Euphytica **88:** 227–230, 1996.

Identification and inheritance of male sterility in groundnut (*Arachis hypogaea* L.)*

Hari D. Upadhyaya & Shyam N. Nigam
Genetic Enhancement Division, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

Received 26 April 1995; accepted 14 September 1995

Key words: Arachis hypogaea, groundnut, peanut, male sterility, inheritance

Summary

Some plants without pods but with gynophores were observed in two F_4 progenies of two crosses of goundnut (*Arachis hypogaea* L.). The flowers on these plants had translucent white anthers with no or a few sterile pollen grains. Three such plants in the succeeding generation were hand pollinated with pollen from a short-duration Indian cv. JL 24. The resulting F_1 hybrid plants (male sterile × 'JL 24') were normal. Chi-square tests for segregation for male fertile and male sterile plants in F_2 and F_3 generations indicated that the male sterility in these crosses of groundnut is governed by two recessive genes. We designate these genes as ms_1 and ms_2 with $ms_1ms_1ms_2ms_2$ being a male sterile genotype.

Introduction

Abnormal looking brachytic plants are occasionally noticed in segregating populations of diverse crosses in groundnut (*Arachis hypogaea* L.). Such plants have been reported in literature under various names: abnormal (Patel et al., 1936), sterile (Hayes, 1933; Katayama & Nagatomo, 1963), sterile dwarf (Husted, 1934), brachytic dwarf (Ashri, 1968; Hull, 1937), and sterile brachytic (Coffelt & Hammons, 1972; Gupta, 1988). These plants were complete (both male and female) sterile (Ashri, 1968; Coffelt & Hammons, 1972; Gupta, 1988). Ashri (1968) ascribed the male sterility in these plants to the lack of pollen grains in anthers, and the female sterility to female gametic sterility and zygotic failure. He artificially pollinated 7 such plants in the greenhouse and only 1 of them developed gynophores which did not produce pods or seeds. Gupta (1988) reported that the sterility of these plants was due to their failure to produce viable pollen grains and ovules.

Expression of such abnormal plants is genetically controlled. Both simple, a single recessive gene (Hull, 1937), or two recessive genes (Ashri, 1968; Hayes, 1933; Husted, 1934; Katayama & Nagatomo, 1963; Patel et al., 1936) and complex inheritance are reported in the literature. Coffelt & Hammons (1972) reported a complex tetragenic F_2 ratio of 243 normal:13 brachytic sterile. Gupta (1988) observed four F_2 ratios: 15:1, 9:7, 54:10, and 243:13 between the normal and brachytic sterile plants in the F_2 generations of different crosses.

In this paper we report male sterility and its inheritance in groundnut. To our knowledge male sterility in the natural state has not been reported previously in groundnut, as all the previous reports relate to complete sterility.

Materials and methods

While harvesting the F_4 progenies of two crosses, ICG 10889 × ICGV 86125 and 'B 4-P4' × ICGS (E) 36, in the 1991 rainy season, we noticed some plants without pods. On closer examination they were found to have a few gynophores. The plants were already 100-day old, and were infected with late leaf spot and rust. We transplanted these plants into 30 × 30 cm plastic pots, and brought them to the glasshouse. The anthers in the

* Submitted as ICRISAT J. A. No. 1812.

Table 1. The chi-square values and probabilities of goodness of fit for a ratio of 15 male fertile:1 male sterile plants in the F_2 and F_3 generations of crosses MS-1, MS-2, and MS-3 with 'JL 24'

Cross	Generation	Number of plants		χ^2	P
		Male fertile	Male sterile		
MS-1 × 'JL 24'	F_2	90	5	0.158	0.500–0.750
Pooled over segregating progenies	F_3	435	34	0.800	0.250–0.500
MS-2 × 'JL 24'	F_2	82	9	2.058	0.100–0.250
Pooled over segregating progenies	F_3	304	24	0.637	0.250–0.500
Total		911	72	1.937	0.100–0.250
Heterogeneity				1.716	0.500–0.750
MS-3 × 'JL 24'	F_2	93	3	1.600	0.100–0.250
Pooled over segregating progenies	F_3	299	21	0.053	0.750–0.900
Total		392	24	0.164	0.500–0.750
Heterogeneity				1.489	0.100–0.250

flowers of these plants were translucent white which contained no or very few sterile pollen grains when studied with acetocarmine under microscope. We pollinated some of these flowers with pollen from 'JL 24', a short-duration cultivar grown in India. The pollination on these late-produced flowers resulted in aerial gynophores. Attempts to vegetatively propagate the branches with gynophores were not successful. In the postrainy 1991/92 season we grew progeny rows of the normal plants from the F_4 progenies in which such plants were noticed in October 1991. The number of male fertile and male sterile plants was counted in both 1991 rainy and 1991/92 postrainy seasons.

Three male sterile plants from the field, one from each of two F_5 progenies of the cross ICG 10889 × ICGV 86125, and one from F_5 progeny of 'B 4-P4' × ICGS (E) 36 cross, were transplanted into plastic pots early in the season. Some flowers on these plants were pollinated with pollen from 'JL 24'. On all the three plants pollinated flowers developed into gynophores and resulted into pods and seeds. The non-pollinated flowers did not result into gynophores. The hybrid seeds obtained from pollinated flowers were sown in plastic pots in the glasshouse. The resulting F_1 plants were normal in phenotype and for pollen grain fertility. They produced normal gynophores, pods, and seeds. The three F_2 populations of the male sterile × 'JL 24' crosses were grown in pots in the 1993 rainy season. The plants were tested for pollen fertility with acetocarmine. The number of male fertile (normal) and male sterile plants was counted in these F_2 populations. Pods from individual normal plants in the F_2 populations were harvested separately. Only 205 out of 265 plants had sufficient seeds to grow the F_3 progenies. The F_3 progenies of these plants were grown in the 1993/94 postrainy season. The pollen grains of male sterile plants were studied after treatment with acetocarmine under the microscope. The number of male fertile and male sterile plants was counted in each segregating F_3 progeny of the three original populations.

Chi-square tests were applied to test the goodness of fit of the expected ratios in all these populations.

Results and discussion

Among the male sterile plants found in this study there was variation for plant morphological characters like plant height and breadth. These plants were slower than the male fertile plants in senescence and retained abundant thick leaves. The anthers of these plants were translucent and white, and contained no or very few sterile pollen grains compared to yellow color with plenty of fertile pollen grains in the male fertile plants.

Table 2. The chi-square values and probabilities of goodness of fit for an expected ratio of 8 segregating:7 non-segregating progenies in the F_3 generation of crosses of MS-1, MS-2, and MS-3 with 'JL 24'

Cross	Number of progenies		χ^2	P
	Segregating	Non-segregating		
MS-1 × 'JL 24'	39	35	0.012	0.900–0.950
MS-2 × 'JL 24'	35	28	0.125	0.500–0.750
Total	74	63	0.026	0.750–0.900
Heterogeneity			0.111	0.500–0.750
MS-3 × 'JL 24'	33	35	0.631	0.250–0.500

Table 3. The chi-square values and probabilities of goodness of fit for an expected ratio of 1:1 between progenies segregating for 15 male fertile:1 male sterile and 3 male fertile:1 male sterile ratios in the F_3 generation of crosses of MS-1, MS-2, and MS-3 with 'JL 24'

Cross	Number of progenies segregating for ratio		χ^2	P
	15:1	3:1		
MS-1 × 'JL 24'	18	21	0.231	0.500–0.750
MS-2 × 'JL 24'	15	20	0.714	0.250–0.500
Total	33	41	0.865	0.250–0.500
Heterogeneity			0.080	0.750–0.900
MS-3 × 'JL 24'	16	17	0.030	0.750–0.900

In the F_4 progeny of the cross ICG 10889 × ICGV 86125 segregating for male sterility (1991 rainy season), 10 out of 11 plants were male fertile and 1 was male sterile indicating a 15 male fertile:1 male sterile segregation (χ^2 = 0.152, P = 0.500–0.750). The progenies of 10 normal plants were grown in rows in the F_5 generation (1991/92 postrainy season) and 8 of them germinated. The plant number in these progenies ranged from 7 to 30. Only 6 out of the 8 progenies segregated for male sterility fitting well to a ratio of 8 segregating:7 non-segregating progenies (χ^2 = 1.509, P = 0.100–0.250) expected among the progenies of plants showing duplicate digenic segregation. Among the 6 segregating progenies with plant number ranging from 7 to 30, 4 progenies showed a high probability of fit to a 3 male fertile:1 male sterile ratio individually and also on pooled basis (χ^2 = 1.523, P = 0.100–0.250, χ^2 (heterogeneity) = 1.153, P = 0.750–0.900) and 2 progenies showed a good fit to a 15 male fertile:1 male sterile ratio (χ^2 = 0.384, P = 0.500–0.750, χ^2 (heterogeneity) = 0.115, P = 0.500–0.750). This also agreed with an expected 1:1 ratio between progenies segregating for 15 male fertile:1 male sterile and 3 male fertile:1 male sterile ratios (χ^2 = 0.667, P = 0.250–0.500).

In the F_4 progeny of 'B 4-P4' × ICGS (E) 36 containing male sterile plants (1991 rainy season), 2 plants were male sterile, and the remaining 6 were male fertile fitting perfectly to a 3 male fertile:1 male sterile ratio. In the F_5 generation, only 2 progenies of the normal plants, segregated for male sterility. The remaining 4 were non-segregating. Of the two segregating progenies, one progeny with 19 plants segregated for a 15 male fertile:1 male sterile ratio (χ^2 = 0.032, P = 0.750–0.900) and the other with 10 plants for a 3 male fertile:1 male sterile ratio (χ^2 = 1.200, P = 0.250–0.500). This indicated that in this cross also the segregation is the same as in the other cross. Such discrepancies often result from small populations, which are frequent in plant-to-progeny rows in groundnut. In such situations, evaluation of progenies in the succeeding generations helps in arriving at the most probable conclusion, as in this case.

Two male sterile plants, one each from the two F_5 progenies of the cross ICG 10889 × ICGV 86125 were designated as MS-1 and MS-2, and a male sterile plant from F_5 progeny of the cross 'B 4-P4' × ICGS (E) 36 was designated as MS-3. These three male sterile plants (MS-1, -2, and -3), when crossed with 'JL 24' produced completely fertile F_1 hybrids, indicating the recessive nature of male sterility in these crosses. In all the three F_2 populations of these crosses (male sterile × 'JL 24'), there was an excellent fit to a 15 male fertile:1 male sterile ratio (Table 1). This indicated that the male sterility in these crosses is governed by two independent recessive genes with double recessive being male sterile.

Of the 265 normal F_2 plants in three crosses (MS-1, MS–2, and MS-3 crossed with 'JL 24'), 205 F_3 progenies were grown. In the MS-1 × 'JL 24' cross, 39 out of the 74 progenies segregated for male sterility (Table 2). This fitted extremely well to a ratio of 8 segregating:7 non-segregating progenies ($\chi^2 = 0.012$, P = 0.900–0.950). Among the 39 segregating progenies, 18 progenies with plant number ranging from 16 to 38, showed an excellent fit to a 15 male fertile:1 male sterile ratio individually and also on pooled basis ($\chi^2 = 0.800$, P = 0.250–0.500, Table 1) and 21 progenies with plant number ranging from 15 to 45, fitted well to a 3 male fertile:1 male sterile ratio, individually as well as on pooled basis ($\chi^2 = 1.299$, P = 0.250–0.500). This agreed with an expected 1:1 ratio between progenies segregating for 15 male fertile:1 male sterile and 3 male fertile:1 male sterile ratios ($\chi^2 = 0.231$, P = 0.500–0.750, Table 3). In the crosses MS-2 × 'JL 24' and MS-3 × 'JL 24' the segregation pattern was similar to that of MS-1 × 'JL 24' (Tables 2 and 3). This supported the preliminary results obtained in earlier in F_4 and F_5 generations suggesting that the male sterility in these crosses of groundnut is governed by two recessive genes. We designate the genes as ms_1 and ms_2. These results need further confirmation with progenies having greater number of plants.

Before this genetic male sterility could be considered for use in a crop improvement program we need to study its stability in diverse environments at different locations, and the extent of seed set through artificial pollination, and through natural out-crossing. Though the maximum natural out-crossing has been reported in groundnut to be 10.0% (USDA, 1963), there are reports which indicate that the irradiation with very low doses (5 kR) of gamma rays can enhance the out-crossing up to 33% in a virginia runner 'M 13' (Dutta et al., 1987). Aspects like the cytological basis of the male sterility need to be studied for better understanding of this phenomenon. Further, the genetic male sterility in groundnut, in general, appears to be associated with abnormal plant phenotype. This could be due either to pleiotropic effect of male sterility genes or tight linkage between genes for male sterility and phenotypic abnormality. Since there is variation in the plant morphological characters such as plant height and breadth in genetic male steriles, the latter appears to be a more plausible reason for this association. If the male sterility genes can be transferred into a normal phenotypic background, an effective population improvement program in groundnut comparable to the program in soybean (Brim & Burton, 1979; Brim & Stuber, 1973; Burton et al., 1983) can be initiated due to reduced labour requirement in hybrid seed production.

References

Ashri, A., 1968. Morphology and inheritance of sterile brachytic dwarfs in peanuts, *Arachis hypogaea*. Crop Sci. 8: 413–415.

Brim, C.A. & J.W. Burton, 1979. Recurrent selection in soybeans. II. Selection for increased per cent protein in seeds. Crop Sci. 19: 494–498.

Brim, C.A. & C.W. Stuber, 1973. Application of genetic male sterility to recurrent selection schemes in soybeans. Crop Sci. 13: 528–530.

Burton, J.W., R.F. Wilson & C.A. Brim, 1983. Recurrent selection in soybeans. IV. Selection for increased oleic acid percentage in seed oil. Crop Sci. 23: 744–747.

Coffelt, T.A. & R.O. Hammons, 1972. Inheritance of sterile brachytic in an intraspecific cross of *Arachis hypogaea* L. Crop Sci. 12: 82–84.

Dutta, M., V. Arunachalam & A. Bandyopadhyay, 1987. Enhanced cross pollination to widen the scope of breeding in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 74: 466–470.

Gupta, R.B., 1988. Genetics of brachytic sterility in groundnut (*Arachis hypogaea* L.). Ind. J. Agric. Res. 22: 41–50.

Hayes, T.R., 1933. The classification of groundnut varieties with a preliminary note on the inheritance of some characters. Trop. Agr. West Indies 10: 318–327.

Hull, F.H., 1937. Inheritance of rest period of seeds and certain other characters in the peanut. Univ. Florida Agr. Exp. Sta. Tech. Bull. 314, 46 pp.

Husted, L., 1934. Genetic and cytological studies on the peanut, *Arachis*. Ph.D. Thesis Univ. of Virginia, Charlottesville, USA, 96 pp.

Katayama, Y. & T. Nagatomo, 1963. Crossing experiments in various strains of peanut. Bull. Fac. Agric. Miyazaki Univ. 9 (1): 99–126 (in Japanese with English summary).

Patel, J.S., C.M. John & C.R. Seshadri, 1936. The inheritance of characters in the groundnut, *Arachis hypogaea*. Proc. Indian Acad. Sci. 3 (B): 214–233.

U.S. Department of Agriculture, 1963. A peanut marker, Agr. Res. 12: 11.