GAMETIC GENE TRANSFER IN INDIAN MUSTARD (*BRASSICA JUNCEA* (L.) COSS.)

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SUMMARY

The concept of gene transfer using irradiated pollen was investigated in *Brassica juncea* (L.) Coss. The M_1 and M_2 generations derived from the pollinations with heavily irradiated pollen (25, 35 and 50 Krad) showed a greater similarity with the maternal parent for both qualitative and quantitative traits. In general, the similarity increased with an increase in radiation dose. The results indicate the random transfer of paternal characters, and their expression in maternal background.

1. INTRODUCTION

The matromorphic development of embryos after prickle pollination has been demonstrated in *Brassica juncea* (Banga and Labana, 1983). A modification of this technique involves the use of highly irradiated pollen not for producing true matromorphs but for the limited transfer of characters from one genotype to another. Evidence has been presented for the success of irradiated pollen technique in effecting the selective transfer of both qualitative and quantitative traits in *Nicotiana* (Virk *et al.*, 1977; Pandey, 1978; Caligari *et al.*, 1981) and barley (Powell *et al.*, 1983). The experiments described in this paper report such an attempt in Indian mustard.

2. MATERIALS AND METHODS

Two different strains of *B. juncea* ($2n = 36$) with homozygous recessive genotypes, one for no false septum (NFS-1) and another for white flower (WF-1) were used as maternal parents. CV. RLM 198 with homozygous dominant genotypes for both these markers was used as the pollen parent. The pollen from RLM 198 was irradiated with 0, 25, 35 and 50 Krad of gamma rays and used to pollinate flowers of NFS-1 and WF-1. Controlled emasculations without pollination were used to detect spontaneous parthenogenesis. The pollen tube penetration in each cross was recorded in fifty random flowers after 96 hours of pollination. Individual harvesting of siliques was done at maturity.

All the matromorphic M_1 plants ($2n = 36$) having the expression of any paternal marker were selfed to produce the M_2 generation. In addition, a sample of 10 plants in remaining matromorphic M_1 populations was also selfed to produce the M_2 . Three hundred individuals each of selected and unselected M_2 populations along with 20 plants each of the parents and F_1 , and 100 F_2 plants were raised in a single plant randomisation with two replications during winter, 1981. Seeds were space planted with a distance of 15 cm plant to plant and 45 cm row to row. Two seeds per hill were sown. Thinning was carried out 20 days after germination. The scoring for

TABLE 1
Results of M_1 progeny obtained from pollinations with irradiated pollen

Maternal parent	Irradiated pollen donor	Dose (Krad)	Frequency of pollen tube penetrations (per 100 pollen)	Total seed set	Total M_1 plants obtained	Frequency of maternal plants	No. of maternal plants with expression of paternal marker
NFS-1	RLM-198	25 (A)	9.5	280	203	33 (16.3%)	7 (21.2%)
		35 (B)	5.3	133	86	29 (33.7%)	8 (27.6%)
		50 (C)	2.7	88	42	20 (47.6%)	5 (25.0%)
WF-1	RLM 198	25 (D)	8.8	219	175	38 (21.7%)	10 (26.3%)
		35 (E)	4.6	126	86	32 (37.2%)	9 (28.1%)
		50 (F)	1.9	56	38	19 (50.0%)	6 (31.6%)

Figures in parentheses indicate percentages of preceding column.

major gene markers (on all 300 plants) and quantitative characters (on fifty random plants) was carried out in both selected and unselected M_2 groups.

3. RESULTS AND DISCUSSION

The plants derived from irradiated crosses were designated M_1 and those from unirradiated crosses as F_1 . The frequency of pollen tube penetration and the yield of viable cross seeds decreased with an increase in radiation dose (table 1). Controlled pollinations with unirradiated pollen produced a large number of viable F_1 seeds. Each F_1 population was uniform and exhibited the dominant character of the pollen parent. The M_1 progenies revealed a varied expression for resemblance to either of the parents. The expression of the dominant paternal marker gene in a largely maternal phenotype was attributed to the gene transfer. The percentage of maternal plants in different M_1 progenies varied from 16.3 to 50.0 per cent whereas that of maternals with the expression of any paternal marker gene was 21.2 to 31.6 per cent. Spontaneous parthenogenesis was ruled out, as controlled emasculations without subsequent pollination failed to set seed.

TABLE 2
Segregation for major gene markers in selected and unselected M_2 families

Population	Number of plants scored in selected/ unselected	Segregation for major genes in			
		Selected	χ^2	Unselected	χ^2
<i>False Vs no false septum</i>					
NFS-1	20	0:20		0:20	
RLM-198	20	20:0		20:0	
F ₁	20	20:0		20:0	
F ₂	100	79:21 (3:1)	0.85	77:23 (3:1)	0.21
A	300	246:54	7.84	16:284	776.55
B	300	258:42	19.36	21:279	739.84
C	300	261:39	23.04	23:277	725.40
<i>Yellow Vs white flower</i>					
WF-1	20	0:20		0:20	
RLM-198	20	20:0		20:1	
F ₁	20	20:0		20:0	
F ₂	100	73:27 (3:1)	0.21	76:24 (3:1)	0.055
D	300	241:59	4.55	26:274	704.02
E	300	249:51	10.24	32:268	662.20
F	300	257:43	18.20	39:261	615.04

Chisquare values were calculated with a null hypothesis of monogenic recessive inheritance.

The segregation ratios for the two marker genes are presented in table 2. The F_2 data supported the hypothesis of monogenic recessive inheritance (3:1). In the absence of the effects of pollen irradiation the progeny plants of each M_1 group were expected to segregate in a 3:1 ratio. However, all the M_2 progenies deviated significantly from the theoretical expectations. The proportion of M_2 plants having the dominant marker gene phenotype was very high in progenies which were derived from selected M_1 plants whereas it was very low in the progenies of unselected M_1 plants. The

deviation of the M_2 segregation in favour of the dominant class in the selected group is explainable if some of the selected plants were homozygous and others heterozygous for the transferred gene. Pandey (1980) has in fact shown the presence of both homozygous and heterozygous M_1 transformants. The occurrence of plants with paternal markers in M_2 progenies derived from M_1 plants without any evidence of transferred markers merits further investigation.

The analysis of the three quantitative traits revealed that means over all the selected M_2 families differed significantly from F_2 means except for secondary branches (table 3). On the other hand, unselected M_2 families

TABLE 3

Analysis of some quantitative traits in M_2 generation of selected and unselected groups

Combination	Days to bloom		Plant height		Secondary branches	
	Selected	Unselected	Selected	Unselected	Selected	Unselected
NFS-1	52.5 $\pm$ 1.02		73.8 $\pm$ 1.03		31.8 $\pm$ 0.83	
RLM-198	87.5 $\pm$ 1.18		151.6 $\pm$ 1.03		19.5 $\pm$ 0.76	
F_1	96.2 $\pm$ 1.15		162.4 $\pm$ 1.02		38.2 $\pm$ 1.39	
F_2	78.4 $\pm$ 3.06		119.9 $\pm$ 3.93		25.6 $\pm$ 2.05	
A	70.3 $\pm$ 2.05	78.6 $\pm$ 2.36	106.5 $\pm$ 1.95	123.2 $\pm$ 1.32	28.9 $\pm$ 2.03	24.2 $\pm$ 1.87
B	69.5 $\pm$ 2.41	75.4 $\pm$ 3.10	103.2 $\pm$ 2.63	119.5 $\pm$ 2.13	28.4 $\pm$ 2.46	27.3 $\pm$ 1.06
C	63.2 $\pm$ 3.39	69.6 $\pm$ 4.75	97.4 $\pm$ 4.85	107.9 $\pm$ 4.56	29.2 $\pm$ 4.53	28.4 $\pm$ 3.85
"t" between groups		2.67**		5.56**		0.93
"t" F_2 vs. group	3.13**	1.05	3.99**	0.71	1.17	0.41
WF-1	73.5 $\pm$ 1.26		125.3 $\pm$ 0.87		12.4 $\pm$ 0.76	
RLM-198	86.3 $\pm$ 1.38		149.5 $\pm$ 1.02		19.9 $\pm$ 0.82	
F_1	87.4 $\pm$ 0.59		161.7 $\pm$ 1.74		19.7 $\pm$ 1.95	
F_2	83.5 $\pm$ 2.14		153.6 $\pm$ 2.56		13.8 $\pm$ 2.54	
D	75.6 $\pm$ 2.85	80.5 $\pm$ 1.35	139.8 $\pm$ 2.36	141.2 $\pm$ 1.36	16.3 $\pm$ 1.85	16.9 $\pm$ 2.68
E	72.4 $\pm$ 2.38	78.9 $\pm$ 1.96	134.5 $\pm$ 2.76	138.3 $\pm$ 2.45	14.8 $\pm$ 2.06	15.7 $\pm$ 3.39
F	68.2 $\pm$ 2.79	76.5 $\pm$ 2.65	132.6 $\pm$ 3.88	135.2 $\pm$ 3.05	13.5 $\pm$ 3.47	15.6 $\pm$ 3.91
"t" between groups		3.36**		1.31		0.49
"t" F_2 bs. group	4.33**	1.99	5.77**	5.17**	0.36	0.71

** Significant at $P=0.01$.

with the exception of plant height in the cross WF-1 $\times$ RLM-198 did not differ from the F_2 means. Though both selected and unselected families deviated towards their maternal parent, they were significantly different from it (t^{**}). Increased radiation dosage led to greater resemblance to the maternal parent. In addition, all the M_2 families revealed heterogeneity for the characters studied. Such situations are unexpected if pollen irradiation had no effect. These results can be explained by assuming the transfer, integration and expression of the random parts of the paternal genome into M_1 and M_2 progenies. The differences in the selected and unselected M_2 families might be due to the influence of paternal parent to varying degree. It is evident (table 3) that the selected group had a comparatively greater influence of the maternal genome, and a deficit of paternal homozygotes, while in the unselected group there was a greater expression of paternal phenotype. Due to this very reason the unselected M_2 progenies behaved more like F_2 . The differences between F_2 and M_2 though not significant were apparent in unselected families also.

Our results are similar to those reported in *Nicotiana* and Barley. Thus the physiological and molecular processes involved in gene transfer may be similar. The interest of a plant breeder in these findings lies mainly in the possibility of effecting specific genome modifications in a quicker and more economical manner, and as a rapid technique for breaking linkages.

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4. REFERENCES

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