Environmental and Experimental Botany, Vol. 26, No. 1, pp. 25–29, 1986.
Printed in Great Britain.

0098–8472/86 $3.00 + 0.00

RADIATION INDUCED POLLEN STERILITY AND ENHANCED OUTCROSSING IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

M. DUTTA,* A. BANDYOPADHYAY, V. ARUNACHALAM, S. L. KAUL and M. V. R. PRASAD

Indian Agricultural Research Institute, Regional Station, Rajendranagar, Hyderabad 500030, India and
*Groundnut Improvement Programme, ICRISAT, Patancheru P.O., Andhra Pradesh 502324, India

(*Received* 4 *February* 1985; *accepted in revised form* 22 *April* 1985)

DUTTA M., BANDYOPADHYAY A., ARUNACHALAM V., KAUL S. L. and PRASAD M. V. R. *Radiation induced pollen sterility and enhanced outcrossing in groundnut* (Arachis hypogaea *L.*). ENVIRONMENTAL AND EXPERIMENTAL BOTANY **26,** 25–29, 1986.—Seeds of six groundnut cultivars with distinguishable morphological features were irradiated with 5, 10, 15 and 20 kR gamma rays and grown surrounded by a pollen parent. The effect of irradiation on pollen sterility and other quantitative characters was studied in M_1. Pollen sterility increased almost linearly with increase in dose in each cultivar. There was significant variety × dose interaction. The correlation between inherent pollen sterility (measured in control) and percent increase in sterility was negative. The induced variation was significant only for five quantitative characters. Fifteen kR was the most effective dose for inducing a high level of pollen sterility without causing significant change in other characters. The correlation between pollen sterility and cross-pollination % was positive and significant implying that induced pollen sterility would enhance cross-pollination.

INTRODUCTION

MUTAGENS have been successfully used to induce different types of sterility in various crop plants. For example, X-ray-induced male sterile mutants were reported in rice,[9] wheat,[5,7] watermelon and cucumber[4] and gamma ray induced male sterile mutants in sugarbeet.[10] However, current emphasis is on inducing partial pollen sterility which would lead to enhancement of natural outcrossing.[12] For example, an increased rate of outcrossing from 0.5–2.4% to 2.4–6.0% was reported by mutagen treatments in oats,[8] and up to 55% by 'Ethrel' in wheat.[6] Use of partial male sterility in hybrid ryegrass production[1] and enhancement of pollen sterility by diethyl sulphate[2] in groundnut have been reported. It is of interest therefore to examine in groundnuts, a self-pollinated legume, the extent of pollen sterility induced by low doses of gamma irradiation and consequent enhancement in outcrossing. Results of such an attempt are reported in this paper.

MATERIALS AND METHODS

Six cultivars with distinct morphological features—NC Ac 12, NC Ac 10 and NC Ac 2144, belonging to Virginia bunch, M 13 to Virginia runner, and GDM and PI 259747 to Valencia sub-groups were chosen. Seventy-five to 200 dry seeds of these cultivars were irradiated at 5, 10, 15 and 20 kR gamma rays (much below LD_{50}, which is about 45 kR) from a ^{60}Co source and then were grown in the 1980 post-rainy season at the Regional Station, Indian Agricultural Research Institute, Hyderabad, India in a split-plot design with two replications. Cultivars formed main-plots and doses (including control) sub-plots. Seeds were sown 20 cm apart in rows of 6.6 m

length with a row width of 60 cm. Each sub-plot consisted of four rows except those for NC Ac 12 and GDM. Sub-plots of NC Ac 12 consisted of two rows each and those of GDM consisted of two rows in one replication and one row in the other. Each sub-plot was surrounded by the pollen parent Gangapuri, a Valencia cultivar.

Pollen sterility using acetocarmine staining, was observed at two stages of plant growth: (a) flowering and (b) 30 days after flowering on five randomly chosen plants in each row of the sub-plots. In the second stage, observation on pollen sterility could be taken in one replication only. Germination percentage and mean number of days to flowering were measured on each sub-plot while seven other characters, *viz.*, number of primary branches, number of secondary branches, number of aerial pegs, number of mature pods, weight of mature pods, number of mature kernels and weight of mature kernels, were observed on 10 random plants per row in each sub-plot.

Plot means were used for split-plot ANOVA. Data on pollen sterility were analysed over the two stages. The following method was used to identify the optimum dose that induced maximum pollen sterility without causing significant change in other characters. First, the characters for which variation among doses was significant were identified. Then for each character the differences between the mean of the control and each of the 4 doses were tested for their statistical significance by a *t*-test. A significant difference was allotted a score of '1' and a non-significant one, a score of 'zero'. The scores over all the characters were added to get a final score for each dose. The dose having the least final score was considered to be optimal.

Cultivars were ranked on pollen sterility % (highest value getting the rank 1) under control and on percentage increase in pollen sterility over control under each dose. Spearman's rank correlation coefficient (r) was calculated between the ranks under control and those under each dose.

To determine cross-pollination %, 1527 plants (over the 6 cultivars) on which detailed observations were taken in M_1F_0 were advanced to the M_3F_2 generation on a plant-to-progeny row basis. A total of 10,821 plants was observed for segregation for pod and seed characters—pod size, pod beak, pod constriction, pod texture and seed coat color. A plant deviating from the seed or pollen parent in any one or more of the pod and seed characters was considered to be a hybrid—criterion A. In addition, two quantitative characters, pod and kernel weight, were observed. The mean and standard deviation (S.D.) of the two characters for the seed and pollen parents were calculated separately. A plant which had a value greater than (mean + S.D.) of the better parent or smaller than (mean − S.D.) of the inferior parent was considered to be a real deviant and given a score of '1'; otherwise a score of 'zero'. The total score across the two characters was computed for each plant. Plants obtaining a score of '2' were considered to be hybrids—criterion B. Plants which were hybrids by both the criteria A and B were taken to be actual hybrids. Cross-pollination % was calculated as the proportion of actual hybrid plants obtained in the M_3F_2 generation out of the plants raised in the M_1F_0 generation for each variety–dose combination. The correlation coefficient between pollen sterility % induced by irradiation and outcrossing % was then calculated.

RESULTS AND DISCUSSION

The variation due to doses was significant for pollen sterility % and five other characters (Table 1). The overall differences among cultivars were significant for 8 characters while cultivar × dose interaction was absent for all of them. Irradiation was effective in enhancing pollen sterility % (Table 2). Pollen sterility remained almost of the same magnitude in both stages, indicating that it was physiologically age-neutral. Although overall variation among cultivars was not significant, there were differences in their inherent sterility (under control) and irradiation doses. Pollen sterility increased with doses in both stages of plant growth. However, significant differences between control and irradiation doses within individual cultivars were found for various characters in stage I above 5 kR only. Thus pollen sterility induced by 5 kR was not sufficient as was also brought out by the non-significant rank correlation. But for the higher doses of radiation, the relationship between pollen sterility of the

Table 1. ANOVA for 10 quantitative characters of groundnuts in the M_1F_0 generation

Source	Cultivars	Error 1	Doses	Variety × dose	Error 2
d.f.	5	5	4	20	24
Germination (%)	*	76.2	NS	NS	77.6[a]
Days to flowering	*	6.7	*	NS	23.8
Primary branches	NS[b]	3.5	NS	NS	0.5
Secondary branches	*	43.1	NS	NS	6.0
Aerial pegs	*	477.2	NS	NS	81.2
Mature pods	*	21.4	*	NS	30.2
Pod weight	*	28.0	*	NS	15.1
Mature kernels	*	61.4	*	NS	20.6
Kernel weight	*	10.5	*	NS	3.4
Pollen sterility (%)	NS[c]	4.4	*	*	3.1

* Significant at 5% level.
[a] Values represent error mean squares.
[b] Non-significant.
[c] Based on pooled analysis over 2 stages.

Table 2. Mean pollen sterility % of groundnuts in 2 stages, under various doses of irradiation

		Doses (kR)					
Cultivars	Stage	0	5	10	15	20	Mean
NC Ac 12	I	6.1	7.2	19.6	25.9	27.9	17.3
	II	8.5	10.0	19.1	22.7	27.9	17.7
GDM	I	14.9	17.4	19.5	18.1	28.0	19.6
	II	13.6	16.1	17.8	20.2	28.2	28.5
PI 259747	I	11.9	15.1	18.9	21.1	31.0	19.6
	II	11.3	13.0	18.2	19.7	27.1	17.9
M 13	I	11.6	13.3	13.6	22.4	29.9	18.2
	II	11.1	13.9	16.6	21.3	26.1	17.8
NC Ac 2144	I	12.4	14.7	16.0	23.0	27.4	18.7
	II	9.5	11.3	17.8	21.3	28.7	17.7
NC Ac 10	I	6.7	9.4	16.4	22.8	23.6	15.8
	II	8.7	12.0	17.6	22.0	23.7	16.8
Overall	I	10.6	12.9	17.3	22.2	28.0	
	II	10.5	12.7	17.8	21.2	27.0	
Rank correlation coefficient between control and respective doses	I		−0.200	−0.600	−0.943*	−0.943*	
	II		−0.257	−0.943*	−1.000*	−0.886*	

* Significant at 5% level.

Table 3. Outcrossing % under various doses of irradiation

Cultivar	Dose (kR) 0	5	10	15	20	Overall	Rank correlation coefficient between pollen sterility and outcrossing (%)
NC Ac 12	10.7	7.7	5.1	12.8	8.3	8.8	0.100
GDM	0.0	7.1	11.1	10.3	11.1	8.0	0.975*
PI 259747	0.0	2.6	3.0	0.0	6.7	2.2	0.670
M 13	4.3	33.3	9.7	30.9	25.4	20.8	0.300
NC Ac 2144	0.0	8.6	11.1	11.7	16.7	8.7	1.000*
NC Ac 10	17.5	12.5	9.1	25.0	30.7	18.7	0.600
Overall	5.4	10.2	6.8	14.1	14.8	11.8	0.900*

* Significant at 5% level.

control and increase in sterility was definite and inverse.

If the induced sterility is to be used in recombination breeding by enhancing outcrossing, the changes induced in other characters of the genotype should be minimal. Since pollen sterility is positively correlated with outcrossing % (Table 3) it should be as high as possible.

The character means under 5 kR were significantly higher than those under the control for 4 out of the 5 characters that were affected by radiation doses (Table 4). They were almost similar to the control at 10 and 15 kR but declined significantly for 4 characters at 20 kR. Overall, the plant phenotypes were not significantly altered under 10 or 15 kR (Table 5). In spite of its beneficial effects in increasing the value of 4 characters, 5 kR was not the desirable dose because of its low induction of pollen sterility. Though 10 and 15 kR had almost similar effects on the cultivars, the latter induced more pollen sterility than the former (Table 2). Therefore, 15 kR was the most effective and desirable dose.

The ultimate utility of the increased pollen sterility depends on how much enhancement in outcrossing it leads to. A significant and positive rank correlation ($r = 0.9$) was found between pollen sterility and outcrossing % (Table 3). The correlation coefficient was significant and positive for the cultivars, GDM and NC Ac 2144

Table 4. Means under various doses of irradiation for 5 characters of groundnuts

Dose (kR)	Character: Days to flowering	Mature pod number	Pod weight (g)	Mature kernel number	Kernel weight (g)
0	47.4	23.6	22.9	24.4	10.2
5	47.2	29.0	27.8	28.9	12.0
10	46.9	23.2	22.7	24.4	10.2
15	47.7	23.6	21.2	23.5	9.4
20	49.1	21.8	18.2	19.0	7.6
Lsd (0.05)	1.3	4.6	3.3	3.8	1.6

Table 5. Significance score of the difference in means under various doses with control

Dose (kR)	Character					
	Days to flowering	Mature pod number	Pod weight (g)	Mature kernel number	Kernel weight (g)	Total score
5	0*	1	1	1	1	4
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	1	0	1	1	1	4

* Score = 1 for significant difference; = 0 for non-significant difference.

and positive though not significant for others. Increased rates of outcrossing were also observed under mutagen treatments in similar studies.[8,11,13]

In view of the significant cultivar × dose interaction for pollen sterility, a single dose may not be universally effective for all the cultivars. Nevertheless low doses of irradiation could enhance pollen sterility to result in increased natural outcrossing in groundnut. While the highest natural crossing reported in groundnut so far is about 6.6%,[3] gamma irradiation of 5 kR could produce as high as 33% outcrossing in a cultivar, M 13 with a mean outcrossing of 21% over doses (Table 3). This enhanced outcrossing can be very effective in groundnut where hand emasculation and pollination are arduous and often do not result in high frequencies of true hybrids. Repeated cycles of irradiation can then be a useful technique for increasing recombination. However, the efficiency of such schemes needs extensive experimental evaluation.

REFERENCES

1. Ahloowalia B. S. (1981) Use of partially male-sterile perennial ryegrass for hybrid cultivar production. *Crop Sci.* **21,** 415.
2. Avadhani K. K. and Rama Rao B. V. (1968) Studies on the effects of diethyl sulphate on TMV-2 groundnut (*Arachis hypogaea* L.) *Indian J. Sci. Indus.* **2,** 77–82.
3. Bolhuis G. G. (1951) Natuurlijke bastaardaring bij de aardnoot (*Arachis hypogaea* L.). *Landbouwk. Tijdschr.* **63,** 447–455.
4. Bowers J. L. (1959) The plant irradiation program in Arkansas. *Arkans. Fm Res.* **86,** 2.
5. Bozzini A. (1974) Radiation induced male sterility in durum wheat. *In Polyploidy and induced mutations in plant breeding.* International Atomic Energy Agency (IAEA), Vienna, 23 pp.
6. Dotlacil L. and Apltauerova M. (1978) Pollen sterility induced by 'Ethrel' and its utilization in hybridization of wheat. *Euphytica* **27,** 353–360.
7. Fossati A. and Ingold M. (1970) A male sterile mutant in *Triticum aestivum*. *Wheat Inf. Serv. Kyoto Univ.* **30,** 8.
8. Grindeland R. L. and Frohberg R. C. (1966) Outcrossing of oat plants (*Avena sativa* L.) grown from mutagen-treated seeds. *Crop Sci.* **6,** 381–382.
9. Imai Y. (1935) The effect of X-rays on the production of sterile rice. *Jap. J. Genet.* **10,** 233.
10. Kinoshita T. and Takahashi M. E. (1969) Induction of cytoplasmic male sterility by gamma ray irradiation in sugar beets. *Jap. J. Breed.* **19,** 445.
11. McKenzie R. I. H., Brunner H., Hsieh S. C. and Mikaelsen K. (1975) Outcrossing rates in wheat, oats and barley produced from mutagen treated seeds. *SABRAO J.* **7,** 79–83.
12. Rutger J. N. and Shinjo C. (1980) Male sterility in rice and its potential use in breeding. Pages 53–66 *in Innovative approaches to rice breeding.* International Rice Research Institute, Los Banos, Philippines.
13. Thurman R. L. and Womack D. (1961) Percentage natural crossing in oats produced from irradiated seeds. *Crop Sci.* **1,** 374.