INHERITANCE OF ANTHOCYANIN PIGMENTATION IN LEAF BLADE OF RICE (*ORYZA SATIVA* L.)

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The occurrence and distribution of anthocyanin pigmentation in the organs of the rice plant is very variable and a very striking feature of the crop. The plants range from fully green in all parts through those with pigmentation present in only one, two or more parts, to a plant fully pigmented in all parts. The genetics of this character has attracted wide interest and attention from early times, and its mode of inheritance in the individual plant parts has been extensively studied.

The investigation reported here deals with number, role and interrelationship of the genes governing the expression of anthocyanin pigmentation in the leaf blade, which may be entirely non-pigmented (green) or fully pigmented; in the latter case the leaf blade may be purple, purple wash or faint purple wash in colour.

A survey of literature shows that previous workers have dealt only with crosses between pigmented and non-pigmented leaf blades, and no investigation dealing with crosses between two pigmented leaf blade types appears to have been reported. The interrelationship of genes controlling pigmentation in two coloured types is reported here for the first time.

Purple leaf blade has been shown by some workers to be caused by the interaction of a basic and two complementary genes, all three of them being essential for the production of purple colour, and ratios like 3:1, 9:7 and 27:37 of purple: green have been reported. Kato quoted by Matsuura (1933), Nakayama (1935) and Ramiah (1953) reported a monogenic ratio of 3 purple: 1 green. Sethi (1934) and Kato quoted by Matsuura (1933) found 9 purple:7 green in their crosses. Takezaki (1923) in a cross between purple and green leaf blade obtained a ratio of 27 purple:37 green. Jones (1930) also obtained a three factor segregation for leaf colour, but obtained three classes of 27 purple:9 purple striped:28 green leaves. Alam (1939) obtained an F_2 ratio of 39 intermediate purple:9 full purple:16 green from a cross between purple lamina and green lamina. He explained this on the basis of 3 genes, one of which is an inhibitor reducing the intensity and distribution of the pigment. Ramiah (1953) states that in one of his crosses (unpublished records) the F_1 had a reduced amount of purple in the lamina, and that in the F_2 he obtained a three factor segregation of 39 intermediate purple:9 purple:12 green leaf with tip and margin purple:4 green.

Presence of an inhibitory factor in certain crosses between purple leaved and green leaved varieties has been shown by some authors. Sethi (1934) obtained a peculiar

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ratio of 10 green:1 purple and the F_3 generation showed ratios varying between 1:1 and 9:1 of green:purple. Kadam (1936) and Dave (1948) obtained ratios of 13 green:3 purple in the F_2 generation from crosses involving the same purple leaf parent *Nagkesar* but different green parents. Dave in another cross using the same purple leaf parent but two other green leaf varieties obtained a ratio of 3 green:1 purple; the monogenic F_2 ratio was confirmed by F_3 results. Yamaguchi (1938) showed that in addition to three genes **S**, **B** and **Pl**, concerned in the production of purple pigment in the lamina, an inhibitory gene **H** was also present which in the homozygous or heterozygous condition caused light colour, **hh** plants alone being dark purple pigmented. Chakravarthy (1940), as quoted by Ramiah (1953), obtained a ratio of 8·3 green:1 purple leaf blade in F_2, and in subsequent generations obtained ratios varying between 4·5:1 and 9:1 of green:purple. Chakravarty (1948) obtained a ratio of 13:3 and 57:7 of green leaf:purple leaf in the F_2 of different crosses between purple and green leaf blade parents. The digenic and trigenic inheritance are explained by him on the basis of a colour factor **L** interacting with one inhibitory factor and two inhibitory factors respectively.

It would thus appear that so far three genes have been reported to be responsible for the production of anthocyanin pigmentation and a fourth, an inhibitory gene, when present suppresses the expression of this character.

MATERIAL AND METHOD

Twelve strains, viz., CP.12, CH.17, CH.17 *mutant*, T.1029/2, T.786, T.136, HS.19, HS.22, T.23-32, GEB.24, 349 *Jhona* and AC.16, all belonging to the *indica* group of *Oryza sativa* L., were employed in this investigation. Particulars of these strains are given below.

CP.12 was received from Madhya Pradesh and is probably the same as *Nagkesar* used by Kadam (1936) and Dave (1948). It has deep purple pigment in all parts except internode, septum, pulvinus, junctura and lemma-palea. While its junctura is non-pigmented, the mid-rib of the junctura, called by us the junctura back, is pigmented. The leaf-blade is entirely purple and shows the same intensity of pigment on both sides. This leaf blade has been termed purple (P) by us.

CH.17 is an introduction from China and has pigment in all the parts except internode, pulvinus and lemma-palea. The purple pigment of the leaf-blade in this variety is lighter than that in CP.12 and gives the appearance of a homogeneous mixture of purple and green colour. The pigment on the upper surface of the leaf-blade is darker than that on the lower surface, but is evenly spread over the entire lamina. This pigmentation has been called by us purple wash (PW) and is different from the purple wash leaf-blade described by Hutchinson and Ramiah (1938). The purple wash of these authors refers to a green leaf-blade with the pigment diffusing slightly from the tip and margin into the lamina.

CH.17 *mutant* is similar to CH.17, from which it arose by natural mutation, except that the pigment is considerably diluted in all the parts. The pigment is very light purple and has been called faint purple wash (FPW) by us.

The other nine strains, i.e., T.1029/2, T.786, T.136, HS.19, HS.22, T.23-32, AC.16, GEB.24 and 349 *Jhona* are non-pigmented (green) in all the plant parts.

The F_2 generation of various cross combinations was grown in the main season (June-December) and was studied as shown below:

Year	Cross combination studied		
1949-50:	T.1029/2	×	CP.12
	T.1029/2	×	CH.17
	CP.12	×	CH.17
1952-53:	CP.12	×	T.136
	CP.12	×	T.786
	CP.12	×	HS.22
	CH.17	×	T.136
	T.23-32	×	CH.17
1953-54:	CH.17	×	349 *Jhona*
	GEB.24	×	CH.17
	CH.17	×	HS.19
1954-55:	CP.12	×	CH.17 *mutant*
	CH.17 *mutant*	×	AC.16
	CH.17	×	CH.17 *mutant*
	CP.12	×	AC.16

The F_3 generation was raised and studied mostly during the second crop season (January to April). In crosses between purple leaf blade and green the F_2 segregated into two distinct classes of purple and green, while in those involving CH.17 *mutant* as one of the parents, the purple wash plants varied in the intensity of pigmentation, which ranged from lighter purple wash to deeper purple wash. The faint purple wash class too showed a certain amount of variation in the intensity of pigmentation, and in the early stages it was sometimes found difficult to distinguish the faint purple wash plants from green. At flowering, however, the two classes were more distinct; moreover all faint purple wash plants always had light purple or faint purple apiculus and stigma.

EXPERIMENTAL RESULTS

Relationship between pigmented and green leaf blades

(a) *Purple × Green*

To study the relationship between purple and green leaf blades, CP.12 was crossed to several green leaf blade types, viz., HS.22, T.1029/2, T.786, T.136 and AC.16. In all cases the F_1 leaf blade was green. The F_2 segregated into two phenotypes viz., purple and green giving ratios of 13:3, 55:9 and 229:27 of green : purple in the various

crosses, *vide* Tables 1, 2 and 3 given below. The intensity of pigmentation in the purple leaf-blade segregates was similar to that found in CP.12.

Table 1. *Families giving digenic ratio of 13 green : 3 purple leaf blade*

Cross			Leaf blade		Total	χ^2	P
			Green	Purple			
CP. 12 × HS. 22 Family 1	..	..	246	49	295	0·887	0·35
CP. 12 × HS. 22 Family 2	..	..	326	82	408	0·486	0·49
Total	..	..	572	131	703		
Expected (13 : 3)	..	..	571·2	131·8	703		

$$\chi^2 = 0·0082 \qquad P = 0·93$$

Table 2. *Families giving trigenic ratio of 55 green : 9 purple leaf blade*

Cross			Leaf blade		Total	χ^2	P
			Green	Purple			
T. 1029/2 × CP. 12	..	..	137	20	157	0·228	0·62
T. 786 × CP. 12	..	..	410	57	467	1·332	0·25
CP. 12 × AC. 16	..	..	422	63	485	0·462	0·51
Total	..	..	969	140 c	1109		
Expected (55 : 9)	..	..	953·05	155·95	1109		

$$\chi^2 = 1·898 \qquad P = 0·17$$

Table 3. *Family giving tetragenic ratio of 229 green : 27 purple*

Cross			Leaf blade		Total	χ_2	P
			Green	Purple			
C.P. 12 × T. 136	..	..	387	49	436	0·221	0·63
Expected (229 : 27)	..	..	390·02	45·98	436		

The F_2 segregations shown in Tables 1, 2 and 3 indicate two, three and four factor pair differences between CP.12 and the green leaf blade parents respectively. One of these factors is an inhibitor suppressing the expression of purple pigmentation.

To confirm the trigenic F_2 segregation given in Table 2, 55 F_3 families from the green leaf blade class and 9 F_3 families from the purple leaf blade class from the cross T.786 × CP.12 were studied. Of the purple leaf blade cultures, four either failed to germinate or had very poor germination and were discarded; the observations were therefore confined to the remaining five families The results are presented in Tables 4 and 5.

Table 4. F_3 *performance of 55 families from green leaf blade groups* (*Cross T. 786 × CP. 12*)
$G = green\ leaf\ blade\quad P = purple\ leaf\ blade$

		Families				χ^2	P
	Green	55G:9P	13G:3P	3G:1P	Total		
Observed	32	9	10	4	55		
Expected (37 : 8 : 8 : 2) ..	37	8	8	2	55	3·301	0·51

Table 5. F_3 *performance of families from 5 purple leaf blade* F_2 *plants* (*Cross T. 786 × CP. 12*)

		Families			χ^2	P
	Purple	9P:7G	3P:1G	Total		
Observed	1	2	2	5		
Expected (1 : 4 : 4) ..	0·556	2·222	2·222	5	0·400	0·82

The F_3 results are in conformity with the expected.

(b) *Purple Wash × Green*

The relationship of purple wash leaf blade with green was studied in crosses of CH.17 with several green varieties viz., T.1029/2, 349 *Jhona*, GEB. 24, T.23-32 and T.136. The F_1 was purple wash, but somewhat lighter in colour than CH. 17. The F_2 segregated into purple wash and green classes in the ratios of 9: 7 and 27: 37 in different crosses. The pigmentation in the purple wash class varied considerably in the intensity of expression. The data are given in Tables 6 and 7.

Table 6.   *Families giving digenic ratio of 9 purple wash : 7 green*

Cross			Leaf blade		Total	χ^2	P
			Purple wash	Green			
T. 23-32 × CH. 17 ..	..	..	459	315	774	2·930	0·087
T. 1029/2 × CH. 17 Fam. 1	..	..	207	165	372	0·056	0·81
T. 1029/2 × CH. 17 Fam. 2	..	..	231	185	416	0·088	0·77
CH. 17 × 349 *Jhona*	..	..	239	169	408	0·899	0·34
GEB. 24 × CH. 17 ..	..	..	207	182	389	1·455	0·23
CH. 17 × HS. 19 ..	..	..	149	140	289	2·586	0·11
Total ..	..	..	1492	1156	2648		
Expected (9 : 7) ..	..	..	1489·5	1158·5	2648		

$\chi^2 = 0·0096$ P $= 0·92$

Table 7.   *Family giving trigenic ratio of 27 purple wash : 37 green*

Cross					Purple wash	Green	Total
CH. 17 × T. 136	..	..	..	..	187	228	415
Expected (27 : 37)	..	..	..	..	175·1	239·9	415
$\chi^2 = 1·40$ P = 0·24							

The F_2 data in Tables 6 and 7 indicate two and three gene differences between CH. 17, the purple wash strain, and the green strains respectively.

In order to confirm the digenic F_2 segregation, 51 F_3 families (30 from purple wash leaf blade group and 21 from green leaf blade group) from the cross T. 23-32 × CH. 17 were raised in the following year. All the 21 families from green leaf blade group bred true for green colour as expected. Of the 30 purple wash leaf blade families, eight failed to germinate or had very poor germination and were therefore discarded. The results of the remaining 22 families are presented in Table 8.

Table 8. F_3 *performance of families from 22 Purple Wash leaf blade group of the cross* (T. 23-32 × CH. 17).   *PW = Purple Wash*

			Families			Total	χ^2	P
			Purple wash	9PW:7G	3PW:1G			
Observed	..	..	3	11	8	22		
Expected (1 : 4 : 4)		..	2·44	9·78	9·78	22	0·602	0·74

The F_3 results confirmed the F_2 performance.

(c) *Faint Purple Wash × Green*

The relationship of faint purple wash with green leaf blade was studied in a cross between AC.16 and CH.17 *mutant*. The F_1 was purple wash like CH.17 and the F_2 segregated into three phenotypes, viz., purple wash, faint purple wash and green. The data are given in Table 9.

Table 9. F_2 *segregation for leaf blade colour in the cross* (AC.16 × CH.17 mutant)

			Leaf blade				
			Purple wash	Faint Purple wash	Green	Total	
Observed	..	..	187	81	229	497	
Expected (27 : 9 : 28)		..	209·7	69·9	217·4	497	
$\chi^2 = 4·84$ P = 0·090							

The results show trigenic difference between the faint purple wash and green parents.

In order to confirm the F_2 hypothesis, 34 families from green leaf blade, 9 families from faint purple wash and 20 families from purple wash leaf blade group were raised in the F_3 generation. All the 34 F_3 families from green leaf blade group bred true for green, as expected.

Of the purple wash families, while they segregated into two or three phenotypic classes as expected, their exact frequencies could not be determined due to poor development of pigment owing to unfavourable environmental conditions of the second crop season during which the F_3 families were raised. Distinction between faint purple wash and green or between faint purple wash and the lower grade of purple wash could not be determined with accuracy. The greens appeared to be in excess which was probably due to misclassification of faint purple wash into green. The results of 9 faint purple wash families are given in Table 10.

Table 10. F_3 *performance of 9 families from Faint Purple Wash* F_2 *plants:*
(*Cross AC.16×CH.17* mutant)

		Families			Total	χ^2	P
		FPW	9FPW:7G	3FPW:1G			
Observed	..	1	2	6	9		
Expected (1 : 4 : 4)	..	1	4	4	9	2·00	0·37

Relationship between the different coloured leaf blade types

(a) *Purple × Purple Wash*

The purple and purple wash leaf blade relationship was studied in crosses between CP.12 and CH.17. The F_1 was purple wash like the CH.17 parent and the F_2 segregated into three phenotypes viz., purple wash, purple and green. The data are given in Table 11.

Table 11. *Segregation of leaf blade colour in* F_2 *generation of Purple × Purple Wash crosses*

Cross		Leaf blade			Total	χ^2	P
		Purple wash	Purple	Green			
CP. 12 × CH. 17 Fam. 2	..	112	30	44	186	1·720	0·41
CP. 12 × CH. 17 Fam. 5/52	..	466	161	173	800	2·567	0·27
CP. 12 × CH. 17 Fam. 4/53	..	118	40	51	209	2·410	0·30
Total	..	696	231	268	1195		
Expected (39 : 12 : 13)	..	728·2	224·1	242·7	1195		

$\chi^2 = 4·267$ P$=0·12$

The data show trigenic difference between purple wash and purple, one of the genes having an inhibiting effect.

To confirm the F_2 hypothesis, 23 families from green leaf blade, 9 from purple leaf blade, and 16 from purple wash leaf blade group were grown in the F_3 generation during the second crop season of 1955-56. Of the 9 families raised from the purple leaf blade group the population in four families was very small and these were therefore discarded. For the same reason three of the 16 purple wash families grown, were rejected. The performance of the remaining F_3 families is presented in Tables 12, 13 and 14.

Table 12. F_3 *performance of 23 families from Green leaf blade group* (*Cross CP.12 × CH.17*)

		Families			Total	χ^2	P
		Green	3G:1P	13G:3P			
Observed	..	11	5	7	23		
Expected (7 : 2 : 4)	..	12·38	3·54	7·08	23	0·756	0·68

Table 13. F_3 *performance of 5 families from Purple leaf blade F_2 plants*
(*Cross CP.12 × CH.17*)

		Families				Total
		Purple	3P:1PW	3P:1G	12P:3PW:1G	
Observed	...	3	0	2	0	5
Expected (2 : 1 : 1 : 2)	..	1·67	0·83	0·83	1·67	5
$\chi^2 = 5·200$ P=0·16						

Table 14. F_3 *performance of 13 families from Purple Wash leaf blade F_2 plants*
(*Cross CP.12 × CH.17*)

	Families							Total
	Purple wash	3PW : 1G	3PW : 1P	12PW : 4G	13PW : 3P	9PW : 4P : 3G	39PW : 12P : 13G	
Observed	1	2	2	1	0	2	5	13
Expected (7 : 6 : 2 : 8 : 4 : 4 : 8)	2·33	2	0·67	2·67	1·33	1·33	2·67	13
$\chi^2 = 8·18$ P=0·22								

The F_3 results are as expected.

(b) *Purple × Faint Purple Wash*

The purple-faint purple wash leaf blade relationship was studied in a cross between CP.12 and CH.17 *mutant*. The F_1 was purple wash like CH.17 and the F_2 segregated into four classes viz., purple wash, purple, faint purple wash and green. The data are presented in Table 15.

Table 15. *Segregation of leaf blade colour in F_2 generation of the cross*
(CP.12×CH.17 mutant)

				Frequencies				Total
				Purple wash	Purple	Faint purple wash	Green	
Observed	..	..	..	352	97	150	137	736
Expected (117 : 36 : 48 : 55)		..		336·4	103·5	138·0	158·1	736
$\chi^2 = 5·00$	P=0·17							

The F_2 data show a four gene difference between purple and faint purple wash, one of which is an inhibitor gene.

To confirm the F_2 hypothesis, 90 F_3 families (48 from green leaf blade, 20 from purple wash leaf blade, 10 from purple leaf blade and 12 from faint purple wash leaf blade) were raised in the second crop season of 1955-56.   Due to poor germination the population in many of the F_3 families was low, and also due to close planting and unfavourable environmental conditions the development of pigment was defective, with the result that in many cases the distinction between green and faint purple wash or between faint purple wash and lower grades of purple wash was not clear, thus making classification difficult.   Though the expected phenotypes were available the frequencies in many cases could not be fitted into any definite ratios.   The results are presented in Tables 16, 17 and 18.

In general the pigmented plants give less seed than the non-pigmented plants with the result that in many cases an adequate population in the F_3 families raised from pigmented leaf blade F_2 plants was not available for study.   This inadequacy of population in the F_3 families further created difficulties in analysing the data.

Table 16. F_3 *performance of 48 families from Green leaf blade class of the cross*
(CP. 12×CH. 17 mutant)

				Families				Total
				Green	3G : 1P	13G : 3P	55G : 9P	
Observed	..	..	..	27	4	11	6	48
Expected (37 : 2 : 8 : 8)		..		..	32·29	1·75	6·98	6·98	48
$\chi^2 = 6·23$	P=0·10							

The F_3 results confirm the F_3 hypothesis.

It will be seen that while some expected ratios have been obtained others are missing. This is mainly due to small populations in each family and also due to the small number of families raised.   The number of families classified as purple wash is high; this is due to difficulty of distinguishing lower grades of PW from FPW, on account of poor development of pigment in the second crop season.

Table 17.   F₃ performance of 20 families from Purple Wash leaf blade group from the cross (CP.12×CH.17 mutant)

							Families						
PW	3PW : 1P	3PW : 1FPW	3PW : 1G	9PW : 3FPW : 4G	9PW : 3P : 4FPW	13PW : 3P	9PW : 4P : 3G	39PW : 9P : 16FPW 12FPW : 13G	27PW : 12P : 12FPW : 13G	39PW : 12P : 13G	117PW : 36P : 48FPW : 35G	Undetermined ratios	Total
Observed ... 7	1	0	1	2	0	0	1	0	0	—	1	6	20
Expected (7 : 2 : 4 : 14 : 14 : 28 : 4 : 4 : 8 : 8 : 8 : 16) 1·19	0·34	2·39	2·39	4·79	0·68	0·68	0·68	1·37	1·37	1·37	2·75		20

Table 18.   F₃ performance of 9 families from Purple leaf blade group from the cross (CP.12×CH.17 mutant)

					Families					
Purple	3P : 1FPW	3P : 1PW	3P : 1G	9P : 7G	9P : {3PW : 4FPW}	12P : 3FPW : 1G	12P : 3PW : 1G	36P : {9PW : 12FPW : 7G}	Undetermined ratios	Total
Observed ... 1	2	0	1	0	1	1	0	1	2	9
Expected on (4 : 2 : 2 : 4 : 4 : 4 : 4 : 4 : 8) 0·78	0·39	0·39	0·78	0·78	0·78	0·78	0·78	1·55		7

The F_3 results confirm the F_2 hypothesis.

Two families in which no ratios could be fitted were discarded, and the expectation on the basis of 7 families is recorded above. The PW and FPW have been put together in two cases due to the difficulty of separating lower grades of purple wash from faint purple wash owing to poor development of pigment.

Table 19. F_3 *performance of 12 families from Faint Purple Wash leaf blade group from the cross* (CP.12×CH.17 *mutant*)

			Families		Undeter-mined ratios of FPW : G	Total
		FPW	3PW : 1G	3FPW : 1G		
Observed	.. ..	3	0	1	8	12
Expected (8 : 1 : 15)	..	4·0	0·50	7·5		12

While the expected phenotypes viz., purple wash, faint purple wash and green were obtained in the F_3 families raised, their exact ratio could not be determined in many families due to insufficient development of faint purple wash pigment and there was consequently an excess of green leaf blade plants.

(c) *Purple Wash×Faint Purple Wash*

The purple wash-faint purple wash leaf blade relationship was studied in a cross between CH. 17 and CH. 17 *mutant*. The F_1 was purple wash like the CH. 17 parent and in the F_2, two phenotypes were obtained. The data are given in Table 20.

Table 20. *Segregation of leaf blade colour in* F_2 *generation of Purple Wash×Faint Purple Wash mutant in the cross* (CH.17×CH.17 *mutant*)

			Leaf blade		Total
			Purple wash	Faint purple wash	
Observed	..	..	368	134	502
Expected (3 : 1)	..	..	376·5	125·5	502
$\chi^2=0·768$	$P=0·38$				

The data show a monogenic difference between the purple wash and purple wash mutant strains.

DISCUSSION

The evidence from the crosses given above shows that for the production and expression of anthocyanin pigmentation in leaf blade six loci are involved. The role and relation-

ship of the genes concerned are given below. In describing the genes the nomenclature recommended by Kadam and Ramiah (1943) has been adopted.

A-a = basic gene for the production of any colour.

C-c = chromogen gene.

Lp-lp = gene responsible for the production of purple pigment in the leaf blade. **Lp** is epistatic over **Lpw**, and is complementary to **A, C** and **Ld**.

Lpw-lpw = gene responsible for the production of purple wash pigment in the leaf blade; and complementary to **A, C** and **Ld**.

Ld-Ld′-ld = multiple alleles producing pigment. **Ld** is complementary to both **Lp** and **Lpw**. **Ld′** is the mutated gene from **Ld**; this gene considerably reduces the expression of the gene **Lpw**, resulting in the production of faint purple wash colour. **Ld′** has no interaction with **Lp** and behaves like **ld** as far as **Lp** is concerned.

Ilp-ilp = inhibitory gene which is epistatic over **Lp** but does not affect **Lpw**.

Thus **A** and **C** are the two fundamental genes for the production of pigment, which, in the presence of **Lp** and **Ld** and absence of **Ilp**, produce purple leaf blade; in the presence of **Lpw** and **Ld** produce purple wash leaf blade (both with and without **Ilp**), while in the presence of **Lpw** and **Ld′** they produce faint purple wash leaf blade (both with or without **Ilp**). On the basis of the above hypothesis the possible genetic constitution and the phenotypic expression of the various parents used in our study are as follows:

Variety	Genotype	Phenotype
CP.12	**AA CC LpLp lpwlpw LdLd ilpilp**	Purple leaf blade.
CH.17	**AA CC lplp LpwLpw LdLd IlpIlp**	Purple Wash leaf blade.
CH.17 *mutant*	**AA CC lplp LpwLpw Ld′ Ld′ IlpIlp**	Faint Purple leaf blade.
T.1029/2	**aa CC lplp lpwlpw LdLd IlpIlp**	Green leaf blade.
AC.16	**aa CC lplp lpwlpw LdLd IlpIlp**	-do-
T.136	**AA cc lplp lpwlpw ldld IlpIlp**	-do-
T.786	Differs from CP.12 in 3 factor pairs one of which is inhibitory.	-do-
T.23-32 349 *Jhona* GEB.24 HS.19 HS.22	These parents differ from CH.17 in two factor pairs, which could be any two of **AA**, **CC**, **LpLp**, **LpwLpw** and **LdLd** gene pairs. Because of the absence of crosses of these types with CP.12, the exact genotype cannot be stated.	-do-

The F_2 and F_3 results of the crosses between the three pigmented leaf blade varieties and also between pigmented and green leaf blade varieties, justify the six factor hypothesis. Crosses between green leaf blade varieties and purple leaf blade variety

CP.12 give green leaf blade in F_1; and the F_2 segregates into either 13:3 or 55:9 or 229:27 of green to purple leaf blade, depending on whether the two parents crossed differ in two, three or four gene pairs, one of which is inhibitory. The inhibitory gene suppresses the expression of **Lp** gene for purple pigment. As mentioned already the genic constitution of CP.12 is **AA CC LpLp lpwlpw LdLd ilpilp**. AC.16 and T.1029/2, both green leaf blade parents are **aa CC lplp lpwlpw LdLd IlpIlp** and T.136 is **AA cc lplp lpwlpw ldld IlpIlp**. T.786 could have the same constitution as AC.16 and T.1029/2, but in absence of a cross with either CH.17 or CH.17 *mutant* its constitution cannot be specifically stated. HS.22 differs from CP.12 in two factor pairs, one of which is inhibitory and the other could be any one of the remaining five gene pairs. In absence of a cross with CH.17 or CH.17 *mutant* its genotype too cannot be definitely stated.

F_2 segregation of 13:3 of green leaf blade: purple leaf blade in rice has been reported by Kadam (1936), Dave (1948) and Yamaguchi (1938). The F_2 segregation of 55:9 and 229:27 of green lamina to purple lamina has not so far been reported in rice. Imai (1927) in the Morning Glory obtained an F_2 ratio of 55:9 of self coloured flowers to white margined flowers and explained this segregation on the basis of two complementary factors and an inhibitory gene F^h; the inhibitor suppressing the white margin. Alam (1939) got an F_2 ratio of 39 intermediate purple:9 full purple:16 green from a cross between purple lamina and green lamina and explained it on the basis of an inhibitor gene which reduces the distribution of pigment. Hsu & Lu (1943) obtained a four factor segregation of 117 purple:139 green which they explained by an inhibitory factor I and an anti-inhibitor **A**. Ramiah (1953) states that in one of his crosses (unpublished record) he obtained an F_2 segregation of 39 intermediate purple: 9 purple: 12 green with tip and margin purple: 4 green. Dave (1948) in another cross between the same purple leaf parent *Nagkesar* but two other green leaf parents *Bhondu* and EB.17 found the F_1 to be green leaf blade in both crosses and the F_2 segregated into 3 green: 1 purple; his F_2 observations were confirmed by F_3 results. He has explained this ratio on the assumption that the purple parent is **LpLp ii** and the green parents are **LpLp II**. The genic constitution of *Nagkesar* would therefore be similar to CP.12 (**AA CC LpLp lpwlpw LdLd ilpilp**), while the constitution of *Bhondu* and EB.17 would be **AA CC LpLp lpwlpw LdLd IlpIlp**. Since only the **Ilp-ilp** factor pair would be segregating the F_1 therefore would be green leaf blade, and in the F_2 a monogenic segregation of 3 green: 1 purple leaf blade will result. Sethi (1934) in one cross obtained a ratio of 10 green: 1 purple leaf blade and in the F_3 obtained ratios varying between 1:1 and 9:1 of Green: Purple. Chakravarthy (1940) quoted by Ramiah (1953), observed an F_1 segregation of 8·3 green: 1 purple, and in subsequent generations the ratio of green to purple varied between 4·5:1 and 9:1; he thought the ratio to be an aberrant one. The F_2 segregation of 229 green: 27 purple observed by us in the cross CP.12 × T.136 is the equivalent of 8·48 green: 1 purple, and in the F_3 ratios of 3:1, 13:3 (equivalent to 4·3:1), 55:9 (equivalent to 6·1:1) and 229:27 (equivalent to 8·48:1) of green:purple would be expected. Thus the segregations of 10 green:1 purple leaf blade observed by Sethi (1934) or 8·3 green:1 purple leaf blade observed by Chakravarthy

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(1940) were probably similar to the ratio of 229:27 purple observed by us. Chakravarty (1948) obtained two ratios of 13:3 and 57:7 of green lamina to purple and explained the trigenic ratio on the basis of a colour factor **L** and two inhibitory factors. It is quite possible that this ratio was similar to 55 green: 9 purple observed by us.

The crosses between green and purple wash resulted either in a 9:7 or 27: 37 ratio of purple wash to green leaf blade, depending on the constitution of the green parents whether they differed from the purple wash parent by two or three factor pairs. An F_2 segregation of 3 purple: 1 green was obtained by Parnell *et al.* (1917) as quoted by Ramiah (1953) and Kato quoted by Matsuura (1933) and Nakayama (1935); that of 9 purple:7 green has been reported by Parnell *et al.* (1917) as quoted by Ramiah (1953) and Sethi (1934), while a ratio of 27 purple: 37 green has been reported by Takezaki (1923).

The cross between green leaf blade (AC.16) and faint purple wash (CH.17 *mutant*) gave a purple wash F_1 and the F_2 segregated into 27 purple wash: 9 faint purple wash :28 green. The green parent therefore has a genetic constitution of **aa CC lplp lpwlpw LdLd IlpIlp**, while CH.17 *mutant* has **AA CC lplp LpwLpw Ld' Ld' IlpIlp**, and since **Ld** is the higher allelomorph of **Ld'** the F_1 would be purple wash and the F_2 would segregate in the ratio stated above. In the F_3, while families raised from green and faint purple wash groups segregated as expected, those from purple wash segregated into purple wash, faint purple wash and green, but owing to poor development of pigment due to adverse environmental factors, it was difficult to classify them properly and these were therefore abandoned. Jones (1930) in a cross between a purplish leaf blade and a green leaf blade found the F_1 to be purple and the F_2 segregated into 27 purple: 9 purple striped: 28 green. The purplish leaf blade referred to by Jones is probably very similar to faint purple wash leaf blade referred to by us, although in our case the faint purple wash leaf blade plant arose as a mutation in a purple wash leaf blade variety CH.17.

The cross between purple lamina and purple wash lamina gave a purple wash lamina in F_1, and the F_2 segregated into three classes *viz.*, purple wash, purple and green in the ratio of 39:12:13; the F_3 results confirmed the F_2 segregation. The genetic constitution of the two parents shows the presence of inhibitory gene **Ilp** in CH.17 parent and its absence in CP.12 and consequently the F_1 is purple wash. In the F_2 three phenotypic classes $\widetilde{A}$ $\widehat{C}$ $\widetilde{Lp}$ $\widetilde{Lpw}$ $\widetilde{Ld}$ $\widetilde{Ilp}$, $\widetilde{A}$ $\widehat{C}$ lp lp $\widetilde{Lpw}$ $\widetilde{Ld}$ $\widetilde{Ilp}$ and $\widetilde{A}$ $\widehat{C}$ lplp $\widetilde{Lpw}$ $\widetilde{Ld}$ ilpilp, will be purple wash; two phenotypic classes $\widetilde{A}$ $\widehat{C}$ $\widetilde{Lp}$ $\widetilde{Lpw}$ $\widetilde{Ld}$ ilpilp, $\widetilde{A}$ $\widehat{C}$ $\widetilde{Lp}$ lpwlpw $\widetilde{Ld}$ ilpilp will be purple because in both cases the inhibitory factor is absent and the genes **A,C,Lp** and **Ld** are present; the remaining three phenotypic classes will be green and these three classes *viz.*, purple wash, purple and green will occur in the ratio of 39: 12: 13.

In the cross between purple and faint purple wash, the F_1 was purple wash due to the presence of the allelomorph **Ld** coming from CP.12 and the presence of inhibitory gene **Ilp** coming from CH.17 *mutant*; the former is dominant over **Ld'** and the latter is epistatic over **Lp,** gene for purple colour. The F_2 segregated into four phenotypic classes *viz.*, purple wash, purple, faint purple wash and green in the ratio of 117:36:48:55.

Three phenotypic classes $\overline{A}\ \overline{C}\ \overline{Lp}\ \overline{Lpw}\ \overline{Ld}\ \overline{Ilp}$, $\overline{A}\ \overline{C}$ lplp $\overline{Lpw}\ \overline{Ld}\ \overline{Ilp}$, and $\overline{A}\ \overline{C}$ lplp $\overline{Lpw}\ \overline{Ld}$ ilpilp were purple wash; two phenotypic classes $\overline{A}\ \overline{C}\ \overline{Lp}\ \overline{Lpw}\ \overline{Ld}$ ilpilp and $\overline{A}\ \overline{C}\ \overline{Lp}$ lpwlpw $\overline{Ld}$ ilpilp were purple, four phenotypic classes $\overline{A}\ \overline{C}\ \overline{Lp}\ \overline{Lpw}$ $\overline{Ld'}\ \overline{Ilp}$, $\overline{A}\ \overline{C}\ \overline{Lp}\ \overline{Lpw}\ \overline{Ld'}$ ilpilp and $\overline{A}\ \overline{C}$ lplp $\overline{Lpw}\ \overline{Ld'}\ \overline{Ilp}$ and $\overline{A}\ \overline{C}$ lplp $\overline{Lpw}\ \overline{Ld'}$ ilpilp were faint purple wash and the remaining seven phenotypic classes viz., $\overline{A}\ \overline{C}\ \overline{Lp}$ lpwlpw $\overline{Ld}\ \overline{Ilp}$, A C Lp lpwlpw $\overline{Ld'}\ \overline{Ilp}$, $\overline{A}\ \overline{C}$ lplp lpwlpw $\overline{Ld}\ \overline{Ilp}$, $\overline{A}\ \overline{C}\ \overline{Lp}$ lpw lpw $\overline{Ld'}$ ilpilp, $\overline{A}\ \overline{C}$ lplp lpwlpw $\overline{Ld'}$ ilpilp, $\overline{A}\ \overline{C}$ lplp lpwlpw $\overline{Ld'}\ \overline{Ilp}$ and $\overline{A}\ \overline{C}$ lplp lpwlpw $\overline{Ld'}$ ilpilp were green in the ratio given above. Since **Ld'** is complementary to **Lpw** and not to **Lp,** the phenotype $\overline{A}\ \overline{C}\ \overline{Lp}$ lpwlpw Ld' ilpilp is green. In the F_3 while families from the green group and the purple group segregated as expected, those from the purple wash and faint purple wash groups did not give a good fit owing to the small number of families available and the difficulty of classifying certain grades owing to the poor development of pigment due to crowding and other environmental factors. According to Gangulee (1954), for the proper development of anthocyanin pigment sufficient light, temperature, sugar feeding etc. in proper proportion are essential. Overcrowding according to him reduces development of pigment. The purple wash and faint purple wash parents are alike genotypically except that the former has a higher order allele **Ld** and the latter a lower order allele **Ld'** and thus a F_2 segregation of 3 purple wash:1 faint purple wash is obtained.

Summary

Three leaf blade pigment patterns viz., purple (Strain CP.12), purple wash (Strain CH.17) and faint purple wash (Strain CH.17 mutant) have been described.

The mode of inheritance of anthocyanin pigmentation in the leaf blade has been studied by making inter-crosses between the three pigmented types and between these pigmented and several green leaf blade types. A six factor hypothesis showing the role of each gene and its relationship with others is presented to explain this inheritance.

The F_2 segregation of 13:3, 55:9 and 229:27 of green: purple leaf, obtained in crosses between CP.12 × HS.22, CP.12 × T.1029/2 or CP.12 × T.786 and CP.12 × T.136 respectively is due to two, three and four gene differences respectively between the parents crossed; one of these genes is an inhibitory factor which suppresses the expression of purple pigment.

The 9:7 and 27:37 ratios of purple wash: green in crosses between CH.17 and green leaf blade varieties are due to the interaction of two and three complementary genes respectively.

The F_2 segregation of 39 purple wash:12 purple:13 green obtained from the cross CP.12 × CH.17 (purple × purple wash) is shown to be due to interaction of complementary factors and presence of an inhibitory gene **Ilp,** the latter being epistatic over gene **Lp** for purple colour. The four factor segregation of 117PW: 36P: 48FPW: 55G in the cross CP.12 × CH.17 mutant, is shown to be due to interaction of four complementary genes one of which is a lower allele **Ld'** and the other an inhibitory factor **Ilp.**

The monogenic segregation obtained from the cross CH.17 × CH.17 mutant is shown

to be due to a single factor difference **Ld-Ld'**.

The occurrence of purple wash leaf blade phenotype along with the faint purple wash and green leaf blade phenotypes in the F_2 of the cross AC.16 × CH.17 *mutant* (G × FPW) has been explained as being due to the presence of the higher allele **Ld** in AC.16 as against the lower allele **Ld'** present in CH.17 *mutant*.

Some of the anomalous ratios like 3 green: 1 purple, 10 green: 1 purple and 8.3 green: 1 purple reported by previous workers have been explained on the basis of the six factor hypothesis.

ACKNOWLEDGEMENTS

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