Genetic Inheritance of Anthocyanin Pigmentation in Rice (*Oryza sativa* L.)

ABSTRACT

Rice (*Oryza sativa*) is an important cereal crop that belongs to the grass family Poaceae. A unique feature of purple-coloured anthocyanin pigmentation is possessed by some of the rice varieties and the accumulation of anthocyanin in plants offers resistance to UV radiation and brings out responses to various biotic and abiotic stresses. In order to study the inheritance of anthocyanin pigmentation, an experiment involving the F_1 and segregated F_2 populations obtained by crossing the two *Oryza sativa* subsp. indica varieties, *viz*., BPT-5204 (non-pigmented) and HY-256 Purple (pigmented), was conducted during three consecutive growing seasons at AICRIP, Agricultural Research Station (Paddy), Sirsi, University of Agricultural Sciences, Dharwad. Hybridization between the female parent, BPT-5204 and male parent, HY-256 Purple, was done during summer 2021 and the seeds obtained from hybridization constituted the F_1 generation and were sown during *kharif* 2021. The same were subjected to selfing to obtain seeds to generate the F_2 population, which were evaluated along with the parents and F_1 plants during summer 2022. Observations on the presence or absence of anthocyanin pigmentation for different morphological traits, *viz*., leaf blade, leaf tip, leaf margin, midrib, juncture and junctura back, were recorded in the parents, F_1's and F_2 populations in such a way that the presence of pigmentation was recorded as purple, while its absence was recorded as non-pigmented or green. Purple-coloured anthocyanin pigmentation was absent in all the characters in the F_1 plant. Similarly, varied segregation for pigmentation was observed and documented in 1276 plants that constituted the F_2 generation. The data was analyzed for colour pigmentation to determine the fitness with diverse segregation ratios and to determine the mode of inheritance by chi-square test, which revealed the involvement of two to three genes with different gene interactions.

Keywords: Anthocyanin, Chi-square, Gene action, Inheritance, Pigmentation

1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important food grain crop and a staple food for almost half of the world's population. It belongs to the grass family Graminae (Poaceae), having chromosome number 2n=24 and a genome size of 389 Mb. It is a natural diploid derived from a single ancestor, *Oryza perennis* and has autogamy as a mode of pollination. Rice cultivation is an essential source of human subsistence in South and South-East Asia. The present average yield of rice in India appears to be low (2.79 t ha^{-1}) as compared to the global average (5.88 t ha^{-1}) in the developed countries of the world. This indicates the possible existence of bottlenecks in rice breeding and cultivation for improving productivity and yield stability in India. This would attract breeders and geneticists interested in rice improvement through genetic manipulation.

Plant colouration is determined mainly by three kinds of plant pigments in addition to chlorophyll *viz*., anthocyanins, betalains and carotenoids, which function in photosynthesis, defence and reproduction [1]. Anthocyanins are important secondary metabolites and form a major subclass of flavonoids that give colour to the tissues of a rice plant [2]. The accumulation of anthocyanin in plants has different functions, *viz*., resistance to UV radiation, participation in hormone regulation and response to biotic and abiotic stress [3]. Under biotic and abiotic stress exposure, anthocyanins sustain plant growth and development as they indirectly protect leaves from damage and maintain normal photosynthetic activity [4].

The accumulation of anthocyanin pigment in rice, followed by its distribution in different parts of the rice plant, is highly variable. Moreover, marker traits like pigmentation may assume greater importance for identification of the true crosses in the crossing programme [5]. Morphological variants with distinct phenotypic expressions and simple inheritance patterns can be used to establish linkages and for indirect selection if they are found to be associated with useful economic traits. Identification of phenotypic markers in the form of anthocyanin pigmentation in rice could help to improve the rice crop yield through selection using the linked traits that contribute to yield improvement without deteriorating its quality, which is an economically friendly approach for crop improvement by utilising the existing genetic variability in the rice germplasm at no additional expenditure.

Genetic inheritance of anthocyanin pigmentation in different morphological regions of rice has been previously studied by Nadaf [6], Surendra [5], Hemaprabha et al. [7], Sahu et al. [8] and Pandey et al. [9]. With this as a backdrop, a phenotype-based classical genetic experiment was conducted in order to study the inheritance of purple-coloured anthocyanin pigmentation in the segregated F_2 population derived by crossing the two rice varieties, viz., BPT-5204 (non-pigmented) and HY-256 Purple (pigmented).

2. MATERIAL AND METHODS

2.1 Experimental Material and Location

Field experiments were conducted at the All India Coordinated Rice Improvement Project (Voluntary Centre), Agricultural Research Station (Paddy), Sirsi, University of Agricultural Sciences, Dharwad, during three consecutive growing seasons, *viz*., summer 2021, *kharif* 2021 and summer 2022. The experimental material consisted of two *Oryza sativa* subsp. indica varieties, *viz*., BPT-5204 and HY-256 Purple, which shared various contrasting characters in terms of pigmentation, morphology, maturity duration, yield level and aromatic nature of grains. BPT-5204, known as Samba Mahsuri, is a semi-dwarf mega rice variety that is devoid of anthocyanin pigment on any of its plant parts (acyanic) and the grains are non-scented. HY-256 Purple is a local cultivar and has the unique feature of purple-coloured pigmentation in most of the aerial plant parts with aromatic grains.

2.2 Experimental Procedure

Pollen grains obtained from the anthers of the male parent, HY-256 Purple, were used to pollinate and fertilise the female parent, BPT-5204, in order to constitute F_1 and F_2 generations. Around 1500 F_2 seeds were directly sown in a dibbling manner at a spacing of 30 cm × 30 cm. Meanwhile, parents and F_1 plants were grown along with the F_2 population for comparison. A total of 1276 F_2 plants survived to maturity. Since the genetic constitution of each F_2 plant is different, all 1276 F_2 plants were harvested individually.

2.3 Data Collection

Visual observations on the presence or absence of anthocyanin pigmentation in different morphological regions, *viz*., leaf blade, leaf tip, leaf margin, midrib, junctura and junctura back, were documented at appropriate growth stages across three generations. The presence of the pigmentation was recorded as purple and its absence as green. A magnifying lens of 10X resolution was used to observe the pigmented characters wherever differences between coloured and colourless conditions were not distinct.

2.4 Statistical Data Analysis

The expected values corresponding to the observed values for each character were calculated based on the genetic ratio (genetic hypothesis) presumed. The chi-square test [10] was applied to test the significance of deviation using the following formula.

$$x^2 = \Sigma \frac{(o-e)^2}{e} \ with \ (n-1) \ df$$

Where,

Σ = Summation over all classes
o = Observed frequencies
e = Expected frequencies and
n = Number of classes

The genetic hypothesis was considered fit only when the calculated chi-square value was less than the table value (3.841) at the 5 per cent level of significance for one degree of freedom.

3. RESULTS AND DISCUSSION

The two *Oryza sativa* subsp. indica varieties, *viz*., BPT-5204 and HY-256 Purple, used in the hybridization programme have shared distinct features for all the traits studied. Observations regarding anthocyanin pigmentation at different morphological regions recorded on female parent BPT-5204, male parent HY-256 Purple in addition to the F_1 plant are presented in Table 1. The absence of purple-coloured anthocyanin pigmentation was noticed in all the characters in the F_1 plant, indicating its recessive nature over no pigmentation. Similarly, the details of the observed frequencies of phenotypic expressions in the F_2 generation for each character, the expected frequencies at one degree of freedom indicating the goodness of fit to the assumed ratios are presented in Table 2 and the details pertaining to each trait are described as follows.

Table 1: Phenotypic expressions for anthocyanin pigmentation in different morphological regions in parents and F_1 plant

Sl. No.	Characters	BPT-5204	HY-256 Purple	F_1 Phenotype
1	Leaf blade	Green	Purple	Green
2	Leaf tip	Green	Purple	Green
3	Leaf margin	Green	Purple	Green
4	Midrib	Green	Purple	Green
5	Junctura	Green	Purple	Green
6	Junctura back	Green	Purple	Green

Table 2: Segregation for pigmentation in different morphological regions in the F_2 population of rice

Sl. No.	Characters	Expected Ratio	Pigmented individuals	Non pigmented individuals	X^2 Value	P value
1	Leaf blade	21:43	414	862	0.0781	0.8 - 0.7
2	Leaf tip	21:43	441	835	1.7698	0.2 - 0.1
3	Leaf margin	21:43	445	831	2.4612	0.2 - 0.1
4	Midrib	21:43	421	855	0.019	0.8 - 0.9
5	Junctura	9:55	159	1117	2.7087	0.05 - 0.1
6	Junctura back	9:55	162	1114	1.9718	0.2 - 0.1

3.1 Leaf blade

The F_1 plant obtained from the cross BPT-5204 × HY-256 Purple was devoid of purple pigmentation in the leaf blade region, specifying its recessive nature over no pigmentation. Out of 1276 F_2 plants evaluated, 414 plants had purple pigmented leaf blades and 862 showed pigment less leaf blades. The phenotypic segregation ratio of 21:43 with respect to pigmentation to no pigmentation was observed in the F_2 population, with a calculated chi-square value of 0.0781 indicating the involvement of one basic gene and one inhibitory complementary gene. However, monogenic governance of anthocyanin pigmentation in leaf blades was noticed by Yadav [11], Kinoshita and Mackaw [12] and Nadaf [6], whereas Dhulappanavar [13,14] and Surendra [5] have reported tetragenic control of pigmentation for the same trait.

3.2 Leaf tip

The genetics of pigmentation at the leaf tip of the F_1 plant exhibited a recessive nature of the anthocyanin inheritance, as indicated by the presence of a pigment-less green leaf tip. Among 1276 F_2 individuals, 441 exhibited a purple-coloured leaf tip, whereas 835 were devoid of pigmentation and had a green coloured leaf tip, which is segregated in the genetic ratio 21:43 with a calculated chi-square value of 1.7698, indicating the governance of leaf tip pigmentation by one basic gene and one inhibitory complementary gene. Similar conclusions were drawn by Dhulappanavar et al. [15], Manjunath [16], Pavithran [17], Surendra [5] and Pandey et al. [9]; on the other hand, Nadaf [6] and Thimmappaiah [18] reported the control of the same trait by three and four genes, respectively.

3.3 Leaf margin

The recessive nature of pigmentation in the form of purple colour was noticed in the leaf margin of a F_1 plant. Anthocyanin pigmentation in the leaf margin region was observed in 445 F_2 segregants, while the remaining 831 F_2 segregants had green-coloured leaf margins. The observed frequencies, when tested for goodness of fit with the chi-square test, showed goodness of fit to the expected phenotypic segregation ratio of 21:43 with a calculated chi-square value of 2.4612, revealing the governance of leaf margin pigmentation through two genes, *viz*., one basic gene and one inhibitory complementary gene. A similar fashion of digenic inheritance was reported by Thimmappaiah [18] and Pavithran [17], on the contrary, the same trait exhibited trigenic control of pigmentation in the studies conducted by Setty and Misro [19], Setty et al. [20], Hadagal et al. [21] and Surendra [5]. Surprisingly, five genes have been reported by Nadaf [6] for leaf margin pigmentation.

3.4 Midrib

The mode of inheritance of purple pigmentation in the midrib region exhibited a recessive nature over no pigmentation in the F_1 generation. In the case of 1276 F_2 genotypes, 421 plants had a purple-pigmented midrib and 855 genotypes had a green-coloured midrib. The proportion of pigmented to non-pigmented individuals in the F_2 population is segregated into the ratio 21:43, with a calculated chi-square value of 0.019 suggesting a digenic nature with the involvement of two genes, *viz*., one basic gene and one inhibitory complementary gene. Digenic inheritance of anthocyanin pigmentation in the midrib region was documented in the studies conducted by Dhulappanavar et al. [22], whereas Nadaf [6] and Surendra [5] reported trigenic governance.

3.5 Junctura

The F_1 plant obtained from the cross BPT-5204 × HY-256 Purple was devoid of purple pigmentation in the junctura region, specifying its recessive nature over no pigmentation. Anthocyanin pigmentation in the junctura region was observed in 159 F_2 segregants, while the remaining 1117 F_2 segregants had green-coloured junctura. The observed frequencies, when tested for goodness of fit with the chi-square test, showed goodness of fit to the expected phenotypic segregation ratio of 9:55 with a calculated chi-square value of 2.7087, indicating that the trait is controlled by three genes, *viz*., two complementary genes and one inhibitory gene. Thimmappaiah [18], Dhulappanavar [23], Hadagal et al. [24] and Surendra [5] noticed the involvement of three genes in governing the pigmentation at the juncture region, whereas Dhulappanavar et al. [25] and Dhulappanavar [26] reported the control of the same trait by four and five genes, respectively.

3.6 Junctura back

Genetic inheritance of purple pigmentation in the juncture back exhibited a recessive nature over no pigmentation in the F_1 generation. In the case of 1276 F_2 genotypes, 162 plants had purple pigmented juncture back and 1114 genotypes were observed with green-coloured juncture back. The proportion of pigmented to non-pigmented individuals in the F_2 population is segregated into the ratio 9:55, with a calculated chi-square value of 1.9718 indicating the involvement of two complementary genes and one inhibitory gene. Anthocyanin pigmentation in the juncture back region was found to be inherited in a trigenic fashion as reported by Ghose et al. [27], on the contrary, the same trait exhibited tetragenic control of pigmentation in the studies conducted by Srivastava and Saran [28], Dhulappanavar [29,30], Nadaf [6] and Surendra [5].

4. CONCLUSION

The presence as well as the distribution of anthocyanin pigmentation in different morphological regions of the rice plant are extremely variable and unique features of the crop. A convoluted system of gene interactions is involved in regulating the anthocyanin pigmentation of different regions of the rice plant, as observed in the present investigation, where the involvement of two to three genes with different interactions was noticed. This varied genetic inheritance might be due to multiple sets of genes conferring the respective characteristics in different source materials, as documented in the previous experiments. Studies on the inheritance of anthocyanin pigmentation emphasise the need for a detailed understanding of genetic stocks of valuable agronomic traits that can be exploited for crop improvement in rice. Similarly, morphological variants accompanied by discrete phenotypic expressions with simple inheritance patterns can be used for identifying genetic linkages and indirect selection if they are found to be associated with useful economic traits.

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