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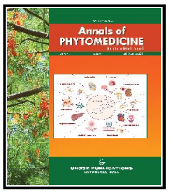

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Therapeutic potential of anthocyanin rich deep pigmented rice landraces and its genetic improvement: A review

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Abstract

The vibrant red, purple and brown hues observed in various plant tissues including rice grains and stems are due to anthocyanins, a subset of flavonoids. Deep pigmented landraces boast diverse anthocyanin profiles, enriching their therapeutic profile with anti-inflammatory and antioxidant properties. The antioxidant properties of black rice are higher than those of red rice due to the presence of cyanidin-3-glucoside, which reduces the risk of diabetes, obesity, breast and liver cancer. So, it is gaining the attention of health-conscious consumers. Genetic improvement of black rice cultivars is essential to meet the growing demand as well as to make it affordable to consumers at a reasonable price. Several genetic studies have mapped the key activator loci for anthocyanin (KALA), Kala 1, Kala 3 and Kala 4 linked with the accumulation of anthocyanin content in purple or black rice grains. However, the complex nature of the trait has to be dissected further for the development of functional markers for its exploitation. Metabolomic studies have shown that black rice accessions from South and Southeast Asian region exhibits noteworthy variability for anthocyanin concentration and antioxidant properties. Therefore, these collections with significant genetic diversity can be effectively utilized to elucidate genomic regions linked to anthocyanin using whole genome re-sequencing techniques to expedite the discovery of functional genes and associated markers for utilization in high-yielding genetic backgrounds.

1. Introduction

Characteristic purple, red and brown colouration in plants is imparted by natural pigments known as anthocyanins, which are classified under flavonoids in polyphenolic compounds. Traditional rice cultivars display a wide variety of anthocyanin profiles. Anthocyanins in rice landraces serve multiple functions including defense against external factors such as UV rays, IR rays, microbial infections and herbivores. Moreover, their anti-inflammatory, anticancerous and antidiabetic properties contribute to rice's nutritional and health promoting qualities (Yamuangmorn and Prom-U-Thai, 2021). It is observed that among the staple food crops, black pigmented rice harbours high anthocyanin content and is enriched with minerals, carotenoids and phytosterols. So, black (purple) rice cultivars with nutraceutical value (Thanyacharoen *et al.*, 2017) are gaining importance among health conscious consumers; although, they are highly prized in the market.

Despite all the uniqueness, these landraces have disadvantages such as low tillering ability, poor yield, lodging nature and poor eating quality. Hence, farmers are hesitant to cultivate these pigmented landraces. As the productivity of pigmented landraces is very low, the cost per kilogram of seed appears to be high. So, it is not affordable to all classes of people as pointed out by its popular name "forbidden rice" or "emperor's rice".

In light of the recent increase in the occurrence of lifestyle related diseases, protecting the most vulnerable, particularly low income individuals in society is crucial. As, rice is a staple food for people in 175 countries and territories worldwide (USDA, 2023) enriching the anthocyanin content in rice grains will be the cheapest and most advantageous option rather than supplementing in the form of medicines which is quite expensive. Therefore, evolving rice varieties with therapeutic value is vital for addressing the present day challenges threatening humankind worldwide. Hence, knowledge on the genetic basis of anthocyanin accumulation and scientific evidence for the therapeutic potential of the trait in pigmented rice is essential for its genetic improvement in rice crops. Numerous studies have delved into the scientific basis of the health promoting properties of anthocyanin content in rice landraces (Dhanushkodi Vellaiyan *et al.*, 2024). Researchers have identified specific genes and processes governing anthocyanin biosynthesis in rice linking variations in anthocyanin composition to the genetic diversity in landraces (Kim *et al.*, 2007). However, the complex nature of the trait has to be dissected using next generation sequencing technologies to identify of functional markers for exploitation in high yielding genetic backgrounds. In this context, this review focuses on the anthocyanin content, antioxidant potential, the therapeutic significance of

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anthocyanin in deep pigmented landraces, its genetics, biosynthesis, QTLs underlying the anthocyanin content to unlock the hidden treasures for exploitation as well as for genetic improvement.

2. Anthocyanin content and antioxidant properties of deep pigmented rice landraces

Antioxidant properties of crop plants were associated with the molecular/structural characteristics of different types of anthocyanins present in them. The presence of catechol and pyrogallol groups in ring B of cyanidin-3-glucosides and the respective aglycones makes them highly reactive to oxygen species. These compounds can reduce oxidative stress either by directly scavenging hydroxyl (OH^-), singlet oxygen (O_2) peroxide (ROO^-), hydrogen peroxide (H_2O_2) and superoxide (O_2^-), radicals or by stimulating the antioxidant enzyme activities and antioxidant protein synthesis (Bendokas *et al.,* 2020).

Deep pigmented landraces were documented to have complex and divergent anthocyanin profiles with cyanidin-3-glucoside being the predominant form accounting for 82.3%, followed by peonidin-3-glucoside (14.6%) and were distributed in free form in whole grains. Other types of anthocyanins, *viz.,* cyanidin-3-galactoside, cyanidin, peonidin, cyanidin-glucoside, cyanidin-3-sambubioside, cyanidin-3-rutinoside, peonidin-3-rutinoside pelargonidin-3-glucosmalvidin and petunidin-3-O-glucoside were also detected in coloured rice but at low levels. Delphinidin- 3-galactoside, delphinidin- 3-arabinoside and cyaniding- 3-galactoside were reported in Chakhao Poireiton a deep pigmented landrace of Manipur with unique nutraceutical value (Jiamyangyuen *et al*., 2019; Zheng *et al*., 2019).

Hu *et al.* (2003) illustrated that the presence of anthocyanin pigments cyanidin-3-glucoside (C_3G) and peonidin-3-glucoside (P_3G) in black rice enhanced its antioxidant activity and free radical scavenging capacity, thereby imparting medicinal value and making them a potential candidate for nutritional and functional food formulation.

Sompong *et al.* (2011) investigated the anthocyanin content and antioxidant properties in nine red and three black genotypes originating from Thailand, China and Sri Lanka and reported that the black varieties had a high concentration of anthocyanins, *i.e.,* cyaniding- 3-glucoside (19.4 to 140.8 mg/100 g DM) and peonidin 3-glucoside (11.1 to 12.8 mg/100 g DM). But, in red rice genotypes, these compounds were the least to be detected. Regarding the antioxidant property, FRAP (ferric reducing antioxidant power) value ranged from 0.9 to 8.1 mmol Fe (II)/100 g with a mean value of 4.0 mmol Fe (II)/100 g amongst the red rice while it ranged between 3.7-7.6 mmol Fe (II)/100 g with an average of 5.6 mmol Fe (II)/100 g amongst the black rice and concluded that black rice possesses high anthocyanin content and antioxidant activities than red rice.

Kiay *et al.* (2019) estimated the bioactive compounds such as total phenolic compounds, anthocyanin and antioxidant capacity in Indonesian black, brown and red colour rice genotypes and identified black rice genotypes as the novel source of antioxidants. The total phenolic content of black rice (10.26 mg/kg gallic acid) was higher than that of red rice (7.48 mg/kg gallic acid) and brown rice (3.97 mg/kg gallic acid). A similar trend was reported for anthocyanin content (black rice-123.33 mg/kg, red rice-120.33 mg/kg, and brown rice - 118.33 mg/kg) and free radical scavenging activity (black rice - 62.67 per cent, red rice -54.78 per cent and brown rice-52.30 per cent). Hence, black pigmented rice varieties enriched with polyphenolic compounds could serve as a natural antioxidant source (Rasaily *et al.,* 2021).

Phytochemical and antioxidant profiling of black, red, and brown rice genotypes of Northern Thailand (Pengkumsri *et al.,* 2015) revealed that black rice Chiang Mai possessed the highest concentration of total phenolic content (305.30 ± 6.15 mg GAE/g of extract), flavonoids (1.93 ± 0.03 mg QE/g of extract) and anthocyanins (1487.25 ± 24.36 mg CE/g) and free radical scavenging compounds and activities (323.21 ± 16.16 mg of TE/g) of extract as per the ABTS assay) in rice bran in contrary to Mali red rice genotype with less Phyto chemical content (total phenolic content (36.14 ± 5.60 mg GAE/g of extract), flavonoids (0.66 ± 0.01 mg QE/g of extract), anthocyanins (least to be detected) and antioxidant activity (< 50 mg of TE/g of extract as per the ABTS assay). Based on the profiling, highly pigmented rice variety was identified to be rich in phytochemicals, anthocyanin and free radical scavenging compounds (Tripathy *et al.,* 2023).

Black pigmented Chakhao landraces of Manipur known for its medicinal value were reported to have total anthocyanin content ranging between 29.8 and 275.8 $mg.100g^{-1}$ DW and radical scavenging activity (RSA) between 17.7 and 65.7 per cent. The RSA of the black pigmented rice was two to eight times higher than that of unpigmented genotypes, owing to the presence of C3G which is a more active pigment against superoxide after delphinidin (Bhuvaneswari *et al*.,2020). Similarly metabolic profiling of pigmented landrace collections of Assam revealed that the total anthocyanin content ranged from 1.006 to 13.90 mg CE/100 g in the studied rice genotypes. DPPH free radical scavenging activity (IC_{50}) varied between 6.61 to 29.37 mg. The HPLC analysis revealed the presence of both cyanidin-3-glucoside and peonidin-3-glucoside in the black pigmented rice genotypes while it was not detected in the red rice genotypes analyzed (Sahewalla *et al.,* 2023).

Biochemical characterization of traditional landraces of Tamil Nadu had exemplified that black pigmented landrace; namely, Chitthan samba (TNAUF00103105) (150.34 mg CE/100 g DW) and Burmablack (TNAUF00103118) (119.9 mg CE/100 g DW) had high anthocyanin content (Figure 1), while it was low in non pigmented landraces like Mutrinakannam (TNAUF00103142) and Puthupatty samba (TNAUF00103159) (1.17 mg CE/100 g DW) and Valasamudon (TNAUF00103166) (1.34 mg CE/100 g DW). Chitthan samba with high anthocyanin was found to possess astonishing antioxidant activity (3699 µg TE/g), while Valasamudon with low anthocyanin content had the least antioxidant activity (115.33 µg TE/g) (Dhanuja *et al.,* 2021). Jangala *et al.* (2022) also confirmed the presence of high anthocyanin content in the black pigmented landrace Chitthan samba (157.70 mg CE/100 g DW).

Variations in anthocyanin content and profiles were suggested to be attributed to the differences in rice variety, anthocyanin localization, the nature of the sample, the measurement method or the growing area (Yamuangmorn and Prom-U-Thai, 2021).

Several life-style related ailments such as cardiovascular diseases, diabetes and cancer are caused to humans due to oxidative stress. Dietary antioxidants can effectively reduce the dangerous reactive oxygen species and balance the body's redox status (Krishnanunni *et al.,* 2015). Tocopherol and anthocyanin compounds found in deep pigmented rice grain extracts, have been established to neutralize reactive oxygen species more effectively (Zhang *et al.,* 2015).

Additionally, animal tests have demonstrated their bioavailability (Tantipaiboonwong *et al.,* 2017). Hence, deep pigmented rice varieties enriched with anthocyanins and distributed in South and South East region of Asia (Figure 2) (Table 1) could act as a natural source of antioxidants thereby reducing the risk of occurrence of chronic and non chronic diseases and aid in improving human health (Prom-U-Thai and Rerkasem, 2020 ; Shao *et al.,* 2011).

Figure 1: **Black rice landraces of Tamil Nadu, India with high anthocyanin and antioxidant activity.**

Figure 2: Distribution of pigmented landraces in South and South East region of Asia.

Table 1: Anthocyanin and antioxidant activity of deep pigmented rice landraces of South and South East region of Asia

Black rice	Country	Anthocyanin (mg CE/g)	Antioxidant activity	Reference
Kavuni	India	2.445 ± 0.02	86.12 ± 0.05%	Ponnappan *et al.*, 2017
KJ-CMU107	Philippines	0.107	-	Fongfon *et al.*, 2021
Lallodo	Indonesia	1.690	-	Hanifa *et al.*, 2020
BREJ Wajaloka	Indonesia	0.00678	-	Fatchiyah *et al.*, 2023
Chakhaoamubi (CB)	China	4.90 ± 0.02	80.5 ± 0.11%	Singh *et al.*, 2022
PIZ	Thailand	0.58	-	Fongfon *et al.*, 2021
Mali Daeng	Thailand	0.312	88.28%	Prajuntasan *et al.*, 2022
Kum Ka Dum	Thailand	0.448	90.31%	-

3. Therapeutic significance of anthocyanin in pigmented rice

3.1 Anticancerous activity

Anticancerous properties of anthocyanins in pigmented rice have been illustrated using *in vitro* cell culture studies, *in vivo* animal models and human epidemiological data. Anthocyanin in pigmented rice selectively inhibits the proliferation of cancer cells with little effect on normal cells (Anticell proliferation) or induces the apoptosis of cancer cells through intrinsic and extrinsic pathways (Induction of apoptosis) or by the suppression of angiogenesis (Antiangiogenesis). Anthocyanins inhibited cancer cell invasion through the reduction of the expression of matrix metalloproteinase (MMP) urokinase plasminogen activator (u-PA) (Anti-invasiveness).

The second most important cancer that causes mortality in females is breast cancer. Approximately, 25% of breast cancer is caused due to the overexpression of HER2 (Human epidermal growth factor receptor 2) which is difficult to diagnose. Treatment of such kinds of tumors in rats using cyanidin-3-glucoside and peonidin-3- glucoside from black rice extracts of Asiatic origin had shown to lower the expression of HER2 as well as ki67 a proliferation marker when compared to control. Moreover, enhanced expression of caspase 3 in treated tumors indicated the apoptotic effect of anthocyanin compounds in black rice (Liu *et al*., 2014). Cytotoxicity evaluation of anthocyanin rich extract from black rice (AEBR) on various breast cancer cell lines *in vitro* by Hui *et al*. (2010) revealed that anthocyanin in black rice (AEBR) induced apoptosis in breast cancer cell line HER2 MDA-MB-453 *via* the intrinsic pathway by activating caspase cascade mechanisms while oral administration of AEBR at the rate of 100 mg/kg/day to MDA-MB-453 cell xenografted nude mice *in vivo* significantly reduced tumour growth by suppressing the expression of various angiogenesis factors in tumor cells. Chen *et al.* (2015) ;

Zhou *et al.* (2017) demonstrated the potential antimetastatic effect of black rice anthocyanins treatment on HER2$^+$ breast cancer cells (*in vitro*) by suppressing RAS/RAF/MAPK and FAK signalling pathways respectively. Likewise, anthocyanin from purple glutinous *indica* rice was identified to have an antimetastatic effect on human oral cancer CAL 27 cells by reduction of MMP-2, MMP-9, and NF-κB p65 expression through the suppression of PI3K/Akt pathway and inhibition of NF-κB levels (Fan *et al.,* 2015). Purple rice's high anthocyanin content has been linked to inhibit human hepatocellular carcinoma cell growth (Banjerdpongchai *et al.,* 2013). Cyanidin-3-glucoside and peonidin-3-glucoside in black rice inhibit the growth of the Lewis lung carcinoma cells *in vivo* and induce apoptosis *in vitro* demonstrating the effectiveness of black rice in the treatment of lung cancer which is the severe form of cancer among all the type of cancers.

Thus, based on *in vitro* cell culture, *in vivo* animal model studies and human cell line culture studies anthocyanin pigment in black rice genotypes has been elucidated as a novel anticarcinogenic compound for the treatment of breast as well as liver cancer. However, a detailed study in this regard is essential for understanding the anticancerous properties of pigmented landraces.

3.2 Antidiabetic activity

Diabetes has emerged as the most common diseases, and it is projected that 73.6 million more people will suffer from diabetes worldwide by 2045. Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetic cases especially in low and middle income countries (Banday *et al.,* 2020). Plant based functional foods, rich in polyphenols have been advocated as an effective approach for the control of T2DM in recent years. Consuming coloured grains rich in anthocyanins can lower blood glucose levels, unlike white rice which elevates them. Extracts of black pigmented rice grain and bran suppress the activity of endogenous α-glucosidase and hinder the conversion of starch into glucose in the small intestine. This serves as a source of resistant starch for coloured rice and provides nutritional benefits for gut microbiota in the colon (Boue *et al.,* 2016). The low glycaemic index and high resistant starch of coloured rice landraces play a key role in the managing and preventing diabetes and related disorders (Meera *et al.,* 2019).

C3G present in the bran of black rice reduces the concentration of blood glucose and improves insulin sensitivity in type 2 diabetic mice by up regulating glucose transporters and down regulating retinol binding protein 4 in adipose tissue thus making black rice a potential antidiabetic source (Guo *et al.,* 2007). Zhong *et al.* (2023) analyzed the effect of black rice anthocyanins on T2DM rats and found that these anthocyanins exhibit antidiabetic properties by regulating the PI3K/AKT signalling pathway and intestinal microbiota. According to Feng *et al.* (2022), anthocyanin rich black rice would be a promising functional food for the prevention and treatment of insulin resistance and diabetic hyperglycaemia as it stimulates GLUT4 glucose uptake *via* upregulation of PI3K/Akt and AMPK/p38 MAPK signalling in C2C12 myotubes. Black rice bran enriched with secondary metabolites such as anthocyanins, γ-oryzanol and proanthocyanidins, were established to control diabetes related molecular signalling and suppress oxidative stress resulting in improvement of glucose levels, and insulin levels. It also increases the defense of pancreatic â cells against apoptosis (Eviana *et al.,* 2023).

Chaiyasut *et al.* (2017) assessed the impact of germinated black rice extract (GBRE) supplementation in a DM induced rat model and pointed out that GBRE with abundant anthocyanin and other phenolic compounds improved the antioxidant status of diabetic rats and reduced the magnitude of DM. Based on the investigation, it was inferred that germinated black rice with other dietary combinations would be an effective approach for preventing and controlling diabetes.

3.3 Anti-inflammatory properties

Anthocyanins in black rice were reported to possess anti-inflammatory properties and antiaging potential by modulating type I collagen gene expression and suppressing H_2O_2-induced NF-κB activation in skin fibroblasts (Palungwachira *et al.,* 2019). Glutinous black rice extracts were demonstrated to possess higher levels of C3G, P3G, antioxidant and anti-inflammatory characteristics than non glutinous black rice extracts. C3G in the black rice extracts was shown to have a significant positive correlation between antioxidant capabilities while P3G showed a high correlation with its anti-inflammatory capabilities (Mapoung *et al.,* 2023).

4. Anthocyanin biosynthesis in rice

Water soluble secondary metabolites called as anthocyanins are divided into three groups: delphinidin, cyanidin and pelargonidin (Ciulu *et al.,* 2018). Beginning with malonyl-CoA and p-coumaroyl-CoA, anthocyanidin biosynthesis takes place in a branch of the flavonoid synthetic route (Holton and Cornish, 1995). Under the direction of chalcone synthase (CHS), three acetate units from malonyl-CoA combine with p-coumaroyl-CoA to form tetrahydroxychalcone, which forms the fundamental building block of anthocyanins.

The enzyme chalcone isomerase (CHI) catalyzes the change of the yellow coloured tetrahydroxychalcone to the colourless naringenin. Flavanone 3-hydroxylase (F3H) then catalyzes the conversion of naringenin to dihydrokaempferol (DHK). Flavonoid 32 -hydroxylase (F32 H) and flavonoid 32 52 -hydrolase (F32 52 H), respectively, hydroxylate the following conversions. DHK in to dihydro quercetin (DHQ) and dihydromyricetin (DHM). The three colourless dihydroflavonols (DHK, DHQ, and DHM) undergo a three-step conversion process to anthocyanins. Leucoanthocyanidins are first produced by dihydroflavonol reductase (DFR) reducing dihydroflavonols. Leucoanthocyanidin oxidase (LDOX) oxidizes them and 3-glucosyl transferase (3GT) glycosylates them to produce anthocyanidin 3-O-glucoside. Anthocyanidin 3-O-glucosides undergo further glycosylation, methylation, and acetylation reactions to provide colourful decorated anthocyanins (Stafford, 1991). It has been reported that rice has eighteen major forms of anthocyanins. The most commonly observed ones in rice were cyanidin-3-glucoside (C3G), peonidin-3-glucoside (P3G), cyaniding-3-rutinoside (C3R) and cyaniding-3-galactoside (Goufo and Trindade, 2014).

In black rice, anthocyanins C3G and P3G were predominant. Nonetheless, P3G was present in relatively smaller amounts than C3G since C3G makes up 64-90 per cent of the overall anthocyanin content, while P3G only makes up 5-28 per cent. However, because of methylation, P3G was more persistent than C3G.

The blacker the rice bran appears, the higher its total anthocyanin content (TAC); conversely, the redder the rice bran appears, the greater its total proanthocyanidin (TPC) concentration (Mackon *et al.,* 2021). Leucoanthocyanidin reductase (LAR) or anthocyanidin reductase (ANR) catalyzes the reduction of leucoanthocyanidin or cyanidin, respectively, to produce proanthocyanin derivatives, which are brown or red in colour. Proanthocyanins are a subclass of flavonoids (Koes *et al.,* 2005).

4.1 Genetic basis of pigmentation of pericarp and its association with anthocyanin content

Since anthocyanin is mostly deposited in the pericarp of the caryopsis, the coloration of the pericarp has been extensively studied to know about its genetic basis. A segregation ratio of 9:3:4 was found for the three phenotypes, *i.e.,* black, brown and white pericarp in genetic experiments carried out using F_2 populations derived from a cross between black and white rice genotypes. Rice genetic analysis had demonstrated that the recessive or dominant nature of genes significantly influences the degree of pericarp pigmentation. The rice pericarp's purple pigment is caused by the presence of two dominant complementary genes, Pb and Pp, whereas brown coloration is caused by the presence of Pb alone. Pb was epistatic to both Pp and pp of the rice pericarp; The Pp allele was incompletely dominant to the recessive pp allele. The absence of Pp allele causes white colour in rice grains. Pb and Pp loci were reported to be found in chromosomes 4 and 1, respectively. The Pb locus has two genes namely Ra and bhlh16 where bHLH16 is involved in proanthocyanin synthesis, while Ra is involved in anthocyanin synthesis (Ham *et al.,* 2015; Rahman *et al.,* 2016).

4.2 Studies on anthocyanin biosynthesis genes in rice

Anthocyanin biosynthesis in rice is controlled by both structural and regulatory genes. Functional enzymes involved in anthocyanin biosynthesis reaction are encoded by structural genes while transcription factors (TFs) that regulate the expression of structural genes are encoded by regulatory genes (Dooner *et al.,* 1991).

The main structural genes that participate in the biosynthesis of anthocyanins were reported to be CHS, CHI, F3H, F3'H, F3'5'H, and DFR. MYB TFs, basic helix-loop-helix (bHLH) TFs and WD40 TFs were shown to be the three major types of genes that regulate anthocyanin biosynthesis in plants. The rice genome has a large number of MYB TFs (Feller *et al.,* 2011; Maeda *et al.,* 2014).

Only three genes, meanwhile, have been linked to the synthesis of anthocyanins. By binding to the promoter regions of OsDFR and OsAns, the MYB TF OsC1 regulates the expression of OsDFR and OsAns, hence taking part in the flavonoid pathway and stress response (Ithal and Reddy, 2004). In spite of Kala3 has been identified as an MYB TF, its precise function is yet unknown. bHLH TFs are extensively found in plants and are involved in a variety of biological processes, including signal transduction and hormone response (Zhang *et al.,* 2015). The rice genome has 167 bHLHs distributed throughout 12 chromosomes. In rice, two R genes Ra and Rb were identified to be located on chromosomes 4 and 1, respectively wherein the Ra locus has two genes Ra1 and Ra2. Moreover, the Pl locus in rice with OsB1 and OsB2 was reported to be identical to Ra and allelic to Ra1. Both OsB1 and OsB2 were involved in the anthocyanin pathway in a complementary way. OSB2 regulates several genes encoding enzymes involved in anthocyanin synthesis, including F3H, DFR, and ANS (Sakulsingharoj *et al.,* 2014). Rc was another bHLH gene located on chromosome 7, involved in proanthocyanin biosynthesis in rice pericarp (Sweeney *et al.,* 2006).

4.3 QTL mapping for anthocyanin content in rice

Maeda *et al.* (2014) mapped three key activator loci for anthocyanin (KALA), Kala1, Kala3, and Kala4 linked with the accumulation of anthocyanin content in purple or black rice grains using F_2 plants derived from a cross between the black rice NIL (derived using the donor Hong XieNuo for high anthocyanin content) and Koshihikari. SSR markers RM 7405 and RM 7419 on chromosome 1 were linked to Kala1, while Kala3 was mapped between the SSR markers RM15191 and RM 3400 on chromosome 3. Kala4 was found between SSR markers RM 1354 and RM 7210 on chromosome 4. Rd locus coding for the DFR enzyme in anthocyanin biosynthesis was included in the chromosomal region of Kala1 but it is regulated by the members of the Kala4 locus (Figure 3). Thus, Rd is similar to Pp, which is believed to be Kala1. As the Kala3 was identified as transcription factor MYB3, in the presence of functional Kala1, Kala3 and Kala4 the rice pericarp colour would be purple. However, in the absence of functional Kala4, the rice pericarp will be white.

Oikawa *et al.* (2015) reported that as Kala 4 and Pb were mapped to the same region, *Pb* was suggested to be an allele of the Kala4 locus. Kala4 upon fine mapping resulted in identification of the gene Os04g0557500 responsible for pigmentation in rice grains. Its duplication along with its insertion in the promoter region of the gene was responsible for the black pericarp phenotype. Kala4 identified as a bHLH gene activates anthocyanin synthesis genes, such as chalcone synthase and dihydroflavonol-4-reductase leucoanthocyanidin reductase and leucoanthocyanidin dioxygenase, by undergoing rearrangement in the promoter region and expressing ectopically in the pericarp region of the grains. As Kala4 and OsB2 were similar in function they may be identical whereas the Pb is likely to be same as the gene Ra and OsB1.

Xu *et al.* (2019) identified a novel quantitative trait loci (QTLs) qANC3 and qPAC12-4 with significant phenotypic variance underlying anthocyanins and proanthocyanidins (ANC and PAC) in rice using recombinant inbred lines (RILs) derived from a cross of red rice Hong Xiang 1 (HX1) and white rice Song 98-131 (S98-131).

Bastia *et al.* (2022) validated the QTLs for qANC3 and qPAC12-2 for anthocyanin and proanthocyanidin content and reported that markers, *viz.,* RM440, RM5638, RM253 and RM5626 on chromosomes 5, 1, 6 and 3 were strongly associated with this trait.

Fathima *et al.* (2022) identified two SSR markers RM228 (Chromosome 10) and RM 297 (Chromosome 1) using the F_2 population of ADT(R)48/Kavuni for anthocyanin content in rice and validated these markers in various forms of Kavuni, pigmented landraces, and improved cultivars.

Mapping studies on the identification of QTLs underlying the anthocyanin content in pigmented rice are at an infant stage. Whole genome resequencing offers a promising approach for the identifying new allelic variants using significant genetic diversity among the pigmented landraces for anthocyanin content in order to expediate the discovery of functional genes and markers influencing anthocyanin content and grain coloring.

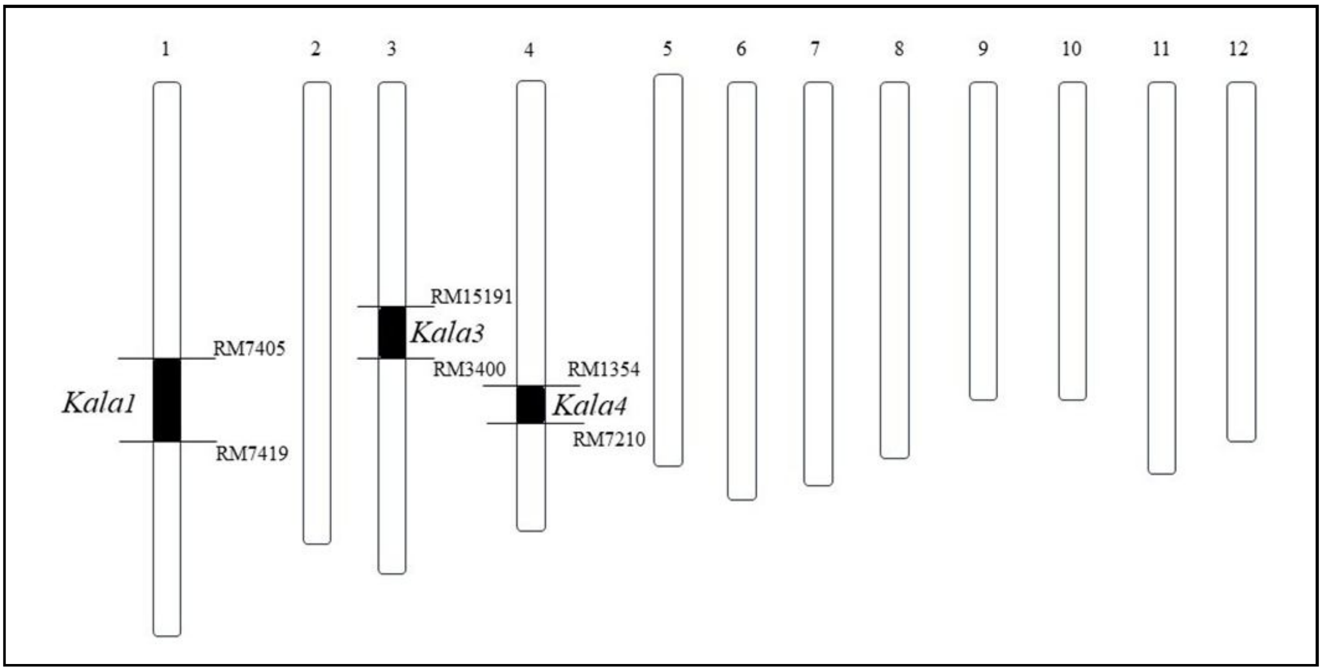

Figure 3: Graphical representation of key activator loci Kala1, Kala3 and Kala4 on chromosome 1, chromosome 3 and chromosome 4.

5. Conclusion

Pigmented landraces have gained momentum in the recent past due to the widespread occurrence of lifestyle related diseases. Based on metabolomic studies, it is evident that black rice varieties possess bioactive compounds and mineral nutrients that are essential for healthy living. Black rice has inherent anthocyanin content and antioxidant properties owing to the presence of cyanidin-3-glucoside, which scavenges the free radicals generated due to oxidative stress. Anthocyanin pigments with anticancerous, antidiabetic and anti-inflammatory properties confer nutraceutical value to deep pigmented landraces. Thus, the incorporation of black rice in their daily diet can be suggested as the cheapest option for poor people to overcome ever rising health related issues. Nevertheless, black pigmented landraces being poor yielders need to be genetically improved without compromising their nutritional and health promoting qualities to ensure food and nutritional security. Systematic assessment of the pigmented landraces with therapeutic significance distributed in South and South East Asian countries like India, Thailand, Sri Lanka, Indonesia and China will help achieve nutritional diversification targeting lower income nations of the world. These collections provide a significant genetic diversity that can be utilized to elucidate the genetics underlying grain pigmentation and associated nutritional and therapeutic features. Fine mapping of genomic regions linked to anthocyanins using third generation sequencing techniques is crucial for the development of high-quality markers facilitating marker-assisted selective breeding of high yielding varieties with medicinal value.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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