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Research article

Morphological markers associated with pericarp colour and its inheritance pattern in black scented rice of Manipur

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Abstract: Rice (*Oryza sativa*) is the most widely consumed staple food for a large section of world's population. Biotechnological developments have led plant breeders to develop for more efficient selection strategies instead of the traditional phenotype-based plant selection method. Rice varieties other than white are usually called red or black rice. Black scented rice is in great demand and mostly grown in Asian countries. The intensity of pericarp in black rice ranges from dark purple to light purple, and the genetic mechanism underlying this colour variation is yet to be identified. Therefore, the inheritance patterns of black rice cultivars and the phenotypic markers associated with black pericarp colour have been studied in this study. Phenotypic investigation of the black scented rice may aid in the breeding of anthocyanin-rich rice varieties and may provide insights into the potential enhancement of this valuable antioxidant in a variety of foodstuffs. The experiments were conducted on F_1 and F_2 individuals developed from the crosses between indigenous black scented rice (*Chakhao*) cultivars of Manipur and the local white landrace cultivars. The segregation ratio of F_2 individuals was analyzed with the chi-square formula. The F_2 population showed some of the morphological markers like purple coleoptiles, purplish-black colour at leaf tip and stalks and the formation of black rings (auricles) were associated with pericarp colours. This, in fact, revealed the inheritance pattern of pericarp colour in black scented rice. The present study provides useful information on the inheritance of pericarp colour in black scented rice of Manipur and possesses the potential for their further genetic improvement.
Keywords: Chakhao Rice - Anthocyanin - Phenotypic markers - Hereditary pattern.

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INTRODUCTION

Rice as a cereal grain is the most widely consumed staple food for a large section of the world's population, particularly in Asian countries. Rice varieties other than white are usually called red or black rice. Black scented rice also known as specialty rice which has several unique properties like unique aroma, colour (red, purple, black), texture (glossiness and stickiness), chemical composition, aesthetic value, waxyness (very low amylose content) and because of its superior processing quality which have increased its demand in the market. In Asian countries, black rice is often consumed after mixing with white rice to enhance the flavour, colour, and nutritional value (such as high protein, total essential amino acids, vitamin B1 and minerals - Fe, Zn, Mn and P) (Yang *et al.* 2008). A spoonful of black rice bran contains not only more health- promoting anthocyanin as antioxidant than that found in a spoonful of blueberries, but also contains less sugar, more fibre and vitamin E. The purple/black colour of black scented rice is due to high anthocyanin content in the pericarp. Total anthocyanin content in Chakhao varieties was found to be 716 mg kg^{-1} of dried powder sample. And the total phenolic content was 539 mg / 100 g of the dried powder sample as Gallic acid equivalent in Chakhao varieties (Asem *et al.* 2015).

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Anthocyanins are the primary pigments in red and black rice which are also considered a group of reddish-purple water-soluble flavonoids that give the attractive red, purple and blue colours of many flowers, fruits, and vegetables. Till date, more than 400 naturally occurring anthocyanin pigments have been identified (Kong *et al.* 2003). Anthocyanin antioxidants play an important role regarding health benefits, such as memory improvement, decreased risk of heart disease and reducing cancer. Anthocyanins also perform a number of functions in a diverse array of plant/animal interactions, which attract pollinators and frugivores repelling herbivores and parasites (Lev-Yadun & Gould 2008). It has been reported that flavonoids and flavonoid derivatives play important roles in the development of the plant, in protection against UV radiation, attracting insects for pollination and also in plant defense responses (Harborne & Williams 2000).

Black scented rice is mainly grown and consumed in Manipur, the North Eastern state of India, where it is called *Chakhao* and occupies an elite status due to its pleasant nutty flavor. It turns into deep purple when cooked because of its anthocyanin content. It is one of the most desired dishes and is commonly served as desserts, breads, flakes, cakes, beverages and as a special snack called *Utong Chak* prepared in bamboo trunks. The black scented rice (*Chakhao*) of Manipur has been used by traditional medical practitioners too. These are sold in the local markets at a premium price. These low yielding rice cultivars are found only in this state of India and very little is known about it. It fetches a high demand in the domestic market, having great potential for export. But the farmers of Manipur have already started neglecting the cultivation of these cultivars as they are very low yielding and economically not profitable.

Rahman *et al.* (2013) reported that the cross between Kewha black rice and Kumgangbyeo white rice showed a segregation ratio of 3 black : 1 white in both F_2 and F_3 generations which revealed that black pigment was dominant over white pericarp. Crosses between two different parents with F_1 similar to one of the parents may also appear under two loci model with two alleles per locus and the results of chi-square analysis show deviations from 9:3:3:1 ratio in F_2 populations.

According to (Acquaah 2009), a cross using two different parents with the F_1 similar to one of the parents may also occur under two loci model with two alleles per locus for different gene actions namely complementary genes with a ratio of 9: 7; or duplicate genes with a ratio of 15:1; or additive genes with a ratio of 9:6:1; or dominant epistasis with a ratio of 12:3:1; or recessive epistasis with a ratio of 9:3:4; or suppression dominant with the ratio of 13:3 inheritance pattern for *Pb* genes in black scented rice.

The present study was undertaken to demonstrate the farmers that the low yielding black scented rice varieties, when crossed with a high yielding local white rice cultivar, could enhance its productivity for higher returns. Further, the study was performed to elucidate the inheritance pattern and the morphological markers associated with the pericarp colour in black scented rice (*Chakhao*) to screen the seed colour prior to seed setting in a rice breeding program. The results of the present study provide insights for the exploration of the black scented rice (*Chakhao*) for commercial cultivation and for the yield enhancement of these cultivars through suitable rice breeding programmes in near future. According to the farmers, black rice landrace varieties are disease resistant and relatively stress tolerant which require less care and energy inputs. Black rice is used in many religious cultural feasts and rituals of the local folks. Besides, it is also used as a medicine for diabetics and traditionally given to pregnant women as a delicacy (Borah *et al.* 2018).

MATERIALS AND METHODS

A field experiment was carried out in the College of Agriculture, Central Agricultural University, Imphal, Manipur, India on clay loam soil with moderate soil fertility. The experiment consisted of two lowland varieties with the desired characters (Table 1). Seeds of the two rice varieties were obtained from the local farmers and were germinated in the petriplates (Fig. 1). Crosses were made between two parents *i.e.* local white rice landraces as donor and the local black scented rice (*Chakhao*) as the recipient. The F_2 generations were developed subsequently and grown with the parents in the field at the same site.

Plant materials

The purple pericarp rice varieties used in the study were *Oryza sativa* var. *japonica* (Chakhao Poireiton), *O. sativa* var. *japonica* (ChakhaoAmubi) and *O. sativa* var. *japonica* (Chakhao Sempak) while the white pericarp rice varieties included *O. sativa* var. *japonica* of local white rice landraces used as wild type controls.

Pot culture of F_1 plants

The F_1 seeds were pre-germinated in a white tissue paper placed in a Petri dish for five days, nursed for 20 days with three seedlings transplanted per bucket. The buckets were filled with sterilized top soil to avoid soil contamination. Sowing of the varieties was staggered over a 3-week period in order to synchronize the flowering in the varieties. The hybrid plants were fertilized at tillering and panicle initiation stage. Standard agronomic operations like irrigation, insecticide application and hand weeding were employed whenever necessary. Some F_1 plants were selfed to produce F_2 generation.

Table 1. Salient features of the parents used in the development of F1 and F2 populations.

Cultivars	Origin	Plant Height (cm)	Duration	Grain Type	Yield (t ha^{-1})	Salient Features
Thangjing Phou	Manipur	105	Medium early	Medium, Bold	3.8	White Rice
Chakhao (P, S, A)	Manipur	140	Late	Long, slender	1.5	Black Rice

Figure 1. Germinated F_1 seeds of crosses.

Assessment of inheritance pattern of pericarp colour

The pericarp colour of mature seeds from the F_1 and F_2 population individuals with purple, brown or white seed pericarp was recorded. The evaluation of the inheritance pattern of purple pericarp was carried out by the segregation analysis using F_1 individuals and a large population of F_2 individuals from crosses among *Oryza sativa* var. *japonica* (Chakhao Poireiton) with purple pericarp as a pollen receptor, and *O. Sativa* var. *japonica* (wild type) with white pericarp as pollen donors. Fertilized seeds for each cross were obtained and the resultant F_1 seeds were grown in the field to produce F_1 plants. The F_1 plants were then allowed to self-fertilize to produce the F_2 seeds, which were collected from a single F_1 plant and grown in the field under natural conditions. The phenotypic data of the F_2 segregations were documented, F_2 seeds from a single panicle were harvested separately from each F_2 plant at the mature stage.

Segregation pattern analysis of pericarp colour was conducted using a large population of F_2 individuals from the cross between *O. Sativa* var. *japonica* (Chakhao Poireiton) with purple pericarp as a pollen receptor and *O. Sativa* var. *indica* with white pericarp as a pollen donor. The genotypes of the parents were determined using the seed pericarp phenotype of the F_1 and F_2 populations. Genomic DNA was extracted from leaf tissues using SDS method (Vivekananda & Thangjam 2018) for further molecular marker analysis.

Testing of rice pigment pattern

The data of seed pericarp pigment based on the scoring yields from F_2 generation population of plants were analyzed using chi-square analysis (Singh & Chaudhary 1979). The calculated chi-square value was compared with the table value of chi-square for appropriate degrees of freedom.

RESULTS AND DISCUSSION

Testing of the rice pigment and the pattern of inheritance were analyzed on three cross combinations between black rice and white rice *i.e.* black rice of Chakhao Poireiton (P/black) × Thangjing Phou (T/white); Chakhao Sempak (S/black) × Thangjing Phou (T/white); and Chakhao Amubi (A/black) × Thangjing Phou (T/white). Rice pigment inheritance pattern was determined from F_2 population.

Based on the pericarp pigment, the individual plants in three F_2 populations (515 individual plants of P×T, 522 individual plants of S×T and 545 individual plants of A×T) were divided into four groups namely: Black (B); Medium Black (MB); Red (R) and White (W) (Table 2). Segregation analysis in F_2 showed that ¾ of the population was black rice (black and medium black) and the remaining ¼ of the population was not black rice (red or white). These findings are consistent with the results of Rahman *et al.* (2013) who observed that the cross of Kewha black rice and Kumgangbyeo white rice showed the segregation pattern of 3 black: 1 white in F_2 and F_3 generations. So, black pigmented pericarp was determined as dominant over white pericarp colour. Crosses using two different parents and F_1 similar to one of the parents may also develop under two loci model with two alleles per locus and the results of chi-square analysis (P×T, S×T and A×T crossing) showed the ratio of 9:3:3:1 in F_2 generation (Table 2).

Table 2. Chi-square analysis of F_2 population in P x T; S x T and A x T crosses.

S.N.	Crossing	F_2 Generation			χ^2	
		Trait	**observed**	**Expected**	**Monogenic (3:1) P Value = 3.80**	**Digenic (9:3:3:1)=(9:6:1) P Value = 7.80**
1	P x T	B	211	289.68	3.64^{ns}	742.64*
		MB	104	96.56		
		R	90	96.56		
		W	110	32.18		
		∑	515	514.98		
2	S x T	B	248	293.62	3.49^{ns}	207.02*
		MB	88	97.87		
		R	74	97.87		
		W	112	32.62		
		∑	522	521.98		
3	A x T	B	222	306.56	3.62^{ns}	226.86*
		MB	112	102.18		
		R	94	102.18		
		W	117	34.06		
		∑	545	544.98		

Note: *significant at α = 5%; ns = not significant at α = 5%; B = Black; MB = Medium Black; R = Red; W = White

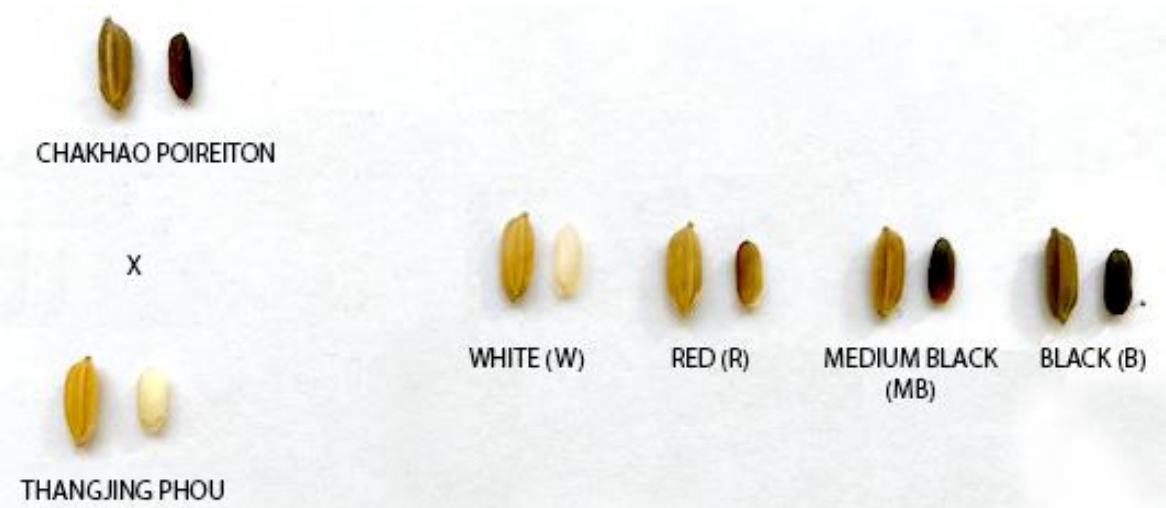

Figure 2. Segregation of F_2 generation between Chakhao Poireiton x Thangjing Phou.

P1 — *PbPbPpPp* Black rice (B) x *pbpbpppp* White rice (W) — P2

↓

F1 — *PbpbPppp* Black (B)

↓

Selfing

F2 — 9 *Pb_Pp_*: Black (B) : 3 *pbpbPp_* : Medium Black (MB) : 3 *Pb_pppp* : Red (R) : 1 *pbpbpppp* White (W)

9 : 6 : 1

Figure 3. Segregation of F_2 plants for pericarp colour in black rice (Chakhao Poireiton) x white rice (Thangjing Phou) cross.

Results of the chi-square analysis on F_2 plants showed that the inheritance of rice pigment character (black) in P×T, S×T and A×T crosses could be controlled by two pair of genes with polymeric gene action having a ratio of 9:6:1 for black: medium black or brown or red: white colour pericarp in the cross Chakhao Poireiton x Thangjing Phou (*P×T*) (Fig. 2). Based on the above results in P×T cross, we could conclude that two genes *Pb* and *Pp* with their dominant alleles together encouraged the formation of black pericarp colour due to formation of anthocyanin pigment. The *Pb* dominant allele when present with *pp* allele in recessive homozygous condition produced red pericarp colour; whereas *Pp* dominant allele when present with *pb* allele in recessive homozygous condition produced medium black (brown) pericarp colour. The alleles *pb* and *pp* in homozygous state inhibited the formation of anthocyanin pigment and thus expressed as white colour pericarp (Fig. 3).

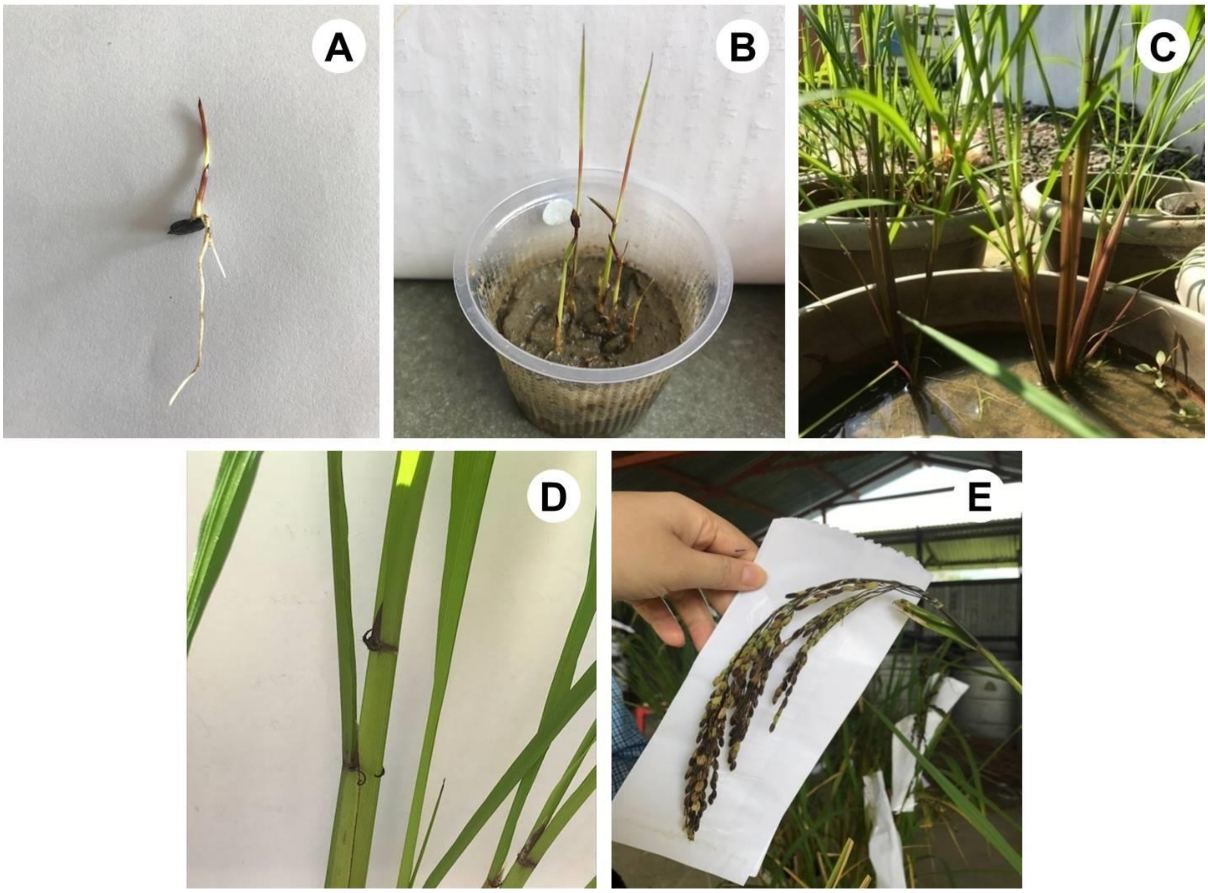

Figure 4. A, Appearance of purple coleoptiles; **B**, Black colour at tender leaf tips; **C**, Expression of black colour in stalk; **D**, F_1 progeny with black ring (auricle); **E**, F_1 panicles showing both black & white colour grains.

The black scented rice (*Chakhao*) cultivars crossed with wild type cultivars *i.e*. F_1 and F_2 progenies showed some of the phenotypic markers were associated with fragrance and pericarp colours, such as the appearance of purplish coleoptiles (Fig. 4A) and black colour at the tip of leaves and stalks (Figs. 4B & 4C). The formation of black rings (auricle) was also observed in black scented rice (*Chakhao*) cultivars (Fig. 4D). The occurrence, as well as the distribution of anthocyanin pigmentation in the grains and panicles of F1 progenies,was also one of the notable features (Fig. 4E). Anthocyanin distribution in different parts of the rice plant is highly variable and is a striking feature of the crop. These features had been the subject of interest in several earlier studies. Such morphological variants (Table 3) with distinct phenotypic expressions and simple inheritance pattern could be used to establish linkages and for indirect selection if found associated with useful traits (Sahu *et al.* 2009). Therefore, in this study, the inheritance pattern of pericarp colour has been elucidated in a very simple way and further, a morphological marker associated with pericarp colour has been identified. This study might help in future rice breeding programmes and also improve the rice seed quality and its anthocyanin contents so that it may be useful in many food companies and natural dye industries.

CONCLUSIONS

The study on inheritance patterns in various crosses helps breeders to choose appropriate breeding methods for genetic improvement of any trait in crop plants. In this study, we have found that the pericarp colour in black rice of Manipur could be governed by at least two genes with two alleles showing a polymeric gene action

Table 3. Morphological data of Chakhao varieties found in Manipur.

SN	Genotype	Ligule Shape	Ligule Colour	Culm Habit	Auricle Colour	Colar Colour	50% flowering	Plant Height (cm)	No. of tillers	No. of panicles	No. of grain/ panicle	Panicle length (cm)	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	100 seed weight (g)	50% Maturity
1	Chakhao Thailand Pong 9	2-cleft	Purple	Erect/ Semierect	Purple	Purple	108	157	12	10	177	22.92	8.41	2.68	1.88	2.52	138
2	Chakhao Poireiton Leimaram	2-cleft	Purple	Erect/ Semierect	Purple	Purple	108	156	16	14	181	23.7	8.7	3.07	2.1	2.18	135
3	Chakhao Sempak	2-cleft	Purple	Erect/ Semierect	Purple	Purple	107	165.2	12	9	198	24.1	8.66	3.08	1.9	2.14	135
4	Chakhao Wahong	2-cleft	Purple	Erect/ Semierect	Purple	Purple	108	167	12	11	192	25.56	8.5	3.57	2.18	2.46	139
5	Chakhao Irengbam	2-cleft	Purple	Erect	Purple	Purple	109	169.8	11	10	205	28.1	8.63	3.1	2.12	2.2	135
6	Chakhao Poireiton Uyumpok	2-cleft	Purple	Erect	Purple	Purple	109	165.2	9	8	200	26.72	9.05	2.88	1.77	1.89	135
7	Chakhao Kotha	2-cleft	Purple	Erect/ Semierect	Purple	Purple	107	175.4	10	9	195	23.56	8.49	3.1	1.89	2.07	135
8	Chakhao Poireiton (9)	2-cleft	Purple	Erect/ Semierect	Purple	Purple	108	169.8	9	8	229	25.9	8.4	2.93	1.87	1.92	135
9	Chakhao Poireiton (19)	2-cleft	Purple	Erect/ Semierect	Purple	Purple	107	152.6	15	13	125	23.04	8.86	2.69	1.86	2.02	135
10	Chakhao Churchandpur	2-cleft	White	Erect	Light purple	Light green	115	154.6	18	18	122	27.26	8.73	3.6	1.99	2.22	140
11	Chakhao Wairi	2-cleft	Purple	Erect	Purple	Purple	108	161	16	14	199	28	8.33	2.73	1.66	1.98	137
12	Chakhao Amubi	2-cleft	Purple	Erect	Purple	Purple	109	166.4	16	13	159	26.32	8.48	3.12	1.95	2.07	136
13	Chakhao Poireiton (T2)	2-cleft	Purple	Erect/ Semierect	Purple	Purple	109	144.4	12	11	199	22.7	9.32	2.94	1.9	2.27	135
14	Chakhao Khurkhul	2-cleft	Purple	Erect	Purple	Purple	108	156.8	10	9	207	26.66	8.6	3.05	1.78	2.19	135

(9:6:1 ratio) in F_2 generation. When both the genes are present with dominant alleles, the phenotype of the pericarp is black, whereas the pericarp could be brown or red when either of them is present singly as dominant allele. However, both the genes in recessive homozygous conditions could produce white pericarp colour. Further, the stalks were found with black ring (auricle) formation which clearly showed the transmission of pericarp colour in the F_1 progeny. The appearance of purplish coleoptiles could also be a morphological marker associated with pericarp colour. Therefore, this study might help in easy identification of rice seed colour before seed setting in rice breeding programmes and improve anthocyanin content in rice grain so that it may be useful in many food companies and natural dye industries.

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