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Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.)

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ABSTRACT

There is renewed interest in breeding for high anthocyanin content in wheat due to its antioxidant potential. A series of adapted spring wheat lines were developed with blue aleurone or purple pericarp. The development of anthocyanin concentration and color of these selected lines was measured during grain filling for two field seasons at Saskatoon, Canada. In addition, the inheritance of the blue aleurone and purple pericarp was studied. Anthocyanin concentration increased rapidly during grain development and then decreased before maturity. Anthocyanin concentration was highest in PIG03008, a purple pericarp wheat. For mature grain, genotypic variation for anthocyanin concentration was statistically significant while the year and genotype by year interaction were not, facilitating the breeding progress. Blue aleurone was shown to be controlled by a single dominant gene in BC populations whereas purple pericarp appeared to be controlled by two loci with a segregation ratio of 11 purple: 5 white in F_2 populations. The results indicate that breeding high anthocyanin blue or purple wheat is feasible.

1. Introduction

Canada's spring wheat (*Triticum aestivum* L.) cultivars are either red or white grained, depending on the market class. It is however possible to breed blue and/or purple pigments into these spring wheats. Neither blue nor purple grain pigmentation originated in common wheat. Zeven (1991) reported that the blue aleurone trait was introgressed into common wheat from blue pigmented *Triticum boeoticum*, *Agropyron tricholphorum*, and *Agropyron glaucum* and most frequently from *Agropyron elongatum*. In Inner Mongolia, blue kernelled *Leymus dasystachys*, which is related to the *Agropyron* genus, was crossed with common wheat to produce blue kernelled wheat (Zeven, 1991). Purple wheat was discovered in tetraploid durum, *Triticum dicoccum*, in east African areas such as Ethiopia and was introgressed into common wheat (Zeven, 1991).

Blue and purple wheat contain anthocyanin compounds in the aleurone and in the pericarp, respectively, whereas red and white wheat contain very small amounts of anthocyanin (Abdel-Aal et al., 2006). When anthocyanins are extracted from whole meal or from bran, blue wheat contains more total anthocyanin than purple wheat (Abdel-Aal and Hucl, 1999; Abdel-Aal et al., 2006; Siebenhandl et al., 2007). Liu et al. (2005) reported that during grain development, purple anthocyanins are found only in the seed coat initially but are found in the pericarp later on in development.

Anthocyanin composition of blue aleurone wheat is relatively simple while purple pericarp wheat has more anthocyanins present (Abdel-Aal et al., 2006). For blue wheat, Abdel-Aal et al. (2006) reported the most abundant anthocyanin to be delphinidin-3-glucoside followed by delphinidin-3-rutinoside, accounting for ~37% and ~32% of the total anthocyanin content, respectively. Cyanidin-3-glucoside and peonidin-3-glucoside have also been detected in blue wheat (Abdel-Aal and Hucl, 2003). Hu et al. (2007) reported that cyanidin-3-glucoside was the principal anthocyanin in aleurone blue wheat with pelargonidin 3-glucoside and cyanidin-3-galactacide also being present. However, in their HPLC chromatogram of anthocyanins, Hu et al. (2007) noted the presence of another two significant peaks that they could not identify but suggested that they could be delphinidin glycosides based on relative retention times.

For purple pericarp wheat, Dedio et al. (1972) reported that the anthocyanins present are mainly cyanidin-3-glucoside and peonidin-3-glucoside with lower quantities of acylated derivates of the

Abbreviations: ABTS, 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid; B, blue; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; F3′,5′H, flavonoid 3′,5′-hydroxylase; GRAS, generally regarded as safe; LSD, least significant difference; NP, non-purple; P, purple; W, white.

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cyanidin and peonidin glucosides and trace amounts of corresponding rutinosides. Abdel-Aal and Hucl (2003) reported that the most abundant anthocyanin in purple pericarp wheat was cyanidin-3-glucoside but also found cyanidin-3-galactoside, peonidin-3-glucoside and a significant number of unknown anthocyanins that could not be identified at the time. Subsequently, Abdel-Aal et al. (2006) reported that the main anthocyanins in purple pericarp wheat were cyanidin-3-glucoside and peonidin-3-glucoside as well as another 10 anthocyanin compounds which were present in lesser quantities.

The inheritance of both the blue aleurone and purple pericarp traits is not definitive. Results are often obscured as interspecific crosses can lead to populations that segregate for fragments or whole donor parent chromosomes (Knott, 1958; Li et al., 1983). Correctly phenotyping blue grains can be especially difficult due to xenia and environmental effects (Hurd, 1959; Keppenne and Baenziger, 1990; Knott, 1958) as well as instability problems which lead to blue streaking or mottling (Hurd, 1959; Mettin et al., 1991). Hurd (1959) concluded that blue aleurone is controlled by two incompletely dominant genes. Knott (1958) stated that the gene(s) for blue aleurone are partially dominant with distinct dosage effects but experienced difficulties determining allele segregation due to the segregation of *Agropyron* chromosome fragments and a whole *Agropyron* chromosome. Knott (1958) noted that pollen carrying the *Agropyron* chromosome was at a disadvantage to the pollen not carrying the *Agropyron* chromosome although it appeared that the eggs functioned normally. Similarly, Li et al. (1983) detected a reduced transmission rate of the *Agropyron* chromosome via male gametes. Mettin et al. (1991) reported that, on average, F_2 populations derived from a blue wheat accession TRI 2401 and white Chinese Spring wheat segregated 74% non-blue and 26% blue but hypothesised that the segregation is not necessarily monogenic as blue aleurone parent TRI 2401 was found to produce monosomic seeds that were missing the alien chromosome. Keppenne and Baenziger (1990) suggested that blue aleurone is controlled by a single dominant gene despite deviations from the expected segregating ratios. In a germplasm registration paper, Metzger and Sebesta (2004) reported that blue aleurone lines Sebesta Blue-1, Sebesta Blue-2, and Sebesta Blue-3 crosses with red and white seeded wheats segregated 3 blue: 1 non-blue, but the authors did not provide supportive evidence.

McIntosh and Baker (1967) hypothesised that two duplicate genes controlled purple pericarp, with purple being dominant when transferring the purple pericarp trait from *Triticum durum* L. to *T. aestivum* L. Piech and Evans (1979) reported two independent genes, possibly on chromosomes 3A and 7B. Gilchrist and Sorrells (1982) reported two incompletely dominant genes for purple pericarp with the penetrance of alleles for the purple pericarp affected by environment and modifier genes. The F_2 segregation ratios included 9 purple (P): 7 non-purple (NP), 11 P: 5 NP, 13 P: 3 NP, and 15 P: 1 NP. Gilchrist and Sorrells (1982) stated that heterogeneity among crosses could be due to the environment and that heterogeneity within crosses may be a result of chromatin loss due to meiotic abnormalities. Subsequently, Griffin (1987) reported that two F_2 populations derived from purple by red wheat crosses segregated in a ratio of 9 P: 7 NP suggesting that purple color is controlled by dominant duplicate genes, with either recessive homozygote epistatic to the effects of the other gene.

Pigmented wheats have proven valuable in measuring gene-flow in wheat (Griffin, 1987; Keppenne and Baenziger, 1990; Matus-Cádiz et al., 2007). Morrison et al. (2004) have suggested a protocol using blue wheat in apomixes studies. Commercially, it was originally thought that pigmented wheat could be used as a visual marker to distinguish food wheats from feed wheats (Myer and Barnett, 1987) or from bio-ethanol wheats. There is the potential to use pigmented wheat for human consumption as generally regarded as safe (GRAS) food colorant or a health food. More importantly, pigmented wheats have high antioxidant activity, due mostly to their high anthocyanin content, and can suppress both hydrogen peroxide-induced oxidation and bacterial lipopolysaccharide-induced nitric oxide in cell cultures (Hu et al., 2007). Antioxidant activity prevents the formation of radicals by scavenging or by promoting their decomposition (Young and Woodside, 2001). This is beneficial as tissue damage to the body by radicals can cause human diseases such as cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes (Fang et al., 2002). Li et al. (2005) reported that purple and blue grained wheats have a much higher scavenging activity of free radicals than white wheats and this was positively correlated with total phenolic content of the bran ($R = 0.86$) and the whole meal ($R = 0.96$). Individual anthocyanin compounds isolated from blue wheat showed substantial scavenging capacity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radicals and inhibition of human LDL cholesterol oxidation (Abdel-Aal et al., 2008).

Progress in breeding pigmented wheat will be aided by a better understanding of the development of pigmentation during grain filling in different pigmented cultivars and the inheritance of the purple and blue pigmented traits. As previously mentioned, the reported inheritance of the purple pericarp and blue aleurone traits is somewhat unclear. The objectives of this study were to characterise pigment accumulation patterns during grain development and to determine the inheritance of the blue aleurone traits and purple pericarp.

2. Experimental

2.1. Pigment accumulation during grain development

Grain from six wheat lines differing in pigmentation was used in this study: *T. aestivum* L. 'AC Barrie' (red pericarp), 'W98616' (white pericarp), 'Purendo 38' (red pericarp, blue aleurone), 'PIG99006' (white pericarp, blue aleurone), 'Laval-19' (purple pericarp), and 'PIG03008' (purple pericarp). The six wheat lines were grown using a four replicate randomised complete block design with 3.7 m long plots consisting of 5 rows spaced 23 cm apart in 2006 and 2007 at the University of Saskatchewan's Seed Farm (Saskatoon, Saskatchewan). The trials were seeded June 2, 2006 and May 25, 2007, at an approximate depth of 2.5 cm and a seeding rate of 304 seeds/m^2. Wheat spikes were tagged during anthesis and at least seven spikes were harvested every five days beginning once the grain reached the milk stage and ending once the grain reached physiological maturity, for a total of seven sampling dates. Only kernels from the first and second florets from the middle spikelets were hand threshed and retained. Kernels damaged by wheat midge (*Sitodiplosis mosellana*) were discarded. Photographs of individual kernels were obtained using a Heward Packard scanner (HP ScanJet 6300C, Singapore). Color values L*, a*, and b* were measured for each freshly harvested sample using a Hunter Lab colorimeter (model No 45/0-L MiniScan XE, Hunter Associates Lab Inc., Reston, VA, USA). The L* value measures brightness with a L* value of 100 being perfectly white and a L* value of 0 being perfectly black. The a* value measures red to green with positive values being redder and negative values being greener, and the b* value measures yellow to blue with positive values being yellower and negative values being bluer. After color was measured, the samples were frozen and then later freeze dried according to Abdel-Aal et al. (2001). Anthocyanins