

Utilization of Wild Relatives in Genetic Improvement of *Arachis hypogaea* L. VI. Fertility in Triploids: Cytological Basis and Breeding Implications¹

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ABSTRACT

The triploid hybrids between *Arachis hypogaea* L. and diploid species of section *Arachis nom. nud.* which had been observed to be sterile at Reading, England, produced pegs and pods at the ICRISAT Center. Cytological investigations of these and other triploid hybrids revealed: (1) spindle abnormalities resulting in restitution nuclei and unreduced gametes, and (2) unequal segregation of chromosomes resulting in haploid to hyperdiploid gametes. Of the 225 F₂ plants derived from triploids, 82% were hexaploid, 8% were tetraploid, and 10% had chromosome numbers from 20-59. This indicates that the above processes occurred both at micro- and mega-sporogenesis and that unreduced gametes were more effective in fertilization resulting in a higher percentage of hexaploid progenies. The implications of these results are that triploids can be used in *Arachis* interspecific breeding for increased recombination between chromosomes and quick recovery of *Arachis hypogaea* like tetraploid lines.

Key Words: Groundnut, Peanut, Triploid, Fertility, Unreduced gametes, Chromosome pairing, Disjunction, Introgression.

The diploid wild species of section *Arachis nom. nud.* have a number of desirable characters such as resistance to important pathogens and pests of *Arachis hypogaea* L. (1,2,14,15). By crossing the *Arachis* section diploid wild species with the tetraploid cultivated *A. hypogaea*, several workers have produced triploid hybrids which were sterile (5,6,7,12). However, all the triploid combinations have produced seeds at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru, India. The production of progenies by three out of seven combinations of triploid hybrids raised by Smartt and Gregory has been reported earlier (12). Meiotic behavior of a male fertile complex triploid *Arachis* hybrid has been reported by Simpson and Davis (9). This paper reports the cytological basis of the seed fertility of the triploids at ICRISAT.

Materials and Methods

Eight diploid wild species ($2n = 20$) all in the section *Arachis* of the genus, and the tetraploid cultivated species *A. hypogaea* ($2n = 40$) were used. The source and identity of these species have been given earlier (10).

Sterile triploid F₁ hybrids ($2n = 30$) of *A. chacoense* Krap. et Greg. *nom. nud.*, *A. cardenasii* Krap. et Greg. *nom. nud.* and *A. species* HELK 410 (PI 338250) crossed with *A. hypogaea* were obtained as cuttings from Reading University, U.K., and planted at ICRISAT. Triploid F₁ hybrids were also produced at ICRISAT by crossing *A. hypogaea* with eight species of section *Arachis*, *A. duranensis* Krap. et Greg. *nom. nud.*, *A. species* GKP-10038 (PI 263133), *A. villosa* Benth., *A. correntina* Krap. et Greg. *nom. nud.* and *A. batizocoi* Krap. et Greg. *nom. nud.* and the three listed above. In each case, a mini-

mum of one fertile triploid hybrid plant derived from *A. hypogaea* subsp. *hypogaea* Krap. et Greg. cv. Robut 331³ as female parent, was cytologically analysed. The seeds obtained from triploid F₁ hybrids were sown in the rainy season of 1980 in the Botanical Garden at ICRISAT Center. The chromosome numbers of two hundred and twenty-five F₂ plants were counted. The cytological techniques used have been described by Singh and Moss (10). Pollen grains were stained in 1% acetocarmine and stainable pollen grains were counted as fertile. Pollen germination on selfed stigma was observed under the microscope in dissected pistils, stained and mounted in 1% acetocarmine. Pollen tube growth through style was studied by fluorescent microscopic technique as described earlier (11). Chromosome associations in triploid hybrids were statistically analysed using one-way analysis of variance.

Results and Discussion

The triploid F₁ hybrids involving three diploid species which were produced and maintained at Reading University, U.K. from 1973 to 1978 were sterile. However, plants derived from cuttings of these sterile hybrids and planted in the field at the ICRISAT Center in June, 1979, produced pegs and pods in 1980. Other triploid hybrids grown at ICRISAT, involving 8 diploid species of section *Arachis*, also produced pegs and pods (Fig. 1).



Fig. 1. a) Triploid *A. hypogaea* x *A. batizocoi* with pegs (arrow); b) Triploid selfed pods.

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Chromosome pairing at metaphase I was nearly identical in all these triploid hybrids (Table 1). The number of bivalents and multivalents indicated that the set of chromosomes contributed by the diploid species usually paired with one and occasionally with both sets of chromosomes of *A. hypogaea* to form more than 10 bivalents and some multivalents in a few pollen mother cells (PMCs) (Table 1). The triploids involving *A. batizocoi* had higher trivalent and lower univalent frequencies at the 5% level of probability than others (Table 1).

result of nonpolarisation of chromosomes and chromatids at AI and AII, producing unreduced gametes, while the latter were probably the products of unequal distribution giving rise to stainable haploid, diploid, and hyperdiploid pollen grains.

The chromosome numbers in 225 F₁ plants from triploid hybrids ranged from $2n = 20$ to $2n = 60$ (Fig. 3), of which 82% were hexaploids ($2n = 60$), a result of fertilization between unreduced gametes, 8% were tetraploid ($2n = 40$), and the remaining 10% had chromosome

Table 1. Chromosome associations, and pollen and pod fertility in F₁ triploids of *A. hypogaea* (4x) x *Arachis* species (2x).

<u><i>A. hypogaea</i> x <i>hypogaea</i></u>	No. of Cells	Mean Chromosome Association				% Pollen	Range of Pods
Cross	analysed	I	II	III	IV	Stainability	Obtained per Plant
<i>A. villosa</i>	25	9.1 ±0.52	8.6 ±0.37	1.0 ±0.22	0.2 ±0.1	19	0-7
<i>A. correntina</i>	21	8.3 ±0.43	9.9 ±0.31	0.6 ±0.16	0.1 ±0.05	20	0-25
<i>A. chacoense</i>	40	9.7 ±0.4	8.7 ±0.25	0.80 ±0.16	0.1 ±0.49	17	0-19
<i>A. species. HLK 410</i>	30	9.2 ±0.43	9.6 ±0.28	0.5 ±0.13	0.1 ±0.03	13	0-16
<i>A. cardenasii</i>	25	8.3 ±0.52	9.7 ±0.27	0.5 ±0.17	0.2 ±0.08	9	0-10
<i>A. species. GKP 10038</i>	25	10.0 ±0.44	8.0 ±0.26	1.2 ±0.18	0.1 ±0.06	11	2*
<i>A. duranensis</i>	20	8.3 ±0.45	9.4 ±0.24	1.0 ±0.17	0.1 ±0.05	18	4*
<i>A. batizocoi</i>	21	6.2 ±0.42	8.7 ±0.49	2.0 ±0.29	0.1 ±0.07	7	3-18
Mean	-	8.8 ±1.31	9.1 ±0.9	1.0 ±0.53	0.1 ±0.18	14	

* Only one plant

At Anaphase I (AI), chromosomal irregularities observed were laggards, bridges, spindle abnormalities and unequal distribution (Fig. 2a,b; Table 2). However, at Anaphase II (AII) there were very few laggards and no bridges. This suggests that the observed frequency of different irregularities at AI was mostly due to the presence of univalents or the nonsynchronous disjunction of bivalents and subsequent lagging of these chromosomes. A majority of these are regularly distributed at AI followed by regular distribution at AII.

The mean pollen stainability in the triploid hybrids was 14% with a minimum of 7% in *A. hypogaea* x *A. batizocoi* and a maximum of 20% in *A. hypogaea* x *A. correntina*. Two classes of stainable pollen grains were observed (Fig. 2c): (1) uniform large pollen grains and (2) small pollen grains of different sizes. The former were probably the products of restitution nuclei formed as a

numbers ranging from 20 to 30 and 41 to 59, probably a result of fertilizations involving haploid, diploid, hyperdiploid, or unreduced gametes. However, the probability of a functional microspore and megaspore effecting fertilization was very low, as was evident from the low pod production and in some plants no pod production by these triploids (Table 1). The production of selfed pods by triploid hybrids and the frequency of hexaploids suggests that the abnormal meiotic division producing unreduced and hyperdiploid, as well as haploid and diploid gametes, occurs at both microsporogenesis and megasporogenesis. The higher percentage of hexaploids in this population indicated that unreduced gametes had competitive advantage over other gametes. This was also confirmed by an observation on pollen germination in 63 self-pollinated flowers of these triploid hybrids. Of the 60% stigma with stainable pollen grains, germinating

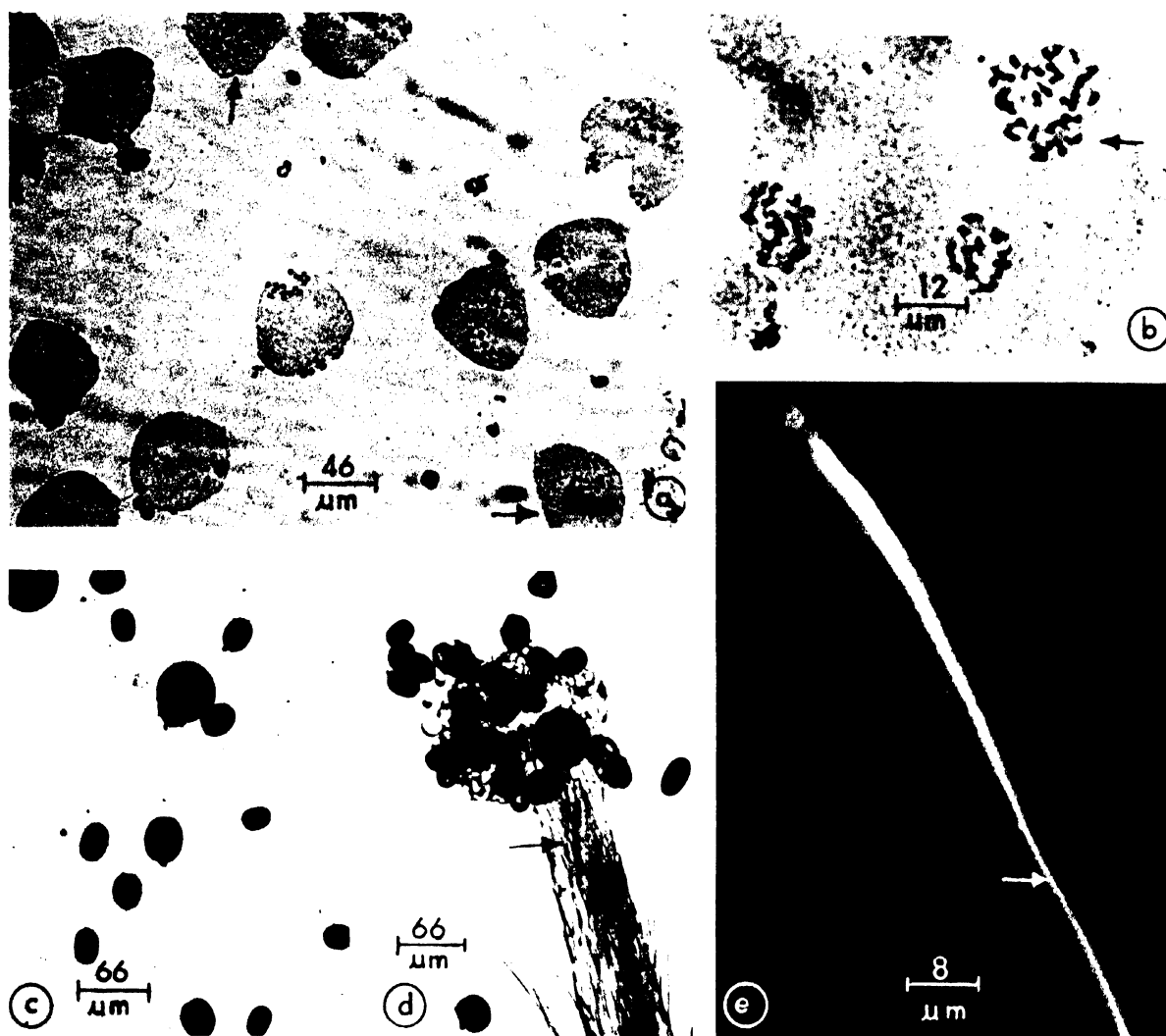


Fig. 2. a) PMCs at AI with fused spindle (arrow); b) A PMC at AI with fused spindle (arrow); c) Unstained pollen and different sizes of stained pollen; d) A stigma with many sterile unstained fertile small and large pollen, and a germinating large pollen grain (arrow); e) A large pollen grain with pollen tube germinating through triploid stigma (arrow).

pollen grains were observed on only 41% of the stigmas. Of these, 33% had large germinating pollen grains and only 8% had small germinating pollen grains (Fig. 2d,e).

In abnormal meiotic division in plants of interspecific origin either the nucleus does not undergo normal disjunction of homologous chromosome (Fig. 2a) at AI and divide mitotically to produce two unreduced gametes, or the product of disjunctional separation (haploid set) divides mitotically but the sister chromatids do not separate to two poles (Fig. 2b). Both lead to the formation of restitution nuclei, unreduced gametes. Whereas unequal segregation of chromosomes during meiosis (Fig. 2a) may lead to the formation of haploid, diploid, and hyperdiploid gametes. In interspecific hybrids such a phenomenon was observed as early as 1927 (3). The meiotic studies in one male fertile and one non-fertile plant of a complex triploid hybrid by Simpson and Davis (9) recorded AI irregularities in a male fertile plant resulting in restitution nuclei. The authors also speculated

the formation of such restitution nuclei in a non-fertile triploid, as they formed stainable pollen grains although to a lesser degree. In plants, changes in the meiotic cycle leading to unreduced gametes have either been attributed to genetical control or to environmental factors (8). The fact that in the present study some of the triploids maintained from 1973 to 1978 at Reading University, U.K. did not produce any pegs or pods, but plants derived from cuttings of these did produce pods at ICRI SAT Center, Patancheru suggests that environmental factors may be affecting the meiotic cycle to produce viable gametes.

Fertility in triploids in interspecific *Arachis* breeding as it eliminates all the steps required to induce fertility by colchicine treatment, and in subsequent backcrosses the need to reduce the chromosome number from hexaploid to tetraploid. Further, the hexaploids produced by colchicine doubling of parental chromosomes have a pre-

Table 2. Chromosome distribution at AI and AII, and pollen fertility in triploids of *A. hypogaea* (4x) x *Arachis* species (2x).

Stage						
% Chromosome Distribution & Stained Pollen						
	No. of Cells analysed	Equal Distribution	Unequal Distribution	Laggards	Bridges	Spindle Breakdown
Stage:						
AI	159	34	19	38	7	2
AII	79	70	20	2	0	8
Products:						
Mean % stainabile pollen (13.75)		Unstainable pollen	4* Stainable variable size pollen (x=2x+)	Unstainable pollen		10** Large stainable pollen (3x)
% chromosome number in F2 plant from triploids			18 (2n=20-59)			82 (2n=60)

* Viable product of unequal distribution
** Viable product of spindle breakdown

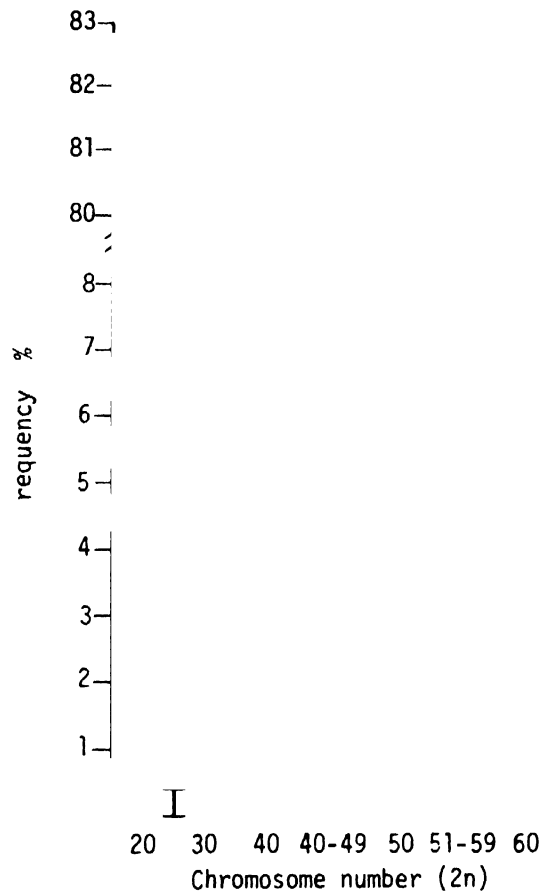


Fig. 3. Frequency of plants with different ploidy levels among 225* plants derived from selfed triploids.
*Three plants showed variation among cells in chromosome numbers (30-60).

dominance of bivalent associations (13) as a result of preferential autosyndetic pairing. Hence, populations produced by this method have lesser genetic exchange between chromosomes from different species. On the other hand, spontaneous hexaploid progenies from triploid hybrids are the products of unreduced gametes, which have resulted from a restitution nuclei (Table 2) formed after first division pairing and genetic exchange at metaphase I, and hence should produce populations with greater degrees of recombination than the artificially induced hexaploids. This advantage has also been realized earlier for a male fertile triploid *Arachis* hybrid (9). Intergenomic recombination also occurs in the formation of tetraploid and hypertetraploid progenies of triploid plants (Fig. 3). These are of great significance in *Arachis* interspecific breeding for quick recovery of stable tetraploid derivatives after combining characters from diploid wild species. Further, the male fertility of the triploid can be exploited in backcrossing to *A. hypogaea* for production of pentaploids, as realised by Simpson and Davis (9), and also for production of tetraploids as in wheat (4). It would be desirable to conduct studies to determine specific conditions which induce fertility in these triploids so that these can be exploited fully in peanut breeding.

Literature Cited

1. Abdou, Y., A. M. W. C. Gregory and W. E. Cooper. 1974. Sources and nature of resistance to *Cercospora atachidicola* Hort. and *Cercosporidium personatum* (Beck. and Cunt.) Dighton in *Arachis* species. Peanut Sci. 1:6-11.
2. Hebert, T. T., and H. T. Stalker. 1981. Resistance to peanut stunt virus in cultivated and wild *Arachis* species. Peanut Sci. 8:45-47.
3. Karpechenko, G. D. 1927. The production of polyploid gametes in hybrids. Hereditas 9:349-368.
4. Kerber, E. R., and P. L. Dyck. 1973. Inheritance of stem rust resistance transferred from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location of the gene involved. Can. J. Genet. Cytol. 15:397-409.

5. Krapovickas, A. and V. A. Rigoni. 1951. Estudios citologicos en el genero *Arachis*. Rev. Investigaciones Agric. 5:289-294.
6. Moss, J. P. 1980. Wild species in the improvement of Groundnuts, pp. 525-535. In: Advances in Legume Science. R. J. Summerfield and A. H. Bunting (eds). Kew (England): Royal Botanic Garden.
7. Raman, V. S. 1976. Cytogenetics and Breeding in *Arachis*. New Delhi (India): Today and Tomorrow Printers and Publisher.
8. Ramanna, M. S. 1979. A re-examination of the mechanisms of 2n gamete formation in potato and its implication for breeding. Euphytica 28:537-561.
9. Simpson, C. E. and K. S. Davis. 1983. Meiotic behavior of a male fertile triploid *Arachis* L. hybrid. Crop Sci. 23:581-584.
10. Singh, A. K. and J. P. Moss. 1982. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. II. Chromosome complements of species in section *Arachis*. Theor. Appl. Genet. 61:305-314.
11. Singh, A. K., J. P. Moss, and D. C. Shastri. 1983. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. I. Technique, pp. 151-157. In: Current Approaches in Cytogenetics. R. P. Sinha and U. Sinha (eds). Patna (India): Spectrum Publ. House.
12. Smartt, J. and W. C. Gregory. 1967. Interspecific cross compatibility between the cultivated peanut *Arachis hypogaea* L. and other members of genus *Arachis*. Oleagineux 22:455-459.
13. Spielman, F. A., A. P. Burge, and J. P. Moss. 1979. Chromosome loss and meiotic behavior in interspecific hybrids in the genus *Arachis* and their implications in breeding for disease resistance. Z. Pflanzenzuchtg. 83:236-250.
14. Subrahmanyam, P., R. W. Gabbous, S. N. Nigam and V. B. Rao. 1980. Screening methods and further sources of resistance to peanut rust. Peanut Sci. 7:10-12.
15. Subrahmanyam, P., J. P. Moss and V. B. Rao. 1983. Resistance to peanut rust in wild *Arachis* species. Plant Dis. 67:209-212.

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These five lines were identified as resistant to ascochyta blight by screening 6594 kabuli germplasm accessions available at ICARDA and 12 749 desi germplasm accessions available at ICRISAT (3,4,6). Screening for ascochyta blight resistance was carried out between 1979 and 1991 in the field and greenhouse at Tel Hadya, the principal ICARDA station in Syria, by inoculating with blight-affected chickpea diseased-debris and spraying a spore suspension of the mixture of six races of *A. rabiei* from Syria (2). In 10 of the 13 seasons in field and in greenhouse evaluation in 1990, the known blight-susceptible cultivar (ILC 263 or ILC 1929) was killed, indicating high disease development. A line was considered resistant when it showed resistance in all the years of testing. The observations of agronomic characters on kabuli lines were recorded at Tel Hadya (36° N, 36° E) in Syria (5) and on desi lines at Patancheru (18° N, 78° E) in India (1). Tel Hadya has a long growing season for chickpea, whereas Patancheru has a short one.

ILC 200 is a kabuli type introduced from the USSR, with pea-shaped and light orange-colored seed. It is a line with late maturity (142 d to flower), medium plant height (60 cm), small seed size (21 g 100 seed⁻¹), and low yield. ILC 6482 is a kabuli type of unknown origin, with ramhead-shaped and beige-colored seeds. It is a late-maturing line (145 d to flower), with medium plant height (50 cm), medium seed size (35 g 100 seed⁻¹), and low yield. ICC 4475 (P 5496) was introduced from Iran and is a black-seeded desi type. It is a line with late maturity (80 d to flower), medium plant height (47 cm), very small seed size (9 g 100 seed⁻¹), and low yield. ICC 6328 (NEC 241) is a desi type introduced from India, with angular shape and black-colored seeds. It is a line with late maturity (73 d to flower), medium plant height (44 cm), small seed size (17 g 100 seed⁻¹), and low yield. ICC 12004 (NEC 2861) is a brown-seeded desi type of unknown origin. It is a line with late maturity (67 d to flower), medium plant height (43 cm), very small seed size (10 g 100 seed⁻¹), and low yield.

None of these five lines have desirable attributes for direct commercial exploitation, but all are valuable as sources of resistance to ascochyta blight in hybridization programs.

Small quantities of seeds of these germplasm lines can be obtained from the Legume Program, ICARDA, P.O. Box 5466, Aleppo, Syria.

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References and Notes

1. Pundir, R.P.S., K.N. Reddy, and M.H. Mengesha. 1988. ICARDA chickpea germplasm catalog: Evaluation and analysis. Patancheru, A.P. 502 324, India.
2. Reddy, M.V., and S. Kabbabeh. 1985. Pathogen variability in *Ascochyta rabiei* in Syria and Lebanon. *Phytopathol. Medit.* 24: 265-266.
3. Reddy, M.V., and K.B. Singh. 1984. Evaluation of world collection of chickpea germplasm accessions for resistance to ascochyta blight. *Plant Dis.* 68:900-901.
4. Reddy, M.V., and K.B. Singh. 1990. Relationship between ascochyta blight severity and yield loss in chickpea and identification of resistant lines. *Phytopathol. Medit.* 29:32-38.
5. Singh, K.B., L. Holly, and G. Bejiga. 1991. A catalog of kabuli chickpea germplasm. ICARDA, Aleppo, Syria.
6. Singh, K.B., M.V. Reddy, and Y.L. Nene. 1984. International testing of chickpeas for resistance to *Ascochyta* blight. *Plant Dis.* 68:782-784.
7. Reddy, M.V., ICARISAT, Patancheru P.O., A.P. 502 324, India; and K.B. Singh, ICARDA, P.O. Box 5466, Aleppo, Syria. Joint contribution from ICARDA and ICARISAT. Registration by CSSA. Accepted 31 Dec. 1991. *Corresponding author.

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REGISTRATION OF SOYBEAN GERmplasm SG1E6

THE SOYBEAN [*Glycine max* (L.) Merr.] population SG1E6 (Reg. no. GP-137, PI 558508) was released on 1 Nov. 1991 by the Agricultural Research Division, University of Nebraska-Lincoln. SG1E6 was derived by repetitively mating elite germplasm to an existing population known as SG1. SG1 was a population random-mated three times after its creation from the matings of 39 female ancestral germplasm strains to four male adapted strains heterozygous for the *ms2* form of genetic male-sterility (3).

The synthesis of SG1E6 commenced in 1985. Seed harvested from male-sterile plants of the 1984 SG1 population were planted in a 1985 intermating block in Lincoln, NE. The SG1 rows were alternated, checkerboard-square fashion, with pure rows of 32 elite strains (Table 1). The SG1 rows segregated for male-fertile (MF) and male-sterile (MS) plants in a 1:1 ratio. The MF plants were rogued as soon as they flowered, when they were distinguishable from MS plants, which have reduced anthers that bear no pollen. Pollen transfer from the MF plants in the elite strain rows to the MS plants in the SG1 rows was mediated by honey bees (a hive was placed near the nursery) and other insects. The nursery was surrounded by a 20-m plant-free border, to minimize insect movement from other soybean fields.

About 400 MS plants bearing seed were gathered from the 1985 intermating nursery. An F₁ seed was collected from the top, middle, and bottom nodes of each MS plant and placed at random into one of three bags. One F₁ seed lot was placed on reserve in a cold room. The other two were planted in a winter nursery in Puerto Rico, where the selfed F₁ plants in each lot were bulk-harvested. One F₂ seed bulk was used to plant the 3MF:1MS rows of the next (1986) intermating nursery. The other F₂ seed bulk was transferred to the breeding program for use in soybean cultivar development.

Repetition of the process facilitated the mating of six sets of elite parents (Table 1) to MS plants in the 1985 (SG1E1), 1986 (SG1E2), 1987 (SG1E3), 1988 (SG1E4), 1989 (SG1E5), and 1990 (SG1E6) mating nurseries. Three procedural modifications were adopted after 1985. First, the total number of rows in the mating nursery was increased, to adjust for the change to a 3MF:1MS segregation ratio, and to ensure the availability of 500 to 1000 MS plants each year. Second, a balanced seed composite of the male parents was planted in each elite row, instead of using replicated pure rows of the elite parents (as was done in 1985). Third, the choice of each year's elite entries was made more objective by selecting: (i) the two highest-yielding public entries in each of six maturity group tests (00, 0, I, II, III, IV) of the Uniform Soybean Tests-Northern States, using multiple-location 2-yr means; (ii) the single highest-yielding public entry in each of six Preliminary Tests (I, IIa, IIb, IIIa, IIIb, IV) of the Uniform Soybean Tests-Northern states, using multiple-location 1-yr means; and (iii) the two highest-yielding proprietary entries in each of the eight and six location-maturity zones of the respective Nebraska and Iowa variety performance trials, using 3-yr zone means. A few entries not meeting these criteria were added to the parental list, to maintain a minimal frequency of genes for disease resistance or morphological variation [e.g., *d1l* for determinant stem growth; *Rps1-k* for phytophthora root rot resistance (*Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin)]. The use of proprietary strains for intermating was discontinued after 1988, primarily because of increasing restrictions in the seed trade on the use of such strains as parental material.