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HETEROTIC EFFECT OF ALLELES AT *P1*-LOCUS IN RICE PLANT

—Genetical Studies on Rice Plant, XXX—¹⁾

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Introduction

Overdominance theory which accounts for the phenomenon of hybrid vigor is based on an idea that there are loci at which the heterozygous is superior to both homozygotes and that vigor increases in proportion to the amount of heterozygous. With making more experiments on this phenomenon, data favoring this hypothesis have been accumulated. But, reports on clear-cut cases of the overdominance, or heterotic effect, in which different alleles in an identical locus actually perform different effects and the sum of their different products indeed is superior to the single product produced by either allele in homozygous state, are not numerous. This has been a deterrent to the general acceptance of this hypothesis.

In the present paper the writers intend to demonstrate a case of cumulative action of divergent alleles in a single locus of gene which is responsible for anthocyanin coloration in leaves and stems of rice plant.

First a brief resume of basic gene system concerning the production of anthocyanin color in rice plant will be made. According to the writers' point of view, the occurrence of anthocyanin pigment in rice plant depends on the complementary effect of genes *C* and *A*: *C* is a basic gene for the production of chromogen and *A* exerts its activation effect on *C* and turns the chromogen into anthocyanin. *C* and *A* both comprise multiple allelic series of genes: six alleles have been found at the *C* locus and four at the *A* locus (NAGAO and TAKAHASHI 1963). They are arranged according to the rank of dominance as $C^B > C^{Bp} > C^{Bt} > C^{Br} > C^{Bm} > C^+$ and $A^E > A > A^d > A^+$. The rank of

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dominancy of each allele at the *C* and *A* loci is in direct proportion to the potency of chromogen production and to the assimilative ability of chromogenic substance in the production of anthocyanin pigment, respectively. In these genic schemes of the anthocyanin color, there is no need of proposing a modifying gene or genes which are responsible to convert color hue or shade in explaining the existence of several color types in rice plant. With regard to the coloration in each part of plant body, the respective distribution gene and genes are in existence. The occurrence and expression of color in these parts are therefore closely related to the gene combination at *C* and *A* loci.

A color type called "Murasaki-ine" which, in Japanese, means purple leaf or purple rice plant is a resultant color pattern by a distribution allele *Pl* at *Pl*-locus, in conjunction with the basic genes of such combination as $C^B A$ or $C^{Bp} A$. The *Pl* allele has been estimated to be concerned with the color distribution over the entire surface of leaf blade, leaf sheath, collar, auricle, ligule and pulvinus (NAGAO 1951). Foreign rice plants are abundant in purple-leaved varieties. Among them, a color type with purple wash in leaf and deep purple color in stem was estimated, by the writers, to be governed by a distribution gene Pl^w in cooperation with the basic genes for anthocyanin production. And a possibility that *Pl* and Pl^w , together with Pl^+ , consist of multiple allelomorphs or that these two are in a relation of pseudoallele has also been pointed out (NAGAO, TAKAHASHI and KINOSHITA 1962).

Experimentals

The best established case of linkage in the 2nd group of rice plant is that between the liguleless gene, *lg*, and *Pl*. MORINAGA *et al.* (1942) first reported this linkage, giving a recombination value of 21%. This was confirmed by many workers including the writers, who announced a recombination value of 31% in the previous paper (NAGAO and TAKAHASHI 1963). Recently the writers added further data and estimated an average linkage intensity of 25.2% (Table 1).

In corroboration of the propriety of the assumption that Pl^w might be one of the allele at the *Pl*-locus, a linkage test between Pl^w and *lg* held in foreign varieties was made, though an indication of linkage has already been obtained through a single cross combination. The result obtained from the present examination is given in Table 1, in which linkage takes place with a recombination value of 23.2%. This value is akin to the value estimated between *Pl* and *lg* within the Japanese varieties. At the same time it has also been ascertained that the liguleless characters both in the Japanese varie-

TABLE 1. Linkage intensities of *Pl* and *Pl^w* with *lg*,
a gene for liguleless character.

Pl-*lg* (9:7) (3:1)

		colored and liguled	colored and liguleless	colorless and liguled	colorless and liguleless	total	recombination value (%)
1		89	17	45	30	181	25.7 ± 3.7
2		101	28	47	35	211	29.8 ± 3.7
3		102	12	59	26	199	24.6 ± 3.5
4		79	19	54	29	181	33.1 ± 4.3
5		87	14	47	33	181	22.4 ± 3.5
6		92	23	56	40	211	28.8 ± 3.6
7		52	8	24	15	99	23.4 ± 4.8
8		54	9	31	21	115	23.4 ± 4.4
9		46	9	29	20	104	25.5 ± 4.9
10		49	9	13	13	84	19.5 ± 4.7
11		50	8	27	21	106	21.0 ± 4.4
12		76	11	32	27	146	18.7 ± 3.5
13		95	19	37	30	181	23.5 ± 3.5
14		67	13	44	26	150	27.8 ± 4.2
15		59	6	32	23	120	16.5 ± 3.3
total	O	1098	205	577	389	2269	25.2 ± 1.0
	C	1089	187	613	380		

$\chi^2 = 4.05$ d.f. = 3 $p = 0.2$

Pl^w-*lg* (9:7) (3:1)

		colored and liguled	colored and liguleless	colorless and liguled	colorless and liguleless	total	recombination value (%)
1		95	15	51	42	203	20.1 ± 3.1
2		53	12	35	22	122	29.3 ± 4.8
3		79	15	52	39	185	23.8 ± 3.5
4		37	2	34	12	85	17.4 ± 4.5
5		38	4	23	13	78	19.7 ± 4.9
total	O	302	48	195	128	673	23.2 ± 1.8
	C	327	52	178	116		

$\chi^2 = 4.93$ d.f. = 3 $p = 0.1$

ties and foreign varieties are identical, since F_1 s from crosses between liguleless varieties of these two groups were liguleless and their F_2 s were similar to their predecessors, giving no liguled segregate.

The color type caused by the presence of Pl^W was introduced into the Japanese varieties through the cross experiment between Japanese tester varieties and foreign varieties. In the process, strains that were keeping the Pl^W allele and were giving rise to fertile F_1 when they were crossed with Japanese varieties were built up. To ascertain that Pl^W and Pl are really allelic, and to examine the actual working realms of their distribution effect, some of these strains with such genotypes as $C^B APl^W$ and $C^{Bp} APl^W$ were crossed with Japanese tester varieties, of which genotypes are $C^B APl$ and $C^{Bp} APl$. Table 2 shows the coloration pattern in three states of genotypes, Pl^W/Pl^W , Pl^W/Pl and Pl/Pl , in cooperation with the basic genes C^{Bp} and A . In this table, “+++” denotes “purple entire” and “+” means “purple wash or streaks”, and it is pointed out that the Pl^W/Pl , the F_1 type, exceedingly abounds with colored realm. In F_2 s of these crosses, three color types in Table 2, a type with Pl^W/Pl^W , a type with Pl^W/Pl , and a type with Pl/Pl ,

TABLE 2. Pattern of anthocyanin coloration in the three genotypic plants, Pl^W/Pl^W , Pl^W/Pl and Pl/Pl .

P ₁ & F ₁	genotype	leaf					stem		pericarp
		blade		sheath	collar	ligule	pulvinus (node)	internode	
		early	late						
H-120	Pl^W/Pl^W	+++	+	+++		+		++	+
H-127	Pl/Pl	+	+++	+++	+	+	+		
F ₁	Pl^W/Pl	+++	+++	+++	+	+	+	++	+

TABLE 3. F_2 segregation mode of color character governed by two alleles at the Pl -locus; Pl^W and Pl .

phenotype	leaf blade	purple			number of individuals	goodness of fit	
	collar and pulvinus	white	purple			χ^2	p
	internode	purple		white			
	pericarp	purple	purple	white			
genotype		$Pl^W Pl^W$	$Pl^W Pl$	$Pl Pl$			
N- 46×H-120		1	2	1	163	0.104	0.9
H-120×H-127		1	2	1	320	3.188	0.2

appeared in a ratio of 1:2:1, suggesting that *Pl^w* and *Pl* are allelic. This is presented in Table 3.

For further verification of the multiple-allelic relation between *Pl^w* and *Pl*, the writers made pedigree culture of the hybrid populations up to F₆ generation. In every generation segregates which seemingly possess such genotypes as *Pl^w/Pl^w* and *Pl/Pl* gave rise to offsprings, all of which is identical with their predecessors. While, segregates which phenotypically was assumed to have a genotype of *Pl^w/Pl* segregated again into the three color types—1 (original parent type, *Pl^w/Pl^w*): 2 (original F₁ type, *Pl^w/Pl*): 1 (original parent type, *Pl/Pl*). As far as the writers have examined, this pattern of segregation holds true in every generation, up to the F₆ generation. Table 4 is an example of the segregation types obtained in the F₅ and F₆ generations. Thus, it may be concluded that the color type with the widest realm of anthocyanin coloration cannot be bred true.

TABLE 4. Segregation types of progenies from three phenotypic segregates of which genotypes are estimated to be *Pl^w/Pl^w*, *Pl^w/Pl* and *Pl/Pl*.

cross combination	geno- type	segregation mode in next generation	number of lines	geno- type	segregation mode in next generation	number of lines	geno- type	segregation mode in next generation	number of lines
N- 44×H-117	<i>Pl^wPl^w</i>	bred true	4	<i>Pl^wPl</i>	1:2:1	9	<i>PlPl</i>	bred true	3
N- 46×H-120	"	"	3	"	"	4	"	"	2
N-122×H-127	"	"	4	"	"	5	"	"	3
total			11			18			8

The distribution effect by two alleles, *Pl^w* and *Pl*, was histologically investigated. This is demonstrated in Figs. 1, 2 and 3. The left top of Fig. 1 is an outward appearance of coloration mode developed in a genotypic plant of *Pl/Pl*, in cooperation with *C^{bp}A*. The striking coloration is seen in leaf blade, leaf sheath, collar and pulvinus, all of which are fully colored with purple shade. Histologically the coloration of leaf blade is due to high density of pigmented cells in the epidermis, including motor cells and cells in stereome of the midrib. In the leaf sheath pigmentation extends to both the epidermal cells and the parenchymatous cells immediately adjacent to the vascular bundles, and in the collar and in the pulvinus epidermal cells form pigment.

The coloration by *Pl^w/Pl^w* is given in Fig. 2. As shown on the left top

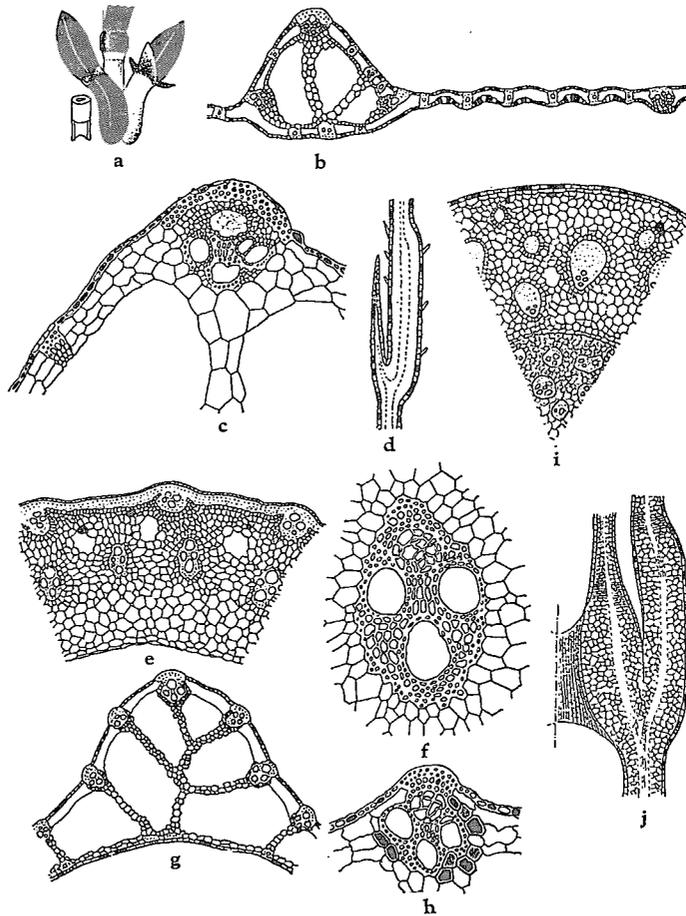


Fig. 1. Histological location of anthocyanin color developed in a genotypic plant of *Pu/Pu*, in cooperation with *C^{Bp}A*.

- a, outward appearance of colored part represented diagrammatically.
- b, cross-section of midrib of leaf blade.
- c, part of b enlarged.
- d, longitudinal-section of collar and ligule.
- e, cross-section of internode.
- f, part of e enlarged showing vascular bundle and adjacent parenchyma.
- g, cross-section of leaf sheath.
- h, part of g enlarged.
- i, cross-section of node.
- j, longitudinal-section of pulvinus and node.

High density of pigmented cells is recognized in the epidermis of both the leaf blade and sheath.

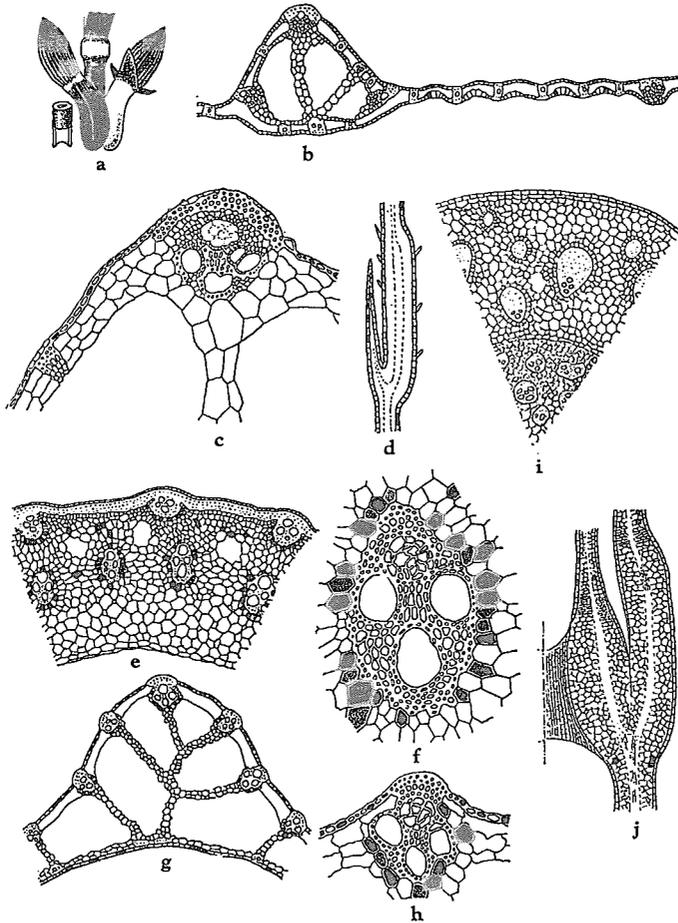


Fig. 2. Histological location of anthocyanin color developed in a genotypic plant of Pl^W/Pl^W , in cooperation with $C^{Bp} A$. a~j, same as Fig. 1.

With the Pl^W allele, color specifically occurs in the internode, of which pigmented cells are located in the parenchyma adjacent to the vascular bundle sheath.

of this figure, the mode of coloration by Pl^W/Pl^W is different from that by Pl/Pl : 1) leaf blade color by Pl^W is washed with purple or almost fade out in the later part of the growing period, while that by Pl is unchanged and remains purple till the end of the season, 2) the Pl^W allele causes internode fully dark purple regardless of its exposure to direct sunlight or not, while the internode color by the Pl allele is expressed only when it is directly

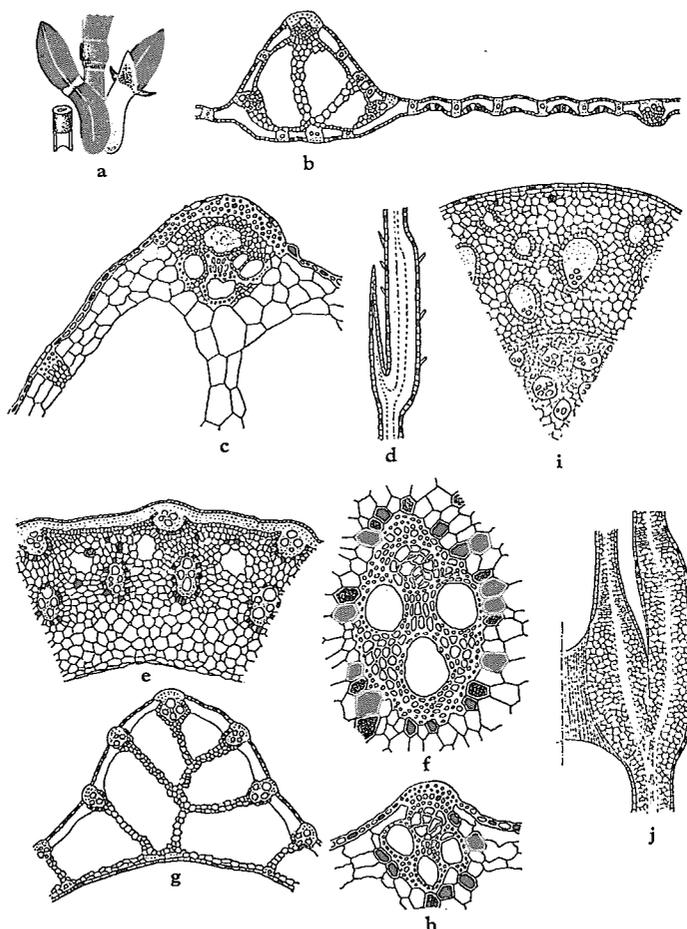


Fig. 3. Histological location of anthocyanin color developed in a heterozygous plant, Pl^W/Pl , in cooperation with $B^{B^p}A$. a~j, same as Fig. 1.

Cumulative effect of alleles, Pl^W and Pl , is demonstrated in the location of cells pigmented with anthocyanin.

insolated, and 3) as to collar and pulvinus colorations, a striking expression resulted as a pleiotropy of Pl and the entirety of their surface is deeply colored, but the effect of Pl^W is rather restricted, appearing to have colored on the upper and lower boundaries of these organs, the middle part being scarcely colored. The internode coloration by the Pl^W -allele derives from the histological cause that three kinds of parenchymatous cell—1) immediately adjacent to the peripheral small vascular bundle, 2) contiguous with large

vascular bundle, and 3) some intervening—are pigmented. This is well illustrated on the right top of Fig. 2.

Fig. 3 accounts for a cumulative effect of *Pl^W* and *Pl* when they are coexistent in a heterozygous state, *Pl^W/Pl*. In this figure it may be particularly needless to mention that the color type and the histological location of pigmental cells in a genotypic plant of *Pl^W/Pl* coincide with an expectation that realm of coloration by the *Pl^W/Pl^W* is overlapped with that by the *Pl/Pl*, or an expectation that the effects of *Pl^W/Pl^W* and that of *Pl/Pl* unite together.

Going back to the inheritance mode of purple leaf character by *Pl^W*, the majority of crosses between the purple leaved, when coexisting with *C^B* or

TABLE 5. Further data of inheritance that proves an existence of the three inhibitors, *I-Pl₁*, *I-Pl₂* and *I-Pl₃*.

phenotype	partial	entire	total	goodness of fit (63:1)	
genotype	<i>Pl^W</i> (<i>I-Pl₁</i> , <i>I-Pl₂</i> , <i>I-Pl₃</i>)	<i>Pl^W + + +</i>		χ^2	<i>p</i>
A- 13×E-44	295	2	297	1.53	0.2
A- 13×E-46	223	5	228	0.59	0.3
H- 69×E-44	217	2	219	0.60	0.3
H-152×H-79	229	4	233	0.04	0.8
total	O	964	977	0.34	0.5
	C	961.73			

homogeneity: $\chi^2=2.41$ d.f.=3 *p*=0.3

phenotype	partial	entire	total	goodness of fit (15:1)	
genotype	<i>Pl^W</i> (up to low loci of <i>I-Pl₁₋₃</i>)	<i>Pl^W + +</i>		χ^2	<i>p</i>
B-12×H-117	O	21	393	0.55	0.3
	C	372			
		24.56			

phenotype	partial	entire	total	goodness of fit (3:1)	
genotype	<i>Pl^W</i> (single locus of <i>I-Pl₁₋₃</i>)	<i>Pl^W +</i>		χ^2	<i>p</i>
N-45×E- 44	224	72	296	0.07	0.7
A-96×H-121	161	44	205	1.37	0.2
total	O	116	501	0.91	0.3
	C	385			
		125.25			

homogeneity: $\chi^2=0.53$ d.f.=1 *p*=0.3

C^{Bp} and A , and the green showed that the former behaved as dominant over the latter. But in certain cross combinations, the leaf blade of their F_1 s were green and the color developed only on the basal portion of the leaf sheath. As briefly reported in the previous paper, this indicates that a sort of inhibiting gene or genes is being involved in the crosses. In F_2 s of these crosses, three color types, with regard to the leaf blade and leaf sheath, appeared. They are, i) purple leaf blade and leaf sheath, ii) green leaf blade with purple leaf sheath, and iii) green leaf blade and leaf sheath. The segregation ratio of i+ii and iii is 9:7 or 3:1, and it has already been known that assortment of these two phenotypic groups depends on the presence or absence of a double dominant combination of basic genes, C and A . The demarcation between i and ii is relatively easy and these two color types segregate in three ways; i:ii=1:3, 1:15 and 1:63. This suggests at least three pairs of inhibitors which depress the leaf blade coloration are involved in these crosses. The symbols of these inhibitors have been designated as $I-Pl_1$, $I-Pl_2$ and $I-Pl_3$. The actual data are given in Table 5.

TABLE 6. Frequency of varieties and their genic constitutions involving genes, C , A , $Pl(Pl^W)$ and $I-Pl$.

group	genotype	number of varieties	leaf			stem		pericarp
			blade	sheath	collar	inter-node	pulvinus (node)	
foreign	$C^B A Pl^W +$	1	purple wash	purple	green	purple	green	purple
	$C^{Bp} A Pl^W +$	1	"	"	"	"	"	"
	$C^{Bm} A Pl^W +$	4	green	green	"	green	"	"
	$C^B + Pl^W +$	3	"	"	"	"	"	reddish brown
	$C^{Bm} + Pl^W +$	1	"	"	"	"	"	"
	$C^B A Pl^W I-Pl^1)$	1	"	purple	"	purple	"	purple
	$C^{Bp} A Pl^W I-Pl$	7	"	"	"	"	"	"
	$C^{Br} A Pl^W I-Pl$	2	"	"	"	reddish purple	"	"
	$C^{Bp} A Pl +$	2	purple	"	purple	green	purple	white
	Japanese	$C^B A Pl +$	2	"	"	"	"	"
$C^{Bp} A Pl +$		3	"	"	"	"	"	"
$C^{Bp} A Pl I-Pl$		2	green	"	"	"	"	"
$C^{Bp} + Pl I-Pl$		1	"	"	"	"	"	"

1) " $I-Pl$ " stands for the presence of one or more of three inhibitors, $I-Pl_1$, $I-Pl_2$ and $I-Pl_3$.

Deducing from the present cross experiment, the following estimation should be noted: 1) in Japanese varieties alleles at the *Pl*-locus are limited to *Pl* and *Pl*⁺, while in foreign varieties, especially in Indian varieties, both the *Pl* and *Pl*^W are held, together with *Pl*⁺, and 2) quite a few varieties, not only in the Japanese but also in the foreign varieties, carry inhibitors for the action of alleles at the *Pl*-locus. The data which gives support to this view is presented in Table 6.

Conclusion and Summary

Pl, which is known as a gene for the purple-leaf character of the Japanese varieties, belong to the 2nd linkage group, and is connected with a distribution effect of anthocyanin color into the leaf blade, leaf sheath and pulvinus, in cooperation with the basic color producing gene combination, *CA*.

In foreign varieties, a color type featured with purple-washed leaf blade, purple leaf sheath and dark purple internode is not a few. This type revealed to have resulted from an existence of a gene *Pl*^W.

Through crosses between Japanese and foreign varieties, *Pl*^W was transferred into the domestic germplasma and some gene stocks with genotypes of *C^BAPl^W* and *C^{Bp}APl^W* that were giving rise to fertile F₁s when they were crossed with ordinary Japanese varieties were built up. These stocks were crossed with Japanese purple-leaved varieties, the genotypes of which were *C^BAPl* and *C^{Bp}APl*, and their hybrid progenies were investigated up to F₆ generation.

In the examination, and in every generation of all the crosses, it was confirmed that *Pl*^W behaves as an allele of the *Pl*-locus, and that a heterozygous state of this locus, viz. *Pl*^W/*Pl*, is superior in its working realm of coloration than those of either homozygotes, *Pl*^W/*Pl*^W and *Pl*/*Pl*. This may be a sort of direct evidence of locus at which the heterozygote lay outside the range of the homozygotes.

In Japanese varieties alleles at the *Pl*-locus are limited to *Pl* and *Pl*⁺, while in Indian varieties both the *Pl* and *Pl*^W are held, together with *Pl*⁺.

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