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Transfer of grain colors to elite wheat cultivars and their characterization

Monika Garg*, Meenakshi Chawla, Venkatesh Chunduri, Rohit Kumar, Saloni Sharma, Nand Kishor Sharma, Navneet Kaur, Aman Kumar, Jaspreet Kaur Mundey, Manpreet Kaur Saini, Sukhvinder Pal Singh

National Agri-Food Biotechnology Institute, Mohali, 160071, Punjab, India

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ABSTRACT

Plant anthocyanins can act as antioxidants and help in prevention of cardiovascular diseases, diabetes, inflammation, cancer, obesity and aging. In the current study, anthocyanin rich blue, purple and black wheat lines with alien chromosome or its arm and adapted to local growing conditions were developed from low yielding exotic donor lines. Selected pigmented lines with commercial potential, had yield and thousand grain weight equivalent to the high yielding cultivars. Higher anthocyanin content was observed in black followed by blue, purple and amber wheat lines. 22 different anthocyanins could be identified from blue, 23 from purple and 26 from black wheat lines. Purple wheat lines exhibited higher peak intensities of acylated anthocyanidins. Chroma and hue values assisted in the efficient and reliable selection of compound grain colors. Cryo-microtome seed sections indicated presence of purple color in the pericarp all around the seed. The blue color was present in the aleurone layer all around the seed except around the embryo.

1. Introduction

Color coded diet (fruits, vegetables, cereals, etc.) rich in phytochemicals e.g. anthocyanins and carotenoids confer innumerable health benefits. Plant anthocyanins and phytochemicals can act as antioxidants and help in prevention of cardiovascular diseases, diabetes, inflammation, cancer, obesity and aging (Chen et al., 2013). Developments in the food industry can lead to the innovation of new products from unconventional colored wheat, with better nutritional and functional properties. Based on the potential of colored grains, a few functional foods have been reported from these wheats (Li et al., 2004, 2007) and there is potential for development and utilization of several such products with added health benefits.

Color in wheat grains is localized in the bran layers. The existence of the interesting genotypes of wheat with a red, purple, blue and amber seed color has been reported in many scientific contributions. The red color is due to the presence of major catechin-tannin and minor anthocyanins (Ficco et al., 2014) in the diploid testa of seed coat. The purple color is due to anthocyanins (Ficco et al., 2014; Abdel-Aal et al., 2006; Hosseinian et al., 2008) in the diploid pericarp layer. Blue color is due to anthocyanins (Abdel-Aal

List of abbreviations: Ag, Agropyron; Ba, Blue aleurone gene; BC, backcross; BW, Black wheat; CIE L*a*b* (CIELAB), Commission internationale de l'éclairage (International Commission on Illumination); Cy-3-Glu, cyanidin-3-glucoside; LC, Liquid chromatography; MS, Mass spectrometry; MQ water, Ultrapure water from Millipore; Pp, Purple pericarp gene; Q-TOF, triple quadrupole time of flight; Th, Thinopyrum; TKW, Thousand-kernel weight.

* Corresponding author. National Agri-Food Biotechnology Institute, C-127, Industrial Area, Phase 8, Mohali, 160071, Punjab, India.

E-mail addresses: mgarg100@yahoo.com, monikagarg@nabi.res.in (M. Garg), oviya.chawla@gmail.com (M. Chawla), chundurivenkatesh@gmail.com (V. Chunduri), rohitsharma20@yahoo.com (R. Kumar), sneha.shrma1@gmail.com (S. Sharma), nkbiotech@yahoo.co.in (N.K. Sharma), kalotinav@gmail.com (N. Kaur), aman.a199@gmail.com (A. Kumar), jaspreetkr87@gmail.com (J.K. Mundey), saini.manpreetkaur87@gmail.com (M.K. Saini), sukhvinder.pal.singh@gmail.com (S.P. Singh).

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et al., 2006; Abdel-Aal et al., 2008) in the aleurone layer.

Genetics of color grains has been studied by several research groups. While genes controlling red (*R*) and purple color (*Pp*) have been reported (Dobrovolskaya et al., 2006; Tereschenko et al., 2012), similar genes for blue aleurone color are still obscure. It has been suggested that inheritance of purple pericarp color was under the control of a single dominant gene in tetraploid wheat (Sharman, 1958) and two incompletely dominant genes in hexaploid wheat (Griffin, 1987). Blue aleurone color gene (*Ba*) has been transferred to wheat from different wild wheat relatives: *Thinopyrum ponticum (Agropyron elongatum)*, *Triticum monococcum* L. spp. *aegilopoides*, and *Th. bessarabicum* (Buresova et al., 2015). Certain studies report two complementary genes (Zeven, 1991; Lan et al., 2008), while others suggested single dominant gene (Knievel et al., 2009; Lan et al., 2008; Kuspira et al., 1989; Singh et al., 2007) for control of blue grain color.

In addition to genotype, environment plays an important role during grain color development. Combinatorial effect of temperature, moisture, light intensity, radiation and oxygen is required for the complete expression of pigments in plants (Chalker-Scott, 1999; Hosseinian et al., 2008). Agronomic measures like magnesium fertilization, time of harvesting, source-sink relationship and position of the grains in the spike affect anthocyanin content in seeds (Bustos et al., 2012). Breeding lines with high anthocyanin accumulation exhibit low yield, conditioned by the negative influence of genes linked to genes for grain color on the chromosome or its segment from the wild species (Martinek et al., 2014). The colored grain trait has not been observed in Indian wheat cultivars. Exotic wheat lines have a very low yield and adaptation to the Indian environment. The main objective of our study was to develop and characterize Indian wheat breeding lines with satisfactory yield level and high anthocyanin content. In this manuscript we have generated several Indian colored wheat lines with good yield potential and further characterized these lines morphologically, biochemically, and microscopically.

2. Material and methods

2.1. Plant material

The Plant material used in this study included blue aleurone wheat TA3972 and purple wheat TA3851 obtained from Wheat Genetics Resource Center, Kansas State University, Kansas, USA (Fig. 1). Black wheat (BW) was obtained from Dr. Hisashi Tsujimoto, Arid Land Research Center, Tottori University, Japan (Fig. 1). Red wheat (Sonora 64) was a released cultivar from India. Blue wheat TA3972 was UC66049 (PI 633834), 20″+1″DS T4BS·4el$_1$ [4B SN64] with blue aleurone (*Ba1*) from *Th. ponticum* is Mexican spring wheat. It was derived from the wheat composite cross I (2n = 44)/Sonora 64 (Qualset et al., 2005). In blue wheat long arm of wheat chromosome 4B had been replaced by translocation of the homoeologous chromosome of *Th. ponticum*. Purple wheat TA3851 was a purple pericarp mutant from Schmidt NE82702 in the winter wheat background, derived from Purple seed//Red Coat/Capitan. Black wheat developed by crossing blue colored, 4E *Ag. elongatum* chromosome substitution line Shou Ien 4E(4D) with purple colored mutant line. Recipient lines include high yielding, disease resistant and locally adapted cultivars PBW550, PBW621 and HD2967 (Fig. 1). Plants were grown in the farms of National Agri-Food Biotechnology Institute, Mohali, Punjab, India (30°44′10″ N Latitude and 76°47′18″ E Longitude at an elevation of 351 m above sea level) in the main season and in the farms of off season nursery facility provided by the Directorate of Wheat Research at Keylong, Himachal Pradesh, India (32°30′27.9″ N Lattitude and 76°59′34″ E Longitude at an elevation of 2971 m above sea level). Crossing, backcrossing and selfing experiments were carried out at both the locations. For yield estimation, four replications of 2.7 m^2 plots were grown in randomized complete block design.

2.2. Extraction and determination of anthocyanins

The grounded wheat samples were extracted with methanol: HCl (85:15 v/v, Hosseinian et al., 2008). Samples were shaken in dark at 1800 rpm for 45 min and centrifuged at 5000 g at 4 °C. The supernatant was collected and dried at 40 °C using a rotary evaporator (Rotavapor® R-215, Buchi India Private Ltd., Mumbai, India) and reconstituted in 1 ml of methanol. Total anthocyanin content was measured at 570 nm. The extract was filtered through a 0.2 μm polytetrafluoroethylene filter for identification of different anthocyanins using a mass spectrometer. Total anthocyanin content per sample (mg/kg) was calculated as cyanidin-3-glucoside:

$$C = A/\varepsilon \times (vol/1000) \times MW \times (1/\text{sample wt}) \times 10^6$$

Where C is concentration of total anthocyanin (mg/kg), A is the absorbance at 570 nm, ε is molar absorptivity (cyanidin-3-glucoside = 25,965 cm^{-1} M^{-1}), vol is total anthocyanin extract volume and MW is the molecular weight of cyanidin-3-glucoside = 449.

2.3. Identification and characterisation of individual anthocyanins

An ultra-performance liquid chromatography system (Acquity, Waters India Pvt. Ltd., Bangalore, India) was employed to achieve separation of anthocyanins. An aliquot of 2 μl methanolic extract of anthocyanins was injected into an Acquity C-18 column (100 mm × 4.6 mm × 3.5 μm) preceded with a guard column of similar stationary phase. The column temperature was maintained at 40 °C and sample manager temperature at 10 °C. The mobile phase A was MQ water: acetonitrile in the ratio of 95:5 containing 0.1% formic acid; mobile phase B was MQ water: acetonitrile in the ratio of 5:95 containing 0.1% formic acid. The flow rate was 0.2 ml/min.

Mass spectrometry (MS) detection was performed on triple quadrupole time of flight (Q-TOF) mass spectrometer (AB Sciex 5600, Haryana, India). TOF-MS (100 ms) and TOF-MS/MS (50 ms) acquisition were combined with an IDA method (AB Sciex) which was used for data acquisition. The threshold was kept at 500 counts per second. Positive electrospray ionization was employed. Ion spray voltage was 5500 V; the source was kept at 550 °C; ion source gas 1, 50 psi; ion source gas 2, 50 psi; curtain gas, 30 psi; collision gas, medium; declustering potential, 80 V; collision energy, 35 V; collision energy spread, 20 V; entrance potential, 10 V. Scan range was fixed from 100 to 1000 m/z. Data processing was done using PeakView software. Identification and peak assignment was based on comparison of MS/MS data with mass spectral databases like METLIN, MassBank and also with the published data (Giusti et al., 1999).

2.4. Grain color estimation

The grain color of bulk seeds was evaluated using ColorFlex EZ Spectrophotometer (Hunter Lab Inc., Virginia, USA). This equipment decomposes color in the L* a* b* color space (CIELAB) with coordinates based on a cube root transformation of the color data and inclusion of all perceivable colors and device independence. In this color space, 'L*' measures brightness, from black (0) to white (100), 'a*' measure chromaticity parameter, red to green with positive values being red and negative values being green, and 'b*' measure chromaticity parameter, blue to yellow with positive