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Transformation of genetical ratios in crosses involving X-ray irradiated pollen grains in rice (*Oryza sativa* L.)

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Abstract

Early reports on radiation dependant-genetical transformation indicated that sublethal levels of irradiated pollen grains allow development of normal hybrid progeny but in subsequent generations they occasionally express paternal characters due to differential inheritance of donor genes. However, detailed genetic analysis has not yet been followed up for confirmatory analysis of genetic transformation especially in rice (*Oryza sativa* L.). Presently different crosses of (normal and treated) Cherumodan (green) × Japan Violet (purple) were effected by using X-ray irradiated pollen grains of the male parent in the treated crosses. The crosses were studied up to F_3 of the control and M_3F_3 of the treated generations for inheritance/differential inheritance for fourteen morphological characters. The results showed transformation of genetical ratios in respect of ten morphological characters in the M_2F_2 populations when compared to the control. Observations recorded in the F_2/M_2F_2 are duly confirmed by the results obtained from the F_3/M_3F_3 generations. It could be concluded that irradiation might have caused allelic differences as to transform genetical ratios concerned as discussed.

Key words: Rice, hybridization, irradiated pollen grains, transformation, genetical ratios

Introduction

The use of irradiated pollen to transfer a single gene or part of the male nuclear component to seed parent has been reported earlier [1-10]. It has also been reported that such transformants were fertile and had maternal phenotypes except for those specific traits transformed from the donor. Various mechanisms were proposed for explaining the phenomenon of this limited gene transfer such as (i) incorporations of paternal DNA fragments into the egg nucleus by pseudo fertilization followed by a parthenogenetic doubling of the egg genome as proposed by Pandey [3, 4], (ii) differential gene transfer, this theory proposes that sublethal levels of radiation allow the development of normal hybrid progeny; in the subsequent generation they occasionally express the paternal characters due to differential transmission of donor chromosomes [6, 7]. (iii) a 'meiotic sieve' that acts at or after M_1 meiosis which limits the transmission of paternal genes as suggested by Snape *et al.* [10]. Sarigorla *et al.* [11] revealed that the seeds from X-rays irradiated pollen have unexpected phenotypes in F_1 and also observed a significant excess of recessive alleles in F_2 and BC generations. Similar observations were also reported by several early workers [6, 8-10, 12]. However, the earlier workers did not give much attention for a detailed genetic analysis for the occurrence of such excess recessive alleles in irradiated pollen used crosses or in the inheritance of such characters [13]. Keeping these lacunae in the basic information, the present study was undertaken to analyse the possible' reasons for the occurrence of such 'excess recessive alleles in the second generation progenies which resulted in irradiated pollen used crosses.

Materials and methods

In the present study two rice varieties, a purple semidwarf plant Japan Violet as male and Cherumodan, a popular green upland variety of Malabar as female were used for hybridization. The female parent had all plant parts green except purple leaf axil. The male parent had all plant parts purple with awn and tip sterility. Florets of Japan Violet panicles were irradiated prior to anthesis with X-rays at a rate of 420 rads/minute by the X-ray machine Maximar - 100 at the Radiation Department of the Calicut Medical College. Three doses 1500, 2000 and 5000 rads X-rays were used for irradiation of pollen grains. Emasculated female panicles were pollinated by dusting the pollen grains over the hairy stigmatic lobes with the help of a fine brush. Thus four crosses *viz.*, 'Cherumodan × Japan Violet-normal, Cherumodan × Japan Violet-1500 rads, Cherumodan × Japan Violet - 2000 rads and Cherumodan × Japan Violet - 5000 rads were made.

Morphological characters such as pigmentation in leaf axil, leaf sheath, leaf blade, leaf margin, leaf tip,

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juncture proper, ligule, auricle, node, internode, stigma, apiculus and two other characters such as awning and tip sterility were considered for the X-ray induced variations. F_1 and M_1F_1 seeds (the seeds derived from irradiated pollen used crosses were designated as M_1 F_1 and those from normal cross as F_1) were harvested 25-30 days after pollination and these were germinated in petridishes and transplanted to well puddled pots for further studies. Controlled emasculations without pollination were used to detect spontaneous parthenogenesis. Inheritance of above mentioned morphological characters were analysed in F_2/M_2F_2 and F_3/M_3F_3 generations based on Chi-square test.

Results and discussion

Observations made on the total number of spikelets crossed, percentage of seed set, number of cross seeds harvested, number of F_1/M_1F_1 plants and F_2/M_2F_2 plants grown are detailed in Table 1. Data on the percentage of seed set, yield of viable cross seeds and percentage of fertility in M_1/F_1 plants showed a decreasing tendency with the increase of radiation dose. It is in conformity with early reports [9, 11]. Spontaneous parthenogenesis was ruled out, as controlled emasculations without subsequent pollination failed to set seeds. Data on the inheritance of anthocyanin pigmentation in different morphological plant parts studied are presented in Table 2. The F_1/M_1F_1 of all four crosses showed the dominance of anthocyanin pigmentation in various plant parts studied, thereby revealing, probably, that there is no single gene transformation or unexpected phenotypes in the present study as reported by earlier workers. However, inheritance of pigmentation in different morphological plant parts in M_2F_2 showed variation in their pattern of inheritance when compared to the respective controls of F_2 while some other characters showed no change in their pattern of inheritance as detailed below.

In F_2/M_2F_2 leaf axil pigmentation showed duplicate factor control, nodal pigmentation showed inhibitory factor control and awning and tip sterility invariably showed monogenic recessive control in all the above four crosses studied, thereby showing that these characters exhibited more genetic stability against X-ray irradiation (Table 2). On the other hand leaf sheath, leaf margin, leaf tip, internode, stigma and apiculus pigmentation showed dominant monogenic control in the normal cross, while digenic complementary interaction in the cross Cherumodan x Japan Violet 5000 rads. Further, leaf margin pigmentation gave 9:7 in 1500 and 2000 rads treated crosses and leaf tip pigmentation gave 9:7 in 1500 rads treated cross in addition to the 5000 rads treatment as detailed above (Table 2). However, in the case of leaf sheath, internode apiculus and stigma pigmentation, only 5000 rads treatment gave digenic complementary ratio 9:7 against 3: 1 in the normal cross or other treated crosses (Table 2). This probably demonstrates the fact that only one of the loci remained heterozygous in normal cross, while X-ray irradiation transformed the other locus also heterozygous by mutating (either of) the complementary genes into recessive" in the treated pollen grains of Japan Violet. However, observations' recorded in respect of leaf blade, juncture proper, ligule and auricle pigmentation showed genetic control by two complementary genes (9:7) in the normal cross. While in the case of juncture proper and auricle pigmentation in 1500,2000 and 5000 rads treated crosses gave trigenic complementary ratio 27:37; in the case of leaf blade both 2000 and 5000 rads treated crosses gave trigenic complementary ratio 27:37 and in the case of ligule only 5000 rads treated cross gave 27:37 (Table 2), instead of the digenic complementary ratio 9:7 of the respective controls. This also demonstrates the differential response of these characters in relation to the different dosages of X-rays treatments. The above

Table 1. Details of seed set obtained by pollination with normal pollen and X-ray irradiated pollen in rice

Pollination dose (rads)/ particulars of cross		Ch × JV (normal)	Ch × JV 1500 rads irradiates pollen	Ch × JV 2000 rads irradiated pollen	Ch × JV 5000 rads irradiated pollen
1	Total spikelets crossed	12.00	20.00	32.00	57.00
2	% of seed set	91.67	50.00	34.37	17.54
3	No. of Cross seeds harvested	8.00	5.00	7.00	5.00
4	No. of cross seed sown	5.00	4.00	7.00	5.00
5	% of cross seed germination	100.00	75.00	71.42	60.00
6	No. of F_1/M_1F_1 plants obtained	4.00	3.00	3.00	3.00
7	% of sterility in F_1/M_1F_1	27.00	53.00	61.00	74.00
8	No. of seeds obtained from the F_1/M_1F_1 plant that followed for further analysis	310.00	204.00	142.00	76.00
9	No. of F_2/M_2F_2 plants obtained	187.00	185.00	87.00	45.00
10	No. of $F_3/M_3,F_3$ families studied	30.00	46.00	32.00	25.00

Ch = Cherumodan; JV = Japan Violet

Table 2. Inheritance of different morphological characters in F_1/M_1F_1 and F_2/M_2F_2 of crosses Cherumodan × Japan Violet (for normal (C), 1500 (T_1), 2000 (T_2) and 5000 (T_3) rads X-ray treatments in rice

S.No.	Characters	Treatments	Parents			Observed F_2 frequency					
			Female	Male	F_1	+	-	Total	Ratio	X^2	P
1	2	3	4	5	6	7	8	9	10	11	12
1.	Leaf axil	C	+	+	+	132.00	12.00	144	15:1	1.07	0.50-0.30
		T_1	+	+	+	171.00	10.00	181	15:1	0.16	0.70-0.50
		T_2	+	+	+	70.00	6.00	76	15:1	0.35	0.70-0.50
		T_3	+	+	+	36.00	2.00	38	15:1	0.06	0.80-0.70
2.	Leaf sheath	C	-	+	+	111.00	33.00	144	3:1	0.33	0.70-0.50
		T_1	-	+	+	126.00	55.00	181	3:1	2.80	0.10-0.05
		T_2	-	+	+	55.00	21.00	76	3:1	0.28	0.70-0.50
		T_3	-	+	+	23.00	15.00	38	9:7	0.28	0.70-0.50
3.	Leaf blade	C	-	+	+	82.00	62.00	144	9:7	0.03	0.90-0.80
		T_1	-	+	+	93.00	88.00	181	9:7	1.74	0.20-0.10
		T_2	-	+	+	38.00	38.00	76	27:37	1.90	0.20-0.10
		T_3	-	+	+	14.00	24.00	38	27:37	0.44	0.70-0.50
4.	Leaf margin	C	-	+	+	100.00	44.00	144	3:1	2.37	0.20-0.10
		T_1	-	+	+	114.00	67.00	181	9:7	3.34	0.10-0.05
		T_2	-	+	+	49.00	27.00	76	9:7	2.09	0.20-0.10
		T_3	-	+	+	19.00	19.00	38	9:7	0.61	0.50-0.30
5.	Leaf tip	C	-	+	+	103.00	41.00	144	3:1	0.93	0.50-0.30
		T_1	-	+	+	114.00	67.00	181	9:7	3.34	0.10-0.05
		T_2	-	+	+	53.00	23.00	76	3:1	1.12	0.30-0.20
		T_3	-	+	+	20.00	18.00	38	9:7	0.20	0.70-0.50
6.	Junctura proper	C	-	+	+	75.00	69.00	144	9:7	1.02	0.50-0.30
		T_1	-	+	+	80.00	101.00	181	27:37	0.30	0.70-0.50
		T_2	-	+	+	33.00	43.00	76	27:37	0.05	0.90-0.80
		T_3	-	+	+	15.00	23.00	38	27:37	0.11	0.70-0.50
7.	Ligule	C	-	+	+	89.00	55.00	144	9:7	1.81	0.20-0.10
		T_1	-	+	+	102.00	79.00	181	9:7	0.01	>0.98
		T_2	-	+	+	39.00	37.00	76	9:7	0.75	0.50-0.30
		T_3	-	+	+	15.00	23.00	38	27:37	0.11	0.80-0.70
8.	Auricle	C	-	+	+	77.00	67.00	144	9:7	0.45	0.70-0.50
		T_1	-	+	+	89.00	92.00	181	27:37	3.62	0.10-0.05
		T_2	-	+	+	33.00	43.00	76	27:37	0.05	0.90-0.80
		T_3	-	+	+	12.00	26.00	38	27:37	1.75	0.20-0.10
9.	Node	C	-	+	+	30.00	114.00	144	3:13	0.41	0.70-0.50
		T_1	-	-	-	25.00	156.00	181	3:13	2.90	0.10-0.05
		T_2	-	-	-	16.00	60.00	76	3:13	0.26	0.70-0.50
		T_3	-	-	-	7.00	31.00	38	3:13	0.002	>0.98
10.	Internode	C	-	+	+	106.00	38.00	144	3:1	0.15	0.70-0.50
		T_1	-	+	+	126.00	55.00	181	3:1	2.80	0.10-0.05
		T_2	-	+	+	54.00	22.00	76	3:1	0.63	0.50-0.30
		T_3	-	+	+	22.00	16.00	38	9:7	0.04	0.90-0.80
11.	Stigma	C	-	+	+	108.00	36.00	144	3:1	0.00	0.70-0.50
		T_1	-	+	+	138.00	43.00	181	3:1	0.15	0.70-0.50
		T_2	-	+	+	54.00	22.00	76	3:1	0.63	0.50-0.30
		T_3	-	+	+	22.00	16.00	38	9:7	0.04	0.90-0.80
12.	Apiculus	C	-	+	+	106.00	38.00	144	3:1	0.15	0.70-0.50
		T_1	-	+	+	127.00	54.00	181	3:1	2.26	0.20-0.10
		T_2	-	+	+	55.00	21.00	76	3:1	0.28	0.70-0.50
		T_3	-	+	+	22.00	16.00	38	9:7	0.04	0.90-0.80
13.	Awning	C	-	+	+	107.00	37.00	144	3:1	0.04	0.90-0.80
		T_1	-	+	+	128.00	53.00	181	3:1	1.76	0.20-0.10
		T_2	-	+	+	61.00	15.00	76	3:1	1.12	0.30-0.20
		T_3	-	+	+	27.00	11.00	38	3:1	0.32	0.70-0.50
14.	Tip sterility	C	-	+	+	107.00	37.00	144	3:1	0.04	0.90-0.80
		T_1	-	+	+	144.00	37.00	181	3:1	2.01	0.20-0.10
		T_2	-	+	+	57.00	19.00	76	3:1	0.00	
		T_3	-	+	+	33.00	5.00	38	3:1	2.84	0.10-0.05

+ - Present, - - Absent, C - Normal, T_1 - 1500 rads, T_2 - 2000 rads, T_3 - 5000 rads of X-rays

Table 3. Breeding behaviour of F_3/M_3F_3 families for different morphological characters studied in crosses Cherumodan × Japan Violet for normal (C), 1500 (T_1), 2000 (T_2) and 5000 (T_3) rads X-ray treated pollen grains used for hybridization

S.No.	Character	Treatment	F_2 ratio	Expected F_3 ratio	Observed breeding behaviour of F_3 families										
					TD	3:1	1:3	9:7	15:1	3:13	27:37	TR	Total	X^2	P
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Leaf axil	C	15:1	7:4:4:1	12.00	7.00			6.00			5.00	30	5.67	0.20-0.10
		T_1	15:1	7:4:4:1	12.00	8.00			7.00			5.00	32	4.91	0.20-0.10
		T_2	15:1	7:4:4:1	14.00	12.00			5.00			1.00	32	3.63	0.50-0.30
		T_3	15:1	7:4:4:1	11.00	5.00			7.00			2.00	25	0.46	0.95-0.90
2	Leaf sheath	C	3:1	1:2:1	9.00	14.00						7.00	30	0.40	0.90-0.80
		T_1	3:1	1:2:1	11.00	14.00						7.00	32	1.50	0.50-0.30
		T_2	3:1	1:2:1	9.00	19.00						4.00	32	2.69	0.30-0.20
		T_3	9:7	-	-	-	-	-	-	-	-	-	-	-	-
3	Leaf blade	C	9:7	1:4:4:7	2.00	6.00		9.00				13.00	30	0.61	0.90-0.80
	(Lamina)	T_1	9:7	1:4:4:7	2.00	5.00		7.00				18.00	32	2.39	0.50-0.30
		T_2	27:37	1:6:12:8:37	1.00	4.00		5.00			5.00	17.00	32	1.25	0.80-0.70
		T_3	27:37	1:6:12:8:37	1.00	4.00		6.00			3.00	11.00	25	3.33	0.50-0.30
4	Leaf margin	C	3:1	1:2:1	6.00	13.00						11.00	30	2.20	0.50-0.30
		T_1	9:7	1:4:4:7	5.00	10.00		6.00				11.00	32	6.14	0.20-0.10
		T_2	9:7	1:4:4:7	3.00	12.00		7.00				10.00	32	3.77	0.30-0.20
		T_3	9:7	1:4:4:7	2.00	8.00		7.00				8.00	25	1.49	0.70-0.50
5	Leaf tip	C	3:1	1:2:1	9.00	14.00						7.00	30	0.40	0.90-0.80
		T_1	9:7	1:4:4:7	4.00	11.00		9.00				8.00	32	5.82	0.20-0.10
		T_2	3:1	1:2:1	9.00	18.0						5.00	32	1.50	0.50-0.30
		T_3	9:7	1:4:4:7	4.00	8.00		7.00				6.00	25	6.63	0.10-0.05
6	Junctura	C	9:7	1:4:4:7	1.00	5.00		11.00				13.00	30	2.87	0.50-0.30
	proper	T_1	27:37	1:6:12:8:37	1.00	6.00		5.00			3.00	17.00	32	4.04	0.30-0.20
		T_2	27:37	1:6:12:8:37	1.00	4.00		2.00			7.00	18.00	32	5.59	0.30-0.20
		T_3	27:37	1:6:12:8:37	1.00	5.00		4.00			3.00	12.00	25	4.50	0.50-0.30
7	Ligule	C	9:7	1:4:4:7	1.00	8.00		9.00				12.00	30	0.84	0.90-0.80
		T_1	9:7	1:4:4:7	4.00	7.00		6.00				15.00	32	2.70	0.50-0.30
		T_2	9:7	1:4:4:7	5.00	10.00		8.00				9.00	32	6.62	0.10-0.05
		T_3	27:37	1:6:12:8:37	1.00	5.00		6.00			4.00	9.00	25	6.64	0.20-0.10
8	Auricle	C	9:7	1:4:4:7	1.00	6.00		8.00				15.00	30	1.00	0.90-0.80
		T_1	27:37	1:6:12:8:37	2.00	5.00		7.00			2.00	16.00	32	7.34	0.20-0.10
		T_2	27:37	1:6:12:8:37	1.00	2.00		6.00			6.00	17.00	32	1.91	0.80-0.70
		T_3	27:37	1:6:12:8:37	1.00	4.00		4.00			5.00	11.00	25	4.17	0.50-0.30
9	Node	C	3:13	1:2:2:4:7	0.00	1.00	4.00			7.00		18.00	30	5.74	0.30-0.20
		T_1	3:13	1:2:2:4:7	0.00	1.00	3.00			8.00		20.00	32	7.07	0.20-0.10
		T_2	3:13	1:2:2:4:7	0.00	3.00	7.00			7.00		15.00	32	2.70	0.70-0.50
		T_3	3:13	1:2:2:4:7	0.00	1.00	4.00			3.00		17.00	25	8.31	0.10-0.05
10	Internode	C	3:1	1:2:1	5.00	16.00						9.00	30	1.20	0.70-0.50
		T_1	3:1	1:2:1	8.00	17.00						7.00	32	0.19	0.95-0.90
		T_2	3:1	1:2:1	6.00	16.00						10.00	32	1.00	0.70-0.50
		T_3	9:7	1:4:4:7	2.00	7.00		9.00				7.00	25	2.84	0.50-0.30
11	Stigma	C	3:1	1:2:1	5.00	16.00						9.00	30	1.20	0.70-0.50
		T_1	3:1	1:2:1	6.00	17.00						9.00	32	0.69	0.80-0.70
		T_2	3:1	1:2:1	6.00	16.00						10.00	32	1.00	0.70-0.50
		T_3	9:7	1:4:4:7	2.00	7.00		9.00				7.00	25	2.84	0.50-0.30
12	Apiculus	C	3:1	1:2:1	5.00	16.00						9.00	30	1.20	0.70-0.50
		T_1	3:1	1:2:1	6.00	17.00						9.00	32	0.69	0.80-0.70
		T_2	3:1	1:2:1	6.00	16.00						10.00	32	1.00	0.70-0.50
		T_3	9:7	1:4:4:7	2.00	7.00		9.00				7.00	25	2.84	0.50-0.30
13	Awn	C	3:1	1:2:1	11.00	12.00						7.00	30	2.27	0.50-0.30
		T_1	3:1	1:2:1	7.00	17.00						7.00	32	0.19	0.95-0.90
		T_2	3:1	1:2:1	7.00	20.00						5.00	32	2.25	0.50-0.30
		T_3	3:1	1:2:1	8.00	12.00						5.00	25	0.76	0.70-0.50
14	Tip sterility	C	3:1	1:2:1	8.00	13.00						9.00	30	0.60	0.80-0.70
		T_1	3:1	1:2:1	7.00	16.00						9.00	32	0.25	0.90-0.80
		T_2	3:1	1:2:1	7.00	18.00						7.00	32	0.50	0.80-0.70
		T_3	3:1	1:2:1	7.00	14.00						4.00	25	1.08	0.70-0.50

*Expected breeding behaviour of F3 families, Table values at 0.5% level; TD - True dominant; Ch - Cherumodan, JV - Japan violet, TR - True recessive; 1 df. 3.84; 2 df 5.99; 3df 7.81; 4 df 9.49; 5 df 11.07

results could be explained on the basis of three complementary loci for the control of these characters [13]. Except the leaf sheath pigmentation ratio 9:7 of 5000 rads treatment all other genetic ratios were confirmed in F_3/M_3F_3 generations as detailed in Table 3. The above ratio could not be confirmed due to less number of progenies in the family. However, certain limitations of the population size observed in certain M_2F_2 of higher treatments could be confounded by the F_3M_3 confirmation studies. Such instances are common consequences of M_1F_1 sterility [14].

Transformation of genetic ratios 3:1 into 9:7 and 9:7 into 27:37 for different morphological characters occurred due to the recessive mutation in the complementary genes or, the formation of increased number of recessive alleles in the pollen parent due to pollen irradiation. In turn, it leads to the segregation of more recessive alleles or the expression of more recessive phenotypes and thus the transformation of genetic ratios. It also reveals the fact that the actual number of genes in the control of a particular character may vary and it can be ascertained by these types of studies.

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