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PLEIOTROPY AND LINKAGE IN RICE (*ORYZA SATIVA* L.). II. PLEIOTROPIC INHIBITORS AND ANTI-INHIBITOR, AND LINKAGE OF ANTHOCYANIN PIGMENTATION GENES: LEAF BLADE, INTERNODE, NODAL RING AND PANICLE BASE

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ABSTRACT

Inheritance pattern of anthocyanin pigmentation in leaf blade, internode, nodal ring and panicle base of rice has been investigated in an intervarietal cross between the upland rice cultivars D 6-2-2 (green variety) and HY-256 Purple (purple variety). Three to five pairs of genes control the pigmentation of leaf blade (F_2 ratio 39 : 25), internode (147 : 109), nodal ring (63 : 961), and panicle base (171 : 853). Two pleiotropic inhibitory genes and one anti-inhibitory gene show variable expression in these plant parts. Linkage has been established between the basic genes for pigmentation in leaf blade, internode and panicle base. This linkage group (Ppba–Pl–Pin) probably forms a part of the II (lg) linkage group of Misro et al. [32], in which the genes Ppba and Pin for pigmentation in panicle base and internode are new additions.

Key words: Rice, anthocyanin, inheritance, pleiotropy, linkage.

Existence of pleiotropic genes of different action, viz., basic, complementary, duplicatory, inhibitory, etc., has been already reported in rice by many workers with respect to pigmentation characters [1–3]. These pleiotropic genes have been found to show variable expression for more than one character [4–9]. Recently, we have reviewed reports on pleiotropy in rice [10]. The present investigation aims to study inheritance and interrelationship of pigmentation, either through pleiotropy or linkage, or both, in four parts of the rice plant and to assign the genes responsible for pigmentation in the respective linkage groups.

MATERIALS AND METHODS

An intervarietal cross of rice between two upland cultivars, D 6-2-2 (green variety, devoid of pigmentation in any plant part) and HY-256 Purple (a variety with purple pigmentation in most of the plant parts), developed at Rice Research Station, Mugad, Karnataka, along with F_1, F_2 and F_3 generations was studied. The F_2 population consisted of 2458 plants and 114 randomly selected plants were carried forward for F_3 studies. In each F_3 family, 74-100 plants were available for observation. Presence and absence of anthocyanin pigment in leaf blade, internode, nodal ring and panicle base were recorded visually by using a 10x hand lens. Joint segregations were worked out taking two characters at a time. The χ^2 test was applied for detection of pleiotropy and linkage. Joint ratios were modified on the basis of pleiotropic common gene(s) between any two particular characters and recombination/crossover values were estimated by applying the product ratio method of Fisher and Balmakund [11] in cases where linkage was detected. Gene symbols recommended by the International Rice Commission [12] have been used.

RESULTS

INDIVIDUAL CHARACTERS

The behaviour of parents, F_1 and F_2 segregation are presented in Table 1. Pigmentation was dominant in leaf blade and internode but recessive in nodal ring and panicle base. In the F_2 generation, pigmentation in leaf blade segregated into the ratio of 39 purple : 25 green, indicating the involvement of a basic gene, an inhibitory gene and an anti-inhibitory gene. Pigmentation in internode showed segregation of 147 : 109 for purple vs. green with an assumption of four interacting genes conditioning pigmentation, of which, one is basic, two are inhibitory–duplicate, and one anti-inhibitory gene. While the pigmentation in nodal ring and panicle base segregated into the pentagenic ratios of 63 : 961 and 171 : 853 for purple vs. green, the segregation pattern of nodal ring pigmentation indicated the role of five genes which consisted of three duplicate genes for formation of pigment and two inhibitory–duplicate genes. Pigmentation in panicle base, however, was found to be under the control of two complementary genes that govern pigment synthesis in this part along with one

Table 1. Behaviour of parents, F_1 and F_2 segregation for pigmentation in four plants in the cross D 6-2-2 x HY-256 Purple of rice

Organ	D 6-2-2	Hy-256 Purple	F_1	F_2 segregation					χ^2
				observed		ratio tested			
				purple	green	purple		green	
Leaf blade	Green	Purple	Purple	1503	955	39	:	25	0.046
Internode	Green	Purple	Purple	1409	1049	147	:	109	0.009
Nodal ring	Green	Green	Green	157	2301	63	:	961	0.235
Panicle base	Green	Purple	Green	425	2033	171	:	853	0.617

Table 2. Joint phenotypic segregation for pigmentation in leaf blade, internode, nodal ring and panicle base in F_2 generation of rice

Character pair	Basis of expectation (C.O.%)	Expected joint ratio	Genes involved in linkage	O/E	Phenotypic frequencies				χ^2
					XY	Xy	xY	xY	
Leaf blade (39:25) with:									
Internode (147:109)	Independent			O	1241	262	168	787	
	1 gene	5733:4251:3675:2725	—	E	860	637.7	551.3	408.8	1006.4
	Linkage (17.71)	1737:759:615:985	—	E	1042.3	455.4	369.0	591.1	294.4
		—	Pl-Pin	E	1240.2	257.6	171.1	788.9	0.14
Nodal ring (63:961)	Independent			O	120	1383.0	37.0	918.0	
	2 genes	2457:37479:1575:24025	—	E	92.2	1405.6	59.0	901.0	17.34
		189:2307:63:1537	—	E	113.4	1384.4	37.8	922.3	0.421
Panicle base (171:853)	Independent			O	421.0	1082	4.0	951.0	
	2 genes	6669:33267:4275:21325		E	250.1	1247.7	160.3	799.8	319.4
	Linkage (1.29)	513:1983:171:1429		E	307.8	1189.9	102.6	857.5	156.3
		—	Pl-Ppba	E	406.6	1091.2	3.85	956.3	0.623
Internode (147:109) with:									
Nodal ring (63:961)	Independent			O	94.0	1315.0	63.0	986.0	
	1 gene	9261:141267:6867:104749		E	86.8	1324.5	64.3	982.1	0.705
		2349:35283:1683:26221		E	88.1	1323.3	63.12	983.4	0.454
Panicle base (171:853)	Independent			O	59.0	1350.0	366.0	683.0	
	2 genes	25137:125391:18639:92977		E	235.7	1175.7	174.7	871.8	408.4
	Linkage (19.99)	432:8976:2304:4672		E	64.81	1346.6	345.6	700.9	2.184
		—	Pin-Ppba	E	58.74	1352.6	351.73	694.8	0.787
Nodal ring (63:961) with:									
Panicle base (171: 853)	Independent			O	90.0	335.0	67.0	1966.0	
	2 genes	10773:164331:53739:819733		E	25.25	385.0	125.9	1921.5	201.22
		2304:8640:1728:52864		E	86.41	324.06	64.81	1982.7	0.73

Table 3. Phenotypes and genotypes of the parents D 6-2-2 and HY-256 Purple in rice for anthocyanin pigmentation

Organ	D 6-2-2		HY-256 Purple	
	phenotype	genotype	phenotype	genotype
Leaf blade	Green	pl pl I-Pl I-Pl ai-Pl ai-Pl	Purple	Pl Pl i-Pl i-Pl Ai-Pl Ai-Pl
Internode	Green	pin pin i-pin$_1$ i-Pin$_1$ i-Pin$_2$ i-Pin$_2$	Purple	Pin Pin I-Pin$_1$ I-Pin$_1$ I-Pin$_2$ I-Pin$_2$
Nodal ring	Green	pnr$_1$ pnr$_1$ pnr$_2$ pnr$_2$ ai-Pl ai-Pl i-Pnrl I-Pl I-Pl	Green	Pnr$_1$ Pnr$_1$ Pnr$_2$ Pnr$_2$ Ai-Pl Ai-Pl I-Pnr$_1$ I-Pnr$_1$ i-Pl i-Pl
Panicle base	Green	ppb$_a$ ppb$_a$ ai-Pl ai-Pl I-Pl I-Pl i-Ppb$_{b1}$ i-Ppb$_{b1}$ i-Pin$_1$ i-Pin$_1$	Purple	Ppb$_a$ Ppb$_a$ Ai-Pl Ai-Pl i-PL i-Pl I-Pbb$_{b1}$ I-Ppb$_{b1}$ I-Pin$_1$ I-Pin$_1$

Note. Both parents are homozygous for the C, A and P loci of the C–A–P complementary gene system.

inhibitory basic and two inhibitory–complementary duplicate genes. The breeding behaviour of pigmentation in the four plant parts in 114 F_3 families, each with 74–100 plants, confirmed the conclusions based on F_2 segregation.

LINKAGE

The combined segregation for the characters under investigation (Table 2) indicated the existence of two pleiotropic common inhibitory genes which govern pigmentation in leaf blade, nodal ring and panicle base, one pleiotropic inhibitory gene governs pigmentation in the internode and panicle base. A pleiotropic anti-inhibitory gene was also detected which caused pigmentation in all these four plant parts. On the basis of common pleiotropic inhibitors and one anti-inhibitor, the combined ratios were modified. The observed frequencies in all the cases showed significant deviations from the expected ones calculated on the basis of the modified joint ratios as revealed by the abnormally high χ^2 values, thus indicating the probability of linkage between the basic genes for pigmentation in leaf blade internode and panicle base. The crossover values, when applied to all such cases, reduced the χ^2 values to a considerable extent and a satisfactory fit could be obtained in these cases. The sequence of the genes as indicated by the crossover values was followed while constructing the linkage map as presented in Fig. 1. The order of genes being Ppba–Pl–Pin. The Kosambi [13] formula was applied to correct map distances and the modified values are given in parentheses.

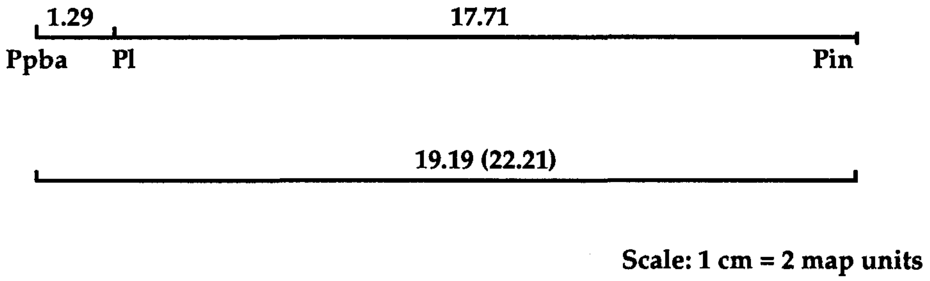

Fig. 1. **Linkage map of rice showing the relative positions of three genes. Calculated crossover values are in percentages. Modified crossover values as per [13] are given in parentheses.**

DISCUSSION

INDIVIDUAL CHARACTERS

Earlier, a few workers have attempted to detect pleiotropic inhibitory and anti-inhibitory genes while studying the inheritance and relationship of anthocyanin pigmentation in different plant parts of rice [3, 8]. Various studies on the inheritance of pigmentation in rice have reported genetic ratios for purple vs. green to be from monogenic to tetragenic for leaf blade as 3 : 1 [14], 3 : 13 [15–17], 9 : 7 [18], 9 : 55 [15], 27 : 229 [15], and 117 : 139[20]; and for internode as 3 : 1 [21, 22], 3 : 13 [8], 9 : 7 [4, 23], 27 : 37 [14, 24, 25], 45 : 19 [26, 27], 54 : 10 [28], 81 : 175 [29], 117 : 139 and 162 : 94 [30]. Only two reports are available on the inheritance of nodal ring pigmentation, which indicate operation of three pairs of interacting genes to give an F_2 ratios of 9 : 55 [31] and 45 : 19 [27]. The variation in F_2 segregation ratios for the same character can be attributed to differences in the parents used and nature of gene action in particular cross combinations. There are, however, no reports on the inheritance of anthocyanin pigmentation in panicle base.

In the present investigation, F_2 segregation ratios conforming to trigenic [39 : 25], tetragenic (147 : 109) and pentagenic (63 : 961 and 171 : 853) for purple vs. green have been obtained in respect of pigmentation patterns in leaf blade, internode, nodal ring and panicle base, respectively. While the segregation ratios observed for pigmentation in leaf blade, internode and nodal ring are additions to the existing knowledge, the observation of 171 : 853 ratio for purple vs. green panicle base has not been reported earlier.

LINKAGE

The analysis of joint segregation for pigmentation in leaf blade, internode, nodal ring and panicle base of rice led to the identification of two pleiotropic inhibitors with variable expression, viz., I-Pl (an inhibitory gene for purple leaf blade) and I-Pin1 (one of the inhibitory duplicate genes for internode pigmentation). A pleiotropic anti-inhibitory gene (Ai-Pl), primarily responsible for pigmentation in leaf blade also acts similarly and differentially in different parts. A pleiotropic inhibitory gene, I-Pl, acts as an independent inhibitor for pigmentation in the nodal ring region and as inhibitory basic gene for pigmentation in panicle base. I-Pin1, on the other hand, shows complementary duplicate action for inhibition of pigmentation in the panicle base. A pleiotropic anti-inhibitory gene, Ai-Pl, acts as a common gene for pigmentation in all the four plant parts studied. This gene acts as anti-inhibitory in the developing pigment in internode, while it shows a differential action in nodal ring and panicle base. Thus, it behaves as a duplicate gene for nodal ring pigmentation and as a basic complementary gene for panicle base pigmentation. These three pleiotropic genes for pigmentation. These three pleiotropic genes for pigmentation led to the genotypic constitution of the two parents (Table 3), where the genotypes for anthocyanin pigmentation had one pleiotropic gene common for leaf blade and internode, and for internode and nodal ring, while the other comparisons showed two pleiotropic genes to be common.

Linkage has been detected between one of the complementary genes, Ppba for panicle base pigmentation, and the basic genes Pl and Pin for leaf blade and internode pigmentation, respectively, as the χ^2 values were high when joint ratios modified on the basis of common pleiotropic gene(s) were applied. Recombination or crossover values estimated by the product ratio method were quite satisfactory in view of the significant reduction in the χ^2 values on linkage basis (Table 2). The sequence of the three genes in the linkage map constructed (Fig. 1) based on the crossover values is: Ppba–Pl–Pin.

Misro et al. [32] reviewed the previous work on linkage in rice up to 1965 while formulating linkage groups in *indica* rices. The groups proposed by them have been taken to be appropriate for the present investigation. The revised II (lg) linkage group [32], corresponding to the Pl group of *japonica*, had twelve genes prior to this investigation [33]. The linkage group of three genes concluded from this study is also tentatively placed in the II (lg) linkage group as the gene Pl is common to the said group. In that case, two new genes (Ppba and Pin) are additions to this group.

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