Nutraceutical Properties of Black Rice and Genetic Regulations

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Rice (*Oryza sativa* L.) is a major cereal crop that is relied by more than half of the world's population as their primary source of nutrition. It is a member of the *Poaceae* or *Gramineae* family. With dozens of varieties being cultivated all over the world, this cereal has a broad genetic diversity. With a production of 117.47 million tonnes, it is grown in an area of 43.86 million hectares in India (DAC&FW, 2019-20). The majority of rice consumed worldwide is white, however numerous unique varieties that include colour pigments, are black rice, red rice, and brown rice. Their names were given by the colours of their kernels, which are black, red, and purple due to deposition of varying degree of anthocyanin contents in various levels of the pericarp, seed coat, and aleurone. Anthocyanin deposition is what gives black rice its dark grain colour (Reddy et al., 1995).

Black rice is one of the coloured rice variants that has drawn the most attention because of its sensory qualities, high nutritional content and most importantly, its advantageous health aspects. Purple rice, forbidden rice, heaven rice, imperial rice, king's rice, and treasured rice are additional names for black rice. Its local name in Manipur, is Chakhao meaning delicious rice. Manipur black rice bagged the GI tag in 2020.

Chakhao is mainly consumed as kheer which is creamy, aromatic and has a nutty flavour. Because of its great nutritional value and healing qualities, many people believe that this rice is a cure for all illnesses. This rice comes in several cultivars that have a long history of cultivation in Southeast Asian nations including Thailand, China, and India. In Manipur, black rice comes in four landrace varieties: Chakhao amubi, Chakhao angouba, Chakhao poireiton, and Chakhao pungdol amubi. In comparison to other rice varieties, black rice is about six times richer in antioxidant activities, high protein content (8.16%), low-fat content (0.07%), is gluten-free, gut-friendly, and a natural cleanser with numerous medicinal properties (Jha et al., 2017). Black rice is rich in lysine and tryptophan, two important amino acids, as well as dietary fibre, functional fats, vitamins B1, B2, and E, folic acid, and phenolic compounds (oryzanols, tocopherols, and tocotrienols). It is low in calories and high in macro and micronutrients including iron, zinc, calcium, phosphorus, and selenium. Compared to other rice varieties growing in northeast India, black rice has a higher protein and nutritional content.

Black rice pigment

The deep purple colour of Black rice is due to the presence of anthocyanin, water-soluble plant pigments that give leaves, fruits, grains, and flowers a variety of appealing colours, the majority of which are red, blue, purple, and dark purple. They are phenolic chemicals that are produced from flavonoids and have significant biological functions in plants. The accumulation of anthocyanin in plants has various functions, including resisting UV radiation, participating in hormone regulation, and responding to biotic and abiotic stress, and is beneficial to human health. Different rice plant parts, but most intriguingly in the rice caryopsis, have been found to contain anthocyanins, which produce an enticing hue. The composition and concentration of the pigments in the rice caryopsis fluctuate, giving the plant's colour a wide range from brown to red to purple to black. The distinction in colour is brought about by various anthocyanin and proanthocyanidin mixtures. The quantity of total anthocyanins (TAC) in rice bran determines how dark it appears; in contrast, the amount of total proanthocyanidins (TPC) determines how red the rice bran appears. Changes are seen at various developmental phases, either in the rice caryopsis or in the extracted pigment between plants accumulating anthocyanin and proanthocyanin, as well as in non-pigmented rice. The pigmentation increases as the rice caryopsis matures.

Biosynthesis of anthocyanin

The genes encoding the proteins involved in the anthocyanin biosynthetic pathway have been extracted from numerous plants, and this pathway has been well characterized. A complex consisting of MYB-type transcription factors (TFs) of SG5 and SG6, basic helix-loop-helix (bHLH) TFs of the IIIf subgroup and WD-repeat proteins, as well as the MYB-bHLH-WD40 or MBW complex, is responsible for regulating the transcription of these biosynthetic genes (Hichri et al., 2011). The C-terminus and variable region of this complex's MYB TF, which has two conserved imperfect repeats (R2 and R3) at its N terminus, are in charge of regulatory action. Through interaction with the R3 region of its R2R3 MYB partner, the bHLH TF is essential for the formation of transcriptional complexes at the promoters of anthocyanin biosynthesis genes (Xu et al., 2015). Finally, rather than serving a direct regulatory role, the WD40 protein functions as a scaffold protein onto which the complex is formed and stabilizes the interaction between the MYB and bHLH TFs. Studies have shown that WD40 proteins, such as the flavonoid biosynthesis regulator *TRANSPARENT TESTA GLABRA1* (*TTG1*), are expressed in tissues that may either accumulate or lack flavonoids and have pleiotropic effects on additional processes, such as the formation of trichomes and root hairs and the production of seed mucilage (Ramsay and Glover, 2005). Similar to WD40 proteins, bHLH TFs can control one or more branches of the flavonoid biosynthesis pathway as well as additional functions such as determining the destiny of epidermal cells, often in a partially overlapping manner. Furthermore, a hierarchy of bHLH TFs controls the anthocyanin biosynthetic pathway so that an upstream bHLH stimulates the expression of a downstream bHLH gene encoding the MBW complex component (Petroni and Tonelli, 2011). MYB TFs have a more limited range of function and are responsible for the differential accumulation of flavonoids in different plant organs and tissues by regulating the specificity of MBW complexes for their cognate target genes in response to environmental factors and developmental stage (Gonzalez et al., 2008). As a result, different combinations of TFs are needed for each type of tissue and stage of development since the expression patterns of distinct MYB genes control the spatial and temporal regulation of the flavonoid biosynthesis pathway. Notably, the majority of rice cultivars lack these flavonoid pigments, presumably as a result of intentional selection for whiter grains. It has been discovered that several rice genes are involved in the manufacture of anthocyanins. For instance, seven potential regulators, including the *R2R3-MYB* gene *OsC1* and the six bHLH genes *OsB1*, *OsB2* (also known as *OsKala4*), *OsRa, OsRb, OsPa,* and *OsPs* were identified and characterized for their role in the spatial and temporal regulation of anthocyanin biosynthesis. Recently, *OsC1*, which is essential for the synthesis of anthocyanins in the leaf sheath, apiculus, stigma, and hull, was shown to be the target of artificial selection for loss of pigmentation during rice domestication, leading to green leaf sheaths in the majority of cultivated rice plants. Oikawa et al. (2015) found that *OsKala4* controls the anthocyanin production in the pericarp. The ectopic expression of *OsKala4* in the pericarp and the subsequent transcriptional activation of anthocyanin biosynthesis genes in this tissue are the results of a rearrangement in the *OsKala4* promoter region. Additionally, Sun et al. (2018) found that *OsC1* and *OsKala4* combined activity stimulates anthocyanin production in several organs, including the apiculus, hull, and leaf sheath, but not in the pericarp. *OsKala4* regulates anthocyanin accumulation in this tissue in collaboration with other MYB-like TFs other than OsC1, as shown by the reddish-brown pericarp that occurs from ectopic expression of *OsKala4* in the absence of *OsC1*. According to earlier research, the introduction of the alleles of three genes such as *OsKala1*, *OsKala3*, and *OsKala4* from the black pericarp "Hong Xie Nuo" into the white pericarp "Koshihikari" causes a complete transformation of the white pericarp into the black pericarp. *OsKala1* and *OsKala4* encode a dihydroflavonol reductase (DFR) and a bHLH TF, respectively. *OsKala3* is believed to encode the MYB TF in the MBW complex (Maeda et al., 2014), although it is still unclear what molecular process causes the rice to be black.

Nutritive value

Iron, vitamins A and B, fibre, protein, necessary amino acids, and other nutrients are all found in black rice. Black rice is very nutrient-dense. This rice is minimal in sugar, salt, and fat and devoid of gluten and cholesterol. Protein content and quality are higher than in any other rice variety. There are 18 amino acids in it. It is helpful for individuals worried about getting enough iron on a plant-based diet because it is a naturally good source of iron. Black rice has a high concentration of minerals, including calcium, sodium, magnesium, and potassium. Studies in the past have shown that the antioxidant activity and phenolic content of black rice is higher than those of white rice.

Conclusion

Black rice is considered to be a rich source of nutrients with various health benefits. Anthocyanin which is present in black rice has many biological functions. By increasing anthocyanin content, resistance to biotic and abiotic stress can be increased. Transgenic breeding targets to increase anthocyanin content in rice to enhance rice quality and nutritional value. Genes involved in the biosynthesis pathway of anthocyanin have been studied and identified. A better understanding of this pathway is necessary to proceed with advanced breeding techniques.

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