Nigerian J. Genet: XII (1997): 9 - 19

GENETIC ANALYSIS OF PIGMENTATION IN A CULTIVAR OF RICE (*Oryza sativa* Linn).

J. O. Faluyi* and F.A.Oloyede
Department of Botany,
Obafemi Awolowo University,
Ile-Ife, Nigeria.

ABSTRACT

Two cultivars of Oryza sativa Linn. were studied for the mode of inheritance of pigmentation of their organs. The studies show that purple leaf blade is conditioned at two loci in such a way that the inhibitor locus must be homozygous recessive for the purple leaf blade gene, Pl to express (ii Pl-) . The collar colour is also controlled by the Pl gene. The coloured state of the apiculus, awn, stigma and outer leaf sheath are conditioned by a single dominant gene. The situation is however different for the auricle and ligule which are conditioned by recessive genes at their respective loci. The inner leaf sheath colouration is under the control of two independent genes in complementary action.

The Pl gene exhibited manifold pleiotropic effect on all the organs except the inner leaf sheath whose colour it inhibits. The colour of the sterile lemma is due exclusively to the pleiotropic action of the P1 gene. The gene for apiculus colour (Pa) is a major gene which acts as a basic gene for colour expression in the inner leaf sheath acting in complementary mode with gene (Psh) and the organs of the junctura complex - ligule and auricle -- which can express colour only when the genes that condition colour in them are present in addition to the gene Pa for purple apiculus colour. Gene Pa, in the homozygous recessive condition is therefore said to be epistatic to the colour genes for the auricle and ligule.

Gene Pa also expresses manifold pleiotropic effect on the stigma, awn, nodal ring and outer leaf sheath This is precisely why this gene is considered the most reliable marker gene of the entire genic system considered. The contribution of pleiotropic action of the various genes was estimated to be between 100% and 22%.

Corresponding Author: Department of Botany,Obafemi Awolowo University,Ile-Ife, Nigeria
e-mail: jfaluyi Oau. net

INTRODUCTION

The first comprehensive investigation of anthocyanin pigmentation in *indica* forms of rice were by Hector (1922) and Pannell, *et al.*, (1922). In both investigations, colour showed complete dominance in the F_1 and in all the organs; they reported monogenic and oligogenic conrol of colour in the apiculus and leaf sheath. Nagao (1951) and Takahashi (1957) implicated complementary gene action in which gene *C* produces a colour precursor and gene *sp* produces the anthocyanin. The inheritance of colour is dominated by multiple allelism, colour producing genes and localization genes. Gene *P* is known to control the spreading of chromogen over the entire apiculus. *Pl* governs the distribution of colour over the leaf blade, sheath, pulvinus, auricle, ligule, internode, node, and rachis; *Pa* conditions colour in the leaf apex, leaf margin, auricle and ligule while *Ps* conditions the localization of pigments in the stigma (Chang, 1962; Kondo, 1963).

Nagao and Takahashi (1956) reported that the C-A-P complementary genic system determine anthocyanin pigmentation in the apiculus of *japonica* rice. Setty and Misro (1973) reported three complementary duplicate genes for apiculus colour in *indica* and *japonica* rice. Nagao and Takahashi (1947) reported the action of two unlinked genes *C* and *SP* acting in the presence of one of the three leaf colour genes- *P l. Pla* or *Plm* to

develop anthocyanin in the leaf. Auricle colour is supposed to be controlled by the same gene that controls junctura colour (Pannell, *et al.*, 1917) while nodal colour is supposed to be under the control of the *C* and *Sp* loci in conjunction with *Pa*, the allele that distributes colour to the node.

In our work on baseline studies in the genus *Oryza*, attention has been focused on pigments as markers. Our land races have many cultivars carrying many distinctly coloured organs. This has been used to delimit the *glaberrima* strains in peasant germplasm (Faluyi and Nwokeocha, 1993) and to isolate hybrids between cultivated rice and endemic wild rices. The genetic studies of these colour markers are going on *parri pasu* with studies of morphological markers [awn, ligule, hairiness of leaf and glumles (Nwokeocha and Faluyi, 1994)]. These markers have been found useful in phylogenetic and hybridization studies.

MATERIALS AND METHODS

Two cultivars, I ITA PURPLE and TOS 15223 were used for this study. They were collected from the Genetic Resources Unit of the International Institute of Tropical Agriculture (I ITA), Ibadan, Nigeria. Seedlings of these cultivars were raised in pots and nursed to maturity at which stage reciprocal crosses were made between the cultivars by physical emasculation and the F_1. plants were advanced to the F_2. A total of 951 F_2 plants were raised in October, 1995 in a 12m x 12m plot at a spacing of 9cm x 15cm. Fertilizer was supplied as N.P.K at the five leaf stage by broadcast.

Pigmentation of all vital organs (leaf, collar, ligule, auricle, lemma, palea, sterile lemma, apiculus, stigma, inner leaf sheath, outer leaf sheath, nodal ring and awn) were observed and recorded at various stages (tillering, booting, grain filling). All organs of each plant were scored for presence or absence of purple pigmentation: acyanic organs were scored as white or green in the plants. 25 green F_2 plants were randomly picked and their panicles bagged to raise pure F_3 seeds. 100 seeds were picked from each of these F_3 seed lots and F_3 plants were raised. These plants were scored for segregation and non segregation for leaf blade colouration 15 days after germination. The observed data were grouped for each organ and for joint segregation forcolour with other organs.

Data were subjected to statistical analysis using Chi-square tests. In testing for inhibitor action of the purple leaf gene on the gene causing colour on the inner leaf sheath. the expected number of plants in the unrecovered purple inner sheath, purple leaf colour class was added to the expected frequency of the purple leaf white inner sheath class and Chi-square test was carried out for a 13 green leaf: 3 purple leaf ratio. When there is pleiotropic effect of the leaf blade colour gene (*Pl*) on say the nodal ring, the purple leaf white nodal ring phenotypic class is not recovered. The expected frequency for this class is added to the expected for the purple leaf, purple nodal ring class and Chi square test is carried out for a 13 green leaf :3 purple leaf ratio.

RESULTS AND OBSERVATIONS

All organs in TOS PURPLE except the inner leaf sheath were purple. The organs of TOS 15223 were not coloured, the leaf was green and
the inner leaf sheath was white. The reciprocal F_1's between these two cultivars had pigmented organs except the leaf and collar which were green and 'white', respectively.

Table I shows the observed pigmentation data for all the organs. A fit for a 13:3 ratio of green leaf to purple leaf plants was obtained for leaf blade, sterile lemma and collar colouration. It is important to note that the same plants were involved in the leaf and sterile lemma purple colouration (780 green / colourless: 171 purple). A fit for a 3 purple: I white ratio was obtained for the colouration of apiculus, stigma, nodal ring, awn and outer innder sheath. Again, it is important to note that the colour class frequencies for apiculus, nodal ring and outer leaf sheath were equal (710 purple: 241 white/green in each case). The only organ that fits the 9 purple: 7 white ratio is the inner leaf sheath. The segregation

ratios for ligule and auricle colouration ratios tested.

Table 2 shows the classification of 25 random F2 green plants into segregating and non segregating classes. The data fit a 7 non-segregating: 6 segregating ratio. It is interesting to know that as few as 28 plants (germination of F3 seeds were poor) were enough to 3:1 ratio and 30 plants fitted a 13:3 ratio.

Table 1: Inheritance of pigmentation of organs in cultivar used.

Organ	Observed			$\sum\chi_i^2$		P
	Pr	Gr/W	3:1/1:3*	13:3	9:7	
LEAF	171	780	-	0.36908	-	$0.5 \leq P \leq 0.9$
S/LEMMA	171	780	-	0.36908	-	$0.5 \leq P \leq 0.9$
APICULUS	710	241	0.05924	-	-	$0.5 \leq P \leq 0.9$
STIGMA	710	241	0.05924	-	-	$0.5 \leq P \leq 0.9$
N/RING	710	241	0.05924	-	-	$0.5 \leq P \leq 0.9$
AWN	218	67	0.3381	-	-	
OTHER SHEATH	710	241	0.05924	-	-	$0.5 \leq P \leq 0.9$
INNER SHEATH	539	412			0.07966	$0.5 \leq P \leq 0.9$
LIGULE*	266	685	6.713	53.076	96.214	$P < 0.005$
AURICLE*	276	675	8.205	65.871	83.819	$P < 0.005$
COLLAR	200	751	7.997	3.247	199.47	$0.005 P \leq 0.1$ (3:13) $P < 0.005$ (3:1,9:7)

*Pr = Purple; Gr/W = green/white; S/Lemma = Sterile lemma; N/Ring = nodal ring. *indicates the organs tested for a 1:3 ratio.*

Table 2: Classification of 25 random (TOS purple x TOS 15223) F greens in F

	Segregation (S)	Non Seregation (S)	P $\sum\chi_i^2$ 7ns:6s	
-	12	13	0.0402	0.5 _ P _ 0.9

Table 3 shows the joint segregation ratios at the loci controlling colour in the various organs. At the purple leaf blade and inner sheath loci, the purple inner sheath class was not recovered but for the other loci segregating with the purple leaf blade gene. The unrecovered class is the purple leaf, acyanic organ phenotypic combination. The two exceptions to this trend are the data presented by the sterile lemma and the collar-- for each organ, the purple leaf blade, acyanic organ; green leaf blade, coloured organ classes which were not recovered. In general, three and two phenotypic states were recovered in the joint segregation of the leaf blade gene and the other loci concerned.

The data for the joint segregation at the apiculus and stigma colour loci and the loci for the other organs are exactly similar. They produced three and two phenotypic classes as the trend with the purple leaf blade gene. The apiculus/stigma gene(s) produced two phenotypic classes with the outer leaf sheath gene, the stigma colour gene and the purple nodal ring gene. For the other organs, this gene(s) produce third phenotypic classes with the white apiculus/stigma, purple organ class not being recovered

Table 4, 5A and 5B show the standard tests carried out to validate inhibitor gene interaction and pleiotropy. The logic for the inhibitor test is that the unrecovered purple leaf; purple inner sheath phenotypic class was expressed as purple leaf, white inner sheath. A fit for a 13 green leaf: 3 purple leaf ratio confirms this logic. Tables 5A and 5B show standard tests for pleiotropic gene effect. The expected frequency for the unrecovered purple leaf, white nodal ring class expresses as purple leaf, purple nodal ring. Table 5B shows the test for pleiotropy between the apiculus gene and the stigma and nodal ring colour genes. The unrecovered classes expressed as purple apiculus, purple stigma/nodal ring and white apiculus, white stigma/nodal ring phenotypic classes. Table 6 shows the Chi-square test for pleiotropy involving the leaf blade colour gene (*Pl*) the apiculus colour gene and the colour genes of other organs. There is widespread pleiotropic effect among these genes; inner sheath colouration however differs in that the *Pl* gene inhibited it while the *Pa* gene showed complementary action on it.

Table 7 estimates the contribution of pleiotropy by the *Pl* gene to colouration in the other organs (apiculus, stigma, nodal ring). 133.73 plants represent the frequency expected from joint segregation of the *Pl* gene (3/16) and the *Pa* gene (3/4). The difference between this frequency and the total number of purple F_2 plants (171) is the number contributed by pleiotropy (22%). This means that the main colour genes for these organs contributed 78% of the purple plants. This assumption was extrapolated to analyse the data for auricle and ligule colour inheritance (Table 8). The observed purple auricle/ligule is assumed to have been underestimated by 78% of 171 plants.

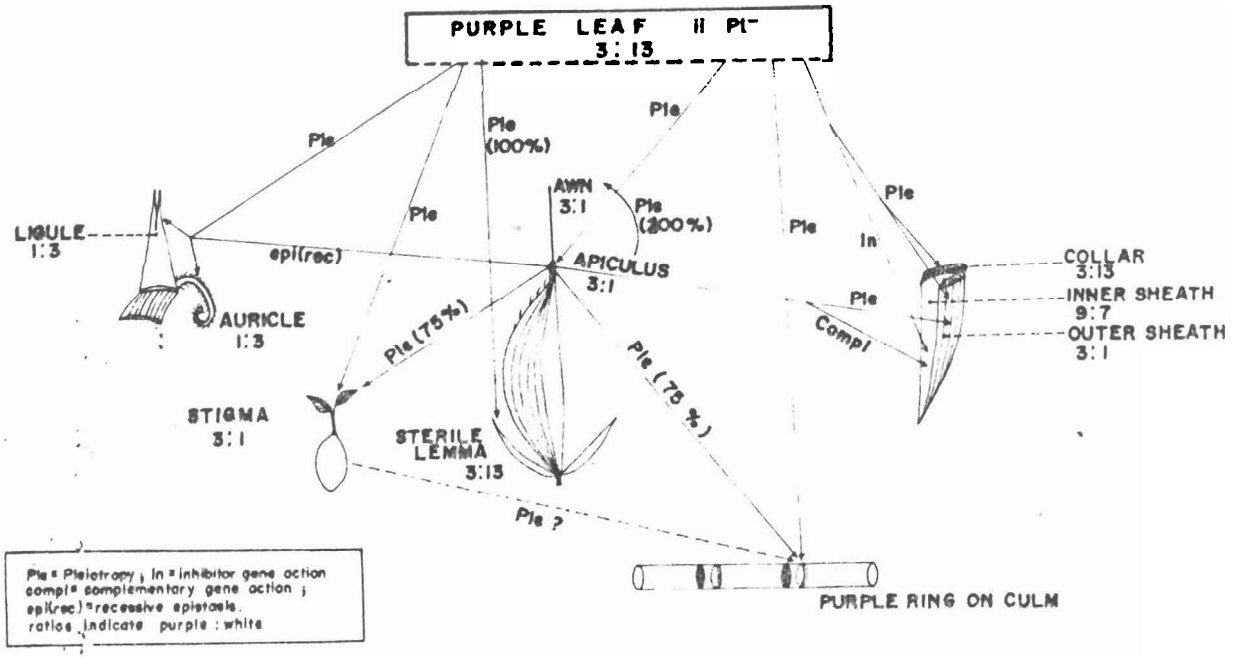
Table 9 explains the pigment states in organs of the F_1. The gene symbols adopted in this work are as reported by the Committee on Gene Symbolization of the Rice Genetics Cooperative (Kinoshita, 1986). Allelic symbols have been avoided.

DISCUSSION

A fit for a 13 green: 3 purple ratio in the F_2 of the TOS PURPLE x TOS 15223 cross indicates that purple leaf blade colour is controlled at two independent loci, one of which carries an inhibitor gene and the other a colour gene (Table 9). The F_3 ratio (Table 2) validates this F_2 ratio. Kadam (1936); Nagao (1951); Nagao *et al.*, (1962); Kondo, (1963) all reported the presence of an inhibitor gene suppressing the effect of the purple leaf blade gene *Pl*. The results obtained for sterile lemma colouration show that it is under the complete pleiotropic action of the *Pl* gene. This is consistent with the scheme of Kinoshita (1986) for *japonica* rice. The situation for collar colouration is similar.

It was observed that the same plants were involved in the segregation for the two character states for the apiculus, stigma, nodal ring, awn and outer leaf sheath. The simplest explanation for this observation is linkage but

since no recombinants were recovered, pleiotropy certainly explains the observations (Table 6). Nagao and Takahashi (1956); Takahashi (1957), Nagai (1959), and Chang (1964) worked out the C-A-P complementary genic system in *japonica* rice. Setty and Misro (1973) shed more light on this system by suggesting that any two of independent genes *Pa, Pb* and *Pc* govern anthocyanin pigmentation in the apiculus The 3 purple: I white ratio confirmed for this organ complex in this work could be consistent with the C-A-P system if, for instance, the acyanic cultivar carries *CCAAPaPapbpbpcpc* and the purple cultivar is conditioned by the genotype *CCAAPaPaPblPbpcpc*.



GENE SYSTEMS CONTROLLING PIGMENTATION IN TOS-PURPLE AND TOS 15223

Fig. 1Gene system controlling pigmentation intos-purple and tos 15223

Table 3: Joint segregation frequencies at the purple blade leaf and other loci

		Outer Sheath		Apiculus		Stigma		S/lemma		Ligule		Auricle		Collar		Node		Inner (Ring)	
	Pr	**Gr**	**Pr**	**W**	**Pr**	**W**	**Pr**	**W**	**Pr**	**W**	**Pr**	**W**	**PrGW/**		**Pr**	**W**	**Pr**	**W**
Pr	171	0	171	0	171	0	171	0	171	0	171	0	171	0	171	0	171	
LEAF																		
Gr	540	240	540	240	540	240	0	780	105	675	115	665	0	780	540	240	540	240
Pr	710	0			710	0			284	442	220	513	314	422	710	0	549	
APICULUS & STGMA																		
W	0	241			0	241			0	225	0	218	0	215	0	241	0	

Table 4: Test for inhibitor effects of Pl on gene for inner sheath colouration

		LEAF COLUR	
		13/16 Gr	3/16 Pr
Inner	Pr 9/16	OBS: =540 EXP: 117/256 x 951 =434.64	OBS: =0 EXP: 27/256 x 951 =100.30
Sheath colour	7/16 W	OBS: =240 EXP: 91/256 x 951 = 338.05	OBS: =171 EXP: 21/256 x 951 = 78.01

Test (13 green: 3 purple)

GREEN LEAF	PURPLE LEAF
OBS: 540 +240 =780	OBS: =171
EXP:434.64 + 338.05 =772.69	EXP: 100.30 + 78.01 =178.31
χ^2= 0.069156	=0.29968
$\sum\chi_i^2$ (13.3) = 0.36884	0.5 P 0.9

Table 5a: Typical test for pleiotropic effect (Pl vs N/RING)

Inner (Ring) Pr	W				
			LEAF COLUR		
		Gr	13/16	Pr	3/16
		Pr	OBS: =540		OBS: =171
0	171	3/4	EXP 39/64 x 951 =579.57		EXP: 9/64 x 951 =133.73
N/RING		W	OBS: =240		OBS: = 0
540	240	1/4	EXP: 13/64 x 951 = 193.17		EXP: 3/64 x 951 = 44.58
0	549	TEST			
			GREEN LEAF		PURPLE LEAF
		OBS:	=780		171
		EXP:579.52 +193.17	=772.69		133.73 +44.58 = 178.31:
			χ^2 = 0.06916		=0.29968
241	0		$\sum\chi_i^2$ (13:3) = 0.36884		$\leq$ P $\leq$ 0.9

Table 5b: Typical test for pleiotropic effect (Pa vs n/ring,stigma,etc)

		APICULUS		
	Pr	3/4	1/4	W
Pr	OBS:	=710	OBS:	= 0
3/4	EXP 9/16 x 951	= 534.94	EXP: 3/16 x 951	=178.31
1/4	OBS:	= 0	OBS:	= 241
W	EXP: 3/16 x 951 = 178.31		EXP: 1/6 x 951	= 59.44
	χ^2 = 0.01481			=0.04443
	$\sum\chi_i^2$ (3:1) = 0.05924		0.5 $\leq$ P $\leq$ 0.9	

0·5≤P≤0·9

0·5≤P≤0·9

Table 6:χ^2 Test for pleitotropy of Pl and Pa genes

	Outer Sheath	Apiculus	Stigma	S/lemma	Ligule	Auricle	Collar	Node (Ring)	Inner Sheath
Pl	0.05933	0.36884	0.36884	0.36903	-	-	0.36884	0.36884	Inhibitor
	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	-	-	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	
Pa	0.05896	0.05896	0.05896	0.05896	-	-		0.05896	
	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	$0.5 \leq P \leq 0.9$	-	-		$0.5 \leq P \leq 0.9$	Complementary

Table 7: Contribution of Pleiotropy: Estimate

No of E plants showing colour due to main genes for:	APICULUS	STIGMA	NODAL RING
	133.73	133.73	133.73
TOTAL F2	177	177	177
NUMBER DUE TO PLEIOTROPY		171- 133.73 = 37.27	
%	37.27/171	=21.8%	

Table 8 : Analysis of auricle/ligule inheritance based on pleiotropic contribution.

AURICLE	W	Pr
OBS:	665	115 + (78/100 x 171) = 248.38
		=223.34
EXP:	670	
	$\chi^2 = 0.0373$	0.1122
	$\sum\chi_i^2$ (1:3) = 0.1495	$0.5 \leq P \leq 0.9$
LIGULE	W	Pr
OBS:	675	105 + (78/199x 171) = 238.38
EXP:	685.03	=228.35
	χ^2 =0.147	=0.441
	$\sum\chi^2$ =0.588	$0.5 \leq P \leq 0.9$

Table 9 : Pigment states in organs of F1

Organ	Colour	Explanation
1. Leaf blade	Green	Inhibitor gene(I) from green cultivar suppresses Pl. *Purple = ii Plpl;, Green-I-Pl, ii Plpl, I- Plpl.*
2 Ligule and auricle	Purple	Purple state not dominant but expresses in F1 because the purple cultivar supplies the *basic gene*, Pa, and the purple auricle/ligule gene. *Purple = Pa-Pau,Pa-Plg-; green = papaPau -, papapaupau, papaPlg-, papaplgplg*
3. Sterile lemma	White	Leaf blade is green because of I-s/lemma controlled by absolute pleiotropy of Pl. Genotype = ii*Pl*-.
4. Inner l/sheath	Purple	Genotype=Pa-Psh-,Pa and Psh(inner 1/sheath gene) came from TOS PURPLE while I came from green cultivar.
5. Collar	White	Genotype = ii*Pl*, under absolute pleiotropic control of Pl. The *I* gene from green cultivar prevents the expression of Pl in the F1.

Gene symbols according to Kinoshita (1986).

Indeed, the C-A-P model has been found to apply to all genotypic ratios (3:1, 15:1, 9:7, 45:19, 54:10, and 162:94) already reported by many workers (Parnel *et al.*, 1917; Chao,1928; Jones 1930; Ghose *et al.*, 1960; Chang, 1964).

The problem with the determination of colour on the ligule and the auricle has to do with the following observed facts: viz i) the organs manifest colour at a prime period, ii) their colour may not be intense and iii) not all culms express colour at the same time. It is also known that if anthocyanoplasts occur singly, the concentration of the pigment may be low (Tunen *et al.*,1991). The extrapolation of the pleiotropic effect of the *Pl* gene to analyse the inheritance of auricle and ligule colour is predicated on the fact that the frequency of the colour classes in these various organs may have been grossly underestimated .It is interesting to note that the Chi-square values for the I purple: 3 white ratio in Table I were the closest to a fit when compared to other ratios.

Table 7 snows that the pleiotropic effect of the *Pl* gene on the other organs is widespread. The inner leaf sheath is however an exception in that the *Pl* gene exerted inhibitor effect on its colour gene. Table 4 shows that the 100:30 plants expected in the purple leaf, purple inner sheath class expressed in the purple leaf, white inner sheath class. The apiculus colour gene also exerts absolute pleiotropic control on the stigma outer leaf sheath and the nodal ring (Table 5B). Table 3 shows that whenever the apiculus is not pigmented, all the organs of the junctura complex and the inner leaf sheath are not pigmented. The gene for apiculus colour can therefore be said to be epistatic in the recessive state to the genes for colour in the above organs. It is clear from this analysis that the apiculus colour gene (*Pa*) is the one acting complementarily with the *Psh* gene to produce colour on the inner surface of the leaf sheath. Setty and Misro (1973) reported pleiotropic effect of the *Pa* gene on the organs of the junctura. In this work, the mode of interaction

has been determined to be recessive epistasis in which the *Pa* state acts as a localization gene for colour on the organs in the junctura complex (Table 9). The pigment states in the organs of the F_1 explained in Table 9 can be understood against this background.

The model in Fig. 1 explains the interaction of the genes involved in the colour expression in the cultivars studied. It is clear from the model that the two main genes involved are *Pl* and *Pa*. All the other organs in the cultivars are given colour by these two genes either directly or through pleiotropy, inhibitor effect, complementary action or epistasis. The *PI* gene exerts exclusive pleiotropic effect on the sterile lemma; 22% is the value already worked out for the pleiotropic contribution of *PI* on other organs (Table 7).

The gene *Pa* for apiculus colouration exercises pleiotropic, complementary and recessive epistatic effects on other organs. The value of 100% for pleiotropic effect of apiculus on the awn suggests that the awn is simply an extension of the apiculus. The value of 75% pleiotropic effect of *Pa* on other organs represents the total F_2 plants that have purple apiculus. The role of this gene in the colouration of other organs is significant. It must be in the dominant state for the organs of the junctura complex to show colour, it acts complementarily with gene *Psh* to colour the inner leaf sheath. These observations are consistent with the role of the *Pa* gene as basic gene, in addition to the C and A genes, for other localization genes governing anthocyanim pigmentation in the respective organs (Nagao, 195I; Takahashi, 1957; Setty and Misro,1973). The role of pleiotropy has been prominently documented in rice (Hadagi *et al.*, 1984; Ramesh, 1984; Yadav, 1984; Yadav and Tomar, 1984).

The value of organ colours as genetic makers in rice has been fully utilized in our work. The pigmentation of the apiculus, awn, stigma and outer leaf sheath are remarkably distinct and they express dominance when they are involved in crosses with accessions having acyanic organs. The complex of coloured organs associated with the apiculus through pleiotropy enhances the marker value of this gene. The wild rices that are indigenous to West Africa except *O. punctata* carry these colour complexes and they are useful in identifying putative hybrids.

ACKNOWLEDGEMENT

We acknowledge the contribution of Chinyere, C. Nwokeocha and Francisca, O.Aladejana. members of the Rice Group for giving us the benefit of their experience in rice genetics. This work was supported by a URC. Grant 1425SA of the ObafemiAwolowo University, Ile-Ife.

REFERENCES

Chang. T.T. 1964. Present knowledge of rice genetics and cytogenetics. IRRI Technical Bulletin 1.

Chao, L.P. 1928. Linkage Studies in rice. *Genetics.13: 133-169.*

Faluyi, J.O. and Chinyere C. Nwokeocha 1993. Agro-botanical studies of some populations of the *sativa-glaberrima* complex of peasant agriculture. *Nigerian Journal of Botany. 6: 1 - 12.*

Ghose K.L.M., M.B. Ghatge and V. Subrahmanyan 1960.Rice in India. Indian council Agric. Research, New Delhi, p. 507.

Hadagal, B.N., A.Manjunath and J.V.Goud 1984. In heritance of anthocyan in pigmentation in a few parts of rice (*Oryza sativa* L.). *Indian J. Genet. 44: 319-324.*

Hector, G.P. 1922. Correlation ofcolour charactersin rice. Hem. Dept. Agric. *India Bot. Ser. II (7): 153-183.*

Jones, J.W.1927. Inheritanceofawnednessin rice. *J.Amer.Soc.Agron. 19: 830-839.*

Kadam, B.S. 1936 Ananthocyan inhibitor in rice. *J. Hered. 27: 405-408.*

Kinoshita, T. 1986. Gene symbols for anthocyanin colouration. *Rice Genetics Newsletter 3: 12- 14.*

Kondo, A. 1963. Fundamental studies on breeding by hybridization between

Japanese and foreign rices. VII. Identification of genes controlling anthocyanin pigmentation in Japanese and foreign rices. *Japan J. Breeding 13:90-92* Nagai,1. 1959 .*Japonica* Rice: Its breeding and culture. *Yokendo Ltd., Tokyo* p.843

Nagao, S.1951. Genetic analysis and linkage relationship of characters in rice. *Advances Genetics.4: 181-212*

Nagao, S. and M. 1 Akahashi 1947. Linkage studies in rice. *Jap. J. Genet. 22: 22-23.*

Nagao, S. and M. Takaha,shi 1956 Genetical studies on rice plant. XXX. The third gene in apiculus colouration. *Japan. J. Botany. 15:141-151.*

Nagao, S. and T. Kinoshita 1962. Genetical studies on rice plant. XXVI. Mode of inheritanceand causual genes for one type of anthocyanin colour character in foreign rice varieties. *J. Fac. Agr. Hokkaido Univ. 52: 20-50.*

Nwokeocha, C.C. and J.O. Faluyi 1993. Botanical indices of ploidy levels in some African accessions of *Oryza punctata* Kotschy ex Steud. *Rice Genetics Newsletter 10 65*

Parnell, F.R . G.N.R. Ayyengar and, K. Ramiah and C.R.S. Ayyengar 1922. inheritance of characters in rice. 1. *Memoirs Department of Agriculture. Indian Banonical Series 9: 75-105.*

Ramesh. B. 1984. Inheritance ofanthocyanin pigment in rice.*Indian J. Genet. 44: 15-17.*

Setty, M.V. and B.Misro (1973). Complementary genic complex for anthocyanin pigmentation inthe apiculus of rice (*Oryza saliva* L.).*Cana.J. Genet Cytol 115:779-789.*

Takahashi. 1957. Analysis of apiculus colour genes essential to anthocyanin colouration in rice. *J. Faculty Agric. Hokkaido Univ. 50: 266-361.*

Tunen, A.J. and J.N.M. Mol. 1991. Control of flavonoid synthesis and manipulation of flower colour. *In: D. Grierson (ed.) Developmental Regulation of Plant Gene Expression.Blackie, Glasgow and London.* p. 94-130.

Yadav, L.N. 1984. Inheritance ofanthocyanin pigmentation is stigma, outer glumes and apiculus of rice (*Oryza sativa* L.) *I. J. Indian Bot. Soc. 63:186-189.*

Yadav, L.N. and N.S Tomar 1984 Inheritance of anthocyanin pigmentation in stigma, outer glumes and apiculus of rice (*Oryza sativa* L.). *II. J. Indian Bot. Soc. 63:190-193.*