8	16.6	10.9	7.0	0.08	0.39**	0.29	0.17
White colored seed	CL3	55.4	13.0*	11.7**	5.4*	0.09	0.25**	0.35*	0.13*
Blue colored seed	CL4	54.0	13.2*	11.7**	5.7	0.09	0.31*	0.32	0.13*
Purple colored seed	CL5	57.2**	13.1*	11.8**	5.9	0.09	0.32	0.34	0.13*
Black colored seed	CL6	57.4**	13.1*	11.9**	4.9**	0.10	0.38**	0.34	0.14
Origin from Canada									
Purendo 38	CL7	55.4	12.9*	11.1	5.7	0.06**	0.33	0.30	0.13*
Konini	CL8	58.4**	12.5**	11.6*	4.2**	0.08	0.33	0.35*	0.13*
Lo2147-3-4	CL9	58.9**	14.4	11.4	6.2	0.07**	0.35	0.30	0.14
F ₁ lines	CL2 X CL7	48.7**	19.8**	10.4	8.6**	0.08	0.28**	0.18**	0.20**
	CL2 X CL8	49.2**	19.3**	10.0**	8.4**	0.09	0.38**	0.22**	0.20**
	CL2 X CL9	49.6*	17.1	9.9**	9.2**	0.08	0.39**	0.24**	0.18*
	CL4 X CL7	49.7*	17.7*	9.6**	8.8**	0.11**	0.31*	0.31	0.19**
	CL5 X CL8	50.0*	16.2	10.5	8.1*	0.11**	0.33	0.27	0.17
	CL6 X CL9	50.8	16.9	9.9**	7.8	0.13**	0.43**	0.34	0.18*
Mean		53.18	15.37	10.9	6.78	0.09	0.34	0.30	0.16

Significant difference from mean value at ** 1% level and * 5% level

73% of F₁ seeds were obtained through hand pollination for CL2 × CL7, CL2 × CL8, and CL2 × CL9 hybrids, respectively. In all three hybrid combinations, the black color control gene(s) of CL2 showed dominance over the CL7 blue color gene(s), CL8 purple color gene(s) and CL9 black color gene(s).

Spectral data of the hybrid seeds from a cross between CL4 and CL7, and CL5 and CL8 had similar seed color to CL7 and CL5, respectively. On the other hand, Canadian spring wheat CL8 had a darker purple color than the Chinese winter wheat CL5. The gene(s) responsible for Chinese winter wheat seed color had a dominant effect in nature, since the reflectance spectra of the hybrid seed CL5 × CL8 was found to be the same with CL5. Blue colored wheat seeds exhibited a reduced effect on anthocyanin content under different growing environments, as compared to purple colored wheat seed. This may be due to the location of the anthocyanins in the aleurone as opposed to the pericarp (Abdel-Aal and Hucl 2003). The blue aleurone wheat CL7 contained higher concentrations of total anthocyanin as compared to the purple wheat cultivar CL8, which may reflect diverse stabilities and characteristics (Abdel-Aal and Hucl 1999, 2003). This stability of the blue-aleurone trait in CL7 was tested over six generations and verified by evaluating grain samples from 200 head-rows (Matus-Cadiz *et al.* 2004). The hybrid plant CL6 × CL9 was a cross between two black seed color substitution lines (CL6 and CL9) and showed different reflectance spectra than the parental lines. By artificial pollination method only 57, 79 and 49% hybrid

seeds were obtained for CL4 × CL7, CL5 × CL8 and CL6 × CL9 crosses, respectively (**Table 1**).

Plant characteristic such as 100-grain weight, number of spikes per plant, spike length and presence of awn were also observed for all parental lines as well as hybrid plants (**Table 1**). In spite of the presence of awn in CL8 and CL9 parents, plants of all hybrids did not have an awn. 100-grain weight of all the hybrids was less than those of their respective parental lines. The CL9 substitution wheat line showed the highest number of spike per plant and longest spike length. Among the hybrid plants the light blue colored hybrid (CL6 × CL9) wheat line produced lighter weight seed with 16.9% protein content. A wide variation of spike length was found among the hybrid plants. Hybrids from the cross between CL2 and CL8 showed the smallest spike length. On the other hand, the crosses CL2 × CL9 and CL6 × CL9 showed similar spike length to CL9. These results indicate the presence of an important gene in CL9 controlling spike length.

NIRS is widely used in the grain industry for determining grain quality. The scope of NIRS application in food and grain quality analysis is very promising. For screening and prediction of main quality parameters in wheat quality breeding, the application of NIRS is really a potential method (Oatway and Helm 2002; Crosbie *et al.* 2007). Comparative graphs of three nutritional components (protein, fibre and starch) for blue, purple, and black wheat genotypes and their respective hybrids are shown in **Fig. 3**. All the hybrid plants showed higher protein content and a lower starch

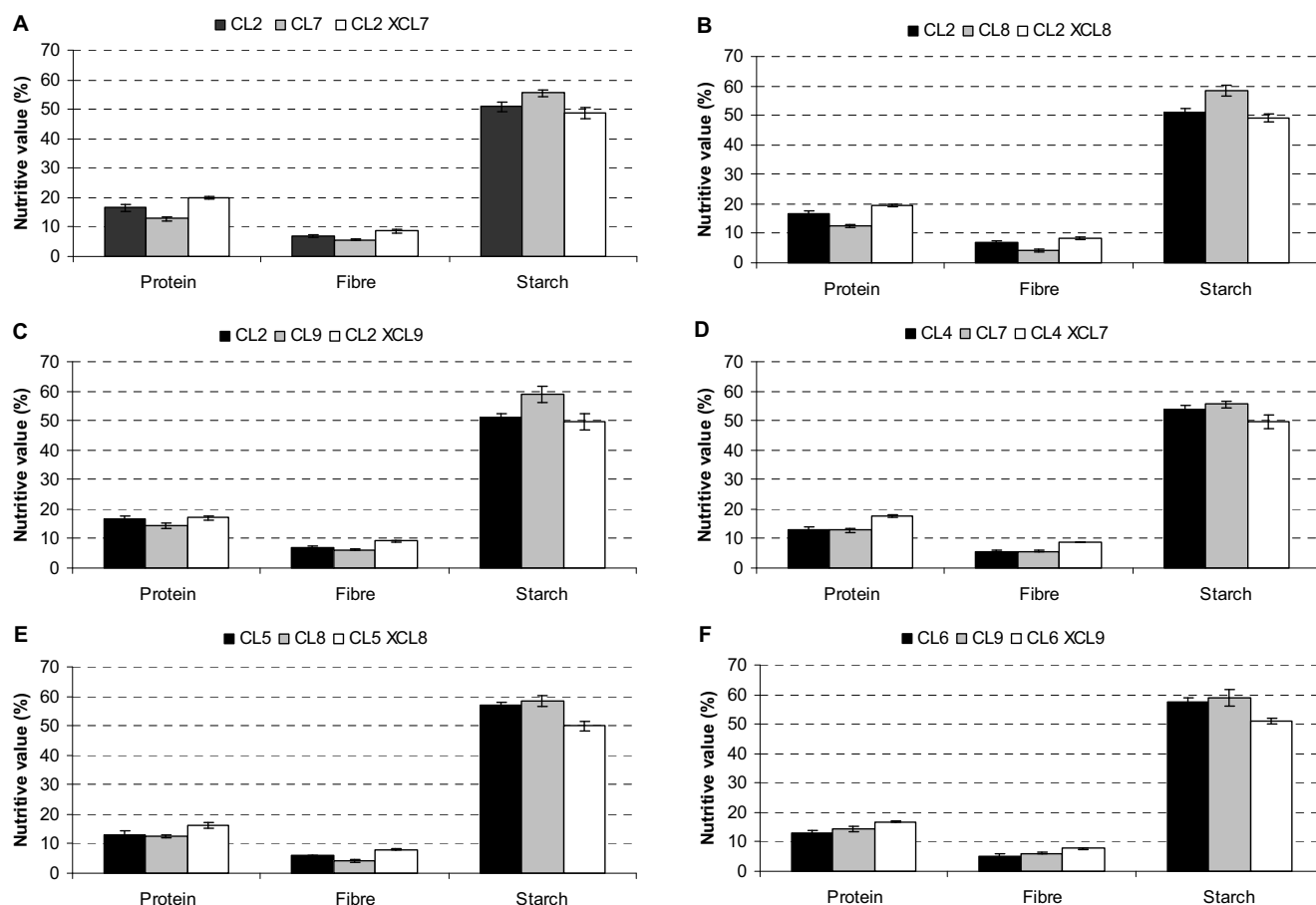


Fig. 3 Graphical representation for predicted nutritional properties of hybrid lines and their respective parents. (A) Nutritive values of CL2, CL7 and hybrid CL2 x CL7; (B) Nutritive values of CL2, CL8 and hybrid CL2 x CL8; (C) Nutritive values of CL2, CL9 and hybrid CL2 x CL9; (D) Nutritive values of CL4, CL7 and hybrid CL4 x CL7; (E) Nutritive values of CL5, CL8 and hybrid CL5 x CL8; (F) Nutritive values of CL6, CL9 and hybrid CL6 x CL9).

Table 3 Correlation between all nutritional properties of colored wheat seeds.

	Starch	Protein	Moisture	Crude fibre	Calcium	Phosphorus	Potassium
Protein	-0.87**						
Moisture	0.85**	-0.86**					
Crude fibre	-0.87**	0.91**	-0.92**				
Calcium	-0.35	0.25	-0.37	0.30			
Phosphorus	-0.16	0.24	-0.36	0.25	0.26		
Potassium	0.63	-0.79**	0.57	-0.73*	0.23	-0.07	
Sulphur	-0.87**	0.98**	-0.90**	0.91**	0.34	0.32	-0.75*

* 5% level of significance and ** 1% level of significance

content than the parental lines. Among the parental lines, CL2 showed the highest protein percentage and hybrid CL2 × CL7 plant showed highest protein content among all these lines, whereas purple colored seed CL8 and black colored seed CL9 showed higher levels of starch content (58.4 and 58.9%, respectively). Similarly, Gao *et al.* (2000) found high levels of polysaccharides (starches), flavonoids and other secondary metabolites in the endosperm of blue-grained wheat. This blue pigments is located in the outer layers of the wheat grain (Abdel-Aal and Hucl 2003). Black wheat contained higher protein levels than the blue and purple wheat lines from both Canadian and Chinese origin. Different crude fibre percentages were observed among the parental lines. Chinese black colored seed, 'Black wheat76', showed higher fibre percentage and lower moisture percentage among all the parental lines, while 'Konini' showed lower crude fibre percentage. From **Table 2** it is clear that all the hybrid plants had high protein, crude fibre and mineral contents but lower starch and moisture content than their corresponding parental lines. A highly significant ($p < 0.01$) positive correlation was found between protein and sulphur content, protein and crude fibre, crude fibre and sulphur content and, starch and moisture content while a strongly

negative correlation was found between crude fibre and moisture content, starch and protein content, starch and crude fibre content, starch and sulphur content, protein and moisture content, protein and potassium content, potassium and sulphur content as well as crude fibre and potassium content (**Table 3**). Higher free radical scavenging ability and phenolic compounds were reported to be present in Chinese black colored wheat than in purple, blue and white colored wheat (Li *et al.* 2005).

In general, black, purple and blue wheat could provide a potential replacement of synthetic color with a nutritional ingredient for the cereal industry based on its anthocyanin content. Further quantification and identification of individual anthocyanins in black, purple and blue wheat would facilitate the use of these grains as natural colorants and functional food ingredients. Breeding for new wheat lines for these quality characteristics without NIRS would be too expensive and time-consuming (Oatway and Helm 2002). This fast, nondestructive and cost-effective NIRS technique is advantageous for colored wheat breeding and prediction of bulked wheat seeds nutritive values. Future research will be important to determine the number of controlling or influencing gene(s) for seed coat color and the possible loca-

tion of the gene(s) involved. A homoeologous series could be developed for black, purple and blue grain color from these lines for future DNA marker analysis.

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