

Chapter 2

Black Rice

2.1 Introduction

Black rice is a type of the rice species *Oryza sativa* L. which is glutinous, packed with high level of nutrients and mainly cultivated in Asia. The pericarp (outer part) of kernel of this rice colour is black due to a pigment known as anthocyanin, an antioxidant. Black rice is also known as purple rice, forbidden rice, heaven rice, imperial rice, king's rice and prized rice. Many people assume this rice as a panacea of many culinary diseases because of its high nutritive value and curative effect. This rice is supposed to enhance the longevity of life, hence it is also known as long life rice. This rice includes several varieties with a long history of cultivation in Southeast Asian countries such as China, India and Thailand (Kong et al. 2008). There are more than 200 types of black rice varieties in the world. Only China is responsible for 62 % of global production of black rice and it has developed more than 54 modern black rice varieties with high yield characteristics and multiple resistances. China cultivates the most black rice followed by Sri Lanka, Indonesia, India and Philippines etc. Thailand occupies the ninth position to black rice cultivation (Ichikawa et al. 2001; Sompong et al. 2011). Interest in black rice is indicated by the number of accessions held in germplasm collections, e.g. China–359, Sri Lanka–50, Indonesia–42, India–30, the Philippines–25 and Bangladesh–24 (Chaudhary 2003). Nutrients such as protein, minerals (Ca, P, Fe, and Zn) and dietary fiber contents are higher in black rice compared to brown and white rice. Demand for this rice is growing fast in the USA and European countries due to its value as a healthy food and its attractive organic food color. Ichikawa et al. (2001) reported that black rice is efficient, and two fold stronger with respect to antioxidant activities of blueberries. Black rice is surely a special breed of rice that is cultivated on earth. This rice is getting popular in recent years because of its high nutritive value and antioxidative properties.

Black rice is actually heirloom rice, means it is open pollinated, was grown at earlier times in the history, and is not grown on a large scale in modern agriculture.

The term ‘black rice’ actually refers to a variety of rice types from the species *Oryza sativa*, and is descriptive of the colour of grain, rather than other properties. Black rice also comes in a number of short grain, long grain and glutinous varieties similar to brown rice. This rice has an incredibly rich history and among its strains has one variety known as “Imperial Rice.” Imperial rice was reserved for the emperor’s consumption only. Black rice, as one would imagine, is deep black in color and mutates into a regal purple hue when cooked. The purple colour is due to the grain’s naturally high anthocyanin content, a trait most typically observed in fruits such as blueberries and blackberries. Black rice has dark purple hues in its outer bran layer that are so intense that the rice appears to be black. Once cooked, the color lightens into that same deep purple/violet found in blueberries. This dark purple color predominantly comes from anthocyanins which are flavonoids that perform as antioxidants in the body.

There exists no other rice with a higher nutritional spectrum near black rice. This rice is free of gluten, free of cholesterol, low in sugar, salt and fat. Black rice is a whole grain, super nutritious type of rice that is high in fiber, anthocyanin, antioxidants, vitamins B and E, iron, thiamine, magnesium, niacin and phosphorous. A huge number of scientific studies show that black rice powder is one of nature’s most well balanced superfood and its abilities are truly remarkable. Black rice anthocyanins (BRACs) are a kind of anthocyanins that are extracted from the aleurone layer of black rice which is a major cereal crop existing since ancient times in China and other Eastern Asia countries (Ling et al. 2002). The anthocyanin components in BRACs are about 26.3 %, and cyaniding-3-O-glucoside and peonidin-3-O-glucoside are the main effective constituents accounting for about 90 % (Chang et al. 2010). Among different compounds of black rice, anthocyanin is the one which mop up harmful molecules and help to protect arteries and prevent the DNA damage. Anthocyanins are the flavonoid pigments of black rice and are the source of antioxidants that have the ability to inhibit the formation or to reduce the concentrations of reactive cell damaging free radicals (Adom and Liu 2002). Black rice extracts could scavenge superoxide anions more effectively than hydroxyl radicals (Nam et al. 2006). This rice has long been consumed in Korea, Japan and China (Ryu et al. 2000; Han et al. 2004). Black rice has been eaten throughout Asia for thousands of years and has a significant history of use in China, India and Thailand. Up until modern times, black rice was not easy to come by. It had been highly treasured and protected in Asia for many centuries. But black rice consumption is more common nowadays. This rice is becoming popular among rice consumers and dieticians day by day mainly because of its high nutritive and medicinal value. Therefore, black rice is becoming the new “IT” organic food that everyone is talking about and the attention it is getting is well deserved (Fig. 2.1).

Dictionary meaning of black rice (Segen’s Medical Dictionary 2012).

A strain of rice that has currency as both a food and medicine. It owes its dark colour to the high concentration of anthocyanins, which are potent antioxidants. It is regarded as a ‘superfood’ that may lower the risk of cancer due to its high concentrations of fibre, B vitamins, niacin, vitamin E, calcium, magnesium, iron, and zinc.



Fig. 2.1 A typical kernel of Chinese black rice with attached hull and dehulled

Black rice is typically sold as unmilled rice, means the fiber rich black husks of the rice are not removed. This rice is commonly used as a condiment, dressing, or as a decoration for different types of desserts in many countries around the world. The unusual colour makes it very popular for desserts and the high nutritional value is an added benefit. This rice is often served with fresh fruit such as mangoes and lychees, especially when drizzled with a fruit or rice syrup. Traditionally this rice has been used in China and Taiwan as dessert rice, but it can actually be used in almost any sweet or savory dish where plain rice would be used. Its pitch is black when raw, but it actually turns a beautiful, shiny indigo when cooked. Its flavor is richer and sweeter than white rice and its texture is slightly sticky. This short grain rice has a slightly nutty flavor, and its texture is smooth and firm. It is suitable for making porridge, dessert, traditional Chinese black rice cake or bread. Noodles also have been produced from black rice. Black rice is usually consumed mixed with white rice in Korea. The grain has a similar amount of fiber to brown rice and like brown rice, it has a mild and nutty taste.

Black rice includes many more varieties of dark colour rice like Forbidden rice, Purple rice, Japonica black rice, Chinese black rice, Indonesian black rice and Thai black rice. The reason they are grouped under the term “Black Rice” is the unusual dark/black colour of the grain. Black rice has diversity in terms of colour due to anthocyanin content and other morphological characters. Looking black rice in the morning is an indication that the whole day will be successful. Based on historical record, black rice was only for the kings of China and Indonesia (forbidden rice). This is because black rice has a double function, namely as a source of staple food with good taste, fluffier and fragrance, as well as an efficacious medicine to cure various illnesses (Kristantini 2009). Black rice is the most popular staple food in

Europe even more than Southeast Asia (Simmons and Williams 1997). This rice is not currently grown on a commercial scale in the US, Europe and other parts of the world but it is hoped that its commercial cultivation will cover the world. Black rice is still a niche rice product, but its popularity seems to be growing.

Black rice special and medicinal values are truly stunning even today with all of our medical knowledge and tools. Thus black rice is a kind of food that can make us healthy and save our life. Black rice is a whole grain and nowadays, whole grain is categorized as one of the potent functional food sources since it contains high amounts of phenolic compounds, especially anthocyanins in pericarp (Abdel-Aal et al. 2006; Ryu et al. 1998a, b; Yawadio et al. 2007). Black rice has long been consumed in Japan and China and is considered to be a healthy food because of its antioxidant content that are able to prevent oxidative stress. Oxidative modification of low-density lipoprotein (LDL) may play an important role in the development of atherosclerosis. Chinese black rice is rich in iron and is considered as a blood tonic. This rice is claimed to be good for the kidney, stomach and liver in China. Previous studies show that black rice was an alternative healthy food for diseases treatment as it contains antioxidative agent, such as anthocyanin (Chutipaijit et al. 2011). This rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki et al. 2004). Compared to white rice, black rice is relatively rich in the mineral contents such as Fe, Zn, Mn and P and has higher variability in mineral content that depend upon varieties and soil types of the planting area (Qiu et al. 1993; Liu et al. 1995; Zhang 2000). Black rice has high nutritional value and it contains the highest levels of anthocyanin. Its dark purple color is primarily due to its anthocyanin content, which is higher by weight than that of other coloured grains. Anthocyanins are a group of reddish purple water soluble flavonoids (Shen et al. 2009) located on pericarp, seed coat and aleurone layer (Sompong et al. 2011; Ryu et al. 1998a, b; Takashi et al. 2001). Black glutinous rice has been also shown to accumulate compounds such as anthocyanins (Zhang et al. 2004a, b; Abdel-Aal et al. 2006) and gamma oryzanol (Rong et al. 1997; Qureshi et al. 2000; Van 2004; Juliano et al. 2005).

2.1.1 Chinese Black Rice

Among all black rice varieties, Chinese black rice is the one which is most popular and it has its own history and medicinal property. In China, the least known and least used rice is Chinese black rice. It is a short grain glutinous rice. This purple cooking rice is grown on the banks of the Yangzi river. Chinese black rice is different from other rice. It is very short with low fat, and is rarely polished. It tastes nuttier than any other rice after cooking. Special characteristics of Chinese black rice is that it is black on the outside and black on the inside. Not all black rices look like this. Chinese black rice and most glutinous rices are exceptionally high in amylopectin, and if cooked too long they disintegrate. That's why Chinese black rice is never milled and always cooked with seed coat or hull intact. Traditionally,

this black rice was steamed in a bamboo tube for two hours. Nowadays, it is boiled for about forty minutes or steamed for an hour. No matter how the rice are cooked, immediately after cooking, it is best to leave this rice sit for about twenty minutes to absorb any remaining water; this allows the grains to separate. Chinese black rice is a food, eaten frequently by many Chinese peoples and people groups, particularly those in and around the Yunnan Province where it is grown mostly. People like this rice because of its sweet flavor. They also like this rice because it remains chewy and has a starchy taste. Some people like it because it does not need to be washed, though many recipes suggest doing so (www.flavorandfortune.com). Those who have celiac disease, their intestinal villae do not allow them to properly absorb gluten, can eat this glutinous black rice. There is no evidence where this glutinous rice acquired its name, the assumption is that it is like gluten when wet and looks very sticky. Wheat flour has lots of gluten, and the gluten in it provides structure to foods when wet and heated. That is why, with a leavening agent, breads rise and when heated (baked) hold their shape. With or without a leavening agent, rice can not make nor can it hold a structure or shape because it has no gluten.

Chinese black rice (Forbidden rice) is medium grained glutinous rice which is different from purple sticky rice. It is not as sweet, although still sweeter than most rice varieties. Black rice can be used in both sweet and savory dishes. It was once known as Emperor's rice because it was only served to Chinese Emperors and was forbidden else where. Black rice is now widely available and even noodles made from black rice are available today. This rice is high in fiber and minerals, more vitamins than regular rice varieties and with nutty flavor makes a great rice pudding and is used in other sweet dishes. This rice is also popular in Indonesia and Thailand and it is not generally used as the main starch in a meal. Black rice is known for its health benefits and was favored by Chinese Emperors as a tonic. Its black colour is not artificial, the plant has distinctive black tops which stand out from traditional rice paddies. There are records of black rice being grown around 150 BC, and it is considered lucky as well as nutritious. Traditionally served in Taiwan and mainland China as a dessert ingredient in the West. This rice is most often found served with main courses. Chinese black rice has a beautiful shiny, indigo colour after cooked.

2.1.2 The Forbidden Rice

Chinese black rice is also known as forbidden rice and it is an ancient grain that has even more impressive health benefits than most other closely related rice varieties, though it is less popular than brown or wild rice. It is not only the rice type that is richest in disease fighting antioxidants, but it also contains dietary fiber, anti-inflammatory properties and has the ability to help and stop the development of diabetes, cancer, heart disease and even weight gain. Black rice has been eaten in regions of Asia for thousands of years, infact for centuries it was reserved for only Chinese royalty. Black rice was reserved in ancient China as the most rare,

nutritious and tasty of all rice. Chinese referred it as “forbidden rice” only to be eaten by nobility. Unfortunately, it fell out of favor through the centuries and is now used in Asia primarily as food decoration and as a component of noodles, sushi and desserts. It is still a complete rarity in the West, but its new status as a super food that can fight cancer and prevent heart disease may bring it out of hiding and back into the favor of peoples.

2.1.3 *The Emperor’s Rice*

Chinese black rice was set aside exclusively for royalty in ancient China. It was thought that consumption of black rice would extend their lives. Royal families and kings used to eat this special rice to retain good health and make themselves fortune. In light of recent scientific studies on the anti aging effects of antioxidants, it seems that this ancient knowledge was on the mark. Black rice is super nutritious when compared to other varieties of rice. This rice is rich in fiber, as well as iron, vitamin E and is a host of other crucial minerals. Black rice has been characterised by the accumulation of phenolic acids, flavonoids and anthocyanins exhibiting antioxidant activities (Kaneda et al. 2006). However, black rice yield is lower than that of white rice which does not contain anthocyanins.

2.2 Origin and Domestication of Black Rice

The origin and spread of novel agronomic traits during crop domestication are complex events in plant evolution. Wild rice (*Oryza rufipogon*) has red grains due to the accumulation of proanthocyanidins, whereas most cultivated rice (*Oryza sativa*) varieties have white grains induced by a defective allele in the Rc basic helix-loop-helix (bHLH) gene. Although the events surrounding the origin and spread of black rice traits remain unknown, varieties with black grains due to anthocyanin accumulation are distributed in various locations throughout Asia. Black grain trait originated from ectopic expression of the Kala4 bHLH gene due to rearrangement in the promoter region. Both the Rc and Kala4 genes activate upstream flavonol biosynthesis genes, such as chalcone synthase and dihydroflavonol-4-reductase, and downstream genes, such as leucoanthocyanidin reductase and leucoanthocyanidin dioxygenase, to produce the respective specific pigments. Genome analysis of black rice varieties as well as red and white grained landraces demonstrated that black rice arose in tropical *japonica* and its subsequent spread to the *indica* subspecies can be attributed to the causal alleles of Kala4. The relatively small size of genomic fragments of tropical *japonica* origin in some *indica* varieties indicates that refined introgression must have occurred by natural crossbreeding in the course of evolution of the black trait in rice (Oikawa et al. 2015). The black (or purple) grain colour has not been observed in any accessions

of *O. rufipogon* (<http://www.gramene.org>). Thus, the black rice trait is most likely newly acquired, incorporated either during or after rice domestication. Compared with the broad distribution of white rice cultivars, black rice cultivars are sporadically distributed throughout Asia. Black rice was used in ancient China before Chinese dynastic times (Newman 2004) and was sometimes called “emperor’s rice” (or “forbidden rice”) since it was used as a tribute food and prized for its rarity in ancient China. It is completely unknown how this aesthetic trait arose and has been maintained for such a long time.

Oikawa et al. (2015) demonstrated that genetically the Kala4 gene involved in the origin of black rice corresponds to Os04g0557500 gene. Kala4 encodes a bHLH transcription factor, which is a rice homolog of the maize R/B gene, previously reported as the OSB2 gene (Sakamoto et al. 2001). Oikawa and his friends further demonstrated that a structural change in the Kala4 promoter induced ectopic expression of the bHLH protein and the subsequent activation of the anthocyanin biosynthesis pathway. With this structural change and rearrangement in Kala4, rice acquired the brand new black grain colour trait, resulting in the birth of black rice. Oikawa also demonstrated that a relatively small genomic segment around the Kala4 gene that originated from tropical *japonica* was transferred into the *indica* subspecies, probably as a result of refined introgression through multiple natural crossbreeding processes. The results described reveal the process that led to the neo functionalization of Kala4 and subsequent spread of the black rice allele of Kala4 to other subspecies of rice. Since closely related structural changes of the Kala4 promoter were observed in the 21 black rice landraces, it is likely that black rice possessed the red trait in the course of its evolution. Subsequently, several structural changes must have occurred and spread to other varieties with some DNA variations (Oikawa et al. 2015).

Domestication Process

Description of Fig. 2.2

The processes involved in the creation of black rice and its spread can be divided into three categories, i.e. domestication (in blue frame), black rice conversion (in black frame) and spread (in orange frame).

2.2.1 Domestication Process

Or-IIIa type *O. rufipogon* is the most closely related ancestral varieties of *japonica* cultivars and landraces (Lu et al. 2008, Huang et al. 2012). The two *O. rufipogon* accessions, W1943 and W1963 which belong to Or-IIIa, do not have LINE1 insertion at intron 2 of the *Kala4* gene (data from Gramene database, www.gramene.org). This observation suggests that the LINE1 insertion occurred after

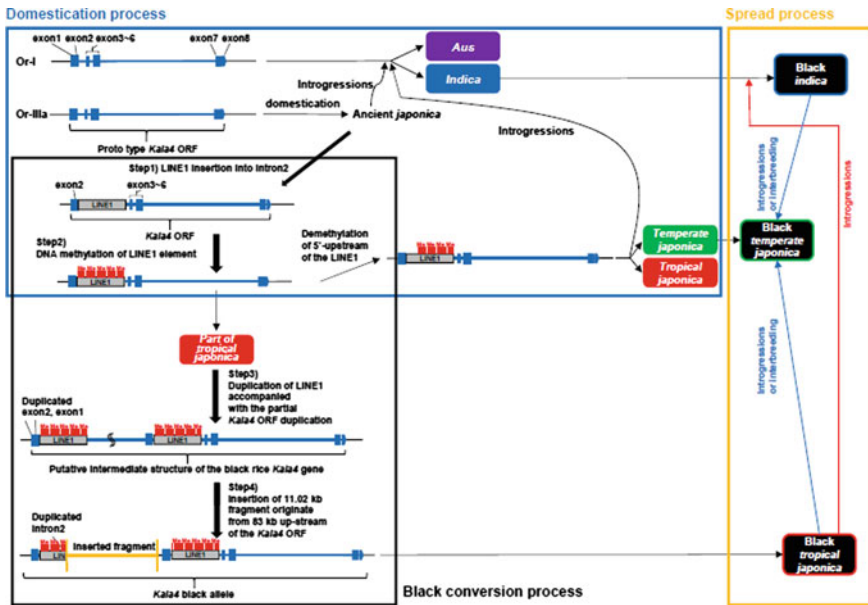


Fig. 2.2 Schematic diagram of a model showing the processes in the origin of black rice and the spread of the *Kala4* black allele (Oikawa et al. 2015)

domestication. Moreover, the LINE1 insertion at this position was observed in both *tropical japonica* and *temperate japonica*. Therefore, the LINE1 insertion probably occurred in the common ancestor before the differentiation to the two *japonica* subspecies (step1) (however, in *temperate japonica* subspecies, the LINE1 insertions were surveyed in only two cultivars). There are relatively few LINE1-inserted cultivars in *indica* and *aus* subspecies, suggesting that the LINE1-inserted allele of the *Kala4* locus was transferred from ancient *japonica* or *tropical japonica* to *indica* and *aus* subspecies.

2.2.2 Black Conversion Process

The difference of the DNA methylation status at intron 2 of the *Kala4* gene is observed in only few varieties of *tropical japonica* subspecies (however, only 2 white *temperate japonica* and no *indica* varieties were investigated). Higher DNA methylation of the LINE1 element has been thought to be necessary for inactivation of LINE1 transposition. Therefore, DNA methylation of the LINE1 element likely occurred immediately after LINE1 insertion (step 2), and then after the nonsense mutation arose in ORF2, the DNA methylation status of the 5'-upstream region of LINE1 was decreased in the common ancestral varieties of most *temperate*

japonica and *tropical japonica*. However, the higher DNA methylation status 5'-upstream of LINE1 remained in some *tropical japonica*, and the variety was the origin of black rice. In this variety, the duplication of the LINE1 sequence accompanied by part of *Kala4* structure likely occurred (step 3), followed by insertion of the 11.02-kb fragment originated from approximately 83-kb upstream of the *Kala4* ORF (step4). As a consequence of these duplication and insertion events, the *Kala4* expression was upregulated in both transcription and translation, resulting in the creation of the black *Kala4* allele.

2.2.3 Spread Process

The black allele of the *Kala4* locus which was generated in *tropical japonica* subspecies, was transferred into *indica* subspecies by introgression (red arrow). The identical alteration of the *Kala4* promoter structure is also observed in black rice of *temperate japonica* subspecies. The black allele of the *Kala4* locus likely was transferred from black *indica* or black *tropical japonica* subspecies by interbreeding (blue arrows).

2.3 Genetics of Black Rice

Molecular genetic background of black rice, however, is not well identified. Among the various coloured rice, black rice is the one which is characterized by dark purple pericarps in seeds with high levels of anthocyanins. The name black rice refer to the kernel colour (black) of the rice which is formed by deposits of anthocyanins in different layers of the pericarp, seed coat and aleurone (Chaudhary 2003). During rice seed development, purple pigments of anthocyanin accumulate rapidly in the pericarp, resulting in the characteristic dark purple grains of black rice (Abdel-Aal et al. 2006; Shao et al. 2011). Previous genetic investigations have shown that cyanidin-3-O-glucoside and peonidin-3-O-glucoside are the two primary anthocyanin pigments deposited in the seed pericarps of black rice (Abdel-Aal et al. 2006; Kim et al. 2011; Zhu et al. 2010). The pericarp pigmentation of black rice requires two genes, PURPLE PERICARP A (Pp, Prpa and Prp1) and PURPLE PERICARP B (Pb, Prpb and Prp2) located on chromosomes 1 and 4 respectively (Hu et al. 1996; Oryzabase, www.gramene.org; Wang and Shu 2007; Wang et al. 2009; Yoshimura et al. 1997). The Pp gene acts in a complementary fashion with the Pb gene for the production of purple pericarps in rice (Hsieh and Chang 1964; Wang and Shu 2007). The presence of at least a dominant Pb allele is an essential factor for colour development in rice pericarps. The Pp allele in rice is incompletely dominant to the recessive pp allele; thus, the number of dominant Pp alleles determines the concentration of cyanidin-3-O-glucoside in black rice (Rahman et al. 2013). R/B homolog genes have been reported to be involved in the regulation of

anthocyanin biosynthesis (Hu et al. 1996, 2000) in rice. Only two loci for the red rice trait have been reported; Rc, which encodes a bHLH transcription factor, and Rd, which encodes dihydroflavonol-4-reductase (DFR) (Sweeney et al. 2006; Furukawa et al. 2007). Red rice accumulates tannins in pericarp/testa. The loss of function of two genes, Rd and Rc, changed the colour to white during rice domestication. Black rice is likely to be related to the red rice but accumulates anthocyanins. Up-regulation of *LDOX* in pericarp/testa due to a gain of function event in *kala4* played a pivotal role for the creation of black rice, and the origin of the functional allele of *kala4* occurred in tropical *japonica* rice (Oguchi et al. 2012).

A whole genome survey of the introgression line using DNA markers suggested three regions on chromosomes 1, 3 and 4 are associated with black pigmentation. The locus on chromosome 3 has not been identified previously. These loci are named as Kala1, Kala3 and Kala4 (Maeda et al. 2014). Lee et al. (2015) used a 135 K rice microarray to identify genes involved in anthocyanin biosynthesis and metabolism in black rice. Using multi-interaction screening method, they screened 427 expression genes, 754 orthologous genes, and 416 pathway-network related genes associated with anthocyanin biosynthesis. Eight candidate genes were also identified by comparing pathway expression genes including ortholog-ontology genes. Transcription factor is related to anthocyanin pigmentation biosynthesis. All black rice strains carry Wx (b) allele and the duplication of the 23 bp sequence motif in the exon 2 of the wx gene. This evidence is a common phenomenon in glutinous rice (Prathepha 2007). To better understand the functional characterization of anthocyanin gene expression in black rice, Kim et al. (2010) performed a detailed computational examination. They identified 12 unknown and hypothetical genes involved in anthocyanin biosynthesis. These genes likely play either a regulatory role in the anthocyanin production process or are related to anthocyanin metabolism during flavonoid biosynthesis. The sequence of the OSB1 gene in black rice was found to differ from that in red and white rice. The sequence of the Rc gene in red rice also differed from that in white and black rice (Lim and Ha 2013). The Os01g0781600 and Os01g0748150 genes were highly induced during the early heading stage, suggesting that these genes may play a role in anthocyanin production in the early black rice heading stage (Kim et al. 2010). Black rice upregulation genes are Os01g0781600, Os10g0315400, Os01g0633500, Os08g0389700, Os01g0615050, Os01g0959100, and Os01g0748150 and down regulation genes are Os01g0246400, Os02g0113800, actin (Kim et al. 2010).

The genetic mechanism involved in a transition from the black coloured seed hull of the ancestral wild rice (*Oryza rufipogon* and *Oryza nivara*) to the straw white seed hull of cultivated rice (*Oryza sativa*) during grain ripening remains unknown. Zhu et al. (2011) reported that the black hull of *O. rufipogon* was controlled by the Black hull4 (Bh4) gene, which was fine mapped to an 8.8-kb region on rice chromosome 4 using a cross between *O. rufipogon* W1943 (black hull) and *O. sativa indica* cv Guangluai 4 (straw-white hull). Bh4 encodes an amino acid transporter. A 22-bp deletion within exon 3 of the bh4 variant disrupted the Bh4 function, leading to the straw white hull in cultivated rice. Transgenic study indicated that Bh4 could restore the black pigment on hulls in cv Guangluai 4 and

Kasalath. Bh4 sequence alignment of all taxa with the outgroup *Oryza barthii* showed that the wild rice maintained comparable levels of nucleotide diversity that were about 70 times higher than those in the cultivated rice. The results from the maximum likelihood Hudson-Kreitman-Aguade test suggested that the significant reduction in nucleotide diversity in rice cultivars could be caused by artificial selection. Zhu et al. (2011) proposed that the straw-white hull was selected as an important visual phenotype of non shattered grains during rice domestication.

The rice cultivar show great diversity in genetic background, composition, granule size and thermal and rheological properties of starches (Sodhi and Singh 2003; Xia et al. 2006). The starch separated from the rice varies significantly in its composition during rice grain development (Ashoka et al. 1985). Black rice varieties are represented in two categories: grain with purple pigmentation on glumes and various colour shades on the pericarp and grains with yellow straw glumes and purple pericarp. In most cases the purple pericarp colour is associated with purple glumes. Based on two growing condition for black rice, rainfed lowland and rainfed upland, chloroplast DNA type is distinct from each other. All rice strains from rainfed lowland is deletion plastotype, but all other rainfed upland strains are non-deletion types (Prathepha 2007) (Table 2.1, Fig. 2.3).

2.4 Health Benefits

American Health Association, the American Cancer Society and the 2005 Dietary Guidelines for Americans recommended an increase in the consumption of black rice to prevent heart disease and certain kinds of cancers (USA Rice Federation 2008). Moreover, the US Food and Drug Administration have recognized black rice as a healthy whole grain capable of reducing the risk of certain diseases. Unfortunately, it is not well accepted among many ones since it is difficult to cook and because of its distinct off taste, dark appearance and hard cooked rice texture. Sprouted brown, red and black rice contain anthocyanins, an antioxidants found in blueberries, grapes and acai that have been linked to a decreased risk of heart disease and cancer (USA rice Federation). Research suggests that plant antioxidants which mop up harmful molecules can help to protect arteries and prevent the DNA damage that leads to cancer (www.independent.co.uk).

Black rice contains many vitamins and minerals, including iron, vitamin A and vitamin B, which are beneficial for overall health and the prevention of heart disease (Chen et al. 2003). The health benefits of black glutinous rice have recently been reported by several investigators. A recent report showed that anthocyanin supplementation in humans improves LDL and HDL levels (Qin et al. 2009) and can delay cancer development in rodents models of carcinogenesis (Thomasset et al. 2009). Black rice may have antiatherogenic activity and may improve certain metabolic pathways associated with diets high in fructose (Guo et al. 2007). These marked health benefits have been attributed to the antioxidant properties of anthocyanin (Wu and Prior 2005). Reactive free radicals contribute to the

Table 2.1 Anthocyanin biosynthesis-related genes in rice and maize

Categories	Proteins	Species	Gene names	Locus IDs	References
Structural genes	Chalcone synthase (CHS)	Maize	Colored aleurone2 (C2) ^a	GRMZM2G151227 (Chr.2)	Wienand et al. (1986)
		Rice	OsCHS1 ^b	Os11g0530600	Reddy et al. (1996)
		Rice	OsCHS2 ^b	Os07g0214900	Shih et al. (2008)
	Chalcone isomerase (CHI)	Maize	CHI1 ^b	GRMZM2G155329 (Chr.1)	Grotewold and Peterson (1994)
		Rice	OsCHI ^b	Os03g0819600	Druka et al. (2003)
	Flavanone 3-hydroxylase (F3H)	Maize	F3H ^b	GRMZM2G062396 (Chr.2)	Deboo et al. (1995)
		Rice	OsF3H-1 ^b	Os04g0662600	Kim et al. (2007)
		Rice	OsF3H-2 ^b	Os10g0536400	
	Flavanone 3'-hydroxylase (F3'H)	Maize	Purple aleurone1 (Pr1) ^a	Os04g0667200	
		Rice	OsF3'H ^b	GRMZM2G025832 (Chr.5)	Sharma et al. (2011)
		Rice	OsF3'H ^b	Os10g0320100	Shih et al. (2008)
	Dihydroflavonol reductase (DFR)	Maize	Anthocyaninless1 (A1) ^a	GRMZM2G026930 (Chr.3)	O'Reilly et al. (1985)
		Rice	Rd ^a	Os01g0633500	Furukawa et al. (2007)
	Leucoanthocyanidin dioxygenase (LDOX)	Maize	Anthocyaninless2 (A2) ^a	GRMZM2G345717 (Chr.5)	Menssen et al. (1990)
		Rice	OsANS1 ^b	Os01g0372500	Shih et al. (2008)
UDP-glycosyl transferase (UGT)	Maize	Bronze1 (Bz1) ^a	Os06g0626700		
	Maize	Bronze1 (Bz1) ^a	GRMZM2G165390 (Chr.9)	Dooner and Nelson (1977)	
	Rice	OsUGT ^b	Os06g0192100	Tanaka et al. (2008)	

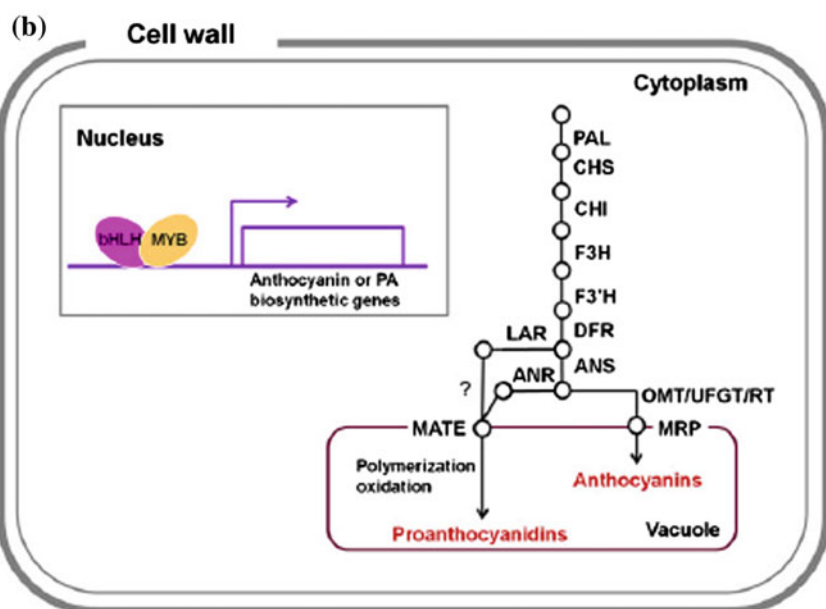
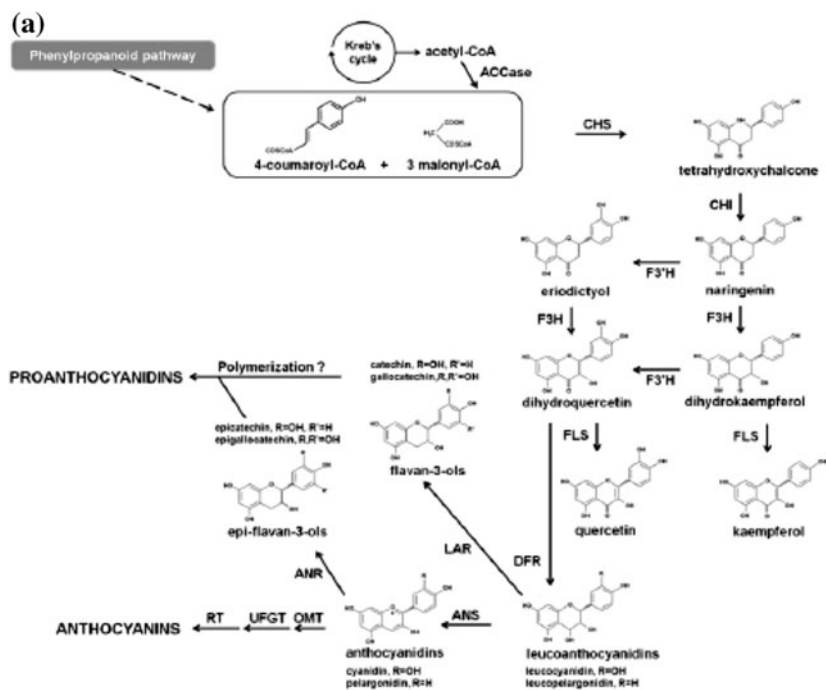
(continued)

Table 2.1 (continued)

Categories	Proteins	Species	Gene names	Locus IDs	References
Regular genes	Basic helix-loop-helix transcriptional factor (bHLH)	Maize	Red (R) ^a	GRMZM5G822829 (Chr.10)	Styles et al. (1973)
			Booster (B) ^a	GRMZM2G172795 (Chr.2)	Chandler et al. (1989)
		Rice	OSB1 ^b	Os04g0557800	Sakamoto et al. (2001)
			OSB2 ^b	Os04g0557500	
	R2R3-Myb transcriptional factor (Myb)	Maize	Colored aleurone1 (C1) ^a	GRMZM2G005066 (Chr.9)	Paz-Ares et al. (1987)
			Purple plant (P1) ^a	GRMZM2G701063 (Chr.6)	Cone et al. (1993)
WD40 repeat		Rice	OsC1 ^b	Os06g0205100	Saitoh et al. (2004)
		Maize	Pale aleurone color1 (PAC1) ^a	GRMZM2G058292 (Chr.5)	Carey et al. (2004)
		Rice	Unidentified		

^aThese genes were genetically identified in maize or rice

^bThese genes were identified based on sequence similarity with other plant species. Although there are many homologous genes annotated in both genomic sequences, only the genes that have been reported as cloned are described in this table



◀ **Fig. 2.3** Schematic representations of anthocyanin and PA biosynthetic pathways in rice. **a** Pathway for anthocyanin and PA biosynthesis. The first committed step is catalyzed by chalcone synthase (CHS) using malonyl CoA and 4-coumaroyl CoA as substrates. Enzymes are as follows: acetyl CoA carboxylase (ACCase), anthocyanidin synthase (ANS), anthocyanidin reductase (ANR), chalcone isomerase (CHI), dihydroflavonol 4-reductase (DFR), flavanone 3-hydroxylase (F3H), flavonoid 3-O-hydroxylase (F3OH), flavonol synthase (FLS), leucoanthocyanidin reductase (LAR), O-methyltransferase (OMT), rhamnosyl transferase (RT) and UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT). **b** Proposal for anthocyanin and PA regulation. Either MYB or bHLH transcriptional factors may bind the promoter region and control the expression of anthocyanin and PA biosynthetic genes. Biosynthetic steps that remain unknown are indicated by a question mark (Lim and Ha 2013)

development of chronic inflammatory proliferative diseases (CIPDs) (Ishihara and Hirano 2002), particularly arteriosclerosis and cancer by causing oxidative damage to essential enzymes, cells and tissues (Klaunig and Kamendulis 2004; Namsh et al. 2006). Especially very high anthocyanin content with superior antioxidant in sticky black rice help to prevent the harmful effects of free radicals (Tananuwong and Tewaruth 2010). The anthocyanins in rice act as antioxidants which can inhibit inflammation throughout the body (Tsuda et al. 1996), act as anticancer agents (Kamei et al. 1995; Martin et al. 2003; Hyun and Chung 2004; Zhao et al. 2004; Chen et al. 2006), promote blood circulation, slow damage and aging of tissues, reduce cholesterol and blood sugar levels (Tedesco et al. 2001; Hirunpanich et al. 2005; Rechner and Kroner 2005), affect pituitary gland function, inhibit gastric acid secretion and inhibit platelet aggregation (Butelli et al. 2008). Defa and Meizu (2006) showed the presence of nutrients especially in sticky black rice as fiber, protein, essential amino acids, B vitamins, minerals etc. that stand out from the others are completely beneficial to human health. The use of food products from rice gave up germs that help to prevent headaches, relieve symptoms of constipation, prevent colon cancer, adjust blood sugar levels, prevent heart disease, lower blood pressure and prevent Alzheimer's disease (Kayahara and Tsukahara 2000). Young and Kim (2007) also found that the antioxidant activity of the extract from rice germ is 1.3–1.6 times higher than the regular white rice. It is advised that rice germs have the ability to improve mental health and immunity in women who are breast feeding (Shigeko et al. 2007). A study was conducted in China on the nutritional composition of sticky rice as “A study on special nutrient of purple glutinous rice”, and Defa found that the presence of nutrients especially in sticky rice as fiber, protein, B vitamins, minerals (Ca, P, Fe, etc.) are much higher than ordinary rice and completely beneficial to human health (Defa and Meizu 2006). A number of studies have showed black rice compounds can reduce low-density lipoprotein cholesterol (LDL), improve lipid profiles, have anti-inflammatory and antioxidative activities, may help to fight heart disease and prevent diabetes (Guo et al. 2007).

Free radicals are unstable and highly reactive molecules. It is normal for bodies to produce these free radicals in small amounts. However many factors like metabolic stress and UV radiation can increase the formation of these free radicals. Free radicals cause oxidative damage within the body which may eventually result

in DNA and protein damage and even cell death. Antioxidants are able to neutralise these free radicals, and can help to prevent oxidative damage. Studies show that antioxidant supplementation can exert a preventative effect against the development of serious conditions like cancer, and may improve overall health. Antioxidant supplementation has been shown to lower markers of inflammation in the body. Inflammation has been a subject of significant research interest in recent times, because it is heavily involved in the pathology of serious conditions including diabetes and cardiovascular disease. Aside from anthocyanins, black rice is rich in tocopherols, another type of powerful antioxidant which is better known collectively as vitamin E. Recent research has shown that antioxidants may work synergistically, meaning that foods containing two or more types of antioxidant may deliver greater health benefits than the sum of each antioxidant alone. Body builders work very hard and place a lot of stress on their bodies. Unfortunately strenuous exercise is known to generate a lot of free radicals. The antioxidants in black rice may assist in neutralising these damaging oxygen species. This not only has general preventative health benefits, but the high antioxidant levels also have the potential to improve post training recovery through their role in reducing inflammation. Additionally, B vitamins, iron and potassium are essential to muscle building. Additionally, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester and 4-carbomethoxy-6-hydroxy-2-quinolone in black rice display antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay (Chung and Woo 2001).

Black rice is one of the most healthy food types of today. It is packed with a wide array of nutrients. Aside from being an extremely rich source of nutrients, black rice is comparatively cheaper and lower in sugar than other superfoods like berries. 21st century food scientists have discovered that black rice has many health benefits. Knowing its health benefits, black rice was first introduced to the United States in 1995. Black rice (*Oryza sativa* L. var. *japonica*) has been used in folk medicine in Asia (Sim et al. 2007). Black rice varieties also have historically been used in Chinese medicine. This rice is high in nutritional value and contains 18 amino acids, iron, zinc, copper, carotene, and several important vitamins. Thus black rice is panacea.

2.5 Pecularity of Black Rice

Black rice varieties have been the subject of exploratory research for its potential biomedical applications. This rice contains an antioxidant, the anthocyanins which are also found in dark hued fruits such as acai berries, blueberries, blackberries, dark grapes and dark cherries. That's why black rice is darker in colour than other rices. Anthocyanins are linked with better heart health, cancer prevention, relieving inflammation, and increasing memory. One tablespoon of black rice contains the same or more anthocyanins than the same amount of blueberries. This makes it a stellar addition to the diet in place of other rices. White rice is digested rapidly

because it is fibre free. Even anybody eat a large amount of white rice, he will soon feel hungry again which causes him to snack and which would likely lead to weight gain. But black rice is digested slowly because of its high fibre content and a man feel hungry with longer time. This rice is naturally high in iron that causes the dense purple colour, and it is also high in fiber, since the bran is left on the rice. A research on physicochemical properties of black rice and white rice varieties were investigated; black rice varieties showed a higher amount of minerals, faster hydrolysis rate, and lower blue value than the ordinary white rice. This study illustrated the wide variation in the physicochemical properties of the black rice varieties analyzed (Kang et al. 2011). Frank et al. (2012) reported that black rice exhibited, in particular, higher levels of fatty acid methyl esters, free fatty acids, organic acids and amino acids compared to non-coloured and red rice. Some major features of this rice are as follows:

2.5.1 Rich in Iron

Black rice has higher iron content and is excellent for those concerned about getting enough iron on a plant based diet. Iron is essential for healthy red blood cell production, for energy expenditure, and even for digestion.

2.5.2 Rich in Protein

Black rice is also higher in protein than other sources of rice. It contains 8.5 grams of protein per cup. It can help keep full, and satisfy rice craving. Getting enough protein is not hard to do on a plant based diet since so many plant based foods contain high quality protein.

2.5.3 A Naturally Gluten Free Grain

Black rice naturally consists of no gluten (protein present in all wheat, rye and barley products). It is approximated that 1 in 7 individuals are responsive to gluten whether they are aware of it or not. But still test negative for celiac disease. After consuming something along with gluten in it, those with a gluten sensitivity suffer most of the same symptoms as individual with celiac disease (a confirmed allergy to gluten), which includes bloating, constipation, diarrhea, nutrient deficiencies, as well as an increased risk for developing leaky gut syndrome. Consuming black rice might help remove digestive problems related to consuming gluten for most people (www.healthbenefitstimes.com).

2.5.4 Black Rice Versus Blueberries

A new healthy rice food on the horizon is the black rice. Emerging research shows that black rice is even richer in antioxidants and is a valuable phytochemicals than blueberries. The special value of the antioxidants in black rice is that they are both water soluble and fat soluble. Vegetables and fruits like blueberries are rich in the water soluble antioxidants (vitamin C), while soybeans contain fat soluble antioxidants (vitamin E). Black rice is exceptional because it contains a rich mixture of both classes of antioxidants (www.circ.ahajournals.org).

2.5.5 Power Up

Black rice is rich in antioxidants particularly anthocyanin which can reduce risk of infection, heart attack and cancer. Anthocyanin is concentrated in the kernels of black rice bran outer layer and gives it dark color. A 1/3 cup serving has as much fiber as a full cup of brown rice, so it is great for digestive system. Black rice is rich in vitamin E (which strengthens the immune system and promotes healthy skin and eyes), iron (which helps make red blood cells), manganese (which aids the nervous and reproductive systems) and other minerals.

2.5.6 Delicious

Black rice is delicious and much more flavorful than white rice. It has a naturally sweet flavor but does not contain sugar. The benefits of black rice compared to white rice is that the fibers will help slow down the starches in the rice which is much better for blood sugar maintainance and helpful for preventing Type 2 diabetes.

2.5.7 Affordable

It is affordable. It is much better to spend 5 dollar more for rice that will keep you full, nourished and healthy than one that's lower in nutrients like processed white minute rice.

2.5.8 Easy to Prepare

Black rice can be prepared like any other rice. It do not need any sophisticated ingredinets thus it is easy to prepare.

2.5.9 Taste and Smell

Black rice has magical aromas. It has deliciously nutty taste, soft texture and a beautiful deep purple colour that makes it a striking presence in any dish. Black rice is praised globally for their health benefit which makes it the perfect rice selection for the best health return.

2.6 Popularity of Black Rice

Within the past 100 years, the nutrient content of foods has been declined significantly. It is due to the depletion of soils caused by chemicals and heavy agricultural methods. The loss of vital minerals of farm soil due to soil depletion has an untold effect on nutrient content of food. Similarly, a move towards hybrid varieties (HYV) that were developed to optimize yields (without concerning nutrient value of food) has diminished the overall nutrition of food. Unhealthy eating habits and an over reliance on heavily processed foods and refined grains has taken a toll on everyone health. The rise in chronic diseases such as heart disease, cancer and diabetes, as well as the alarming increase in obesity rates are all indicators of this negative trend. Scientific research has confirmed a diet rich in whole grains, fruits, vegetables and high quality proteins is an important factor in maintaining overall good health. Either selections of nutrient dense lands whose soils are rich in nutrients than other lands or growing nutrient packed rice landraces in organic way make rice naturally more healthful. Traditional and heirloom varieties are naturally more nutritious than high yielding varieties. Black rice is cherished for its exceptional nutritional value, as well as its sustainability. Other rice variety can not meet nutrient content compared to black rice though it is grown in simple organic land.

Human micronutrient deficiencies are relatively severe in areas where rice is the major staple. Often, calorie demand is met by an increase in rice without a corresponding increase in other foods such as legumes or fish. Increasing the density of provitamin A carotenoid, iron, and zinc in rice can alleviate these deficiencies, especially among the urban and rural poor people who have little access to alternatives such as enriched foods and diversified diets. Research is under way to fortify rice with micronutrients in areas where these are inadequate in the diet. It is still debated whether such micronutrient increases in the endosperm are sufficient to significantly affect human nutrition (Ricepedia). Besides these fortified rice, there is a hope on black rice which is a rich source of many micronutrients and full of antioxidants. Thus black rice is a remedy of fatigue health.

Though the popularity of black rice and knowledge about its health benefits is growing in western nations including the US, it still remains much less popular than white and brown rice. As more people learn about black rice's benefits and demand it, black rice will probably become more widely available at larger chain supermarkets and restaurants. Today this type of rice is picking up popularity and

popping up in more health food stores across the US, Australia, and Europe, as people discover the numerous health benefits that whole grain black rice has to offer. Black rice has experienced times when it nearly disappeared because of the popularity of white rice varieties, but resurgence in popularity has brought new life to this ancient and venerable grain. The “forbidden rice” of Imperial China, a scarce grain was said to be permitted only to the royalty. Today it is a guilt free Asian grain that is gaining steam because of its delicately nutty taste and its substantial health benefits. It is less sticky than white rice, less chewy than brown, and loaded with nutrients that can help to grow old and wise like Confucius. Black rice has soared in popularity in recent years as consumers become aware of its high levels of antioxidants and nutrients. The amount of the antioxidant anthocyanin that is in a spoonful of black rice bran (or 10 spoonfuls of cooked black rice) is equal to that in a spoonful of fresh blueberries. When an old grain is new: “It’s rich colour, aromatic nutty flavor, and high levels of antioxidants make this rice special” (www.crops.org). Black rice was initially introduced to the US in 1995.

2.7 Black Rice Cons, Side Effects and Negatives

There are no real drawbacks to eating black rice. It is a nutrient rich, low fat, source of long lasting carbohydrates and is tasty and versatile. The only negative mounting evidence is that the benefits of antioxidant consumption may be overstated. Antioxidant consumption does not directly increase the level of antioxidant in the blood plasma as was first thought. New research suggest molecules like anthocyanins may still modulate oxidative damage but in different ways. One of the more popular theories is that plant flavonoids like anthocyanins do not act directly, but work as signalling molecules telling certain cells in the body to alter their expression of particular genes. It is the products of these genes which then exert protective effects against free radicals in the body. The inhibitor of tyrosinase activity in black rice bran has been investigated and ethyl acetate extract is found to be the most potent inhibitor against tyrosinase activity by 80.5 % at a concentration of 0.4 mg/mL (Miyazawa et al. 2003).

2.8 Ways to Call Black Rice in Different Languages

Africa	swart rys
Albanian	oriz i zi
Arabic	أسود الأرز
Armenian	Մուր քիմիս
Azerbaijani	qara düyü
Basque	Arroz beltza

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Belarusian	чорны рыс
Bulgarian	черен ориз
Catalan	arròs negre
Chinese	黑米
Croatian	crna riža
Czech	černá ryže
Danish	sorte ris
Dutch	zwarte rijst
Estonian	must riis
Filipino	itim na bigas
Finnish	musta riisi
French	riz noir
Galician	arroz negro
Georgian	შავი ბრინჯოსი
German	Schwarzer Reis
Greek	Μαύρο ρύζι
Haitian creole	nwa diri
Hebrew	אורז שחור
Hindi	काले चावल
Hungarian	fekete rizs
Icelanding	svartur hrísgrjón
Indonesian	beras hitam
Irish	rís dubh
Italian	riso nero
Japanese	黑米
Korean	검은 쌀
Latvian	melns rīsi
Lithuanian	juodųjų ryžių
Macedonian	црн ориз
Malay	beras hitam
Maltese	iswed ross
Nepali	काले चावल
Norwegian	sort ris
Persian	برنج سیاه و سفید
Polish	czarny ryż
Portugese	arroz preto
Romanian	negru de orez
Russian	черный рис
Serbian	црна рижа
Slovak	čierna ryža
Slovenian	črni riž

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Spanish	arroz negro
Swahili	mchele mweusi
Swedish	svart ris
Thai	ข้าวสีตํ่า (kao neow dom)
Turkish	siyah pirinç
Ukrainian	чорний рис
Urdu	سیاہ چاول
Vietnamese	màu đen gạo
Welsh	reis du
Yiddish	שוואַרץ רייז

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