

# Utilization of Wild Relatives in Genetic Improvement of *Arachis hypogaea* L. VI. Fertility in Triploids: Cytological Basis and Breeding Implications<sup>1</sup>

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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 F<sub>2</sub> 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 F<sub>1</sub> hybrids ( $2n = 30$ ) of *A. chacoense* Krap. et Greg., *nom. nud.*, *A. cardenasii* Krap. et Greg. *nom. nud.* and *A. species HLK-410* (PI 338280) crossed with *A. hypogaea* were obtained as cuttings from Reading University, U.K., and planted at ICRISAT. Triploid F<sub>1</sub> 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* sub. sp. *hypogaea* Krap. et Greg. (cv. Robut 33-1), as female parent, was cytologically analysed. The seeds obtained from triploid F<sub>1</sub> 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 F<sub>2</sub> 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 F<sub>1</sub> 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).

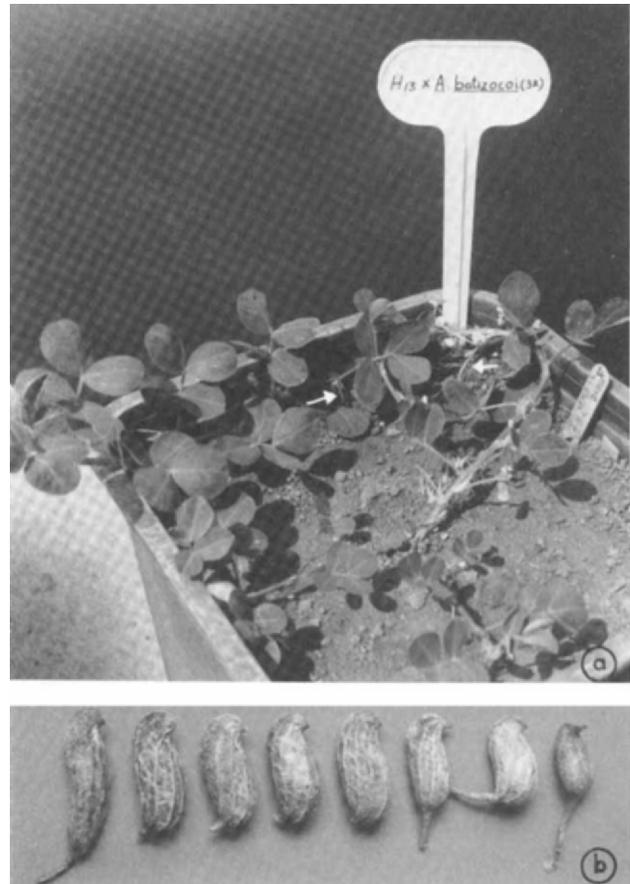


Fig. 1. a) Triploid *A. hypogaea* x *A. batizocoi* with pegs (arrow); b) Triploid selfed pods.

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

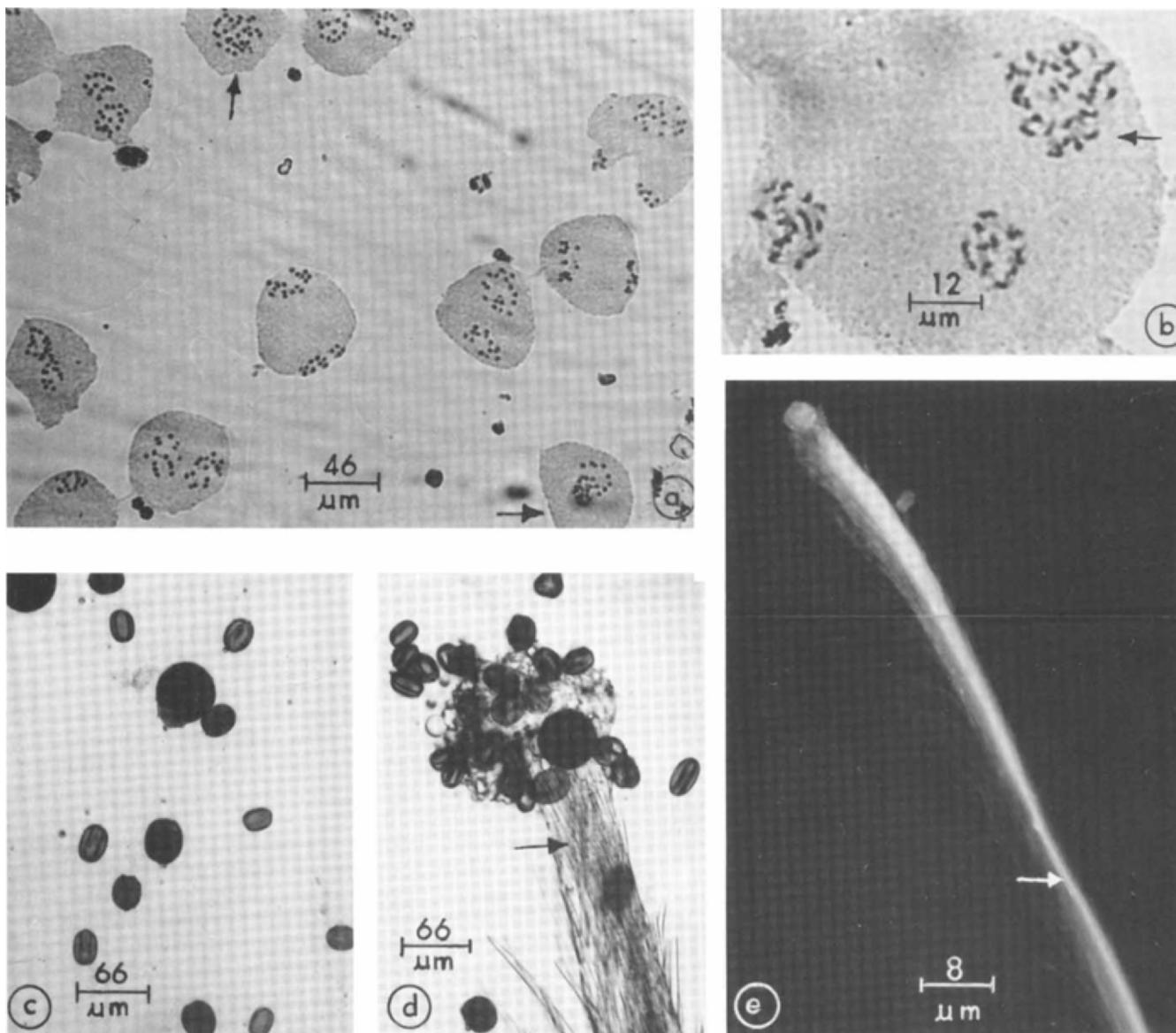


Fig. 2. a) PMCs at AI with fused spindle (arrow); b) A PMC at AII with fused spindle (arrow); c) Unstained pollen and different sizes of stained pollen; d) A stigma with many sterile unstained pollen stained fertile small and large pollen, and a germinating large pollen grain (arrow); e) A large pollen 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 ICRISAT Center, Patancheru suggests that environmental factors may be affecting the meiotic cycle to produce viable gametes.

Fertility in triploid  $F_1$  hybrids is of great importance in the use of 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
<b>Stage:</b>						
AI	159	34	19	38	7	2
AII	79	70	20	2	0	8
<b>Products:</b>						
Mean % stainable 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

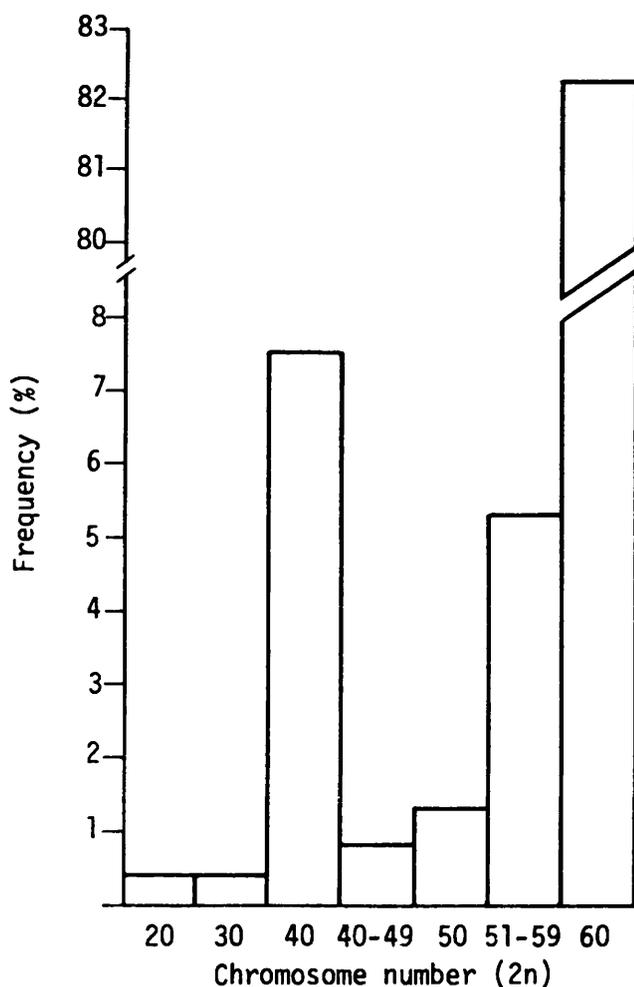


Fig. 3. Frequency of plants with different ploidy levels among 225\* plants derived from selfed triploids.

\*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 realized 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.

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