Utilization of Wild *Arachis* Species at ICRISAT

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One of the possibilities for increasing the yield of groundnut, particularly in the Semi-Arid Tropics, is breeding varieties with resistance to pests and diseases. Some progress has been made in this field, but the improvements that can be made by breeders are limited by the availability of genes within *A. hypogaea.* Collections of wild species from South America have made available a wider range of genes, especially genes for disease resistance. The richness of *Arachis* germplasm collection offers a great opportunity for anyone interested in the improvement of this crop (Bunting et al. 1974, Simpson 1976; Smartt et al. 1978a, b; Gregory and Gregory 1979).

However the cytotaxonomy of the genus *Arachis* is such that it is difficult for a breeder to use wild species in groundnut improvement (Gregory and Gregory 1979; Moss 1980; Stalker 1980). The two major constraints to utilization of wild species are differences in ploidy level and incompatibility between some wild species and *A. hypogaea.* The small size of chromosomes *of Arachis* and the difficulty experienced by some workers in making good cytological preparations has deterred many people from attempting cytogenetic techniques in the improvement of *Arachis,* despite their successful application to many other crop plants, especially wheat and tobacco. The groundnut cytogenetics program at ICRISAT has attempted to overcome these difficulties, and to produce interspecific hybrids and to manipulate the ploidy level to produce tetraploid lines incorporating desirable characters which can then be utilized by breeders in the improvement of groundnut.

The program on utilization of wild species in *Arachis* was initiated at Reading University (U.K.) with three species which were known to cross with *A. hypogaea* and were resistant to leaf spots. These were *A. cardenasii* Krap. and Greg., nomen nudum, *A. chacoense* Krap. and Greg., nomen nudum, and *Arachis* species Coll. HLK 410 which were reported as immune to *Cercosporidium personatum,* highly resistant to *Cercospora arachidicola,* and resistant to both (Abdou 1966; Sharief 1972; Abdou et al. 1974; Hammons, personal communication).

The groundnut cytogenetics program at ICRISAT was initiated in April 1978 with the object of making the fullest possible use of the genus *Arachis.* Cooperation with the Genetic Resources Unit, pathologists, entomologists, and microbiologists has increased the number of wild species at ICRISAT and our knowledge of the desirable genes which they contain. Cytogenetic analysis provides information to improve the efficiency of incorporation of wild species genes into *A. hypogaea.* Thetechniques have been described by Singh et al. (1980).

In addition to *A. hypogaea,* section *Arachis* contains one other tetraploid. *A. monticola,* and several diploid species. All these species are cross compatible with *A. hypogaea* (Smartt and Gregory 1967; Stalker 1980). Nine diploid species and the two tetraploids have been studied in detail and a chromosome with a secondary constriction and a small satellite seen as chromosome 3 in *A. villosa, A. correntina, A. chacoense,* and *Arachis* species Coll. No. 10038, and a chromosome with a secondary constriction and a large satellite seen in *A. batizocoi* and in *A. duranensis* as chromosome 2, in *Arachis* species 338280 as chromosome 6 and in A *cardenasii* as chromosome 9. Chromosomes with secondary constrictions had only been reported previously in *A. batizocoi.* The small pair of chromosomes in *A. cardenasii* are larger than in the remaining species but still smaller than the smallest chromosomes of *A. batizocoi. A. monticola* and *A. hypogaea* are close karyomorphologically, though *A. monticola* has two pairs of chromosomes with secondary constrictions whereas *A. hypogaea* has only one, and the chromosome with a secondary

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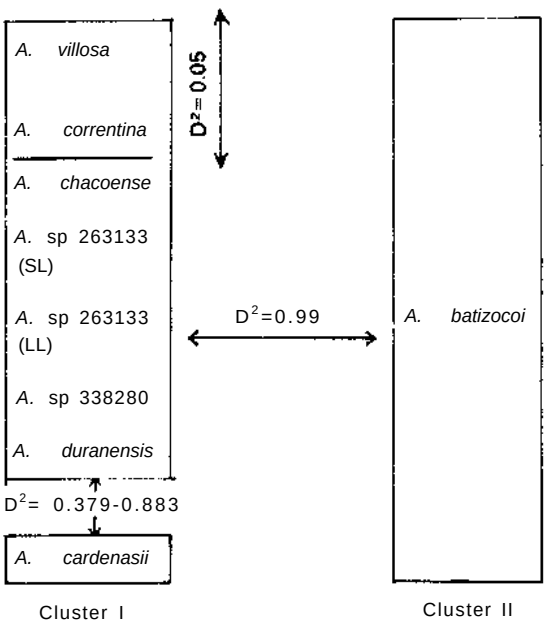
constriction in *A. cardenasii* is comparable to one of those of *A. monticola* and that of *A. hypogaea.*

Mahalanobis D^2 analysis and canonical analysis, using the arm ratio of each of the ten chromosomes of the diploid taxa investigated, resulted in two clusters within these. *A. batizocoi* is the only species in one of these clusters; there are eight taxa in the other cluster, which can be further subdivided (Fig. 1).

All the species of the section *Arachis* are cross-compatible, and differences in crossability are too small to be satistically significant. All the available nine diploid taxa have been crossed in all possible combinations and a large number of F_1 hybrids have been analysed cytologically. Results from these studies substantiate our grouping of the diploid species. The F_1 hybrids resulting from the cross of two species belonging to the two different clusters show a high frequency of univalents and a high pollen sterility, while F_1 hybrids between two species of the same cluster show a low frequency of univalents and a low pollen sterility.

D^2 distances among species of section *Arachis*



Average intracluster D^2= 0.335

Figure 1. Cluster diagram.

These basic studies are designed to assist us in the main objective of the cytogenetics unit, i.e., the utilization of the wild species for the improvement of groundnuts. The two go hand in hand because many of the plants produced in the course of utilization of wild species are analyzed in detail, and plants which are analyzed are then used in crossing programs. The two main thrusts of the subprogram are the use of compatible species to transfer currently available genes, and the study of the barriers to hybridization and the means of breaking them to make the whole gene pool within *Arachis* available to breeders in the future.

Breeding in Compatible Species

The incorporation of genes from wild species involves the transfer of one or more wild species genomes to a hybrid where they can undergo recombination with *A. hypogaea* genomes, and subsequent transfer of the desired gene or genes into *A. hypogaea* with the elimination of all undesirable characters from the wild species. Five routes have been adopted to achieve these objectives (Fig. 2).

Triploid Route

Smartt and Gregory (1967), Moss and Spielman (1976), Raman (1976), and Moss (1977) have all produced hexaploids by chromosome doubling in a triploid hybrid. As early as 1976, ICRISAT received hexaploids from the program at Reading University. These hexaploids combined the genomes of *A. cardenasii, A. chacoense* and *Arachis* species No. 338280 with *A. hypogaea,* and were screened for leaf spot resistance at ICRISAT, which is mainly infested by late leaf spot (C. *personatum).* Resistant plants were selected from each type of hexaploid, and have been backcrossed to different cultivars of *A. hypogaea* (Moss 1980). These progenies are now in the fourth generation of backcrossing and tetraploid and near tetraploid plants are being screened for disease resistance. Many hexaploids were also resistant to rust. There is no correlation between leaf spot or rust resistance and defoliation in hexaploids derived from resistant wild species, as some hexaploids susceptible to disease do not defoliate. Conversely, some hexaploids with few small lesions suffer severe defoliation (Moss et al.

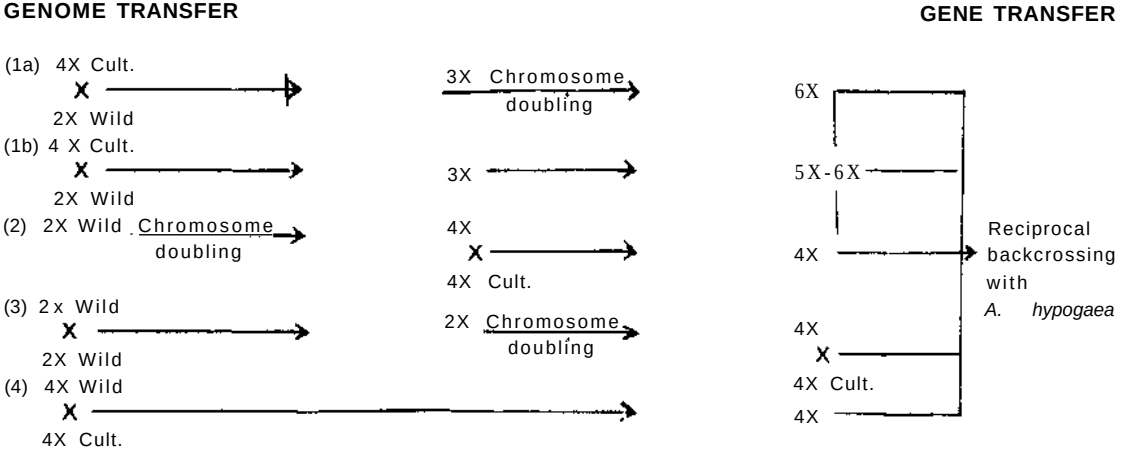


Figure 2. Utilization of diploid and tetraploid wild species.

1979). The fertility of the hexaploids and backcrosses ranges from nil, in some sterile but vegetatively vigorous plants, to highly productive. Some plants produce many pegs per node and pegs per plant, but few pods, whilst others have good reproductive efficiency.

Crosses between *A. hypogaea* and five diploid species have produced 92 pods (Table 1). Five triploids have been established from sprouts and the remaining seed will be used to produce hexaploids.

The artificial induction of polyploidy in a triploid to restore fertility, can be difficult and time consuming, so the development of a technique whereby large numbers of cuttings of the sterile triploid can be treated has increased the production of hexaploids (Spielman and Moss 1976; Nigam et al. 1978). Triploids can produce fertile gametes through the formation of a restitution nucleus and by segregation giving 2n or near 2n gametes. Studies of Anaphase I of meiosis show that 30, 20 and hyper-20 chromosome cells occurred; triploids have produced seed in the field at ICRISAT.

This process may be environmental and/or genotype specific; for instance high temperature in India may be one of the reasons for the formation of unreduced gametes, and the triploids differ in the frequency with which they produce seed. This latter may be due to the different wild species used, or an effect of the different *A. hypogaea* genotypes which are known to affect the amount of pairing in hexaploids (Spielman et al. 1979).

Autotetraploid Route

The production of an autotetraploid from a diploid wild species enables all hybridization and genome transfer to be done at the tetraploid level, and increases the dosage of wild species genes. Autotetraploids have been produced in seven taxa (Table 2). Of these only *A. batizocoi* (4x) has produced seed; many plants of the others remained sterile and eventually died. However, three autotetraploids were successfully used as male parents in crosses with *A. hypogaea* (Table 3). The resultant progenies are morphologically similar to *A. hypogaea,* but cytologically unstable, and sterile. A number of generations of selfing of the autotetraploids will increase the frequency of balanced gametes, and the likelihood of fertile hybrids with *A. hypogaea.* Hybrids will be backcrossed to *A. hypogaea* to restore fertility whilst selecting desirable recombinants.

Amphiploid Route

Chromosomedoubling in a hybrid between two diploid wild species produces an amphidiploid which combines the two wild species, which is thesame ploidy level as tetraploid *A. hypogaea,* and increases the number of genomes in the hybrid and therefore the number of possible recombinants. If the two wild species used are the ancestors of *A. hypogaea,* the amphidiploid will be a synthetic *A. hypogaea,* and this may be the most promising tetraploid derivative of the

Table 1. Crossability between *A. Hypogaea* and diploid species of section *Arachis.*

Cross	No. of Pollinations	No./% of pegs	No./% of pods
A. hypogaea x *A. correntina*	63	24/38	22*/35
A. hypogaea x *A. batizocoi*	64	20/31	16/25
A. hypogaea x *A. villosa*	87	38/44	26/30
A. hypogaea x *A. duranensis*	58	19/33	14*/24
A. hypogaea x *A.* sp 338280	44	15/34	14/32
Total	316	116/37	92/29

* Sprouts produced.

Table 2. Production of autotetraploids from wild diploid *Arachis* species.

Species	Seedlings treated	Plants survived	2x plants	4x plants
A. villosa	17	14	11	4
A. correntina	18	16	10	2
A. chacoense	5	1		
A. sp 338280	19	12	3	3
A. sp 263133	8	5	4	1
A. duranensis	7	6	2	2
A. cardenasii	15	14	1	3
A. batizocoi	26	21	11	10

Table 3. Crossability between *A. hypogaea* and autotetraploids of section *Arachis.*

Cross	No. of pollinations	No./% of pegs	No./% of pods
A. hypogaea x *A.* sp 338280 (4x)	132	16/12	13*/10
A. hypogaea x *A. villosa* (4x)	139	30/22	20*/14
A. hypogaea x *A.* sp 263133 (4x)	113	19/17	17/15
Total	384	65/17	50/13

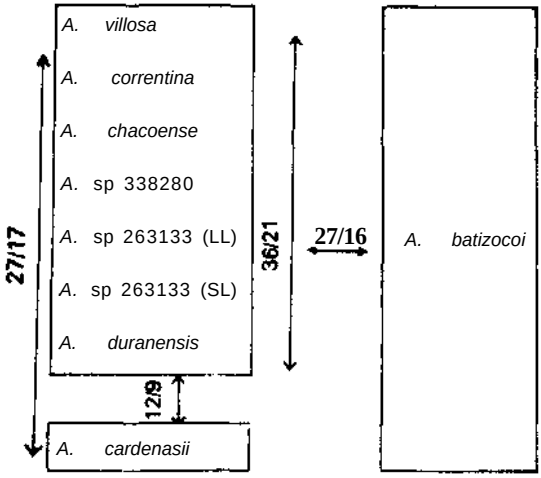
* Sprouts produced.

wild species with regard to the genetic improvement of *A. hypogaea.* Intracluster crosses are much more successful than intercluster crosses (Fig. 3).

Fifty-one amphiploids have been raised from 17 different cross combinations of wild species, including *A. batizocoi* (Table 4), and eight different combinations have been successfully crossed with *A. hypogaea* (Table 5). The resultant progenies are being analyzed morphologically and cytologically; their behavior is similar to the progenies obtained through the autotetraploid route. The amphiploids involving three species are the most fertile.

Use of Tetraploid Wild Species

A. monticola has been crossed with *A. hypogaea* and fully fertile hybrids have been produced.



Total no. of pollinations	5348
Total no. of pegs	1453
Total no. of pods	901

Figure 3. Percentages of peglpod production in interspecific crosses in section Arachis.

Gene Transfer

By Chromosome Pairing

Our cytological analysis of the diploid wild species, F1 hybrids and triploids resulting from crosses of wild diploid species with A *hypogaea* has indicated that there is chromosome homology or homoeology among the genomes of all the wild diploid species studied and between these genomes and A *hypogaea.* This means that there is a good probability of gene transfer through chromosome pairing in these hybrids and backcrosses producing new gene combinations in progenies, enabling selection of the desired plants.

Induced Translocation

Such chromosome pairing may not always occur, either because of the incorporation of a nonhomologous genome, or because the genetic background of the hybrid prevents pairing of homologous genomes. In such cases translocation of chromosome segments has to be induced through mutagenesis.

Barriers to Hybridization and Means of Breaking Them

A *hypogaea* has not been successfully and repeatably crossed with any species outside section *Arachis* (Gregory and Gregory 1979). Many species in other sections have potential as gene sources in groundnut improvement (Moss 1980). Tetraploid wild species are found in section *Rhizomatosae* (Gregory and Gregory 1979) and these species are of special interest as they are immune to many pathogens. Several attempts have been made by many people over the years to achieve an intersectional hybrid but these have not been successful (Gregory and Gregory 1979). Recent advances in the knowledge of the physiology and development of pollen, factors involved in pollination and advances in the technology of

Table 4. Number of amphiploids established in section *Arachis.*

♀ Parents \ ♂ Parents	*A. villosa*	*A correntina*	*A sp 338280*	*A duranensis*	*A. sp 263133*	*A chacoense*	*A. batizocoi*	*A. chacoense x A. cardenasii*
A villosa			6				2	
A. correntina	**4**		1					5
A sp 338280	2							2
A. duranensis	1	1			3	5		
A. sp 263133			2	3				
A. batizocoi	2		1			8		

Table 5. Crossablllity batween *A. hypogaea* and amphlploids.

Cross	No. of pollinations	No./% of pegs	No./% of pods
A. hypogaea x *[A. correntina* x *[A chacoense x A. cardenasii)]*	160	26/16	22*/14
A. hypogaea x *[A.* sp. 338280 x *(A. chacoense x A. cardenasii)]*	164	42/26	32*720
A. hypogaea x *{A. correntina x A. villosa)*	199	62/31	42*721
A, hypogaea *x* *{A. villosa x A.* sp 338280)	135	15/11	15*/11
A. hypogaea x (A *duranensis x A. chacoense)*	96	9/9	7»/7
A. hypogaea x (A *batizocoi x A. villosa)*	29	8/28	7/24
A. hypogaea x (A *batizocoi x A. duranensis)*	18	7/39	5*/28
A. hypogaea x *[A. batizocoi x A. chacoense)*	10	7/70	3*/30
Total	811	176/22	133/16

* Sprouts produced.

tissue culture have increased the possibility of producing hybrids between species which were previously considered to be genetically isolated (Heslop Harrison 1978; Vasil 1978, 1980; Shivanna et al. 1979; Sala et al. 1980).

In June 1979 a project was initiated to investigate the barriers to intersectional hybridization. Fluorescent microscopic comparison of the compatibly and incompatibly pollinated pistils showed that in the former, the pollen tubes were smooth with small callose patches distributed evenly along the lengths of the pollen tubes. In the incompatibly pollinated pistils, however, the callose depositions along the pollen tube were uneven and in larger quantities indicating a retarded growth of the tubes. However, a small frequency of incompatible pollinations induced peg initiation and elongation, though these usually dried and degenerated before they penetrated the soil.

A number of techniques have been tested for their efficiency to overcome such incompatibility. Plant growth hormones applied to the ovary were found to increase the frequency of peg formation in incompatible crosses. The initial trials, using cytokinin at 10^{-6}M applied to cotton webs wrapped round the ovaries, were followed by trials using four concentrations of kinetin and three of benzylamino purine, as well as auxins and gibberellic acid. The effects of these treatments are shown in Table 6. Kinetin, naphthaleneacetic acid and gibberellic acid have all significantly increased the pegging percentage.

Some of these pegs have been left to form pods in the soil. Others have been excised from the plants for aseptic culture of the tip of the peg, or the ovule, or the embryo. We have so far been able to culture immature embryos successfully into seedlings using Murashige and Skookg's 1962 medium. Our attempts to culture pegs according to Ziv and Zamsky (1975), or ovules according to Martin (1970), even from compatible crosses, have not given satisfactory repeatable results. We were able to induce normal embryogeny in one ovule culture up to the cotyledonary stage of the embryo by using an aqueous peg extract in the medium.

While excising the embryos from seed for culture, the cotyledons were also cultured. We observed that the end of the cotyledon proximal to the embryo is a highly embryogenic tissue which gives rise to roots, shoots, embryoids or whole plants depending upon the hormonal balance in the medium. We have also been trying to regenerate plants from leaflet segments and have been able to induce roots but not shoots or embryos, although we have tried four different basic media, White (1943), Murashige and Skoog (1962), Gamborg et al. (1972) and Kao and Mickayluk (1975), with a range of auxins and kinetins, as well as supplementing with coconut milk, casein hydrolysate, yeast extract, malt extract or gibberellic acid.

Conclusion

Considerable progress has been made with a

Table 6. Effact of soma plant growth hormones on pegging after pollination of tetraploid species of section *Anchls* with *Arachis* sp PI No. 276233 of section *Rhizomatotae.*

		♀ *Arachis monticola*		♀ *A. hypogaea* var. Robut33-1	
Cross	Treatment	No. of pollinations	Pegs formed (%)	No. of pollinations	Pegs formed (%)
Compatible (self)	None	201	34.33	156	34.62
Incompatible (control)	None	314	17.20	147	15.65
Incompatible	Kinetin, 10^{-4} M			21	14.29
"	Kinetin, 10^{-5} M	90	15.56	82	25.00
"	Kinetin, 10^{-6} M	75	18.67	55	25.46
"	Kinetin, 10^{-7} M	95	14.75	87	40.23
"	Benzyl Adenine, 10-^{5}M	134	22.39		
"	Benzyl Adenine, 10-^{6}M	191	20.42		
"	Benzyl Adenine, 10-^{7}M	98	19.39		
"	Indole Acetic Acid (25 ppm)	107	13.08	53	24.53
"	Napthalene Acetic Acid (25 ppm)	129	17.83	52	42.31
"	2, 4-Dlchloro Phenoxy-acetlc Acid (25 ppm)	73	17.81	18	22.22
*	Gibberellic Acid (25 ppm)	37	48.65		
"	Kinetin 70-^{4}M + Indole Acetic Acid (25 ppm)			89	24.72

number of different ways of utilizing wild species. The number of plants produced, and the range of variation they show, indicate that there is good potential for transferring desirable characters from wild species. Although the wild species were originally considered solely as sources of disease resistance, the progenies from interspecific crosses have potential as a means of expanding the gene pool of *Arachis* with respect to a number of other desirable characters.

Results of attempts to break the barriers to hybridization hold promise for utilization of characters from species outside section *Arachis.*

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