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PLEIOTROPY AND LINKAGE IN RICE (*ORYZA SATIVA* L.). III. LINKAGE INVOLVING BASIC AND INHIBITORY GENES FOR PIGMENTATION: LEAF BLADE, SHEATH PULVINUS, GLUME (EMPTY), NODAL SEPTUM AND BLACKENING OF LEMMA/PALEA

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ABSTRACT

Inheritance pattern of anthocyanin pigmentation in leaf blade, sheath pulvinus, glume (empty) and nodal septum and a nonanthocyanin pigmentation character, namely, blackening of lemma/palea, was studied in a cross between the upland rice cultivars D 6-2-2 and HY-256 Purple. Three to five pairs of interacting genes have been found to govern pigmentation in these plant parts. An anti-inhibitory gene for pigment synthesis in leaf blade is pleiotropic by producing pigment in glume (empty) and nodal septum also. Linkage has been established between the basic genes for pigmentation in leaf blade, nodal septum and sheath pulvinus and the inhibitory genes for pigmentation in glume (empty) and blackening of lemma/palea. This linkage group (Pl–Pm_a–Pu–I-H–I-Pg) probably forms a part of the II (lg) linkage group of Misro et al. In that case, the four genes Pm_a, Pu, I-H and I-Pg are new additions to this linkage group.

Key words: Inheritance, anthocyanin, anti-inhibitor, pleiotropy, linkage, rice.

Genetics of anthocyanin pigmentation in rice is complicated as many genes interact to develop colour in various plant parts such as coleoptile, leaf sheath, leaf blade, ligule, auricle, junctura, internode, glume, apiculus, lemma, nodal ring, nodal septum etc. Earlier studies have reported basic genes for anthocyanin and nonanthocyanin pigmentation in different plant part and other modifier complexes [1, 2]. Interaction of genes has been worked out in many cases. Pleiotropic genes play an important role in determining pigmentation in different plant parts [3–7]. In the present paper, inheritance and relationship of pigmentation in five plant parts of the rice plant are reported and genes responsible for pigmentation in these plant parts are assigned to different linkage groups.

MATERIALS AND METHODS

A cross between two upland rice cultivars, namely, D 6-2-2 (green variety without pigmentation in any of its plant parts) and HY-256 Purple (a variety with purple pigmentation in most of its parts), was made in 1987 and F_1, F_2 and F_3 generations studied in subsequent seasons. 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 or absence of anthocyanin pigmentation in the leaf blade, sheath pulvinus, glume (empty) and nodal septum, and blackening of lemma/palea during flowering were recorded visually by using 10X hand lens. Joint segregations were worked out taking two characters at a time. The χ^2 test was applied to detect pleiotropy and linkage. Joint segregation ratios were modified on the basis of common pleiotropic genes for any two particular characters, and recombination/crossover values were estimated by applying the product ratio method developed of Fisher and Balmakund [8] in those cases where linkage was detected. The gene symbols recommended by the International Rice Commission [9] have been used.

RESULTS

INDIVIDUAL CHARACTERS

The phenotypes of parents, F_1 and F_2 segregations are presented in Table 1. Pigmentation was dominant in leaf blade and nodal septum but recessive in sheath pulvinus, glume (empty), and blackening of lemma/palea. In the F_2 generation, pigmentation of leaf blade segregated into the ratio of 39 purple : 25 green, indicating the involvement of one basic gene, one inhibitory, and one anti-inhibitory genes. Pigmentation in nodal septum segregated into 81 purple : 175 green, which would be expected if four

Table 1. Phenotype of parents, F_1 and F_2 segregation for pigmentation in four plant parts 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		expected ratio		
				purple	green	purple	green	
Leaf blade	Green	Purple	Purple	1503	955	39 :	25	0.046
Sheath pulvinus	Green	Green	Green	52	2406	21 :	1003	0.051
Glume (empty)	Green	Purple	Green	604	1854	255 :	769	0.143
Nodal system	Green	Green	Purple	779	1679	81 :	175	0.003
Blackening of lemma/palea	Absent	Absent	Absent	262 (black)	2196 (white)	27 : (black)	229 (white)	0.333

complementary genes are involved. The pentagenic ratios of 21 : 1003 and 255 : 769 for purple vs. green were obtained for pigmentation in sheath pulvinus and glume (empty), respectively. The segregation pattern of sheath pulvinus pigmentation indicated the role of one basic gene for pigment synthesis with two independent inhibitory duplicate genes and two inhibitory complementary genes. Pigmentation in glume (empty), however, was found to be under the control of four duplicate genes pigment synthesis and one inhibitory gene. The nonanthocyanin blackening of lemma/palea at flowering showed segregation in the ratio of 27 : 229 for presence vs. absence, suggesting four interacting genes, three of which are complementary and one inhibitory. The breeding behaviour of pigmentation for these five characters in 114 F_3 families each of 74–100 plants confirmed the F_2 segregation.

LINKAGE

The combined segregation for the characters under investigation (Table 2) indicated the existence of a pleiotropic anti-inhibitory gene, Ai-Pl, primarily responsible for leaf blade pigmentation, which was involved in the anthocyanin pigmentation of glume (empty) as duplicate gene and of nodal septum as complementary gene. On the basis of pleiotropic common gene, the combined ratios were modified. The observed frequencies in all the cases showed significant deviations from the expected ones on the basis of the modified joint ratios as revealed by the high χ^2 values, thus indicating the probability of linkage between the basic genes for pigment synthesis in leaf blade, nodal septum and sheath pulvinus, and inhibitory genes for glume (empty) pigmentation and blackening of lemma/palea. The crossover values, when applied to all these cases, reduced the χ^2 values to a considerable extent and a satisfactory fit was obtained in all the cases. The gene sequence, as indicated by the crossover values (Fig. 1) was: Pl–Pm_a–Pu–I-H–I-Pg. The Kosambi [10] formula was applied to correct the map distances and the modified values are given in parentheses.

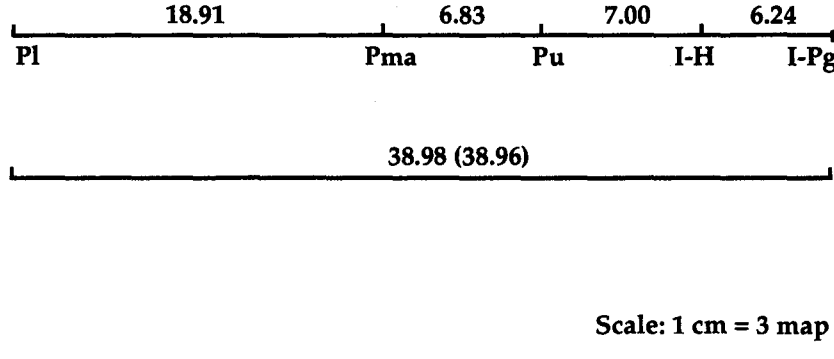

Fig. 1. Linkage map showing the relative positions of five genes. Crossover values are given in percentage. The modified crossover values as per [10] are given in parentheses.

Table 2. Phenotypic joint segregation of anthocyanin pigmentation in leaf blade, sheath pulvinus, glume (empty) and nodal septum and nonanthocyanin blackening of lemma/palea at flowering in F_2 generation

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:									
Sheath pulvinus pigmentation (21:1003)				O	35	1468	17	938	
	Independent	819:39117:525:25075	—	E	30.72	1467.13	19.59	940.46	0.971
	Linkage (30.87)	—	Pl–Pu	E	33.83	1464.01	16.58	943.58	0.095
Glume (empty) pigmentation (255:769)				O	413	1090	191	764	
	Independent	9945:29991:9375:19225	—	E	373	1124.85	239.1	721.05	17.604
	1 gene	2493:7491:1587:4813	—	E	374.01	1123.83	238.08	722.07	16.828
	Linkage (40.42)	—	Pl-I-Pg	E	147.18	1080.66	194.92	765.24	0.203
Nodal septum pigmentation (81:175)				O	538	965	241	714	
	Independent	3159:6825:2025:4375	—	E	473.93	1023.92	303.79	656.36	30.092
	1 gene	972:1524:324:1276	—	E	583.29	914.55	194.43	765.72	20.947
	Linkage (25.75)	—	Pl–Pma	E	535.68	962.17	242.05	718.1	0.046
Blackening of lemma/palea (27.229)				O	118	1385	144	811	
	Independent			E	157.97	1339.87	101.27	858.89	32.333
	Linkage (33.02)	—	Pl–I–H	E	116.13	1381.71	143.12	817.04	0.088
Sheath pulvinus pigmentation (21:1003) with:									
Glume (empty) pigmentation (255:769)				O	3	49	601	1805	
	Independent	5355:16149:255765:771307	—	E	12.55	37.85	599.55	1808.05	10.56
	Linkage (9.26)		Pu–I–Pg	E	2.96	47.45	609.14	1798.45	0.184
Nodal septum pigmentation (81:175)				O	21	31	758	1648	
	Independent	1701:3675:81243:175525	—	E	15.95	34.46	761.75	1645.81	1.968
	Linkage (6.83)	—	Pu–Pma	E	20.33	30.08	757.8	1650.19	0.126

(Contd.)

Table 2. (contd.)

Character pair	Basis of expectation (C.O %)	Expected joint ratio	Genes involved in linkage	O/E	Phenotypic frequencies				χ^2
					XY	XY	xY	xY	
Blackening of lemma/palea (27:229)				O	21	31	758	1648	
	Independent	567:4809:27081:229687	—	E	1	51	261	2145	4.515
	Linkage (7.00)	—	Pu–I–H	E	0.96	49.45	258.28	2149.31	0.087
Glume (empty) pigmentation (255:769) with:									
Nodal septum pigmentation (81:175)				O	252	352	527	1327	
	Independent	20665:44625:62289:134575	—	E	193.67	418.43	584.05	1261.85	37.051
	1 gene	5184:11136:15552:33664	—	E	194.43	417.67	583.29	1262.61	36.087
	Linkage (13:21)	—	I–Pg–Pma	E	254.71	357.39	523.01	1322.89	0.154
Blackening of lemma/palea (27:229)				O	1	603	261	1593	
	Independent	6885:58395:20763:176101	—	E	64.56	547.54	194.68	1651.22	92.838
	Linkage (6.24)	—	I–Pg–I–H	E	1.01	611.09	258.24	1587.66	0.155
Nodal septum pigmentation (81.175) with:									
Blackening of lemma/palea (27:229)				O	16	736	246	1433	
	Independent	2187:18549:4725:40075	—	E	82.02	695.7	177.22	1503.06	89.611
	Linkage (7.49)	—	Pma–I–H	E	15.77	761.96	243.47	1436.8	0.041

DISCUSSION

INDIVIDUAL CHARACTERS

Various studies on the inheritance of anthocyanin pigmentation showed the segregation ratios for purple vs. green to vary from monogenic to pentagenic for different plant parts considered in this investigation, viz., for leaf blade 3 : 1 [11], 3 : 13 [12–14], 9 : 7 [15], 27 : 37 [16], 9 : 55 and 27 : 229 [12] and 117 : 139 [17]; for sheath pulvinus 3 : 1 [15], 9 : 7 [11, 18, 19], 15 : 1 [20], 81 : 175 [21], and 162 : 94 [22]; for glume (empty) 3 : 1 [18, 23, 24], 3 : 13 [25], 9 : 7 [2], 15 : 1 [26], 9 : 55 [5, 27], 27 : 37 [3, 28], 81 : 175 [22], 117 : 139 [29, 30], 207 : 49 [24] and 243 : 781 [22]; and for nodal septum 3 : 1 [15, 31], 9 : 7 [3, 28], 15 : 1 [26], 27 : 37 [32], 54 : 10 [33], and 81 : 175 [34]. The variation in F_2 segregation ratios for the same character can be attributed to differences in the genotypes of the parents used and nature of gene action in those combinations. There are, however, no reports on the inheritance of blackening of lemma/palea at flowering, which is a nonanthocyanin pigmentation character.

In the present investigation, the F_2 segregation ratios for purple vs. green

were: trigenic (39 : 25) for leaf blade, tetragenic (81 : 175 and 27 : 229) for nodal septum and blackening of lemma/palea, and pentagenic (21 : 1003 and 255 : 769) for sheath pulvinus and glume (empty). While the segregation patterns observed for pigmentation in leaf blade, sheath pulvinus, glume (empty) and nodal septum provide additional support to the previous reports, the 27 : 229 ratio for black lemma/palea is reported for the first time.

LINKAGE

The analysis of joint segregation for pigmentation in these five plant parts led to the detection of a pleiotropic anti-inhibitory gene, Ai-Pl, which is primarily responsible for pigmentation in the leaf blade. This gene has differential action in the pigmentation of glume (empty) and nodal septum. It acts as a duplicate gene for glume pigmentation and as a complementary gene for pigmentation of nodal septum. Based on the proposed hypothesis of genetic control of pigmentation in these parts and operation of a pleiotropic gene, the genetic constitution is of the two parents has been derived (Table 3).

Table 3. Phenotype and genotype for pigmentation characters of the parents D 6-2-2 and HY Purple of rice

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
Sheath pulvinus	Green	pu pu i-Pu_1 i-Pu_1 i-Pu_2 i-Pu_2 i-Pu_{3a} i-Pu_{3a} i-Pu_{3b} i-Pu_{3b}	Green	Pu Pu I-Pu_1 I-Pu_1 I-Pu_2 I-Pu_2 I-Pu_{3a} I-Pu_{3a} I-Pu_{3b} I-Pu_{3b}
Glume (empty)	Green	pg_1 pg_1 pg_2 pg_2 pg_3 pg_3 ai-Pl ai-Pl I-Pg I-Pg	Purple	Pg_1 Pg_1 Pg_2 Pg_2 Pg_3 Pg_3 Ai-Pl i-Pg i-Pg
Nodal septum	Green	Pma Pma Pmb Pmb pmc pmc ai-Pl ai-Pl	Green	pma pma pmb pmb Pmc Pmc Ai-Pl Ai-Pl
Blackening of lemma/palea	Absent	ha ha hb hb hc hc i-H i-H	Absent	Ha Ha Hb Hb Hc Hc I-H I-H

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

Linkage was detected between the basic genes, Pl and Pu for leaf blade and sheath pulvinus pigmentation, the complementary gene Pm_a for nodal pigmentation, and the inhibitory genes I-Pg and I-H for glume pigmentation and blackening of lemma/palea. The χ^2 values were high when joint segregations were tested on the basis of independent

assortment and modified on the basis of pleiotropic common gene. Recombination values estimated by the product ratio method were satisfactory as is evident from the considerable reduction in the χ^2 values on linkage basis (Table 2). The sequence of the five linked genes (Fig. 1) based on the crossover values is: Pl–Pm_a–Pu–I-H–I-Pg.

Misro et al. [36] reviewed the studies on linkage in rice up to 1965 while formulating linkage groups of *indica* rices. The groups proposed by them are equally applicable in the present investigation. The revised II (lg) linkage group [36] corresponding to the Pl group of *japonica* had twelve genes prior to this investigation [37]. The present linkage group of five genes have been placed tentatively in the same II (lg) linkage group as the gene Pl was common to both. In that case, four genes, i.e. Pm_a, Pu, I-Pg and I-H have been added to this group.

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