International Journal of Plant Breeding ©2009 Global Science Books

Inheritance of Grain Color Controlling Genes in Diverse Wheat Crosses using Near-Infrared Spectroscopy

Sanjib Nandy[1] • Qin Chen[1*] • Hi Yan Li[1] • Faiz Ahmad[2] • Robert Graf[1] • Gerald Kereliuk[1] • Shan Cheng Sun[3]

[1] Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, T1J4B1, Canada
[2] Brandon University, Biology Department, Brandon, MB, R7A 6A9, Canada
[3] Institute of crop Genetics, Shanxi Accademy of Agricultural Sciences, Taiyuan 030031, China

Corresponding author: * **chenqi@agr.gc.ca**

ABSTRACT

Wheat seeds with black, purple and blue color are more uncommon pigmentation than the red and white pigmentation. The purpose of this work was to study the genetic of seed color in wheat lines, originally from two different genetic backgrounds (China and Canada). The genotypes of the blue and black seeded parents seem to be under the effect of two genes located in two alien chromosomes from the J and J^s. The purple color was controlled totally by the genes located in wheat chromosome. The gene(s) responsible for Chinese winter wheat seed color was shown to have dominant based on reflectance spectra of the hybrid seed. Genetic models from F_2 population also support the presence of different genes controlling colored winter Chinese wheat grains and Canadian spring wheat grains. Interaction between two alien chromosomes may have led to the instability in blue grain color and resulted in light blue to bluish yellow grain. Duplicate dominant epistasis was playing a key role for expression of blue coloration and duplicate gene action was observed for black color controlling gene.

Keywords: black grain, black wheat76, blue grain, genome, NIR, purple grain
Abbreviations: NIRS, Near Infrared Reflectance Spectroscopy

INTRODUCTION

Purple seeded wheat lines from *Triticum durum* L. and blue seeded wheat lines from *Agropyron elongatum* (Host.) Beauvoir have long history in North America, Europe, and Asia (Zeven 1991). 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. The 'J' genome was very valuable source of genes in *Th. bessarabicum* (Savul. & Rayss) [Syn. *Elymus farctus* subsp. *bessarabicus*] (genome J, 2n = 14) contributing genes for wheat seed color (Li *et al*. 2003), while 'J^s' refers to modified 'J-' type chromosomes distinguished by the presence of 'S' genome specific sequences close to the centromere. Both 'J' and 'J^s' genomes were present in *Thinopyrum* sp. (Chen *et al*. 1998).

The Chinese black colored wheat, Black wheat76 (Heilixiaomai 76), was developed from existing blue line (Blue1 or "No.1 Lan"), *T. durum* and a purple line (Sun *et al*. 1999) in China through the application of distant hybridization and chromosome engineering. The Blue 1 was developed from *Agropyron glaucum* (Desf. ex DC.) Roemer & Schultes [Syn. *Th. intermedium* (Host) Barkworth & D.R. Dewey; *Agropyron intermedium* (Host) Beauvois; *Elytrigia intermedia* (Host) Nevski; *Elymus hispidus* (Opiz) Meldris] (Zeven 1991) and purple line was developed from a wild *Elymus dasystachys*, which is related to *Agropyron* genus (commonly grown in Asian environment). Genetics of seed pigmentation of colored wheat especially black grain wheat was very complex, (Sun *et al*. 2003). As pointed out by Gilchrist and Sorrells (1982), the purple pigment is located in the pericarp, which follows a maternal inheritance. Sometimes these colors might be deep purple to brownish purple or almost black. Deep purple and almost black grain colors were found in *T. durum* cv. 'Tukur Sinde' (black wheat) and Charcoal wheat varieties, respectively (Zevan 1991).

Purple, blue and black wheat could provide a potential replacement of synthetic color for the cereal industry. Visual measurements are most effective when it is based on standard samples, light, background color, texture and observer's experience (van Deynze and Pauls 1994), but consistency of the results for color will vary from lab to lab. Several instrumental methods were developed to measure the intensity of the color (Liu *et al*. 2005). Reflectance spectroscopy measures accurately the amount of light reflected by samples at different wavelengths from the visible to infra-red spectrum. The Near Infrared Reflectance Spectroscopy (NIRS) is widely used in the grain industry for determining grain admixture. Therefore, NIRS has been widely used for the analysis of seed color trait of intact seeds for cereals as well as oilseeds plants (Velasco *et al*. 1999). Very limited number of research has been presently undertaken for estimation of genetic nature of seed color. The objective of the study was to investigate the genetic architecture of grain color using near infrared reflectance spectroscopy technique.

MATERIALS AND METHODS

Plant material

Four distinct colored hybrid lines (white, blue, purple and black) were developed by crossing with white grained *T. aestivum* cv. 'Victo' (2n=42, CL1) and 'Black wheat76' (2n=42, CL2) through cytogenetical method in China. Grain samples of these Chinese white grained wheat (2n=42, CL3), blue grained winter wheat (2n=42, CL4), purple grained winter wheat (2n=42, CL5) and black grained winter wheat (2n=42, CL6) were developed by Sun *et al*. (1999) and collected from him for this study.

Received: 24 July, 2008. Accepted: 26 December, 2008.

Original Research Paper

Table 1 Light reflectance values in different wavelength for parental lines and F_1 hybrids.

Wheat lines	Code	Seed color	Light reflectance value (Log 1/R)		
			Red (662 nm)	Green (572 nm)	Blue (438 nm)
Chinese origin					
Victo	CL1	White	0.362	0.440	0.532
Black wheat76	CL2	Black	1.032	1.173	1.222
Chinese white grained winter wheat	CL3	White	0.421	0.461	0.496
Chinese blue grained winter wheat	CL4	Blue	0.915	0.918	1.001
Chinese purple grained winter wheat	CL5	Purple	0.911	1.078	1.166
Chinese black grained winter wheat	CL6	Black	1.068	1.133	1.199
Canadian origin					
Purendo-38	CL7	Blue	0.889	0.905	1.019
Konini	CL8	Purple	0.909	1.112	1.197
Lo2147-3-4	CL9	Black	1.026	1.112	1.193
F_1 progeny					
CL5 × CL8		Purple	0.914	1.062	1.166
CL2 × CL8		Black	0.909	1.112	1.197
CL4 × CL7		Blue	0.836	0.878	1.032
CL2 × CL7		Dark black	1.127	1.221	1.262
CL6 × CL9		Purplish black	0.979	1.053	1.146
CL2 × CL9		Black	1.070	1.165	1.221

Another blue colored wheat line, a composite spring type blue-aleurone, awnless wheat cultivar 'Purendo-38' (*T. aestivum*, 2n=42, CL7) was developed in Northern America (Abdel-Aal and Hucl 1999). The stability of the blue-aleurone trait in 'Purendo-38' was tested over six generations (Matus-Cadiz *et al*. 2004), whereas purple grained 'Konini' was developed in 1980 (Bezar 1982). Recently a black color substitution line 'Lo2147-3-4' (*T. aestivum*, 2n=42, CL9) was developed as a result of a cross between spring type blue-grained wheat (CL7) and purple grained wheat 'Konini' (*T. aestivum*, 2n=42, CL8) in Canada. Seeds of blue-grained wheat (CL7), purple grained wheat (CL8) and black grained wheat (CL9) were collected from Canada and used for the study of cross-compatibility of winter and spring colored wheat from different origin. 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 ('Black wheat76' × 'Purendo-38', 'Black wheat76' × 'Konini', and 'Black wheat76' × 'Lo2147-3-4'). Another three hybrids were developed by crossing with the same colored Chinese winter wheat and Canadian spring wheat e.g. blue grain (CL4 × CL7), purple grain (CL5 × CL8) and black grain (CL6 × CL9). In all the cases Chinese winter wheat was used as the maternal parent. The F_1 hybrid plants were self-pollinated to produce F_2 seeds. Every F_1 hybrid lines were sown in two rows with 6 individuals per row, in a greenhouse.

All these lines were grown in a greenhouse with 10 replications in the spring season. Winter wheat seeds were kept for one month at 1°C for vernalisation before the sowing of spring wheat seeds. Conventional techniques were used for emasculation and crossing. Only lines with well formed and mature seed samples were used for this analysis. F_1, F_2 along with all parental seeds were harvested and evaluated for their color. Olympus BX51 microscope was used for chromosome identification from root tips of F_1 hybrid lines.

Near Infrared Spectroscopy analysis

First the seed colors of these F_1, F_2 and parental plants were visually assessed and classified into different groups (Black, Purple Yellow and Blue). In addition to the visual analysis, NIR spectroscopy was used to confirm the different seed color. Seed color was measured with the near infrared reflectance spectroscopy (NIRS) Foss Model 6500 Feed and Forage Analyzer (Foss NIRSystems Inc. Silver Spring, MD, USA. Model 6500). The spectra were collected using ISIScan^{TM}. The NIRS instrument determined a graph of the food material by measuring log (1/R) values, where R represents reflectance.

Statistical analyses

The Chi-squared test was used to test the fit of observed segregations with the theoretical ratios. Statistical analyses such as Chi-squared test for Mendelian genetic ratios were performed using SAS software (SAS Institute 2003).

RESULTS

The average values of light reflectance (R) in terms of log (1/R) for the parental lines and F_1 hybrids under different wave lengths are given in **Table 1**. The average values of light reflectance at 662 nm (red spectrum) and 438nm (blue spectrum) wavelength were 1.032 and 1.222 for black seeded CL2, 1.068 and 1.199 for CL6 and, 1.026 and 1.192 for CL9. Light reflectance values for purple seeded wheat at red and blue spectrum were found to be 0.911 and 1.165 for CL5 and, 0.909 and 1.197 for CL8. In the case of blue seeded wheat light reflectance values at red and blue spectrum were 0.915 and 1.009 for CL3 and, 0.889 and 1.019 for CL7. These two parental lines also showed a difference in reflectance values beyond the red spectrum. F_1 seeds from the CL2 crossed with CL7, CL8, and CL9 were all black in color, which indicated maternal control of the seed color. The spectral data (log 1/R) from NIR results in visual range also showed that all hybrids (CL2 × CL8, CL2 × CL7, CL2 × CL9) spectra were similar or close to CL2 spectrum at 400-750 nm wavelength (**Table 1**). Spectral data of the hybrid seeds from cross between CL4 and CL7, CL5 and CL8 were found to have similar seed color to CL7 and CL5, respectively. The hybrid plant (CL6 × CL9) was a cross between two black seed color substitution lines CL6 and CL9, which showed different reflectance spectra than the parental lines. F_2 seeds were separated on the basis of visual assessment and then confirmed from NIR graphical results. After hand pollination 60.69%, 48.53%, 72.85%, 56.94%, 79.09% and 49.35% of hybrid seeds were obtained for CL2 × CL7, CL2 × CL8, CL2 × CL9, CL4 × CL7, CL5 × CL8, CL6 × CL9 crosses, respectively, whereas more than 75% seeds were found for all parental lines in natural pollination.

CL5 × CL8

Genotypes CL5 and CL8 both were purple seeded wheat but they showed different light reflectance values at different wavelength. CL8 was more reddish than CL5. The F_2 generation of the cross CL5 × CL8 showed a distribution of 1871 pooled purplish black-seeded to 483 purple seeded and 155 reddish yellow seeded plants fitting a 12:3:1 segregation ratio (χ^2= 0.419, p<0.5) (**Table 2**).

Table 2 Observed and expected segregation ratios, χ^2 and p values in F_2 generations in six crosses between Chinese colored winter wheat and Canadian colored spring wheat.

Crosses	Seed color	Black		Purple		Yellow			Blue		Expected	χ^2 value	P value
		Dark	Light	Dark	Light	Reddish	Reddish	Bluish	Dark	Light			
CL5 ×CL8	Ch. Purple× Can. Purple	-	-	1871	483	-	155	-	-	-	12:3:1	0.419	<0.5
CL2 ×CL8	Ch. Black × Can. Purple	922	-	-	220	-	85	-	-	-	12:3:1	1.34	<0.5
CL4 ×CL7	Ch. Blue × Can. Blue	-	-	-	-	-	-	130	1426	862	1:15	3.149	<0.05
CL2×CL7	Ch. Black × Can. Blue	548	184	-	175	333	-	-	80	-	9:6:1	0.566	<0.5
CL6 ×CL9	Ch. Black × Can. Black	1206	-	93	-	-	-	-	-	-	15:1	1.833	<0.1
CL2×CL9	Ch. Black × Can. Black	1909	170	-	165	332	-	-	-	-	13:3	0.499	<0.1

* Ch. = Chinese, Can. = Canadian

CL2 × CL8

The F_2 generation of the cross between CL2 and CL8 segregated into 922 black, 220 purple and 85 reddish yellow seeded plants, which fitted a 12:3:1 segregation ratio (χ^2= 1.34, p<0.5). These reddish yellow seeded plants showed similar light reflectance values at different wavelength with the reddish yellow seeded F_2 plants from the cross CL5 and CL8.

CL4 × CL7

Genotype CL4 and CL7 both were blue seeded wheat but they showed different light reflectance values at different wavelength. CL7 seeds were more yellowish than the CL4 seeds. The F_2 populations segregated into 2288 pooled blue and 130 pooled bluish yellow seeded lines fitting a 15:1 segregation ratio (χ^2= 3.149, p<0.05).

CL2 × CL7

The F_2 generation of the cross CL2 × CL7 segregated into 732 pooled black, 508 purple and 80 blue seeded plants which fitted a 9:6:1 segregation ratio (χ^2= 0.566, p<0.5). It was also found that pooled purple seeded plants ranged from light purple to reddish purple seed color.

CL6 × CL9

The black seeded parents CL6 and CL9 showed same graphical pattern for different light reflectance values at different wavelength. The F_2 population of the cross CL6 × CL9 showed a distribution of 1206 dark black to 93 dark purple seeded plants fitting a 15:1 genetic ratio (χ^2= 1.833, p<0.1).

CL2 × CL9

Both the parents CL2 and CL9 were black seeded wheat but they showed different light reflectance values at different wavelength. The CL2 seeds showed light purplish black in color than the CL9. A 13:3 (pooled black: pooled purple) segregation ratio (χ^2= 0.499, p<0.1) was observed in F_2 population of the cross between CL2 × CL9. The light black F_2 seeds gave similar light reflectance values at different wavelength to CL9 parents.

DISCUSSION

The present results indicated that the inheritance of seed color in hexaploid wheat is complex in nature and having features of both qualitative and quantitative inheritances. The trait is controlled by few major genes making it possible for visual classification, with a continuous variation from dark black to almost purplish black in the pooled black seeded group, light to dark purple in pooled purple seeded group, reddish yellow to yellow in the pooled reddish yellow group and bluish yellow to almost yellow in the pooled bluish yellow seeded group. The dark black seed color of CL2 showed dominance over the blue, purple and black seeded wheat plants from Canadian origin. In accordance with the results of the three crosses, a genetic model was proposed to explain the observed inheritance of the black seed color from Chinese origin. Because Chinese black colored CL2 (Heilixiaomai 76) was developed from *Agropyron glaucum* and *Elymus dasystachys* in China through distant hybridization, whereas blue seeded wheat lines in North America were developed from *Agropyron elongatum*. The J genome was observed in Chinese winter wheats (CL2, CL4 and CL6) and J^s genome was observed in Canadian spring wheats (CL7 and CL9). Genotypes CL1, CL3, CL5 and CL8 don't have any alien genome (Chen *et al*. unpublished data). No chromosomal changes were observed in all F_1 (2n=42) hybrids (**Fig. 1**).

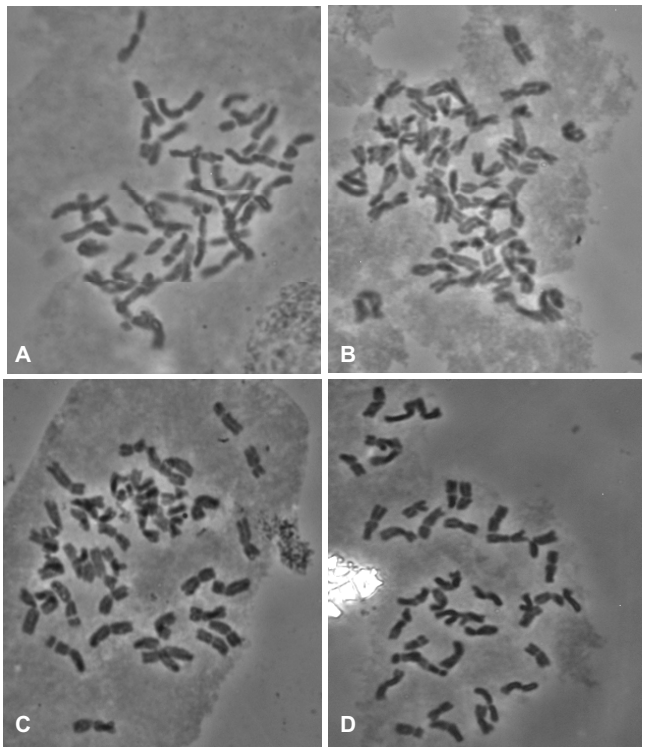

Fig. 1 Chromosome identification from root tips for four hybrid lines using an Olympus BX51 microscope at 100X magnification. (**A**) Hybrid CL2 × CL8, (**B**) Hybrid CL2 × CL9, (**C**) Hybrid CL4 × CL7, (**D**) Hybrid CL5 × CL8.

This genetic model can easily support the presence of J

	CL5 × **CL8**	**CL4** × **CL7**	**CL6** × **CL9**
	(Purple) (Purple)	(Blue) (Blue)	(Black) (Black)
	ppWW PP ww	J_LJ_L J_wJ_w $J^s_lJ^s_l$ $J^s_wJ^s_w$	J_bJ_b pp $J^s_bJ^s_b$ PP
F_1	pPWw	$J_LJ_wJ^s_lJ^s_w$	$J_bJ^s_b$ pP
	(Purple)	(Blue)	(Purplish black)
F_2	p-W-, PPW-, P-ww, ppww	J_LJ_w--, $J_LJ^s_w$--, $J^s_lJ^s_w$--, $J^s_lJ_wJ^s_lJ_w$	J_b-p-, J_b-PP, $J^s_bJ^s_b$P-, $J^s_bJ^s_b$pp
	9 3 3 1	9 3 3 1	9 3 3 1
	Dark purple (p-W-, PPW-)	**Blue** [Dark blue (J_LJ_w--)	**Black** [Dark black (J_b-p-, J_b-PP, $J^s_bJ^s_b$P-)]
	Purple [Light purple (P-ww)]	Light blue ($J_LJ^s_w$--, $J^s_lJ^s_w$--)]	**Purple** [Dark purple ($J^s_bJ^s_b$pp)]
	Reddish yellow (ppww)	**Bluish yellow** ($J^s_lJ_wJ^s_lJ_w$)	
	12:3:1	15:1	15:1

	CL2 × **CL8**	**CL2** × **CL7**	**CL2** × **CL9**
	(Black) (Purple)	(Black) (Blue)	(Black) (Black)
	J_BJ_B pp wwPP	J_BJ_B pp $J^s_lJ^s_lJ^s_wJ^s_w$	J_BJ_B pp $J^s_bJ^s_b$ PP
F_1	J_B w pP	$J_BJ^s_l$pJ^s_w	$J_BJ^s_b$ pP
	(Black)	(Dark black)	(Black)
F_2	J_B-p-, J_B-PP, P-ww, ppww	J_B-p-, $J^s_lJ^s_l$ p-, J_B-$J^s_wJ^s_w$, $J^s_lJ^s_lJ^s_wJ^s_w$	J_B-p-, J_B-PP, $J^s_bJ^s_b$p-, $J^s_bJ^s_b$ PP
	9 3 3 1	9 3 3 1	9 3 3 1
	Black [Dark black (J_B-p-, J_B-PP)]	**Black** [Dark black (J_B-p-)]	**Black** [Dark black (J_B-p-, J_B-PP)
	Purple [Light purple (P-ww)]	**Purple** [Light and Reddish purple	Light black ($J^s_bJ^s_b$ PP)]
	Reddish yellow (ppww)	($J^s_lJ^s_l$p-, J_B-$J^s_wJ^s_w$)]	**Purple** [Light purple ($J^s_bJ^s_b$pp)
		Blue [Dark blue ($J^s_lJ^s_lJ^s_wJ^s_w$)]	Reddish purple ($J^s_bJ^s_b$pP)]
	12:3:1	9:6:1	13:3

Fig. 2 Proposed genetic model of seed color inheritance in F_2 populations derived from six crosses between Chinese colored winter wheat and Canadian colored spring wheat.

genome may be control of black color in CL2 and J^s genome controlled seed color in Canadian spring black and blue wheat plants. From F_2 analysis, the genotypes of purple, blue and black seeded parents seem to be controlled by two genes. It was reported that purple color controlling genes were located in chromosome 3A or chromosome 7B (Kuspira *et al*. 1989). Let the gene symbols for Black color controlling genes are B and b; purple color controlling genes are P and p; blue color controlling genes are L and l; white color controlling genes are W and w. J_l or J_L means blue gene in J genome, J_b or J_B means black gene in J genome, J^s_l or J^s_L means blue gene in J^s genome and J^s_b or J^s_B means black gene in J^s genome. Proposed genetic model of seed color inheritance have described in **Fig. 2**. Spectral data of the F_1 hybrid seeds from cross between CL5 and CL8, CL4 and CL7 were found to have similar seed color to CL5 and CL7, respectively (**Table 1**). A continuous variation of seed color was observed in F_2 population for these two crosses. The color of CL8 showed more reddish than the CL5 and the seed color of F_1 hybrids of this cross was similar to CL5 parent. Let, purple genotype of Chinese origin was 'ppWW' for CL5, and genotype of purple seeded Canadian wheat was 'PPww' for CL8. The Chi-squared test of the F_2 populations also suggested that two genes were controlling purple color. Dominant epistasis was observed for purple color in the cross between purple seeded wheat CL5 and CL8. Different segregating seeds in F_2 progeny were only possible when the color responsible gene(s) is present in different genomes. The genetic model also confirms this interaction (**Fig. 2**) and purple color controlling genes of these parents (CL5 and CL8) were located in other than J genome. Kuspira *et al*. (1989) observed that red testa gene and purple culm genes were located in 3A and purple coleptile gene was located in chromosome 7B. Dominance of purple alleles was also found in F_1 plants in *T. durum* (Sharman 1958). Two genes controlling purple pericarp were found by Capron (1918) in a cross between *T. polonicum* and *T. elboni*. At the hexaploid level, those dark purple grained plants would have one of the genotypes 'p-W-' or 'PPW-' and light purple grained plants would be 'P-ww', whereas the non-purple type would be 'ppww'. Presence of 'ww' gene in pair can suppress the expression of purple color production in seed coat and thus seeds seem to look purple to reddish yellow. So that, reddish yellow colored F_2 seeds were produced by the 'ppww' genotypes. Similarly reddish yellow seeded F_2 wheat plants were also found in the cross between CL2 and CL8. On the other hand, CL5 was derived from the purplish black seeded cultivar CL2 (having the J genome) and white seeded cultivar 'Victo' (did not have the J genome) through cytogenetical method. The purple color controlling gene in CL5 must have come from CL2 and that gene(s) were present in wheat genome. Two independent purple color controlling genes possibly located on chromosomes 3A and 7B (Kuspira *et al*. 1989). In this study the purple color F_2 seeds were also produced in both of the cross CL2 × CL7 and CL2 × CL8. Spectral data of the F_1 hybrid seeds from cross between CL2 and CL7, CL2 and CL8 were found to have similar seed color to CL2 (**Fig. 3**). A duplicate dominant epistasis was observed from the cross between CL2 and CL7. The presence of the 'J' genome produced dark black color F_2 seeds when a cross was made between black seeded wheat and purple seeded wheat or blue seeded wheat (**Table 2**).

Let the blue seeded Chinese parent for CL4 be 'J_LJ_L J_wJ_w' and the blue seeded parent for Canadian spring wheat CL7 be '$J^s_lJ^s_l$ $J^s_wJ^s_w$' (**Fig. 2**). Two types of seed color in F_2 generation were observed in the cross of blue seeded CL4 and CL7 parents. The chi-square value agreed with the expected genetic ratio of 15:1 (pooled blue and bluish yellow seeded plants) for the segregation of two loci. These results confirmed the genetic hypothesis of blue color controlling gene had duplicate dominant epistasis. Two types of seed

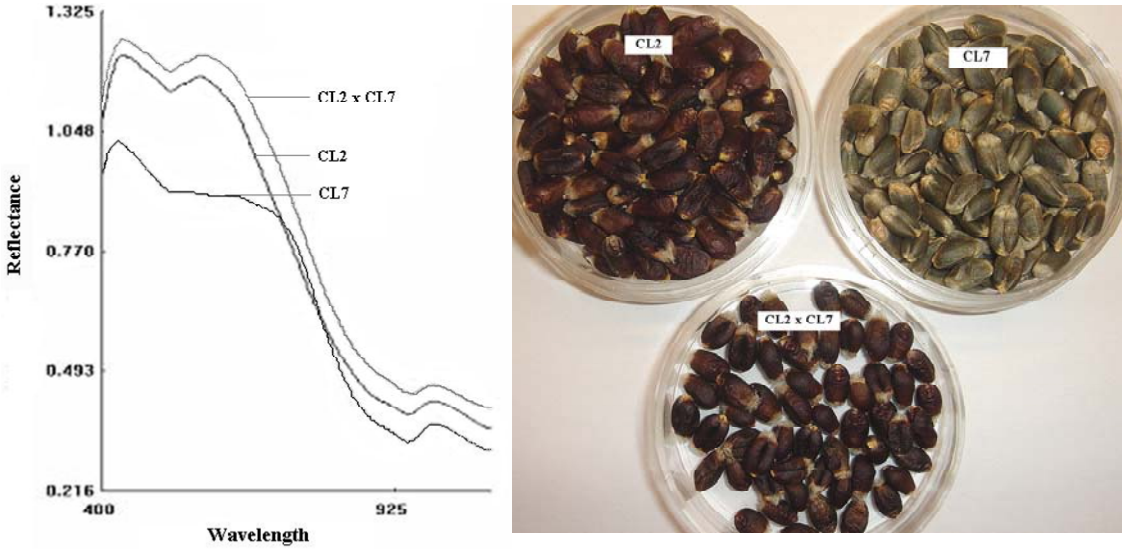

Fig. 3 Reflectance spectra of near-infrared spectroscopy and photograph for Black wheat76 (CL2), Purendo-38 (CL7) and F_1 (CL2 × CL7) hybrid wheat seeds.

color only possible when blue color was controlled by two different genomes (J and J^s) in CL4 and CL7 parents, respectively. The color of CL4 seeds was darker than CL7 and the blue color of F_1 seed was similar to CL4. Further analysis in pooled F_2 blue color seeds could be divided into two different groups 1426 dark blue seeded and 862 light blue seeded, which also agreed with a 9:6:1 (Dark blue: light blue: bluish yellow) genetic ratio. Two independent genes with duplicate dominant epistasis have been playing a key role for expression of blue coloration in wheat seeds. Previously, Piech and Evan (1979) reported that the 'blue color controlling gene' was located on a homoeologues group-4 chromosome of wheat, but the expression of this gene was influenced by 'red pericarp controlling gene'. It is possible that plants derived from blue grains may be unstable for grain color due to an irregular distribution of the chromosome or fragment derivatives carrying 'blue color controlling gene' during meiosis (Zevan 1991). Seed with a single copy of the gene for blue aleurone pigmentation have a light blue color, which is difficult to distinguish and sometimes may be misclassified as red (Knott 1958, Kepenne and Baenziger 1990). Recently Li *et al*. (2003) described that the blue grained lines with alien J and J^s chromosome translocation were not reciprocal translocation and they were derived from independent chromosomes of *Th. Ponticum*. However, Hurd (1959) believed that two complementary dominant genes conditioned the blue grain color. In this study, the intensity of the blue color may be affected by the presence of dominant gene in J genome for the cross CL4 and CL7. In accordance with the proposed genetic model presence of two loci in a J genome (JJ - JJ, JJ – JJ^s, JJ – J^sJ^s) may have caused dark blue color in wheat seeds and remaining showed bluish yellow seeds (JJ^s- JJ^s). Analysis of the segregation of grain color and presence of J and J^s chromosome composition also supported the recent discovery of J-J^s translocation chromosomes in the blue grained lines. The J^s-J chromosome led to the instability in blue grain color and resulted in light blue to bluish yellow grain (Li *et al*. 2003). Dark blue colored wheat plants would have one of the genotypes 'J_L-J_w-', whereas the non blue or bluish yellow colored grains would be '$J^s_lJ^s_lJ_wJ_w$'. However, Li *et al*. (1986) stated that blue color controlling gene showed a clear dosage effect by the color that ranged from blue, light blue to non blue or red as shown in our study. F_2 seeds of the cross between CL2 and CL7 also produced a continuous variation between black to blue and purple in this study. This also shows that two complementary genes were playing key role of controlling black color in CL2, but the intensity of black color may depend upon the dose effects of genes. The continuous variation probably resulted from the action of some minor genes together with environmental effects. It was found that blue colored wheat exhibited a reduced effect on anthocyanin content in the aleurone layer under different growing environment as compared to purple wheat (Abdel-Aal and Hucl 1999). Temperature fluctuation may also disrupt the biochemical processes involved in the production of pigments in wheat seeds. It might be possible that the black colored- and blue colored- genes are very tightly linked to each other in alien chromosome of CL2 because blue colored genotype CL4 was developed from CL2.

Let, the genotype of the black seeded Chinese parents for CL6 and CL2 were 'J_bJ_b pp' and 'J_BJ_B pp' (**Fig. 2**), respectively and the genotype of the Canadian black seeded parent CL9 was '$J^s_bJ^s_b$ PP'. The NIR spectral data showed that, Chinese black colored wheat line CL6 was darker than Canadian black colored wheat line CL9 and the purplish black color of F_1 seed ($J_bJ^s_b$ pP) was more similar to Chinese parent CL6. Two different genomes J and J^s maybe responsible for variation in black color intensity for CL6 and CL9 parents, respectively. This effect is possible when black color controlling gene from J^s genome is a recessive gene. The genotypes of F_1 hybrids were therefore 'J_B w pP' for CL2 × CL8, '$J_BJ^s_l$ pJ^s_w' for CL2 × CL7, '$J_BJ^s_b$pp' for CL2 × CL9, which all produced dark black seeds similar to CL2. This result indicated that black color controlling gene in CL2 was dominant in nature. A duplicate gene action was observed in the cross between CL6 and CL9 for black color controlling gene. The Chi-squared analysis results agreed with the expected genetic ratio of 15:1 (black and purple) for the segregation at two loci in F_2 population. The average light reflectance value in dark purple F_2 seeds for this cross in 662 nm wavelength was higher than both of the parents. This indicates that one pair of gene was playing a key role for expressing the purple color in F_2 seeds. The purple color controlling gene seems to be present in other than J or J^s genome, because black grained CL9 developed from cross between blue grained CL7 (having J^s genome) and purple grained CL8 (no alien chromosome), whereas black grain CL6 developed from cross between black grained CL2 (having the J genome) and white grained CL1 (no alien chromosome). The purple color controlling gene in CL2 was present in the wheat genome.

Three different types of colored wheat seeds in F_2 generation (viz. dark black, light black and purple) were produced by the cross between black seeded CL2 and CL9. A genetic ratio of 13:3 (pooled black and purple) was observed for this cross. Among the 2079 pooled F_2 black colored wheat seeds, 170 light black seeds were similar to CL9 wheat seed color and remaining 1909 dark black seeds

were similar to CL2 wheat seed color. This result also confirmed that two different black color controlling gene 'J_BJ_B' and '$J^s_bJ^s_b$' maybe present in J and J^s genome respectively. These segregation ratios postulated that the genotype of black seeded parent CL2 may carry three genes, two dominant genes in J genome and one purple gene in wheat genome. However, Sun *et al*. (2003) reported that black kernel color of CL2 was controlled by two incompletely dominant genes for dark purple pericarp and testa and the segregation ratio in F_3 population fitted 9:7 (dark: white).

In future a powerful approach would be to use the phenotypic values of seed color obtained from the NIR technique and perform molecular marker analysis. GISH technique using genomic DNA probes from *Thinopyrum elongatum* (Host) D.R. Dewey (genome E, 2n=14), *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve (genome J, 2n=14), and *Pseudoroegneria strigosa* (M. Bieb.) Á. Löve (genome S, 2n=14), will be useful to examine in the genomic constitution of Canadian colored spring wheat and Chinese colored winter wheat (Chen *et al*. 1999; Fedak *et al*. 2000). It is anticipated that such an approach will help to explain more fully the inheritance of this complex trait and thereby identify the location of genes responsible for the development of black, purple and blue seeded wheat cultivars.

REFERENCES

Abdel-Aal EM, Hucl P (1999) A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry* **76**, 350-354

Bezar HJ (1982) Konini, Speciality bread wheat. *New Zealand wheat review* **15**, 62-63

Caporn AC (1918) On a case of permanent variation in glum length of extracted parental types and the inheritance of purple colour in the *Triticum polonicum* × *T. eloboni*. *Journal of Genetics* **7**, 259-280

Chen Q, Conner RL, Laroche A (1995) Identification of parental chromosomes of the wheat-alien amphiploid *Agrotana* by genomic *in situ* hybridization. *Genome* **38**, 1163-1169

Chen Q, Conner RL, Laroche A, Fedak G, Thomas JB (1999) Genomic origins of *Thinopyrum* chromosomes specifying resistance to wheat streak mosaic virus and its vector, *Aceria tosichella*. *Genome* **42**, 289-295

Chen Q, Laroche A, Thomas JB (1998) Genome analysis of *Thinopyrum intermedium* and *Th. ponticum* using genomic *in situ* hybridization. *Genome* **41**, 580-586

Fedak G, Chen Q, Conner RL, Laroche A, Petroski R, Armstrong KC (2000) Characterization of wheat-*Thinopyrum* partial amphiploids by meiotic analysis and genomic *in situ* hybridization. *Genome* **43**, 712-719

Gilchrist JA, Sorrells ME (1982) Inheritance of kernel color in charcoal wheat. *Journal of Heredity* **73**, 457-460

Hurd EA (1959) Inheritance of blue kernel colour in wheat. *Canadian Journal of Plant Science* **39**, 1-8

Keppenne VD, Baenziger PS (1990) Inheritance of the blue aleurone trait in diverse wheat crosses. *Genome* **33**, 525-529

Knott DR (1958) The inheritance in wheat of a blue endosperm color derived from *Agropyron elongatum*. *Canadian Journal of Botany* **36**, 571-574

Kuspira J, Maclagan J, Bhambhani RN, Sadasivaiah RS, Kim NS (1989) Genetic and cytogenetic analysis of the A genome of *Triticum monococcum* L.V. Inheritance and linkage relationships of genes determining the expression of 12 qualitative characters. *Genome* **32**, 869-881

Li HY, Chen Q, Conner RL, Li HJ, Shi YZ, Laroche A, Graf RJ (2003) Inheritance of blue grain color and its association with J and J^s translocation chromosomes in Wheat-*Agrotana* hybrid lines. *Cytologia* **68**, 307-315

Li Z, Mu S, Zhou H, Wu J (1986) The establishment and application of blue grained monosomic in wheat chromosome engineering. *Cereal Research Communication* **14**, 133-122

Liu XP, Tu XJ, Chen BY, Fu TD (2005) Identification and inheritance of a partially dominant gene for yellow seed color in *Brassica napus*. *Plant Breeding* **124**, 9-12

Matus-Cádiz MA, Hucl P, Horak MJ, Blomquist K (2004) Gene flow in wheat at the field scale. *Crop Science* **44**, 718-727

Piech J, Evan LE (1979) Monosomic analysis of purple grain color in hexaploid wheat. *Zeitschrift für Pflanzenzüchtung* **82**, 212-217

Sharman BC (1958) Purple pericarp: a monofactorial dominant in tetraploid wheat. *Nature* **181**, 929

Sun Q, Sun B, Wang JH (2003) Genetic of seed pigment of black kernel wheat. *Seed* **37**, 33-34 (in Chinese with English abstract)

Sun SC, Sun Y, Yuan WY, Yan WZ, Pei ZY, Zhang MR (1999) Breeding and qualitative analysis for black wheat 76 of superior quality. *Acta Agronomica Sinica* **25**, 51-54 (in Chinese with English abstract)

Van Deynze AE, Pauls KP (1994) Seed colour assessment in *Brassica napus* using a near infrared reflectance spectrometer adapted for visible light measurements. *Euphytica* **76**, 45-51

Velasco L, Becker HC (1998) Estimating the fatty acid composition of the oil in intact-seed rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. *Euphytica* **101**, 221-230

Yang GH, Zhao XQ, Li B, Liu JZ, Zheng Q, Tong YP, Li ZS (2003) Molecular cloning and characterization of a DFR from developing seeds of blue grained wheat in anthocyanin biosynthetic pathway. *Acta Botanica Sinica* **45**, 1329-1338

Zeven AC (1991) Wheat with purple and blue grains: a review. *Euphytica* **56**, 243-258

Zhang ZY, Xin ZY, Larkin PJ (2001) Molecular characterization of a *Thinopyrum intermedium* group 2 chromosome (*2Ai-2*) conferring resistance to barley yellow dwarf virus. *Genome* **44**, 1129-1135