Theor Appl Genet (1987) 74:466–470

Enhanced cross pollination to widen the scope of breeding in groundnut (*Arachis hypogaea* L.)

M. Dutta, V. Arunachalam and A. Bandyopadhyay

Indian Agricultural Research Institute, Regional Station, Rajendranagar, Hyderabad-500 030, India

Received August 30, 1986; Accepted November 20, 1986
Communicated by B. R. Murty

Summary. Six groundnut genotypes belonging to the Virginia and Valencia sub-groups were irradiated with gamma rays at doses of 5, 10, 15 and 20 kR, much below LD_{50}, and grown surrounded by a pollen parent in a split-plot design. The succeeding two generations were checked for the occurrence of hybrids by examining the segregation for pod and seed characteristics and the two quantitative characters, pod and seed yield. Cross-pollination up to 20.8% was observed in 'M13', a Virginia cultivar. There was a genotype-dose interaction for the extent of cross-pollination. Cross-pollination was higher in Virginia than Valencia genotypes and more frequent under 15 and 20 kR than under other doses, in general. The observed substantial enhancement of cross-pollination encourages the use of seed irradiation at proper doses as a method for increasing recombination in plant breeding programmes.

Key words: *Arachis hypogaea* – Gamma irradiation – Cross pollination – Outcrossing

Introduction

Efforts to increase productivity in self-pollinated crops stimulated, in its wake, a search for male sterility and other outcrossing mechanisms (Rachie et al. 1975). Such mechanisms play a role in accelerating recombination in recurrent selection cycles.

Natural outcrossing of up to 6% has been reported to occur under field conditions in groundnut (Kushman and Beattie 1946; Hammons and Leuck 1966; Pompeu 1974; Anonymous 1978), yet it is not high enough to ensure adequate recombinational variability for effective selection.

Mutagen treatment has been reported to enhance outcrossing rates in such self-pollinated crops as oats (Grindeland and Frohberg 1966), wheat (Rana and Mathur 1967) and barley (Hermelin and Brunner 1976). Of the mutagen treatments, gamma irradiation of seeds is relatively easy. However, the dose of irradiation should be kept low, much below LD_{50}, to keep the frequency of mutations and consequent confounding variability also low. Outcrossing resulting from such a treatment is usually measured using genetic markers and ideally these markers need be distinctive and conditioned by single dominant genes with complete penetrance to enable unambiguous hybrid detection in the F_1 generation. In general, most of the characters in an allopolyploid like groundnut are conditioned by more than one gene. A majority of single dominant genes were found to be unsuitable as markers for estimating the frequency of natural outcrossing in groundnut (Hammons 1964). When yield improvement is desired, the genetic markers should also preferably combine genes for productivity (Norden 1980). Further, such markers can have variable expressivity in various cultivars. It is then difficult to measure outcrossing using them.

Taking all these factors into account, a study was formulated in groundnut using six cultivars as seed parents and a cultivar, distinctly different from these six, as the pollen parent. The main objective was to examine whether outcrossing could be enhanced by gamma irradiation. If so, it will have definite implications in breeding for productivity.

Materials and methods

Six seed parents with distinguishing characteristics (Table 1) were selected along with a pollen parent, 'Gangapuri'. Seventy-five to 200 dry seeds of each cultivar were irradiated with 5, 10, 15, and 20 kR gamma rays, respectively, from a ^{60}Co source available at the Osmania University, Hyderabad (India). They had been grown during the 1980 post-rainy season at the Regional Station, Indian Agricultural Research Institute, Hyderabad, surrounded on all sides by the pollen source.

One thousand five hundred and twenty-seven single plants of the M_1F_0 generation were raised to the next two generations on a plant-to-progeny basis; 10,821 single plants and 655 progeny bulks (consisting of 10,515 plants) were carefully

Table 1. Distinguishing features of the varieties used to estimate cross-pollination

Cultivar	Group	Branching	Leaf	Flower	Pod	Seed
'NC Ac 12'	Virginia bunch	Profuse	Yellow, medium, thick	Yellow	2 seeded, slightly beaked, constricted	Pink, medium
'M13'	Virginia runner	Profuse	Dark green, small, thick	Light yellow	2 seeded, large	Light pink, large
'NC Ac 2144'	Virginia bunch	Profuse, ribbed	Krinkled (corduroy), dark green, medium	Yellow	2 seeded, slightly beaked, large	Pink, medium
'NC Ac 10'	Virginia bunch	Profuse	Dark green, small, thin	Yellow	2 seeded, medium	Pink, medium
'GDM'	Valencia (dwarf)	Few, compact	Dark green, small, thin	Yellow	2–3 seeded, long	Red, small
'PI 259747'	Valencia	Few, hairy	Bluish green, hairy, large, thin	Orange	2–3 seeded, slightly beaked, deeply grooved, medium	Dark brown, compressed, small
'Gangapuri'	Valencia	Few, pigmented	Light green, large, thin	Yellow	4 seeded, smooth, long	Red, small

examined in the M_2F_1 generation for a number of pod and seed attributes – pod size, beak, constriction, texture and seed coat (testa) colour. In 527 plant progeny and 56 progeny bulks, at least one plant was different from the seed and pollen parents for one or more of those attributes. They were thus suspected to be hybrids and forwarded to the M_3F_2 generation by a fair sampling process.

A large number of plant families were examined for the above pod and seed attributes. A progeny in which at least one plant deviated from the seed and pollen parent in one or more of the attributes was counted to be a hybrid progeny – criterion A.

In addition, segregation for two characters, pod and seed yield, was also examined. For this purpose, the mean (m) and standard deviation (s) of the two characters in the control plots of seed and pollen parents were calculated separately. A plant which had a value greater than (m+s) of the better parent or smaller than (m–s) of the inferior parent was considered to be a real deviant and given the score 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 possible hybrids – criterion B. A progeny in which at least one plant was a hybrid by both criteria A and B was taken to be an actual hybrid. Every hybrid progeny thus detected was different from their parents for at least a minimum of three characters (and maximum of six). Cross-pollination percent was then equal to 100× (number of hybrid progeny obtained in M_3F_2/number of single plants in M_1F_0 from which M_3F_2 was derived).

Results and discussion

A disquietening feature of the study is how to be sure that the estimates of outcrossing percent do not include a large or small proportion of mutations. First of all, the study could not have been based on simple genetic markers, as traditional approaches would recommend. For example, even testa colour is not simply inherited. 'Gangapuri' has red testa, supposedly dominant. Crosses between 'Gangapuri' and 14 parents in direct and reciprocal combinations (Arunachalam et al. 1980) did not provide supporting evidence. On the other hand, the dark brown testa of 'PI 259747' was dominant in some crosses and persisted in a high frequency even up to the F_7 generation (Koteswara Rao, pers. commun.). The inheritance of testa colour depends very much on the parental material. While screening for white testa peanut phenotypes, Hammons and Branch (1982) found four accessions to be recessive at all five loci that conditioned testa colour. F_2 populations from marker-identified natural crosses of each of these lines to a tester genotype homozygously dominant at four of the testa colour loci fitted the ratio 225 tan: 31 white. Branch and Hammons (1980) were also able to establish in some other material incomplete dominance for each of the two genes, R_1, R_2 that controlled testa colour. They could establish absence of linkage and incomplete dominance at each locus. Some other studies reported that red and white testa colour segregated in a 3:1 ratio (Jadhav and Shinde 1979) and that the various shades of red testa were governed by two major genes with a possible third locus present (Ashri 1969, 1970).

We examined the frequency of progeny resembling the seed parent in testa colour and those showing intermediate testa colour from the 583 progeny that were suspected to be hybrids for pod shape, constriction, beak, texture and size in the M_2F_1 generation. It may be pointed out here that the remaining 944 out of 1,527 progeny in the M_2F_1 resembled the seed parent in all characters including testa colour. It was found in almost all cases, regardless of the irradiation dose and seed parent, that the progeny resembled the seed parent in testa colour (Table 2) with a few progenies having a colour intermediate between the seed and pollen parent (but mostly inclining towards the seed

Table 2. Testa colour of progeny suspected to be hybrids on pod shape, constriction, beak, texture and size

Dose kR		Seed parent						Total
		'NC Ac 12'	'M13'	'NC Ac 2144'	'GDM'	'PI 259 747'	'NC Ac 10'	
0	s	6	13	5	5	6	33	68
	i	0	1	0	1	0	1	3
	t	6	14	5	6	6	34	71
5	s	13	53	14	12	26	31	149
	i	1	0	0	0	2	1	4
	t	14	53	14	12	28	32	153
10	s	10	23	12	10	12	16	83
	i	1	1	0	0	3	0	5
	t	11	24	12	10	15	16	88
15	s	13	44	15	6	12	27	117
	i	0	5	0	0	0	0	5
	t	13	49	15	6	12	27	122
20	s	20	45	12	14	7	51	149
	i	0	0	0	0	0	0	0
	t	20	45	12	14	7	51	149
Total	s	62	178	58	47	63	158	566
	i	2	7	0	1	5	2	17
	t	64	185	58	48	68	160	583

s = number of progeny resembling seed parent in testa colour; i = number of progeny whose testa colour was a shade different from that of seed parent, and classified intermediate; t = total

parent). This clearly established that in the M_2F_1 progeny (with distinct differences in some other pod attributes), M_2 variability was absent for testa colour. Due to the extensive evidence we assembled earlier for the complex inheritance pattern reported for testa colour, we chose to examine F_2 segregation for more than one character to decide on the extent of possible cross-pollination. In addition, the following points provided further possible safeguards to avoid mix-ups between F_2 and mutational segregation:

a) The cultivars used in this study were pilot-tested with various doses of gamma irradiation. The LD_{50} was determined to be 45 kR. Ten and 15 kR, under which high outcrossing percentages was observed, were thus doses one-third of, or below the LD_{50}. Such doses may not be expected to induce high frequencies of mutations for at least three characters simultaneously in a polyploid like groundnut.

b) Pollen sterility percentage induced by irradiation in M_1F_0 was found to be positively correlated with the estimated cross-pollination percentage ($r = 0.9$, significant at 5% level).

It is known that induction of pollen sterility is a mechanism with which to promote cross-pollination (Thurman and Womack 1961; Grindeland and Frohberg 1966; McKenzie et al. 1975).

Table 3. Number of plants deviating from the parents for one or more pod and seed attributes in M_2F_1 generation and their progeny that segregated in M_3F_2 generation counted as true hybrids

Dose (kR)	Deviate based on distinct differences for characters					Deviants segregating in M_3F_2 generation
	1	2	3	4	Total	
0	45	25	1	–	71	16
5	88	54	8	3	153	38
10	52	32	4	–	88	21
15	71	47	4	–	122	46
20	97	48	4	–	149	48
Total	353	206	21	3	583	169

c) Deviant plant progeny were isolated in the M_2F_1 generation. Their progeny, in the next (M_3F_2) generation, which segregated for three characters (one pod/seed attribute under criterion A, pod and kernel weight under criterion B mentioned for hybridity) or more were taken to contribute to cross-pollination (Table 3). This process eliminated an overestimation of the extent of outcrossing. In general, F_2 segregation would be expected to be higher than M_3 segregation. Further, the

Table 4. Extent of cross-pollination in different groundnut cultivars under various irradiation doses

Cultivar		Dose (kR)					Overall
		0	5	10	15	20	
'NC Ac 12'	A	3	4	3	6	3	19
	B	6	9	10	12	12	49
	H	3	3	2	5	3	16
	P	10.7	7.7	5.1	12.8	8.3	8.8
'M 13'	A	3	36	8	27	24	98
	B	5	40	16	39	31	131
	H	3	24	7	22	18	74
	P	4.3	33.3	9.7	31.0	25.4	20.8
'NC Ac 2144'	A	0	2	3	5	5	15
	B	1	10	9	9	6	35
	H	0	2	3	5	5	15
	P	0	3.4	5.6	8.3	13.9	5.4
'GDM'	A	0	1	4	3	3	11
	B	2	3	5	2	2	14
	H	0	1	3	3	3	10
	P	0	3.6	11.1	10.3	11.1	7.3
'PI 259747'	A	0	2	2	0	3	7
	B	6	22	14	5	7	54
	H	0	2	2	0	3	7
	P	0	3.0	3.2	0.0	6.7	2.2
'NC Ac 10'	A	14	6	4	11	20	55
	B	33	30	14	26	56	155
	H	10	6	4	11	16	47
	P	17.6	12.2	7.3	25.0	30.7	18.3
Overall	N	325	313	310	312	267	1,527
	P	4.9	12.1	6.8	14.7	18.0	11.1

A = Based on pod and seed characteristics; B = based on pod and seed yield; H = hybrids by both A and B; N = number of single plants in M_1F_0 from which M_3F_2 was derived; P = cross-pollination %

frequency of mutation if it occurred at all, could not be high since in none of the M_3 families were all or most of the plants found to be deviants from either parent.

d) Moreover, at doses 10 to 15 kR, mutations cannot occur at a very high frequency. At worst, therefore, the values of cross-pollination reported may be marginally reduced. This does not impair in any way the import of the results that will now be presented and discussed.

The cultivars used in the study were true breeding. 'NC Ac 10', 'NC Ac 12' and 'NC Ac 2144' were obtained from the germplasm maintained at ICRISAT and have not been grown extensively in India. The fairly large number of deviants under 0 kR in the first two, indicated they may have yet to attain complete homogeneity.

Cross-pollination up to 20.8% was found to occur in 'M13', a Virginia Runner cultivar (Table 4). It was low (2.2%) in 'PI 259747', but moderate (8%) in 'GDM', both belonging to the Valencia sub-group. The results suggest that cross-pollination was induced more in the Virginia than in the Valencia types. Virginia types, in general, produce more flowers which span a longer period than Valencias. Flowers around the basal nodes only produce pods in Valencia types. Thus, the distribution of flowers over space and time in seed parents may have an important bearing on the extent of outcrossing. The presence of cultivar-dose interactions suggests the need for the proper choice of both seed parent and irradiation dose to obtain high cross-pollination.

Estimates of cross-pollination percent were based on a large sample of more than 23,000 plants in the M_3F_2 generation. Further, 4,875 plants of seed parents (in the 30 variety-dose combinations) were surrounded by 13,110 plants of the pollen parent in the M_1F_0 generation, providing nearly a 1 : 3 ratio of seed : pollen plants. Since every seed plant could not ideally be alternated with a pollen plant due to a paucity of seeds, it may be argued that cross-pollination percentage has been under-estimated. However, the main focus of the study was to find whether cross-pollination could be enhanced by seed irradiation.

Cross-pollination achieved through seed irradiation is very useful for tackling special breeding problems like incorporation of resistance in a productive cultivar. In this case, for example, the seed parent can be irradiated at a proper dose and grown in isolation surrounded by resistant genotypes to allow cross-pollination by them. Desirable recombinants can be selected and allowed to increase in frequency gradually through recurrent cycles of irradiation and selection. Breeding lines combining yield and resistance could thus be produced in a shorter time with greater ease than is possible through conventional pedigree or back cross breeding. The confounding of variation induced by radiation with that due to outcrossing is to be considered as unavoidable.

Acknowledgement. We are grateful to Professor A. Ashri whose constructive comments have helped us to substantially improve an earlier version of this paper.

References

Anonymous (1978) Role of insects in cross-pollination. ICRISAT Ann Rep 1977–78, p 152

Arunachalam V, Bandyopadhyay A, Nigam SN, Gibbons RW (1980) Heterotic potential of single crosses in groundnut (*Arachis hypogaea* L.). Oleagineux 37:415–420

Ashri A (1969) A second locus controlling red testa in peanuts, (*Arachis hypogaea* L.). Crop Sci 9:515–517

Ashri A (1970) Further evidence for a second testa gene in peanuts, *A. hypogaea* L. Oleagineux 25:393–394

Branch WD, Hammons RO (1980) Inheritance of variegated testa color in peanuts. Crop Sci 20:660–662

Grindeland RL, Frohberg RC (1966) Outcrossing of oat plants (*Avena sativa* L.) grown from mutagen-treated seeds. Crop Sci 6:381–382

Hammons RO (1964) Pedigreed natural crossing – A new genetic technique. In: Proc 3rd Nat Peanut Res Conf. Alabama (USA), pp 49–53

Hammons RO, Leuck DB (1966) Natural cross-pollination of the peanut, *Arachis hypogaea* L. in the presence of bees and thrips. Agron J 58:396

Hammons RO, Branch WD (1982) Pedigreed natural crossing to identify peanut testa genotypes. Peanut Sci 9:90–93

Hermelin T, Brunner H (1976) Outcrossing in mutagenically treated plants. In: Induced mutations in cross-breeding. IAEA, Vienna, pp 149–155

Jadhav GD, Shinde NN (1979) Genetic studies in groundnut (*Arachis hypogaea* L.). Indian J Agric Res 13:93–96

Kushman LJ, Beattie JH (1946) Natural hybridization in peanuts. J Am Soc Agron 38:755–756

McKenzie RIH, Brunner H, Hsieh SC, Mikaelsen K (1975) Outcrossing rates in wheat, oats and barley produced from mutagen treated seeds. SABRAO J 7:79–83

Norden AJ (1980) Peanut. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy, Madison Wis, pp 443–456

Pompeu AS (1974) Rate of natural crossing in groundnut (*Arachis hypogaea* L.). Cien Cult (Sao Paulo) 26:782–783

Rachie KO, Rawal K, Frankewoiak JD, Akinpelu MA (1975) Two outcrossing mechanisms in cowpeas, *Vigna unguiculata* L. Walp. Euphytica 24:159–163

Rana RS, Mathur HC (1967) Enhanced outcrossing following mutagenic treatments in Einkorn wheat. Indian J Genet 27:452–456

Thurman RL, Womack D (1961) Percentage natural crossing in oats produced from irradiated seeds. Crop Sci 1:374