Australian Journal of Crop Science AJCS

AJCS 5(5):589-594 (2011) ISSN:1835-2707

Segregation ratios of colored grains in crossed wheat

Zifeng Guo[1,2], Ping Xu[1], Zhengbin Zhang[1]*, Daowen Wang[3]*, Miao Jin[4], Anping Teng[5]

[1]Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, CAS, Shijiazhuang 050021, China
[2]Graduate University of the Chinese Academy of Sciences, Beijing 100049, China
[3]Institute of Genetics and Developmental Biology, Beijing, 100101, China
[4]Yangling Vocational and Technical College, Xi'an 712100, China
[5]Guizhou Seed administer station, Guiyang 550001, China

*Corresponding authors: zzb@sjziam.ac.cn (Z.B.Zhang), dwwang@genetics.ac.cn (D.W.Wang), norequire@sohu.com (Z.F. Guo), webofsci@sohu.com (P. Xu)

Abstract

Colored-grain wheat is one kind of new germplasm resource in cereal crops, some of which are rich in beneficial anthocyanins. The genetics of wheat with blue and white grains or those with purple and white grains have been investigated. However, as far as we know, the genetics of wheat with purple and blue grains has not been reported. We bred new lines of colored-grain wheat by crossing einkorn wheat (*Triticum boeoticum*, AA) and French rye (French *Secale cereale*, RR) as male parents (mixed pollination) with a high-quality bread wheat line Y1642 (derived from common wheat and *Agropyron elongatum*, AABBDD) as the female parent. We selected colored-grain wheat lines in the F_6 generation that exhibited different colored grains in the same spike to study the genetic patterns of colored-grain wheat. In addition to normal grain color segregation ratios, we found some unconventional segregation ratios such as 1:37 (blue to white). Furthermore, we found blue and purple grains in the same spike of blue-grain or purple-grain wheat lines. For blue wheat grain the color is due to the blue aleurone layer. The coloration of a purple wheat grain is located in the pericarp and testa. This unique phenomena may be due to the influences of environmental factors as well as genetic factors. Besides, the colored-grain wheat lines used in this work may be genetically unstable. Some unique segregation ratios are also observed in the genetic segregation of grain color. The results will be useful for genetics and breeding in colored-grain wheat.

Keywords: Blue-grain wheat, Purple-grain wheat, Inheritance, Segregation ratios.

Introduction

Recently, nutritious and functional foods have received great attention (Krystallis et al., 2008). It has been shown that colored plants, including fruits, vegetables and grains, are rich in anthocyanins and micronutrients (Yamasaki et al., 1996; Moyer et al., 2002; Peng et al., 2006). Plant anthocyanins can act as an antioxidant and show antibacterial and anti-cancer activity. They are also useful in curing systemic inflammation (Kong et al., 2003). In addition, one of the active ingredients of anthocyanin can inhibit the oxidation of low-density lipoprotein and platelet aggregation, maintain the normal osmotic pressure of blood vessels, and reduce the vulnerability of capillaries (Mazza, 2007). Colored wheat grains are rich in anthocyanins and other nutrients (Escribano-Bailon et al., 2004; Humphries et al., 2004). Among them, Chinese black wheat grains are rich in amino acids, proteins and some micronutrients (Li et al., 2006). The biochemistry, genetics and breeding of colored-grain wheat in China and other regions of the world have been explored (Li et al., 1982; Li et al., 2002; Zheng et al., 2006a; Zheng et al., 2006b). However, the composition of colored-grain wheat has not been fully understood. Elucidation of the genetic patterns of colored-grain wheat can promote the breeding efficiency, improve the nutritional valuet, and increase the consumption. Wheat grain shows different colors such as white, red, green, blue, purple and black (Tammam et al., 2008; Ali et al., 2009; Atta and Shah, 2009; Kamal et al., 2010; Zhang et al., 2010). Numerous different grain colors are found in wheat and related species. For example, rye is diploid (RR) and has blue-green grains; Purple grains occur in tetraploid wheat from Ethiopia (AABB) and in a bread wheat accession (AABBDD) apparently native to China; *Triticum boeoticum* (AA) is a source of purple (or blue) grains (Zeven, 1991). And, the intensity of the color is also different. This variation is particularly prominent in purple and blue wheat. Purple wheat grains are sometimes divided into light purple, medium purple, and deep purple (or black); blue wheat grains are also divided into light blue, medium blue, and dark blue (Li et al., 1982). Previous studies indicate that blue grain coloration in wheat and related species is controlled by some genes (Morrison et al., 2004). Some researchers (Knott, 1958; Li et al., 1982; Zeven, 1991) reported blue grains segregated from crosses between *Agropyron elongatum* and common wheat (*Triticum aestivum* L.). They found that chromosome 4E of *Agropyron elongatum* carries the blue aleurone gene. These results suggested that blue grains are controlled by two complementary, incompletely dominant genes in wheat. The blue-grain gene has a significant dose effect and the hereditary pattern of blue grains is stable and independent, apparently controlled by a pair of genes. It is generally believed that blue-grain wheat exhibits the genetic pattern of xenia (i.e., traits of the endosperm are influenced by genes from the male parent). Purple wheat grains result from a purple pericarp, and the pericarp develops from the female parent, so purple-grain wheat exhibits a maternal inheritance pattern (Zhang, 2001). The purple genes of purple-grain wheat may come from the female parent. The segregation ratios of purple wheat grains have been studied by various groups. It was found that segregation ratios of 9:7 and 11:5 (purple to white) had the same frequencies in eight groups and in four environments (Gilchrist and Sorrells, 1982). Nilsson-Ehle discovered three different segregation ratios, 3:1, 15:1 and 63:1 (purple to white) (Nilsson-Ehle, 1911). However, to the best of our knowledge, wheat with purple and blue grains in the same spike has not been reported. The objective of this work is to explore the genetic segregation patterns of the colored-grain wheat lines and to obtain more useful genetic information for the breeding of colored-grain wheat. Herein, we showed many different segregation ratios within a single wheat line. Also, as far as we know, spikes with blue and purple grains are reported for the first time.

Materials and methods

Plant Materials

Experimental materials used in this work were obtained from the wheat-breeding nursery in the Luancheng experimental station for agro-ecosystems. When the wheat grains were ripe, we examined spikes in wheat lines with different grain colors and collected spikes with different grain colors in the same spike. Ears of wheat with blue and purple grains or with blue and white grains in the same spike were discovered and selected in blue-grain wheat lines in plot A. Ears of wheat with blue and purple grains in the same spike were discovered and selected in blue-grain wheat lines in plot B and plot C and in purple-grain wheat lines in plot D. The colored-grain wheat lines used in this experiment were bred and selected in the Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, CAS. The lines were F_6 generations derived from the cross by mixed pollination of einkorn wheat (*Triticum boeoticum*, AA) and French rye (French *Secale cereale*, RR) as male parents with the high-quality bread wheat line Y1642 (derived from common wheat and *Agropyron elongatum*, AABBDD) as the female parent. These lines bred in 2001 have an independent genetic background from other colored-grain wheat lines. Sampled spikes were required to be complete and were bagged immediately. Experimental records were made at the same time in order to prevent the loss of wheat grains. In the laboratory, we sorted the grains by color and counted the number of grains of each color from spikes of each wheat line.

Statistical analysis

In this work, four replications were used. Summary statistics for wheat grain colors were calculated and chi-square analysis of the segregation ratio of different grain colors was also performed.

Results

Segregation ratios of blue and white grains from the same spike in different wheat lines

As shown in Tables 1 and 2, 41 spikes that are randomly selected with blue and white grains are found in the blue-grain wheat line in plot A. Eleven different segregation ratios of blue to white grains exist in these 41 spikes. Their distributions are shown in Figure 1. Thirteen spikes (31.7%) exhibit a segregation ratio of 2:1 (blue to white, the same hereinafter), which is the largest section. Segregation ratios of 5:1, 5:3, 7:9, and 1:37 are only found in one spike (2.4%). The results suggest that no single segregation ratios in the same spike accounts for the majority of cases in this blue-grain wheat line. We speculate that this line is an unstable strain and that grain color will continue to segregate. These complex grain color segregation ratios in blue-grain wheat appear consistent with the genetic model of xenia. Previous studies have proved that the genetic patterns of blue-grain wheat are consistent with xenia (Mettin et al., 1991; Zeven, 1991; Metzger and Sebesta, 2004). However, some other studies proposed different explanations. The inheritance models of the blue aleurone and purple pericarp traits are not definitive. Because interspecific crosses can lead to populations that segregate for fragments or whole donor parent chromosomes (Knott, 1958; Li et al., 1983; Knievel et al., 2009), the results are often obscured. Knievel et al. (2009) found that blue aleuronic populations grown under controlled environmental conditions fit a one-gene model, while F_2 populations did not. Thus, wheat grain color is controlled by complex mechanism which requires further investigations.

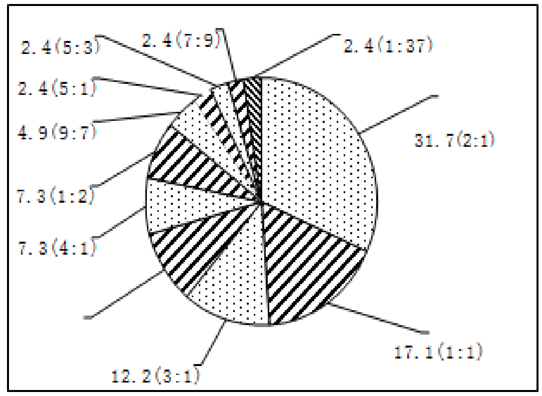

Fig 1. Percentages of various segregation ratios of blue and white colored-grain wheat

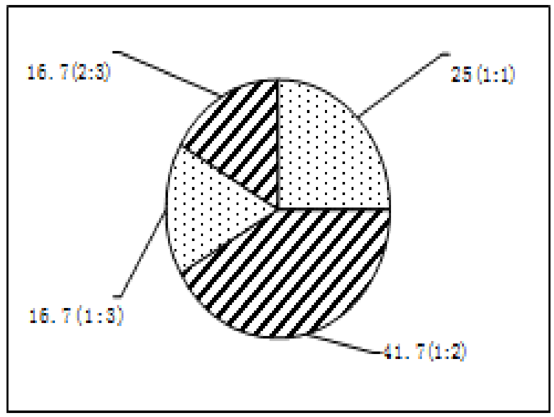

Fig 2. Percentages of various segregation ratios of blue and purple colored-grain wheat

Segregation ratios of wheat with blue and purple grains

Twelve spikes with blue and purple grains were found in four plots. Tables 3 and 4. show that there are four different segregation ratios in four plots. Their distributions are shown in Figure 2. Although the segregation ratio of 1:2 accounts for the largest proportion, it does not reach 50%. Therefore, we speculate that these lines are unstable. In addition, the number of different segregation ratios in wheat with blue and purple grains in the same spike is remarkably less than that with blue and white grains. Interestingly, no spike with purple and white grains was found in this experiment. This suggests that the genetic pattern of purple-grain wheat is more stable. It seems that the purple grain trait is dominant to the white grain trait and follows a maternal inheritance pattern.

Table 1. Statistics and chi-square analysis of wheat with blue and white grains in the same spike

Sample number	Blue grains	White grains	Ratio (Blue/White)	Expected ratio	χ^{2*}
A1	17	16	1.062	1:1	0.300
A2	12	14	0.857	1:1	0.154
A3	10	8	1.250	1:1	0.222
A4	13	10	1.300	1:1	0.391
A5	17	15	1.133	1:1	0.125
A6	19	18	1.055	1:1	0.027
A7	17	15	1.133	1:1	0.125
A8	17	10	1.700	2:1	0.167
A9	22	9	2.444	2:1	0.258
A10	19	8	2.000	2:1	0.167
A11	23	13	1.769	2:1	0.125
A12	17	8	2.125	2:1	0.020
A13	16	8	2.000	2:1	0.000
A14	20	10	2.000	2:1	0.000
A15	22	9	2.444	2:1	0.258
A16	21	12	1.750	2:1	0.136
A17	24	12	2.000	2:1	0.000
A18	22	13	1.692	2:1	0.229
A19	30	15	2.000	2:1	0.000
A20	18	9	2.000	2:1	0.000
A21	13	20	0.650	1:2	0.545
A22	9	22	0.409	1:2	0.258
A23	10	21	0.476	1:2	0.016
A24	25	9	2.778	3:1	0.039
A25	18	6	3.000	3:1	0.000
A26	22	7	3.143	3:1	0.011
A27	22	8	2.750	3:1	0.044
A28	30	11	2.727	3:1	0.073
A29	12	8	1.500	3:2	0.000
A30	26	16	1.625	3:2	0.063
A31	21	14	1.500	3:2	0.000
A32	19	13	1.462	3:2	0.005
A33	18	5	3.600	4:1	0.043
A34	27	7	3.857	4:1	0.007
A35	21	6	3.500	4:1	0.083
A36	12	9	1.333	9:7	0.007
A37	18	13	1.385	9:7	0.041
A38	14	18	0.778	7:9	0.000
A39	31	6	5.167	5:1	0.005
A40	25	15	1.667	5:3	0.000
A41	1	37	0.0270	1:37	0.000

$*\chi^2_{0.05} = 3.84$; $\chi^2_{0.01} = 6.63$

Spikes with blue and purple grains are found not only in three blue-grain wheat lines (plots A, B, and C) but also in one purple-grain wheat line (plot D). Spikes with blue and purple grains and those with blue and white grains simultaneously existed in the blue-grain wheat line (plot A). Within a single wheat line, many different segregation ratios have been observed. It is unclear whether these genetic patterns are caused by xenia and other effects of cross-pollination among different colored-grain wheat lines or whether they are resulted from gene segregation.

Discussion

Different genetic segregation patterns of grain color of wheat

Mettin et al. (1991) reported that on average F_2 populations derived from blue wheat accession 'TRI 2401' and white Chinese spring wheat segregated 74% non-blue and 26% blue. However, they hypothesized that the segregation was not necessarily monogenic because the blue aleurone parent 'TRI 2401' produced monosomic seeds that were missing the alien chromosome. The blue grain trait of wheat lines 'D87065' and 'D87089' (derived from *Thinopyrum*) was controlled by two pairs of complementary genes, whereas the blue grain trait of wheat line '92-1' (derived from rye) was also controlled by two pairs of complementary genes (Lan et al., 2008). However, the blue grain trait of the F_2 line '7083L-16', whose genetic background is still not clear, fits a one-gene model. The dark blue color of 'Hedong Wumai 526' grains is related to the genetic phenomenon of xenia. It has been reported that the blue grain color of 'Hedong Wumai 526' is determined by endosperm genetic traits (Sun et al., 2003). The segregation ratio of the F_2 generation is 9:7 (blue-black to white), and the blue grain trait is controlled by two pairs of complementary genes. Some studies have suggested that the grains of 'Luozhen l' and 'Black grain wheat 76' are dark purple and that the aleurone layers of these strains do not contain purple pigment. Their purple pigments are present only in the testa and pericarp. The deep purple grain trait of 'Luozhen l' is controlled by one dominant gene; the grain colors of its F_3 population fit a segregation ratio of 3:1 (dark purple to white). The grain color of 'Black grain wheat 76' (deep purple) is apparently controlled by two pairs of complementary genes; the grain colors of its F_3 population fit a segregation ratio of 9:7 (dark purple to white) (Chang et al., 2002; Sun et al., 2003).

Table 2. Percentages of colored-grain wheat spikes with different segregation ratios

Segregation ratio	Number of spikes	Percentage of spikes (%)
2:1	13	31.7
1:1	7	17.1
3:1	5	12.2
3:2	4	9.8
4:1	3	7.3
1:2	3	7.3
9:7	2	4.9
5:1	1	2.4
5:3	1	2.4
7:9	1	2.4
1:37	1	2.4

The variable intensity of wheat grain colors related to different genetic models and environmental factors

Variable color intensity of wheat grains may be caused by many factors such as minor genes and can be analyzed by the quantitative trait model. The color intensity of blue grains is influenced by a genetic dosage effect (Zheng et al., 2006a; Zheng et al., 2006b). It has been proposed that blue aleurone is controlled by a single dominant gene, despite of deviations from the expected segregation ratios (Keppenne and Baenziger, 1990). The blue grain trait is primarily genetically controlled but is also influenced by environmental factors such as light, temperature, and fertilization and by interaction effects between genotype and environment (Li et al., 2003; ur Rehman et al., 2009; Islam, 2010). Two duplicate genes controlled the purple pericarp trait, with purple being dominant, when transferring the purple pericarp trait from *Triticum durum L.* to *T. aestivum L.* Gilchrist et al. (1982) crossed charcoal wheat (purple grains) with white wheat. They found that segregation ratios fluctuated between 9:7 and 11:5 (purple to nonpurple) depending on environmental conditions. Under the same environmental conditions, segregation ratios also fluctuated among crosses, ranging from 9:7 to 13:3 (purple to nonpurple). Knievel et al. (2009) reported that the purple grain trait fits a genetic model of one pair of genes in spring wheat from Canada. Based on the segregation ratio of 11:5 (purple to white) found in their experiments, they proposed that the pericarp color of purple-grain wheat fits a two-locus model and purple grain color is related to gene dosage effects. In addition, our long-term observations suggest that wheat grain color intensity is related to seed maturity. In the same purple-grain wheat strains, fully mature grains have deeper purple coloration, while immature grains are lighter purple. Some parts of the seeds that have not developed completely lack coloration.

Analysis of normal grain color segregation ratios and genetic effects

In plot A, there are seven spikes with a grain color segregation ratio of 1:1 (blue to white) (Table 1) and three spikes with a segregation ratio of 1:1 (blue to purple) (Table 3). These results indicate that the grain color of these spikes fits a genetic model of one pair of genes. The parental genotypes of these spikes may be Rr and rr (R refers to dominant genes, and r refers to recessive genes). Because these spikes are selected from blue-grain wheat lines, in which the blue gene (R) is dominant to the purple gene (r) and white gene (r). Similarly, it is the same for the grain color segregation ratios of 3:1 (blue to white, Table 1) and 1:3 (purple to blue, Table 3). The parental genotypes of these spikes may be Rr. The grain color segregation ratio of 9:7 (blue to white) may be due to a gene complementation phenomenon. Under a gene complementation model, two independent dominant genes in the homozygous or heterozygous dominant state jointly determine one trait; when only one pair of genes is dominant or both pairs are recessive, they determine other traits. The genotypes of this segregation ratio can be expressed as 9 (A_B_): 7 (3A_bb +3 aaB_ +1 aabb) (A and B represent dominant genes, and a and b represent recessive genes). Therefore, it can be concluded that blue grain color is a dominant trait and white is a recessive trait in our cases.

Analysis of unconventional grain color segregation ratios

Some grain color segregation ratios observed in this work cannot be explained by the known genetic models, which include the ratios of 5:1 (blue to white), 4:1 (blue to white), 2:1 (blue to white), 1:2 (blue to purple) and 1:37 (blue to white). These segregation ratios are rare or not reported in early studies on colored-grain wheat. Possible explanations for these unconventional segregation ratios are proposed below. The genetic backgrounds of wheat grain color are varied. Xenia occurs when genes from pollen can directly affect the phenotype of seeds or fruits after outcross pollination. Different transmission capacity of gametes in xenia may affect the segregation ratios of blue-grain wheat, resulting in unconventional segregation ratios. Lethal genes can also affect the segregation ratios of some genetic traits (Lobo, 2008). Transposons are segments of DNA that can move to different positions within the genome of a single cell. These mobile segments of DNA are sometimes called 'jumping genes'. Transposable elements of various types have been found in all organisms, including all plants investigated to date. Transposons make up over 50% of the genome in many species with large genomes. Transposons can rearrange genomes and alter the structure and expression of individual genes through transposition, insertion, excision and chromosome breakage (Diao and Lisch, 2006). Therefore, it is possible that the different grain colors of colored wheat are related to transposons. No colored grains were found in the female parent used in this experiment, but some were found in its offspring. None of the parent strains exhibited different grain colors in the same spike, but many wheat lines with different grain colors in the same spike were found among their offspring. We found many wheat lines with blue grains and white or purple grains in the same spike, but no line is observed with blue and purple grains together. Knievel et al. (2009) reported that the blue and purple grain traits are both dominant to non-pigmented grain. As far as we know, the relationship between the blue and purple traits hasn't been reported. In this work, the blue grain trait is dominant to the purple grain trait. Because the purple pericarp derives from the female parent, the genetic pattern of purple wheat grains may conform to a maternal genetic model (Bradbury et al., 1956; Yuan and Sun, 1993).

Chromosomal locations of grain color genes and molecular genetic research in wheat

Grain color genes in wheat have been studied but the results are very different. Two dominant complementary genes were identified for purple grains at the hexaploid level and localized the genes on chromosomes 3A and 7B (Piech and Evans, 1979). Near-isogenic lines carrying genes for purple grain color derived from the Australian cultivar 'Purple Feed' and the Canadian cultivar 'Purple' in the genetic background of the red-grained wheat cultivar 'Saratovskaya 29' were also investigated (Arbuzova et al., 1998; Dobrovolskaya et al., 2006). Using monosomic analysis, they mapped the genes for purple pericarp (Pp1, Pp2 and Pp3) to chromosomes 7B, 6A and 2A, respectively.

Table 3. Statistics and chi-square analysis of wheat with blue and purple grains in the same spike

Sample number	Blue grains	Purple grains	Ratio(Blue /Purple)	Expectedratio	χ^{2*}
A42	16	17	0.941	1:1	0.300
A43	16	19	0.842	1:1	0.257
A44	18	19	0.947	1:1	0.027
A45	14	30	0.467	1:2	0.045
A46	10	19	0.526	1:2	0.017
A47	11	22	0.500	1:2	0.000
C1	12	21	0.571	1:2	0.136
C2	13	23	0.565	1:2	0.125
C3	8	21	0.381	1:3	0.103
C4	7	20	0.350	1:3	0.012
B1	14	22	0.636	2:3	0.314
D1	10	15	0.667	2:3	0.000

*$\chi^2_{0.05} = 3.84$; $\chi^2_{0.01} = 6.63$

Table 4. Percentages of colored-grain wheat spikes with different the segregation ratios

Segregation ratio	Number of spikes	Percentage of spikes (%)
1:1	3	25.0
1:2	5	41.7
1:3	2	16.7
2:3	2	16.7

The use of different materials is one reason why such different results have been obtained. The mapping and cloning of grain color genes is an important direction for future research because such information will be the key to studying grain color gene functions and exploring nutritional function in wheat.

Conclusions

We observed some complex and unique phenomena in the genetic segregation of grain color and proposed possible explanations for the observations. Some segregation ratios of grain color are reported for the first time. In addition to normal grain color segregation ratios, we observed rare segregation ratios such as 1:37 (blue to white). These grain color segregation ratios may be caused by the complex genetic background from the three parental lines. The colored-grain wheat lines used in our experiment are genetically unstable. However, further study of these complex and unique genetic phenomena is required to provide more information for colored-grain wheat breeding.

Acknowledgements

This project was funded by the National Basic Research Program of China (2007CB108800).

References

Ali S, Shah SJA, Khalil IH, Raman H, Maqbool K, and Ullah W (2009) Partial resistance to yellow rust in introduced winter wheat germplasm at the north of Pakistan. Australian Journal of Crop Science 3:37-43.

Arbuzova VS, Maystrenko OI, and Popova OM (1998) Development of near-isogenic lines of the common wheat cultivar 'Saratovskaya 29'. Cereal Research Communications 26:39-46.

Atta BM, and Shah TM (2009) Stability analysis of elite chickpea genotypes tested under diverse environments. Australian Journal of Crop Science 3:249-256.

Bradbury D, Macmasters MM, and Cull IM (1956) Structure of the mature wheat kernel. 4. Microscopic structure of the germ of hard red winter wheat. Cereal Chemistry 33:373-391.

Chang LW, Yen WJ, Huang SC, and Duh PD (2002) Antioxidant activity of sesame coat. Food Chemistry 78:347-354.

Diao XM, and Lisch D (2006) Mutator transposon in maize and MULEs in the plant genome. Yi Chuan Xue Bao 33:477-487.

Dobrovolskaya O, Arbuzova VS, Lohwasser U, Roder MS, and Borner A (2006) Microsatellite mapping of complementary genes for purple grain colour in bread wheat (Triticum aestivum L.). Euphytica 150:355-364.

Escribano-Bailon MT, Santos-Buelga C, and Rivas-Gonzalo JC (2004) Anthocyanins in cereals. Journal of Chromatography A 1054:129-141.

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

Humphries JM, Graham RD, and Mares DJ (2004) Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. Journal of Cereal Science 40:151-159.

Islam SMS (2010) Effect of embryoids age, size and shape for improvement of regeneration efficiency from microspore-derived embryos in wheat (Triticum aestivum L.). Plant Omics 3:149-153.

Kamal AHM, Kim KH, Shin KH, Choi JS, Baik BK, Tsujimoto H, Heo HY, Park CS, and Woo SH (2010) Abiotic stress responsive proteins of wheat grain determined using proteomics technique. Australian Journal of Crop Science 4:196-208.

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

Knievel DC, Abdel-Aal ESM, Rabalski I, Nakamura T, and Hucl P (2009) Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (Triticum aestivum L.). Journal of Cereal Science 50:113-120.

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

Kong JM, Chia LS, Goh NK, Chia TF, and Brouillard R (2003) Analysis and biological activities of anthocyanins. Phytochemistry 64:923-933.

Krystallis A, Maglaras G, and Mamalis S (2008) Motivations and cognitive structures of consumers in their purchasing of functional foods. Food Quality and Preference 19:525-538.

Lan SQ, Li XP, and Liu YP (2008) Genetic of seed pigment of blue kernel wheat. Acta Agriculturae Boreali-Sinica 23:12-14.

Li JL, Li JB, Li ZH, Zhang XG, and Hou MC (2003) Overview of the special color grain wheat. Seed World 3:18.

Li WD, Beta T, Sun SC, and Corke H (2006) Protein characteristics of Chinese black-grained wheat. Food Chemistry 98:463-472.

Li XP, Hou HJ, Liu YP, Lan SQ, and Zhu YY (2002) Studies of grain nutritional quality on wheat with Blue or Purple Kernels. Acta Agriculturae Boreali-Sinica 17:21-24.

Li ZS, Mu SM, Jiang LX, and Zhou HP (1983) A cytogenetic study of blue-grained wheat. Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding 90:265-272.

Li ZS, Mu SM, Jiang LY, Zhou HP, Wu JH, and Yu L (1982) A Study on blue-grained monosomic Wheat. Acta Genetica Sinica 9:431-439.

Lobo I (2008) Mendelian ratios and lethal genes. Nature Education 1:1.

Mazza GJ (2007) Anthocyanins and heart health. Ann Ist Super Sanita 43:369-374.

Mettin D, Schlegel G, and Lehmann C (1991) Instability of the blue grain color in a strain of Triticum-Aestivum L. Genome 34:745-750.

Metzger RJ, and Sebesta E (2004) Registration of three blue-seeded wheat genetic stocks exhibiting xenia. Crop Science 44:2281-2282.

Morrison LA, Metzger RJ, and Lukaszewski AJ (2004) Origin of the blue-aleurone gene in Sebesta Blue wheat genetic stocks and a protocol for its use in apomixis screening. Crop Science 44:2063-2067.

Moyer RA, Hummer KE, Finn CE, Frei B, and Wrolstad RE (2002) Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. Journal of Agricultural and Food Chemistry 50:519-525.

Nilsson-Ehle H (1911) Kreuzungsliniersuchungeii an hafer und weizen. Lunds Univ. Arsskr. N. F. Afd 2:73-84.

Peng CL, Lin ZF, Lin GH, and Chen SW (2006) The anti-photooxidation of anthocyanins-rich leaves of a purple rice cultivar. Science in China Series C-Life Sciences 49:543-551.

Piech J, and Evans LE (1979) Monosomic analysis of purple grain colour in hexaploid wheat. Zeitschrift f. Pflanzenz 82:212-217.

Sun Q, Sun BQ, and Wang JH (2003) Genetic of seed pigment of black Kernel wheat. Seed 4:33-34.

Tammam AA, Abou Alhamd MF, and Hemeda MM (2008) Study of salt tolerance in wheat (Triticum aestium L.) cultivar Banysoif 1. Australian Journal of Crop Science 1:115-125.

ur Rehman A, Habib I, Ahmad N, Hussain M, Khan MA, Farooq J, and Ali MA (2009) Screening wheat germplasm for heat tolerance at terminal growth stage. Plant Omics 2:9-19.

Yamasaki H, Uefuji H, and Sakihama Y (1996) Bleaching of the red anthocyanin induced by superoxide radical. Archives of Biochemistry and Biophysics 332:183-186.

Yuan WY, and Sun SC (1993) Survey on blue grain and purple grain wheat. Journal of Triticeae Crops 2:12-14.

Zeven AC (1991) Wheats with purple and blue grains – a review. Euphytica 56:243-258.

Zhang ZB (2001) *Wheat genetics*. Chinese agriculture pressing, Beijing.

Zhang ZB, Xu P, Jia JZ, and Zhou RH (2010) Quantitative trait loci for leaf chlorophyll fluorescence traits in wheat. Australian Journal of Crop Science 4:571-579.

Zheng Q, Li B, Mu SM, Zhou HP, and Li ZS (2006a) Physical mapping of the blue-grained gene(s) from Thinopyrum ponticum by GISH and FISH in a set of translocation lines with different seed colors in wheat. Genome 49:1109-1114.

Zheng Q, Li B, Zhang XY, Mu SM, Zhou HP, and Li ZS (2006b) Molecular cytogenetic characterization of wheat-Thinopyrum ponticum translocations bearing blue-grained gene(s) induced by r-ray. Euphytica 152:51-60.