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Inheritance of anthocyanin pigmentation in interspecific cross of rice (*Oryza sativa* L. × *O. rufipogon* Griff)

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ABSTRACT

An inheritance study of the anthocyanin pigmentation in the inter-specific cross of *O. sativa* × *O. rufipogon* was conducted in the greenhouse of the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, including the parents, F1 and subsequent F2 segregants in the rice growing season of the year 2012. The study was done in the segregating generation of the inter-specific cross of *O. sativa* cv. Pokhreli Palele × *O. rufipogon*. The inheritance of anthocyanin pigmentation pattern in the different plant parts was found to be complicated. The segregation of pigmented: non-pigmented for basal leaf sheath, stigma and leaf apex was digenic with complementary gene action (9: 7). Digenic inheritance of the pigmentation in the awn and lemma and palea was found with the segregation ratio of 11 pigmented: 5 non-pigmented. A tetragenic ratio with inhibitory gene action (81 pigmented: 175 non-pigmented) was observed for the internode colour. A pleiotropic gene action of one of the basal leaf sheath pigmentation with that for the stigma and internode pigmentation was found.

INTRODUCTION

Oryza rufipogon Griff., is a valuable source of resistant genes to various biotic and abiotic stresses. These resistant genes can be easily transferred to cultivated rice (*Oryza sativa* L.) through wide hybridization (Ali et al. 2015). Broad variability in kinds and distribution of pigments can be used to document cultivars, in genetic studies, as marker traits, and to incorporate desirable traits into breeding programs (Maurya et al. 2001). The anthocyanin pigmentation in rice is an example of many dispersed genes controlling a single trait (Reddy 1996) that indicates three to five genes (Nadaf et al. 1994).

Kadam (1997) concluded that the two duplicate anthocyanin sheath genes in the presence of the chromogen gene produce colour in the sheath group, which consists of sheath, internode, stigma and apiculus. There is a considerable variation in the intensity and distribution of anthocyanin pigmentation in the leaf sheath with domination of the purple in the F1 (Hsu and Lu 1999; Eruotor 1993).

For the stigma and sheath Kadam and D'cruz (2000) have reported the basic genes with other specific genes develop colour. According to Siddiq et al. (1996), internodal pigmentation follows a tetragenic inheritance involving one basic, one inhibitory and two complementary genes. Inheritance of the pigment in the stigma showed a trigenic ratio involving one complementary and two duplicate genes (Siddiq et al. 1996).

There is a wide diversity in the distribution, intensity and location of pigments in the lemma and palea. The black colour of lemma and palea is dominant to brown and is caused by four complementary genes (Kadam and D'cruz 1995). The non-anthocyanin blackening of lemma and palea at flowering showed four interacting genes, three of which are complementary and one inhibitory (Nadaf et al. 1994).

According to Nadaf et al. (1994) linkage has been established between the basic genes for pigmentation in leaf blade and blackening of lemma/palea. Pigment in lemma/palea and internode always go together except in very rare instances (Parnell et al. 2003). In a study of patterns of anthocyanin distribution of world collection, Misro et al. (2000) concluded that the gene for pigmentation in the leaf sheath is closely

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linked with that for pigmentation in apiculus and stigma. The distinct phenotypic appearance and simple inheritance pattern can be used to set up linkages and for indirect selection if found linked with useful traits. Present study investigated genetic basis of anthocyanin pigmentation in basal leaf sheath, leaf apex, stigma, internode, awn and Lemma and Palea colour.

MATERIALS AND METHODS

The F1 plants from the cross between *O. sativa* cv. Pokhreli Pahele (a Nepalese landrace) and *O. rufipogon* (common wild rice), and the subsequent F_2 generation in were grown in the glass house of Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal, as the main season crop. The choice of the parents was based on the contrasting characteristics in the preliminary study at the previous year (Table 1). There were 270 plants in the study with 224 F_2 plants, 16 *O. rufipogon*, 16 *O. sativa* (Pokhrei Pahele), and14 F_1 Plants.

Table 1. The different contrasting characters in the *O. sativa* (Pokhreli Pahele) and *O. rufipogon* under the preliminary investigation

Characters	Pokhreli Pahele	*O. rufipogon*
Basal leaf sheath colour	Green	Purple
Stigma colour	Dull white	Purple
Stem colour	Light gold	Purple lines
Leaf apex	Non-pigmented	Pigmented
Lemma and palea colour	Straw	Black

All the plants of both the parents, F_1 and F_2 were characterized using the descriptor for rice (IRRI-IBPGR 1980). Characterization of the following traits was done for basal leaf sheath colour, leaf colour, stigma colour, internode colour, awn colour , lemma and palea colour.

Segregation of the F_2 materials for the Characterized traits was tested for goodness of fit using chi-square (χ^2) test. The observation was classified into different groups. The expected ratio was calculated by using the following formula for more than 1 degree of freedom.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

In case of single degree of freedom the chi-square test was done by using the Yate's corrected formula (Steel and Torrie 1980).

$$\chi^2 = \sum \frac{\left\{|(O-E)| - \frac{1}{2}\right\}^2}{E}$$

Where, 'O' is the Observed frequency and 'E' is the Expected frequency. The Microsoft Excel 2007 program was used for data entry and analysis.

RESULTS AND DISCUSSION

Anthocyanin Pigmentation in Different Parts

There was presence of the pigment in the different plant parts like basal leaf sheath, stigma, leaf apex, internode, awn, lemma and palea in *O. rufipogon* but not in the Pokhreli Pahele (Table 1). The F_1 showed the pigment in all plant parts investigated which was present in the *O. rufipogon*. The segregation of the pigmentation in the F_2 showed the presence of the pigment with high frequency in all organs studied except internode.

Basal Leaf Sheath Colour

In the F_1 purple pigmentation was observed in the basal leaf sheath colour. A dominance type of gene action with a digenic ratio of 9:7 of the pigmented to non-pigmented for the basal leaf sheath pigmentation was observed (Table 2). Hector (2002) also found the 9: 7 ratio of the pigmented to non-pigmented. Therefore involvement of the two complementary genes for the pigmentation in the basal leaf sheath in the *O. rufipogon* is concluded.

Stigma Colour

In stigma colour also dominance type of inheritance was concluded. The F_2 segregation of the pigmented to non-pigmented stigma confirmed 9: 7 ratios. Therefore the complementary gene action for the stigma colour in *O. rufipogon* is concluded.

Internode Colour

Dominance type of gene action was observed for the internode colour. Pigmentation in the internode segregated into 81 pigmented: 175 non-pigmented, which we can expect that the tetragenic segregation with the complementary gene action (Table 2). The interaction of the four genes with one basic gene and three inhibitory gene action was observed for the internode pigmentation. Three pair of gene involvement also has been reported by Hsu and Lu (1999) but tetragenic inheritance has been reported by Siddiq et al. (1996). It is concluded that

Table 2. Segregation for the occurrence of the pigment in different plant parts in F_2 of *O. sativa* cv. Pokhreli Pahele × *O. rufipogon*

Plant Parts	Observed		Ratio expected	χ^2	P value
	Pigmented	Non-pigmented			
Basal leaf sheath	120	104	9:7	0.55	0.3-0.5
Leaf apex	127	97	9:7	0.018	0.7-0.9
Stigma	120	104	9:7	0.55	0.3-0.5
Internode	81	143	81:175	1.86	0.1-0.2
Awn	147	77	11:5	0.88	0.3-0.5
Lemma and Palea	148	76	11:5	0.52	0.3-0.5

the inheritance of the pigmentation in the internode in the *O. rufipogon* is governed by the four pairs of genes.

Leaf Apex Pigmentation

The presence of the colour in the F_1 confirms the dominance type of gene action for the pigmentation in leaf apex in the interspecific cross. The F_2 showed the segregation ratio of 9:7 for pigmented to non-pigmented which shows that the trait is governed by two genes with the complementary gene action. For the leaf blade pigmentation Nadaf et al. (1994) observed a 39 pigmented: 25 non-pigmented segregation in the F_2 was in the trigenic segregation.

Awn Colour

The Pokhreli Pahele is an awnless variety and the *O. rufipogon* is an awned variety. The pigmented awns were present in the F_1 showed that the pigmentation in the awn was a dominant character. Segregating ratios of the 11 pigmented to 5 non-pigmented in the F_2 in *O. sativa* cv. Pokhreli Pahele × *O. rufipogon* (χ^2 = 0.88, P = 0.3-0.5), indicated that the inheritance of colour was through the involvement of two genes. Strickberger (1996) has also reported such type of gene action for the pigment glands in cotton plants (*Gossypium hirsutum*), where the glandular were dominant to the glandless. The ratio observed was the 11 glandular to the 5 glandless.

The genotype of the Pokhreli Pahele for this trait can be designated as $\mathbf{ac_1\ ac_1\ ac_2\ ac_2}$ and for the *O. rufipogon* as $\mathbf{Ac_1\ Ac_1\ Ac_2Ac_2}$. When $\mathbf{Ac_1}$ and $\mathbf{Ac_2}$ present together produce pigment, absence of dominant allele at one gene pair produce pigmented plants only when the dominant allele at other gene pair is homozygous. The $\mathbf{Ac_1ac_1ac_2ac_2}$, $\mathbf{ac_1ac_1Ac_2ac_2}$, and $\mathbf{ac_1ac_1ac_2ac_2}$ give the non-pigmented awns. The ratio observed in this study is unique for the rice pigmentation.

Lemma and Palea

The dominance type of gene action for the lemma and palea pigmentation can be concluded. In F_2 the segregation for pigmented to non-pigmented was in the ratio of 11: 5 (χ^2 = 0.52, P= 0.3 - 0.5), which indicates that the two genes are involving for the pigmentation in the Lemma and palea as in the awn colour. The dominance type of gene action of the black lemma and palea pigmentation to the brown has also reported by Kadam and D'Cruz (2000). Nadaf et al. (1994) described the four gene interaction where there were the complementary and one was the inhibitory. In *O. rufipogon* the pigmentation for the lemma and palea is concluded to be governed by two genes.

Linkage in pigmentation

All the plants with the pigmented basal leaf sheath showed the pigmented stigma. When the basal leaf sheath was devoid of pigmentation then the stigma was also without the pigmentation (Table 3). When basal leaf sheath colour was pigmented only then internode colour was pigmented. All the plants with pigmented internode show the pigmented basal leaf sheath, but all the plants with pigmented basal leaf sheath do not show the pigmented internode. Based upon the reanalyzed data Kadam (1997) has concluded that the two sheath gene cause pigmentation in the sheath group which consists of basal leaf sheath, internode, stigma and apiculus. Chao (1982) has reported one of the complementary genes for production of stigma colour may be same as one of the complementary genes for production of colour of leaf sheath. Therefore it can be concluded that one of the two complementary genes causing the basal leaf sheath colour is also complementary to the pigmentation in the stigma colour and the internode colour.

When Lemma and palea was non-pigmented only a few plants were pigmented in the basal leaf sheath (Table 3). When Lemma and palea and awn were non-pigmented only a few accessions were pigmented in basal leaf sheath. Therefore the presence of linkage or pleiotropy between lemma and palea and basal leaf sheath is suggested. When leaf colour was pigmented only a few accessions of F_2 plants were non-pigmented for the awn but vice

Table 3: Observed pigmentation in different Plant Parts (+, Presence; -, absence)

Basal leaf sheath color	Stigma colour	Leaf colour	Stem color	Awn color (early)	Lemma-palea colour	Number of plants
+	+	+	+	+	+	45
+	+	+	+	+	-	3
+	+	+	+	-	+	5
+	+	+	+	-	-	1
+	+	+	-	+	+	25
+	+	+	-	-	+	2
+	+	-	+	+	+	8
+	+	-	+	+	-	1
+	+	-	+	-	+	7
+	+	-	+	-	-	2
+	+	-	-	+	+	7
+	+	-	-	+	-	1
+	+	-	-	-	+	1
+	+	-	-	-	-	1
-	-	+	-	+	+	11
-	-	+	-	+	-	25
-	-	-	-	+	+	2
-	-	-	-	+	-	13
-	-	+	-	-	-	1
-	-	-	-	-	+	27
-	-	-	-	-	-	22

versa were prominent. Therefore the genes governing the leaf pigmentation also determine the awn colour. So a close association between these two traits with one gene common and presence of inhibitory gene action for the leaf apex colour is assumed.

When there was pigmentation in the internode most of the accessions showed the pigmentation in the awn and lemma and palea (Table 3). So a close association between the pigmentation of internode with the lemma and palea and awn is assumed. The lack of pigmentation of the internode when the other part was pigmented was due to the inhibitory gene action (Table 2). For the pigmented internode there was the presence of pigmentation in the stigma and basal leaf sheath but not all the pigmented stigma and basal leaf sheath present the pigmented internode. This shows the similar basic genes acting for the internode colour but its expression is reduced by other inhibitor genes.

CONCLUSION

The F_1 showed pigmentation in all of the plant parts where *O. rufipogon* was pigmented. The segregation in the F_2 generation was found to be very complex. The inheritance of anthocyanin pigmentation pattern found in the different plant parts was more complicated. The segregation of pigmented: non-pigmented found for basal leaf sheath, stigma and leaf apex was digenic with complementary gene action (9: 7). For the awn and lemma and palea pigmentation, a digenic ratio of 11 pigmented: 5 non-pigmented was observed. Inhibitory gene action with four genes (81 pigmented: 175 non-pigmented) for the internode colour was found. One of the basal leaf sheath pigmentation gene was found to be pleiotropic for the stigma and internode pigmentation. There is the linkage in the basal leaf sheath colour and the stigma colour.

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