

## Blue Wheat: Genetics, Healthy Value and Food Processing

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### Review Article

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**Abstract:** Wheat is the universally accepted staple food crop. Originally wheat does not have blue grain trait. The blue wheat varieties were usually developed from distant hybridization, and alien chromosome fragments were integrated into the wheat genome. At present, many scientists involved in molecular genetics and food related studies of blue grained wheat due to its high anthocyanin content which show organoleptic properties and potential health-promoting effects. Blue wheat is considered as the potential functional food which can address the nutritional aspects of the human without having much effort. To increase the dietary anthocyanin content, we can propose blue wheat for the bakery industry other than normal commercial wheat varieties. Present review provided the updated knowledge and will be supportive to initiate further research events of plant breeding, food processing and related health issues.

**Keywords:** Blue wheat, Anthocyanin, Distant hybridization, Functional food.

### INTRODUCTION

Wheat is a universally accepted staple food crop and owing to its well-documented health benefits, receiving continuous attention of the world. Cultivated bread wheat is a hexaploid and consist of 17 gigabase of DNA [1] in seven pairs of chromosomes in each three genomes of A, B and D. Except the two most common colors of red and white, different colors such as blue, purple, black and yellow wheat were reported [2-3].

Previous decades, many experiments have been carried out on different colored horticultural crops, paying little attention to the development of colored grain as a functional food. Interestingly, at present, many scientists involved in molecular genetic studies of blue grained wheat due to its high anthocyanin content [4].

Due to the aqueous nature of anthocyanin pigments in wheat, it can be utilized as a natural food colorant. Once separated, the anthocyanins can be used to protect and color foods and cosmetic products. Anthocyanins contribute to maintaining the health of human being, presenting substantial antioxidant capacities, with antimutagenic and antitumor properties, positive effects on cardiovascular diseases[5-8].

### Phenotype, Genetics and Breeding

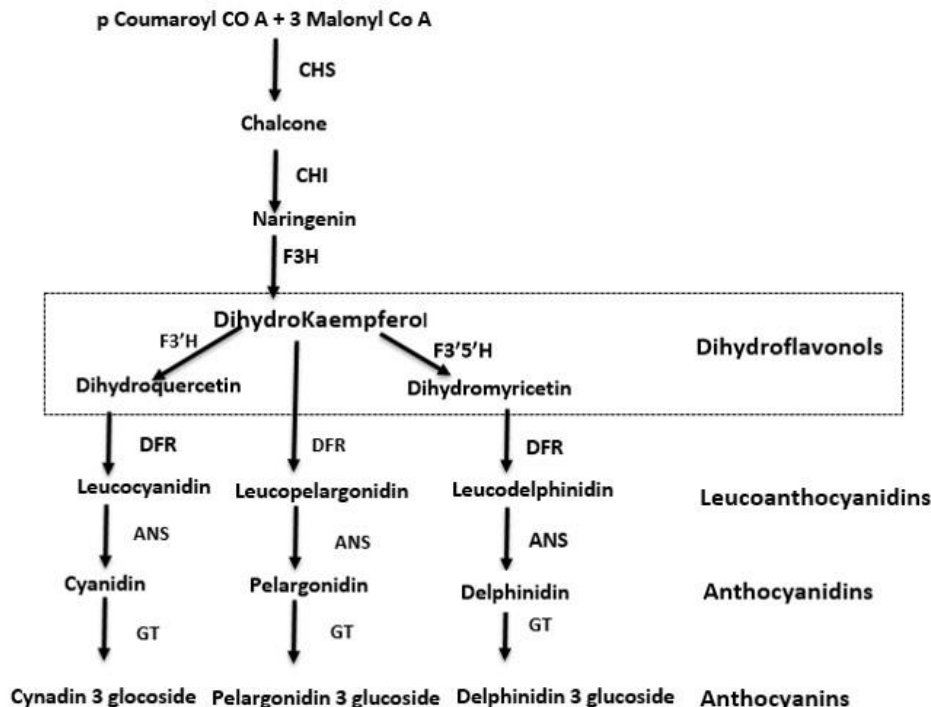
Originally, wheat does not have blue grain trait. The blue wheat varieties were developed from distant hybridization, and alien chromosome fragments were integrated into the wheat genome. The blue color

was introduced into hexaploid wheat from blue colored diploid wild einkorn wheat *Triticum boeoticum*, *Agropyron glaucum*, *Agropyron tricholporum* or tall wheatgrass *Agropyron elongatum* [2]. Several European hexaploid wheat lines of 'Berlin', 'Probstdorf', 'Tschermak', and 'Weihenstephan' received the blue grain trait through a complete replacement of 4A chromosome pair whereas, 'Brunn' and 'Moskau' blue lines were 4B chromosome substitutions [9]. Those both 4B and 4A chromosomes were substituted by alien diploid chromosome, 4A of *Triticum monococcum* or *T. boeoticum*. Further, they proposed the gene for blue aleurone trait in 'Xiao Yan' a Chinese variety, could be on 4D chromosome, which has been substituted by a chromosome pair of *Ag. elongatum*. Based on the results of fluorescence and genomic in-situ hybridization (FISH and GISH), the transferred chromosomal segments of *Th. ponticum* in blue grained wheat can be grouped into six groups by the length, position, and number of transferred segments. This results, suggested the great diversity among the individual blue grained gene sources. Various types of

introgressions present suggested that they can stimulate the blue aleurone trait [10].

The closely related two colors of blue and purple in wheat is caused by anthocyanins deposited in the aleurone layer and in pericarp (outer cover) respectively. The anthocyanin accumulation process progressed from the pericarp to the aleurone layer and visible on caryopsis at 25 days post anthesis [11]. Various concentrations of anthocyanin were detected in different stages of seed development, and the highest concentration was found in the mid-grain-development stage [11-12]. The presence of delphinidin-3-glucoside in the aleurone layer is characteristic for blue caryopses [13]. Delphinidin is a type of anthocyanidin found in the anthocyanin biosynthesis pathway which is a division of the whole flavonoid biosynthesis pathway. Both structural and regulatory genes participate for the anthocyanin biosynthesis. The structural genes, encode enzymes for the production of the flavonoid precursors, as well as involved in the formation of particular decorated anthocyanin molecules. Regulatory genes contain the genes encoding regulatory factors that control the expression of structural genes. They are mainly arranged as a complex formed by MYB, basic helix-loop-helix (bHLH) and WD40 repeats transcription factors [14]. Anthocyanin biosynthesis is well documented and after various steps, the colorless chemical compounds converted into colored

anthocyanin (Figure 1) [15]. Delphinidin is recognized as responsible for the bluish color, whereas pelargonidin and cyanidin are in charge of red and purple colors in plants respectively [13, 16, 17]. It is believed that black grain color in wheat is due to the combination effect of anthocyanin genes in both pericarp and aleurone layer [17]. The genetics of wheat anthocyanins has been studying more than fifty years. Majority of the structural and regulatory genes related to anthocyanin in red and purple wheat has been discovered. However, we could not purely unveil the secret of blue wheat yet. There are several arguments about the genes for blue trait in wheat. Knott [18] stated that the blue seed color is due to the different effects of partially dominant gene/genes. This was further described as blue color is a responsibility of two complementary dominant genes [19]. Several scientists proposed that blue color of wheat aleurone is governed by a single dominant gene [20]. The blue aleurone gene (*Ba*) originated from the 4E chromosome of *Thinopyrum ponticum* is considering as the incompletely dominant gene responsible for the wheat blue trait [21]. According to the recent concept, a single dominant gene is handling the blue trait. Furthermore, genes for the blue aleurone (*Ba*) trait have been identified in several wheat related species such as *Th. ponticum* (*Ba1*), *T. monococcum* (*Ba2*), *T. boeoticum* (*Ba2*), and *Th. Bucrium* (*BaThb*) [20-23].



**Fig-1: Simplified Anthocyanin biosynthesis pathway.** Pathway enzymes are indicated in abbreviated as follows; PAL: phenylalanine ammonia-lyase; CHS: Chalcone Synthase; CHI: Chalcone Isomerase; F3H: Flavanone 3-hydroxylase; F3'5'H: Flavonoid 3'5'-hydroxylase; F3'H: Flavonoid 3'-hydroxylase; DFR: Dihydro Flavonol-4-Reductase; ANS: Anthocyanidin Synthase; GT: Glycosyltransferase

The interesting fact on blue wheat is its segregation. Blue grain trait can be used as a marker to evaluate the out-breeding rate in wheat and to select for apomixes [24, 25]. In wheat, each kernel is an embryo produced from an individual fertilization and much number of offspring can be recorded in a single spike. All earlier studies agreed that the blue color in wheat is due to xenia phenotypes [26, 27]. It has been observed whole blue spikes as well as both white and blue seeds in one individual spike in a blue plant population derived from blue and white wheat cross. Similarly, when the various colored wheat lines crossed with blue wheat, different segregation ratios obtained and some of the segregation ratios were difficult to explain using the Mendelian genetics [2]. The heredity of blue grain trait is not definitive. Results are frequently changed along with different wide crosses which can direct segregation of the population to whole donor parent chromosomes or fragments [28]. Some transitional colors were also found in blue wheat and this phenomenon may be caused by dose effect of grain color gene [29]. A possible explanation for different color intensities of blue wheat is given by Knott [18]. According to his explanation, the aleurone layer is a one portion of the triploid endosperm tissue. Therefore, several allele combinations can be occurred, especially lack of blue color can be due to the absence of the *Ba1* gene, light blue seed color is due to one dose of gene, medium-blue seed is due to two doses and 3 doses produce dark blue seed [18]. Color intensities and genomic constitution of blue grained wheat genotypes has been explained in detailed by Burešová *et al.* [10]. Transposons are movable DNA segments which can alter the genome of a plant. They can change the genome by interfering the structure and expression of genes by adding, breaking, transposition and excision. Therefore, transposons also can alter the segregation ratios of several traits [30]. The knowledge of the available genetic resources for blue wheat and its biosynthesis could be utilized for the breeding of new varieties with plenty of relevant genes.

### Healthy Value of Blue Wheat

For a healthy life, the estimated daily anthocyanin consumption should be 3-215 mg/day [31]. Blue wheat differs from purple wheat by its composition and presence of different unique anthocyanins [13]. It contained higher amount of anthocyanin in bran (460 mg/Kg), followed by flour (200 mg/ Kg) and whole meal (160 mg/ Kg). These quantities are higher than that of purple and red wheat [32]. A higher milling yield of blue wheat such as 7.5, 14.6, and 55.0% for bran, shorts, and flour respectively, was found in blue wheat while 0.9, 11.8, and 42.7% respectively for that of purple wheat [33]. Furthermore, a higher concentration of anthocyanins in blue wheat, than red and purple durum wheat, has been found by Ficco *et al.* [4]. This result indicated that the antioxidant value of blue wheat is much higher than other colored wheat varieties. High Performance Liquid Chromatography of the anthocyanin components

indicated 5 to 8 main anthocyanins in blue wheat compared to 3 anthocyanins for red and purple wheats.

The blue grained wheat was signified by delphinidin 3-O-rutinoside, delphinidin 3-O-glucoside, and malvidin 3-O-glucoside. Furthermore, it contained lower levels of cyanidin 3-O-rutinoside, cyanidin 3-O-glucoside, peonidin 3-O-arabinoside, peonidin 3-O-galactoside, and peonidin 3-O-glucoside. In the same experiment, the predominant anthocyanin compounds in purple wheat were found as cyanidin 3-O-glucoside, peonidin 3-O-galactoside and malvidin 3-O-glucoside [4]. Significant changes were found in the total anthocyanins content from one year to another, suggesting that its synthesis is affected by environmental factors. Great extent of the environmental effects was observed in purple wheat compared to blue wheat, and the reason may be the location of the pigments in the wheat seed such as pericarp or aleurone layer [13]. It is believed that, Delphinidin is the most powerful angiogenic inhibitor in the anthocyanins, which help in cancer anticipation therapies and treatments [34]. Further, it can obstruct the formation of tumors, by hindering the initiation of the mitogen-activated kinase [35]. The antioxidant potential of anthocyanins mainly depends on the phenolic structure of the molecule. Furthermore, the structure related several factors such as the number of hydroxyl groups, acylation, glycosylation, and hydroxylation and methylation pattern influenced the antioxidant property [36]. The highest antioxidant potency recorded in delphinidin followed by petunidin, malvidin and cyanidin, peonidin and pelargonidin respectively [37].

### Food Processing

At present, many food products have been commercially produced from high anthocyanin containing plant materials or directly fortifying with anthocyanin extracts. These are commercially available under different brands of cereals, soft drinks, baby foods, and dairy products [38-40]. As wheat is one of the most important staple food in the world and numerous wheat based products are popular among the people, the nutritional and health benefits of blue wheat can be easily acquired by simply adding the blue wheat flour into their bakery products. Improvement of anthocyanin content in wheat relates to available genetic resources and breeding programs. To enhance the anthocyanin consumption, high anthocyanin contained wheat varieties should be suggested as the flour for baking other than normal commercial wheat.

Antioxidant activities and aroma quality in anthograin liqueur which was made using purple wheat have been examined. The results showed the antioxidant activities and phenolic acid content were comparatively higher in anthograin liqueur than the check samples of vodka and whisky. The aroma quality also acceptable by customers [41]. Similarly, as blue

wheat contains higher anthocyanin than purple wheat same experiment can be adopted to check the antioxidant properties, aroma quality and consumer acceptability which will help to offer the added health benefits due to anthocyanins.

The advantage of colored grain other than usual anthocyanin sources (vegetables and fruits) is colored grains can be stored easily and treated with stable products due to its comparatively long shelf life [32]. Due to the health and safety issues, the natural colorants replacing synthetic dyes in the food industry have increased remarkably. Blue wheat has been identified as a potential source for natural anthocyanin dye production [42]. It could be used in foods like bread, biscuits, muffins and dairy products such as yoghurt, ice cream and frozen desserts. A powder derived from the wheat could be used in skincare products. This will open new market opportunities for farmers and food processors.

It is believed that the natural anthocyanins are in equilibrium with the colored flavylium cation and the colorless hydrated form. This phenomenon is used in the food industry, by adding anthocyanins in acidic food to ensure the prevalence of the flavylium cation. Both whole meal and isolated form of anthocyanins in blue wheat are heat stable at the lower level of pH such as pH 1, while their breakdown is somewhat lower at pH 2 compared to higher pH of 5 [13]. In beverages, addition of Sulfur dioxide is commonly practiced and due to that color of the anthocyanin can be bleached at first and then partially restored when Sulfur dioxide becomes oxidized [43]. It is reported that anthocyanins in blue wheat showed higher heat stabilization after adding sulfur dioxide compared to no sulfur dioxide [13]. Blue wheat powder can be used as a high anthocyanin contained dietary supplement [42]. All above studies suggested the use of blue wheat as a functional food, food colorant, or a dietary supplement.

The major challenge of applications of anthocyanin in food processing industry is to keep the original bioactivity qualities of anthocyanin in the final food product as anthocyanin will degrade with heat, pH and several other environmental and chemical factors [44-47]. Accordingly, these food products should maintain the original sensory attributes, such as texture and flavor, while keeping the bio activity of the added anthocyanin. Microencapsulation which is a newly developed food processing technology will help to minimize or avoid such effects of the bioactive compounds through microstructural modifications [48]. Several thermal food processing techniques such as pasteurization and blanching can also distinctly interfere with the anthocyanin content of final products. Volden *et al.* [49] reported that reduction of anthocyanin content in red cabbage due to several food processing techniques of steaming, boiling and blanching as 29%, 41% and 59% respectively. It is

reported that, blanching is practicing in the fruit juice industry to retain the anthocyanin activity of their products. Anthocyanins can be degraded with polyphenol oxidase and mild heating inactivate the activity of polyphenol oxidase [50]. Present information indicates that thermal food processing techniques can be affected to the level of anthocyanin in the final food product. However, the published information on thermal stability of anthocyanins in processed foods is limited. Generally, anthocyanins begin to hydrolysis with sugar moieties such as rutinose and glucoside at the thermal degradation process. Due to this reaction anthocyanins start to deglycosylation which lead to produce several anthocyanidins and subsequently produce chalcones. These chalcones subsequently degrade into carboxyaldehydes and phenolic acids [51]. Slavin *et al.* [52] proposed that thermal degradation is not affect significantly on the antioxidant properties of anthocyanins as the thermally degraded products also have some antioxidant properties.

### Conclusion and Future Prospects

The facts found in this review suggest the possible utilization of blue wheat as commercial functional foods. As blue wheat flour contained higher concentration of anthocyanins, the nutritional and health benefits can be easily acquired by simply adding the blue wheat flour into their bakery products. However, new processing techniques should be found in order to keep the quality of final product with desired benefits of anthocyanin. Improvement of anthocyanin content in wheat relates to available genetic resources and breeding programs. From the genetic point of view, the location and number of genes for blue wheat is not yet clear or not definitive. Notable progress has been made in identifying genes for red and purple wheat using RNA sequencing. Hopefully, RNA Sequencing technique and high throughput SNP mapping will be helpful to identify and map the gene/s responsible for blue aleurone trait in hexaploid wheat without taking much time.

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