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Linkage studies in rice, *Oryza sativa* L. X-ray induced transformation of genetic control and linkage associations of morphological characters

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ABSTRACT

An untreated cross of Cherumodan x *Japan Violet as control was studied against the treated crosses, wherein 1500, 2000 and 5000 rads X-ray irradiated pollen grains of the male parent were used for crossing. The crosses were studied upto the respective F_3/M_3F_3 generations for inheritance and interrelationships of 14 morphological characters. The results indicated transformation of genetical control and linkage associations as the genetical consequences of the possible chromosomal aberrations/genic transformations.*

Key words: *Genetical analysis, X-rayed, pollen grains transformation, inheritance, linkage relationships*

The rice genetic system is highly complex due to differential patterns of intricate gene interactions, linkage associations and pleiotropic relationships. Linkage associations of 209 genes have been recently documented by locating them in the 12 linkage groups in rice (Kinoshita 1998). However, the present report refers to transformations in the pattern of inheritance and linkage relations of morphological characters that resulted from the use of irradiated pollen grains in differential crosses of Cherumodan x Japan Violet.

MATERIALS AND METHODS

The differential crosses of Cherumodan x Japan Violet studied upto the respective F_3/M_3F_3 generations were: the untreated control and crosses 2-4 wherein 1500, 2000 and 5000 rads X-ray treated pollen grains were used for pollination respectively. Cherumodan and Japan Violet are well known rice genotypes with 80-85 and 90-95 days duration, respectively and the former has all plant parts green except leaf axil, the latter is a purple marker for anthocyanin pigmentation and has tip-sterility.

The expectant spikelets to bloom were treated with X-rays (Pandey 1978) emitting 420 rads/min with the Maximar-100 of the Medical College, Calicut. The dosages were calibrated to 1500, 2000 and 5000 rads. Pollen grains collected from the irradiated spikelets of the male plant were dusted over the stigmatic surface of the emasculated flowers of the respective female parent during 8-9 a.m. The plants were harvested at 30 days maturity and the cross seeds were grown to raise the respective F_1/M_1F_1 generation. F_2/M_2F_2 and F_3/M_3F_3 generations were grown and studied during subsequent seasons, following Misro (1963).

Chi-square tests on individual segregations and combined segregations of character pairs, on expected independent assortment, were applied for inheritance and interrelationships of characters respectively. Wherever the joint Chi-square value appeared significant, linkage was estimated on the F_2/M_2F_2 data on minimum discrepancy (Haldane 1953 and Richharia *et al.,* 1966).

RESULTS AND DISCUSSION

The results on inheritance and linkage relationships of 14 morphological characters studied are presented in Tables 1-2.

Inheritance. Six morphological characters (Psh Plm Pla Pin Ps Pa) showed 3:1 in the control. The ratio was transformed into 9:7 in certain treated crosses but invariably with the 5000 rads X-ray treated cross. Four morphological characters (P1 Pjp Plg Pau) gave 9:7 in the control while the treated crosses, especially 5000 rads X-ray treated, gave 27:37 involving three complementary genes. However, no transformation of genetical ratios was observed in the treated crosses with Px Pn an and tst. These gave 15:1, 3:13 and 3:1, the latter for an and tst in all crosses (Table 1).

The genetical transformations of 3:1 into 9:7 and 9:7 into 27:37 in the treated crosses appear to be probable, as the complementary ratios reported earlier (Santhoshlal 1995, Pavithran 1981) support the transformed ratios. Transformation of genetical ratios and genic interrelationships through the use of X-rayed pollen grains in differential crosses appears to be a new report. Early studies on gene transfer through sublethally irradiated pollen grains do not seem to have considered such genetical transformations of inheritance patterns and interrelationships of morphological characters (Pandey 1978, 1983). The present situation is explained on the basis of two or three altered complementary loci instead of one or two in the normal cross for the control of characters. The additional heterozygous condition/s might have arisen as a result of recessive mutation of the respective homozygous dominant loci in the male parent, probably, mutation of the activator and/or localization gene. The genes Px1/2 Pn/I-Pn an and tst appeared stable against the X-ray treatments of pollen grains. The above F_2/M_2F_2 data have been confirmed by the breeding behaviour of the respective F_3 and M_3F_3 families.

Independent assortment. Treated crosses of Cherumodan x Japan Violet showed linear increase in the incidence of independent assortment of characters compared to the control. The control showed 26 instances of independent assortment of characters, 1500 rads X-ray treated cross 27, 2000 rads X-ray treated cross 33 and 5000 rads X-ray treated cross showed 44 instances (Table 2). Awn and tip-sterility showed independent assortment with all other characters and also mutually, while Px Pn and Plg showed variation from the control (Santhoshlal 1995).

Linkage relationship. Linkage relationships of morphological characters also showed transformation in the treated crosses (Table 3). However, Px, an and tst did not show any linkage with any other character in all the crosses. The control showed 27 instances of

Table 1. Inheritance of morphological characters in relation to genetical transformation induced by X-ray treated pollen grain used in crosses of Cherumodan X Japan violet

				F_2/M_2F_2 of Crosses							
Morph.	Parents		F_1/M_1F_1	1		2		3		4	
Characters	F	M	in all crosses	F_2/M_2F_2 population P-G	Ratio	F_2/M_2F_2 population P-G	Ratio	F_2/M_2F_2 population P-G	Ratio	F_2/M_2F_2 population P-G	Ratio
Leaf axil	+	+	+/+	132/12	15:1	171/10	15:1	70/6	15:1	36/2	15:1
Node	-	-	--/-	30/114	3:13	25/156	3:13	16/60	3:13	7/31	3:13
Awn	-	+	+/+	107/37	3:1	128/53	3:1	61/15	3:1	27/11	3:1
Tip sterility	-	+	+/+	107/37	3:1	144/37	3:1	57/19	3:1	33/5	3:1
Leaf sheath	-	+	+/+	111/33	3:1	126/55	3:1	55/21	3:1	23/15	9:7
Leaf margin	-	+	+/+	100/44	3:1	114/67	9:7	49/27	9:7	19/19	9:7
Leaf tip	-	+	+/+	103/41	3:1	114/67	9:7	53/23	3:1	20/18	9:7
Internode	-	+	+/+	106/38	3:1	126/55	3:1	54/22	3:1	22/16	9:7
Stigma	-	+	+/+	108/36	3:1	138/43	3:1	54/22	3:1	22/16	9:7
Apiculus	-	+	+/+	106/38	3:I	127/54	3:1	55/21	3:1	22/16	9:7
Leafblade	-	+	+/+	82/62	9:7	93/88	3:1	38/38	27:37	14/24	27:37
J. Proper	-	+	+/+	75/69	9:7	80/101	27:37	33/43	27:37	15/23	27:37
Ligule	-	+	+/+	89/55	9:7	102/79	9:7	39/37	9:7	15/23	27:37
Auricle	-	+	+/+	77/67	9:7	89/92	27:37	33/43	27:37	12/26	27:37

P - Purple
G - Green
+ - Presence
- - Absence

1. Normal cross
2. 1500 rads treated pollen
3. 2000 rads treated pollen
4. 5000 rads treated pollen

F- Female
M- Male

Table 2. Linkage relationships of morphological characters observed in the control and X-ray treated crosses of Cherumodan and Japan Violet

Character pairs / F_2	Cross	Combined F_2 segregation				C.O %	Linkage phase
		AB	Ab	aB	Ab		
Psh – P1							
3: 1 9:7	1	82	29	0.0	33	2.02	C
3: 1 9:7	2	93	33	0.0	55	1.01	C
3: 1 27: 37	3	38	17	0.0	21	1.01	C
Psh – Pla							
3 :1 3:1	1	101	10	2.0	31	6.74	C
Psh – Pjp							
3: 1 9:7	1	75	36	0.0	33	2.5	C
3: 1 27:37	2	80	46	0.0	55	1.01	C
3: 1 27: 37	3	33	22	0.0	21	3.05	C
Psh – Plg							
3: 1 9:7	1	88	23	1.0	32	3.05	C
3: 1 9:7	2	98	28	4.0	51	4.08	C
3: 1 9:7	3	39	16	0.0	21	3.56	C
Psh – Pau							
3: 1 9:7	1	77	34	0.0	33	2.53	C
3: 1 27:37	3	33	22	0.0	21	3.05	C
Psh – Pn							
3: 1 3:13	1	29	82	1.0	32	10.56	C
3: 1 3:13	2	23	103	2.0	53	15.15	C
Psh – Pin							
3: 1 3:1	1	100	11	6.0	27	12.82	C
3: 1 3:1	2	125	1.0	1.0	54	2.02	C
3: 1 3:1	3	49	6.0	5.0	16	16.93	C
Psh – Ps							
3:1 3:1	1	103	8.0	5.0	28	10.55	C
3:1 3:1	3	49	6.0	5.0	16	16.93	C
Psh – Pa							
3 :1 3:1	1	101	10	5.0	38	11.12	C
3 :1 3:1	2	120	6.0	7.0	48	8.35	C
3 :1 3:1	3	50	5.0	5.0	16	15.74	C
Plm – Pla							
3:1 3:1	1	98	2.0	5.0	39	5.13	C
Plm – Pjp							
3:1 9:7	1	75	25	0.0	44	1.51	C
Plm – Plg							
3:1 9:7	1	85	15	0.0	44	15.74	C
Plm – Pau							
3 :1 9:7	1	77	23	0.0	44	1.01	C
Plm – Pn							
3: 1 3: 13	1	30	70	0.0	44	3.04	C
Plm – Pin							
3:1 3 :1	1	98	2	8.0	36	6.19	C
Plm – Ps							
3: 1 3:1	1	97	3	11.0	33	8.35	C
Plm – Pa							
3:1 3:1	1	97	3	9.0	35	7.80	C
Pla – Pjp							

Contd...

3:1 9:7	1	75	28	0.0	41	1.51	C
3:1 27:37	3	33	20	0.0	23	2.53	C
Pla – Plg							
3:1 9:7	1	84	19	5.0	36	4.08	C
3:1 9:7	3	39	14	0.0	23	3.05	C
Pla – Pau							
3:1 9: 7	1	73	30	4.0	37	8.35	C
3: 1 27: 37	3	53	20	0.0	23	2.53	C
Pla – Pn							
3:1 3:13	1	28	75	2.0	39	11.12	C
3:1 3:13	3	16	37	0.0	23	4.61	C
Pla – Pin							
3:1 3:1	1	96	7	10.0	31	12.82	C
3:1 3:1	3	51	2	3.0	20	8.90	C
Pla – Ps							
3:1 3:1	1	99	4	9.0	32	9.45	C
3:1 3:1	3	50	3	4.0	19	11.12	C
Pla – Pa							
3:1 3:1	1	96	7	10.0	31	12.82	C
3:1 3:1	3	51	2	4.0	19	9.45	C
Pin – Ps							
3:1 3:1	1	104	2	4.0	34	5.13	C
3:1 3:1	3	52	2	2.0	20.	7.27	C
Pin – Pa							
3:1 3:1	1	101	5	5.0	33	8.35	C
3:1 3:1	2	122	4	5.0	50	5.66	C
3:1 3:1	3	52	2	3.0	19	8.35	C
Ps – Pa							
3 :1 3:1	1	105	3	1.0	35	3.56	C
3 :1 3:1	3	54	0	1.0	21	3.05	C

linkage associations, 1500 rads X-ray treated cross 7 instances, 2000 rads X-ray treated cross 17 and 5000 rads X-ray treated cross showed none (Table 3). Linkage relations showed a diminishing trend towards higher dosage of X-ray treatment, probably, because genetical transformations occurred as a result of chromosomal aberrations like inversion and/or translocation leading to alterations of linkage relationships. The present observations are particularly significant in respect of the fact that besides usual mutant expressions, such genetical alterations or transformation are revealed only through comprehensive genetical analysis of the character concerned. Such mutational transformation may have significant evolutionary consequences as they alter the order of genic sequences considerably. Such situations are often ignored or unnoticed in the pursuit of macromutations/micromutations, especially in the case of hazarduous environmental mutagenesis which might lead to sever genetic consequences.

REFERENCES

Haldane JBS 1953. A class of efficient estimates of a parameter. Proc Int Stat Confr India.p.53

Kinoshita T 1998. Report of the committee on gene symbolization, nomenclature and linkage groups. II. Linkage mapping using mutant genes in rice. RGN 15: 13-74

Misro B 1963. Studies on the inheritance of anthocyanin pigmentation in rice. Doctoral Thesis, Utkal University, Orissa, India.p.65

Misro B 1981. Linkage studies in rice (*Oryza sativa* L.) X. Identification of linkage groups in indica rice. Oryza 18: 185-195

Pandey KK 1978. Gene transfer in *Nicotiana* by means of irradiated pollen. Genetica 49: 56-69

Pandey KK 1983. Evidence of gene transformation by the use of sublethally irradiated pollen in *Zia mays* and theory of occurrence of chromosome repair through somatic recombination and gene conversion. Mol

Gen Genet 191: 358-365

Pavithran K 1986. Inheritance of characters in rice, *Oryza sativa* L. In New Frontiers in Breeding Researches. Proc V Int Congr SABRAO Thailand pp 197-211 (Eds.)

Pavithran K 2005. Genetics and Linkage groups in rice. In Rice in Indian Perspective Ed. Dr SD Sharma and Nayak SK, Today and Tomorrow Printers and Publishers New Delhi p.534

Richharia RH, AK Ghose, SVSP Rao and B Misro 1966. Formula for the estimation of linkage from F_2 data. Bull CRRI 5: 1-36

Santhoshlal PS 1995. Genetical and Transformation studies in rice, *Oryza sativa* L. Doctoral Thesis, University of Calicut, Kerala, India.