Wide Hybridization in Legumes at ICRISAT

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Abstract

A major objective of the Legumes Program at ICRISAT is to overcome constraints to production of groundnut (Arachis hypogaea), *chickpea* (Cicer arietinum) *and pigeonpea* (Cajanus cajan). *Sources of resistance to some pests and diseases have been identified among wild relatives of these crops. Attempts have been made to cross* Cajanus cajan *with* Atylosia *species, and to investigate causes of, and develop techniques to overcome, intergeneric incompatibility. Pods formed following some interspecific hybridizations contained shriveled seeds, and hybrid embryos have been cultured. Seeds have been obtained from some interspecific crosses with chickpea. Hormone treatment at pollination has been used to produce hybrid embryos from crosses between A. hypogaea and distantly related tetraploid wild species. Ovule and embryo culture has been used to produce hybrid callus and shoots, but effective roots have not been induced. Hybrid shoots have been grafted onto groundnut seedlings. Compatible diploid wild species have been used as ~~irces of resistance, and a number of high-yielding, disease-resistant lines have been produced ~~owing ploidy and genome manipulations.*

Introduction

Work on chickpea and pigeonpea started at Patancheru in 1972, when ICRISAT was founded, and, on groundnut in 1976 after groundnut became the fifth ICRISAT mandate crop.

The Legumes Program consists of breeders, cytogeneticists, pathologists, agronomists, physiologists, and entomologists with responsibility for one, or more, of the three crops. The objective of the program is to overcome constraints to production of the three crops. Breeders in cooperation with other scientists are producing new genotypes adapted to conditions in the semi-arid tropics, and with resistances to major pests and diseases.

᛫ has been considerable success in all three ᛫᛫᛫᛫᛫ ᛫roundnut varieties ICGS 11 and ICGS 44 have been released for cultivation in India. In pigeonpea, ICPL 87 (Pragati) has been released, and the chickpea breeders have produced varieties for release in both desi and kabuli seed types. The wild relatives of all three crops are sources of a few desir-

able genes, but their traits are seldom easy to transfer to cultivated taxa by conventional techniques.

The priority has been to transfer genes for resistances not presently available in the cultivated germplasm. Wherever resistance is available, the incorporation of genes from exotic species widens the breeders' options, and it can lead to more stable resistance. This paper reports the work that has been done at ICRISAT to make genes from wild species available to breeders.

Pigeonpea (*Cajanus cajan*)

Cajanus is a monotypic genus, but there are a number of compatible and incompatible species in *Atylosia*, a genus taxonomically very close to *Cajanus*. They are of interest to breeders, because of their potential as sources of resistance to pests and/or diseases, drought tolerance, early maturity, high protein content, and/or annuality (Table 1).

A number of *Cajanus* × *Atylosia* crosses have been

Table 1. Sources of characteristics sought for pigeonpea improvement.

Character	Source of gene
1. Insect resistance (*Heliothis*)	*Atylosia scarabaeoides*
2. Disease resistance	
Wilt (*Fusarium*)	*A. platycarpa*
	A. volubilis
Blight (*Phytophthora*)	*A. platycarpa*
	A. sericea
Sterility mosaic virus	*A. volubilis*
3. Drought tolerance	*A. albicans*
	A. lineata
	A. scarabaeoides
	A. sericea
	A. volubilis
4. Early maturity, annuality	*A. platycarpa*
5. High protein	*A. albicans*
	A. lineata
	A. scarabaeoides
	A. sericea

attempted, but only *Cajanus cajan* × *A. acutifolia* and *C. cajan* × *A. reticulata* formed mature seeds. Neither of these wild species contain desirable traits, but they do cross with other species. Therefore one approach is to use these species as a bridge to gain access to the desired genes. This will further our understanding of the genomes involved as well as generating a range of interspecific derivatives probably crossable with *Cajanus cajan*.

Nineteen combinations were attempted at ICRISAT (Table 2). The bridge-cross most likely to succeed is *C. cajan* × *A. acutifolia* and *A. cajanifolia* × *A. acutifolia*, as both crosses produce mature seed, though *A. cajanifolia* is not a species with a desirable trait. *A. acutifolia* is a possible means of accessing *A. platycarpa* and *A. scarabaeoides*, but only if shriveled seeds can be grown, or young embryos rescued.

Studies of seed formation, from *A. platycarpa* × *A. scarabaeoides* crosses, showed that endosperm degeneration began about 7 days after pollination, when the embryo is too small to dissect and culture. The largest hybrid embryos grew to a maximum length of 1 mm about 19 days after pollination, compared with 5-6 mm in *C. cajan* at that age. Pigeonpea embryos have been successfully cultured (Kumar et al. 1985), so embryo culture was attempted in two combinations important as bridge-crosses *A. platycarpa* × *A. acutifolia*, and *A. platycarpa* × *A. scarabaeoides*. Embryos were excised 16-19 days

after pollination, when they were 0.7-1.0 mm long, and had reached the heart-shaped stage. The medium used was Gamborg's B5 (Gamborg 1984) with 0.8% agar and 2.0% sucrose. When 1 mg L⁻¹ 2,4 dichlorophenoxy acetic acid (2,4-D) was added, profuse callus was formed. When embryos were placed on the same basic medium but with 0.25 mg L⁻¹ kinetin and 0.1 mg L⁻¹ naphthalene acetic acid (NAA), less callus was formed than on the medium containing 2,4-D. Some shoots were formed, though not at a frequency high enough for a practical program on gene transfer.

Nurse culture

An investigation of nurse-culture techniques was undertaken with the objective of increasing the frequency of rescue of hybrid embryos. Embryos were placed on B5 medium, with selfed embryos or endosperm placed immediately adjacent brid embryo. Embryo development was impaired when maternal tissue was present, as necrosis spread from the ovular wall to the embryo, but when all maternal tissue was removed, hybrid embryos grew, and the frequency of root and shoot formation was increased (Table 3). Over all crosses, 48% of embryos produced callus or shoots and roots with nurse tissue. In another experiment, nurse endosperm gave 45% response, compared with 35% where the embryo was used as a nurse.

Dark treatment

Hybrid embryos from *A. platycarpa* × *A. spp* crosses were cultured on B5 medium and kept in the light, or in the dark, for 1 week after culture. Fifty-seven percent of embryos kept in the dark responded favorably compared with 30% kept in the light.

Chickpea (*Cicer arietinum*)

There are a number of wild *Cicer* species with characters that would be useful in chickpea improvement (Table 4). *C. reticulatum*, which has resistance to ascochyta blight (ICRISAT 1980) has been crossed with *C. arietinum* (Pundir and van der Maesen 1983). Four chickpea genotypes were used as female parents, and three crosses were successful, giving 9%, 8%, and 7% hybrid seeds. Hybrids produced were normal in morphology and fertility.

Table 4. Characters in wild *Cicer* species useful for chickpea improvement.

Species	Character
C. judaicum	Resistant to fusarium wilt
	Resistant to gray mold
	Resistant to ascochyta blight
C. reticulatum	Promising against ascochyta blight
	Acceptable seed size (10 g 100⁻¹ seeds)
C. pinnatifidum	Resistant to aschochyta blight
C. montbretii	Resistant to aschochyta blight,
	3-7 seeds per pod
C. bijugum	Acceptable seed size (9 g 100⁻¹ seeds)
C. cuneatum	Good vigor, 3 seeds per pod, resistant
	to fusarium wilt
C. microphyllum	Cold tolerance

Source: Adapted from van der Maesen and Pundir (1984).

C. arietinum × *C. cuneatum* was attempted with seven genotypes of chickpea, and the reciprocal with two genotypes. The only successful combination was *C. arietinum* 'G-130' × *C. cuneatum*, and that combination produced only one seed from 304 pollinations (Pundir and van der Maesen 1983).

All other crosses of cultivated chickpea with *C. pinnatifidum, C. bijugum, C. chorassanicum,* and *C. judaicum* were unsuccessful. However, a number of crosses between wild species were successful. These were *C. pinnatifidum* × *C. judaicum*, and the reciprocal, *C. pinnatifidum* × *C. bijugum*, and *C. judaicum* × *C. bijugum*.

The future emphasis in our chickpea research will be to repeat these crosses and attempt others, using mentor pollen, hormone treatment, and embryo rescue techniques.

Groundnut (*Arachis hypogaea*)

The genus *Arachis* has been divided into seven sections based on morphological and cross-compatibility studies, and sections subdivided into series (Gregory and Gregory 1979). There are 22 described species and possibly another 40 distinct species among recent collections (Smartt and Stalker 1982, Stalker 1985).

The cultivated groundnut, *A. hypogaea*, belongs to section *Arachis* and is a tetraploid, 2n=4x=40. It is readily crossable with a closely related tetraploid wild species, *A. monticola*, and produces fertile hybrids. A cultivar Spancross, has been developed from such a cross in the USA (Hammons 1970). *A.*

monticola has been proposed as a wild subspecies of *A. hypogaea* (Singh and Moss 1982, 1984a, Smartt and Stalker 1982). Other closely related species in section *Arachis* are diploid, 2n=2x=20. The majority of these species have the 'A' genome, only one species, *A. batizocoi*, has the 'B' genome, and a new collection, *A. spinaclava* has the 'D' genome (Stalker 1985). The A genome species have been further subdivided on chromosome morphology using Mahalonobis D^2 analysis (Singh and Moss 1982). *A. hypogaea* has been crossed with both A and B genome species, and has been concluded to have AABB genomic formula. Hybrids have not been produced with *A. spinaclava*.

There are no confirmed reports of species in sections other than *Arachis* having been crossed with *A. hypogaea* by conventional means. A number of intersectional crosses involving diploid species in section *Arachis* have been successful (Gregory and Gregory 1979), but none has been used as a bridge-cross to transfer genes to *A. hypogaea*.

Many of these taxa are resistant to important pests and diseases that cause economic losses in many groundnut growing areas (Moss et al. 1987) (Table 5). The primary interest at ICRISAT was to transfer resistance to *Phaeoisariopsis personata* (Berk. and Curt.) v. Arx, late leaf spot, from species in section *Arachis*, and resistance to a number of viruses and insect pests from section *Rhizomatosae* (Moss 1985a,b).

Therefore there were two main thrusts. The first was centered on species, in section *Arachis* to transfer disease resistance and to explore the effects of genomes on gene transfer, as Smartt et al. (1978) had proposed that susceptibility in one genome could not be overcome by transferring a resistance gene into the other genome. The second thrust was to understand the barriers to hybridization, and to discover means of overcoming them, with the objective of utilizing taxa in section *Rhizomatosae* in the genetic improvement of cultivated groun

Intersectional gene transfer

When *A. hypogaea* is pollinated by *A.* sp 276233 (*Rhizomatosae*) pollen germinates and penetrates the stigma. There are many abnormalities of the pollen tubes; tubes are irregular in shape, vary in width, often with swollen tips, and with callose plugs. However, some pollen tubes reach the base of the style, and reports of some pegs being formed indicated that they may be effective at fertilization.

Table 5. Immune (I), resistant (R), and tolerant (T) reactions of wild *Arachis* species to pests and pathogens (data from ICRISAT screening and various authors).

Section series/ Species	Pathogen								Pest			
	RUS	LLS	ELS	PSV	GRV	PMV	TSW	PCV	THR	APH	MIT	JAS
Arachis Annuae												
A. batizocoi ·	I											
A. duranensis	I			R					R			R
A. spegazzinii	I											
Arachis Perennes												
A. helodes								T				
A. villosa	I			R						R		R
A. correntina	I			I		R	R			R	R	R
A. cardenasii	I	I				R	R					R
A. chacoense	I	R*	R/I			R	R		R	R		
A. stenosperma	R	R	R									
Arachis spp				R¹								
Ambinervosae												
Arachis spp												R¹
_____ae												
A. repens		R	I	R	R				R		R	
Extranervosae												
A. villosulicarpa	I	I	I*								R	
A. macedoi									R			
Arachis spp										R¹		
Triseminalae												
A. pusilla	I					R	R		R			R
Erectoides Tetrafoliolatae												
A. benthamii				R								
A paraguariensis	I	R							R			
Arachis spp				R¹					R⁴			R¹
Erectoides Procumbensae												
A. rigonii												R
A. appressipila	I	R										
Arachis spp		R²	R									
Rhizomatosae Eurhizomatosae												
A. glabrata	R	I/R	R	R*	R	R			R	R	R	
A. hagenbeckii	I	R	I						R*			R
is		R/I										R¹¹

RUS = Rust, *Puccinia arachidis*
LLS = Late leaf spot, *Phaeoisariopsis personata*
ELS = Early leaf spot, *Cercospora arachidicola*
PSV = Peanut stunt virus
GRV = Groundnut rosette virus
PMV = Peanut mottle virus

TSWV = Tomato spotted wilt virus
PCV = Peanut clump virus
THR = Thrips, *Scirtothrips dorsalis*
APH = Aphids, *Aphis craccivora*
MIT = Mites, *Tetranychus* sp
JAS = Jassids, *Empoasca* sp

* = Conflicting reports may be due to misidentification, or variation in the wild species, the pathogen, or the test conditions.
1,2,3, superscripts = number of species or unnamed accessions.

Mentor pollen or mentor pollen leachate was applied to styles to increase the frequency of fertilization (Sastri and Moss 1982, Moss and Sastri 1986). These treatments were combined with kinetin applied to the base of the hypanthium, and it was found that kinetin alone increased the number of pegs. Subsequently gibberellic acid (GA) was found most effective, up to 82% of pollinations with GA treatment producing pegs (Table 6). However, many of these pegs do not form pods, but application of auxin subsequently increases the rate of pod formation (Nalini and Sastri 1985a). The most effective treatment was indole acetic acid (IAA) at 50 or 100 ppm applied to the developing peg 15-25 days after pollination. This sequence of treatments produces up to 32 pods for every 100 pollinations, but if left on the plant the embryos do not develop fully, and pods contain shriveled seeds.

The timing of the second hormone application, and the nature of the hormone used, has an influence on the size of the ovules as well as on the number of pods produced. Ovules up to 4.8 mm long were excised from pods of *A. hypogaea* cv Robut 33-1 pollinated with *Arachis* sp 276233, after treatment with 87.5 ppm GA at pollination and 100 ppm IAA 20 days after pollination (Table 7).

A range of cultivars of *A. hypogaea* were used as female parents, and different accessions of rhizomatous species used as male parents. There was little difference in the frequency of peg formation after gibberellin treatment, but the frequency of pod formation, without further hormone treatment, varied depending on the genotypes used (Table 6). The percentage pod production per peg ranged from 0 to 42%, *A. hypogaea* MK 374 × *Arachis* sp 276233 being the best combination for pod production (Table 6) from a single hormone treatment with GA at pollination. There were also differences in the sizes of the ovules dissected from the pods. Ovule lengths ranged from 1.6 mm to 4.8 mm (Table 7). Ovules from the three crosses longer than 3 mm could be dissected, and the embryo excised and cultured, but ovules shorter than 3 mm were cultured intact (Nalini and Sastri 1985b).

Thus, although the hormone treatme duce the highest numbers of pegs did not produce the largest ovules, there was a range of hormone treatments in most hybrid combinations that gave

Table 6. Peg and pod production after GA treatment in *A. hypogaea* × *Arachis* sp crosses.

Female parent	TMV 2			Robut 33-1			MK 374			Chico		
Male parent	a	b	c	a	b	c	a	b	c	a	b	c
A. sp 276233	408	77	27	491	82	18	648	68	42	58	66	9
A. sp 9649	11	73	0	82	44	6	26	42	15	26	73	19

a = Number of pollinations. b = pegs per pollination (%). c = pods per peg (%).

Table 7. Ovule length (mm) from pods obtained in three *A. hypogaea* cultivars crossed with *Arachis* sp 276233 with subsequent hormone treatments.

Hormone treatment[1] (ppm)	DAP[2]	MK 374	TMV 2	M 13	Robut 33-1
Nil		1.6			
GA		2.6	2.3	2.8	
GA; IAA (10)	10		3.8		2.8
GA; IAA (10)	15	3.1		2.8	2.1
GA; IAA (25)	10			2.5	2.4
GA; IAA (25)	15	2.0			2.2
GA; IAA (50)	15	2.4			2.1
GA; IAA (100)	15	2.8			2.1
GA; IAA (100)	20				4.8

1. GA = Gibberellic acid (87.5 ppm aqueous) applied to bases of flowers soon after incompatible pollinations.
   IAA = Indole acetic acid at different concentrations, in lanolin, applied to peg bases on different days after pollination.
2. DAP = days after pollination.

an acceptable frequency of ovules that could be cultured. The present technique is to pollinate all flowers on a plant over a period of about 20 days, treating them with GA at pollination and IAA or naphthalene acetic acid (NAA) about 20 days later, and then to harvest the plant. This technique has been applied to other wide crosses in *Arachis*, and pods have been produced from intersectional combinations where there has been no success previously. Pods have been produced in crosses of *A. hypogaea* with species in sections *Erectoides* and *Extranervosae* as well as *Rhizomatosae*. Ovules were excised and cultured on MS medium. When benzyl amino parine (BAP)-NAA were used, ovules survived longer and formed callus, but kinetin-IAA media stimulated embryo growth better than BAP-NAA. Embryos were dissected from ovules larger than 3 mm, but were often abnormal. Embryos were cultured on MS + 2.0 mg L⁻¹ NAA and 0.5 BAP mg L⁻¹. This stimulated callus ꞏ, and subsequently shoots were produced. _____ ᵥere grafted onto *A. hypogaea* seedlings, where vegetative growth was good.

Gene transfer from wild diploids in section *Arachis*

There are a number of options for transferring genes from wild diploids to a cultivated tetraploid, but they can be broadly divided into direct hybridization followed by ploidy manipulations, and ploidy manipulations including hybridization of the wild species before crossing with the cultivated species (Singh 1986a).

Direct hybridization produces a triploid. At ICRISAT, the eight original species, including *A. cardenasii*, a species resistant to late leaf spot, were crossed with *A. hypogaea* (Singh and Moss 1984a). Triploids were treated with colchicine to produce hexaploids, which have been backcrossed to *A. ꞏa*. The chromosome number was reduced in successive backcross generations. The fertility was low in early backcross generations. Ten cytologically stable tetraploid progenies were produced (ICRISAT 1983).

Triploids, although previously reported to be sterile, were observed to form pods under certain conditions at ICRISAT (Singh and Moss 1984b). Progeny from selfed triploids were mostly hexaploids resulting from fusion of unreduced gametes, but plants with other ploidy levels were also produced, including tetraploids that were represented by about 8% of the progenies. They were the result of fusion

between balanced gametes resulting from unequal segregation. Analysis of meiosis in triploids indicated that recombination had occurred between chromosomes from the wild and cultivated parents. Stable, tetraploid, disease-resistant plants have been selected from progenies of selfed triploids (ICRISAT 1983).

Ploidy manipulations of wild species, before crossing to *A. hypogaea*, include the production of autotetraploids and of amphidiploids.

Autotetraploids have been produced from A-genome species and the only B-genome species, *A. batizocoi* (ICRISAT 1983, Singh 1986b). These were crossed with *A. hypogaea*. The resultant hybrids were backcrossed to *A. hypogaea*. Fertility was low in the early generations. Nevertheless about seven pods per 100 backcross pollinations were produced and stable, fertile, disease-resistant plants were selected in subsequent backcross generations' progenies.

Amphidiploids have been produced by colchicine treatment after crossing A-genome species with *A. batizocoi*, and also after intercrossing A-genome species (Singh 1986c). The amphidiploids were crossed with *A. hypogaea*, and selected hybrids were backcrossed to the cultivated parent. In the amphidiploids, one or both genomes are homologous with the *A. hypogaea* genomes, as indicated by chromosome pairing that should facilitate gene transfer from the common genome. However, even in hybrids between *A. hypogaea* (AABB) and amplidiploids with the AABB genome combination, many univalents (I in Table 8) were observed.

The frequency of trivalents and quadrivalents in these hybrids indicates that intergenomic A-B pairing does occur. In successive backcrosses to *A. hypogaea*, fertile stable disease-resistant plants have been selected at frequencies of around 1%, indicating that amphidiploids are a practical means of transferring genes from wild species.

Agronomic characters of derivatives of *A. hypogaea* × wild species hybrids

The hybrids and backcross derivatives that are cytologically stable, fertile, and disease-resistant have been selected for a number of agronomic characters, including yield of pods, kernels, and of haulm, which is valuable as animal feed: and a number of advanced lines have been bred (ICRISAT 1985) (Table 9). These have been distributed to breeders in many countries, who now have access to genes from wild species in their breeding programs.

Table 8. Chromosome associations in amplidiploids and their F₁ hybrids from crosses with A. hypogaea.

Amphidiploid or hybrid	Genomic formula	I	II	III	IV	VI or VII
A. villosa × Arachis sp HLK-410	AAAA	2.36	13.6	0.68	2.0	
A. hypogaea × (A. villosa × Arachis sp HLK-410)	AAAB	9.72	12.5	1.36	0.27	
Arachis sp HLK-410 + A sp 10038	AAAA	3.45	14.0	0.41	1.80	
A. hypogaea × (Arachis sp HLK-410 × Arachis sp 10038)	AAAB	10.04	12.0	0.8	0.88	
A. batizocoi × A. correntina	AABB	4.50	16.6	0.5	0.20	
A. hypogaea × (A. batizocoi × A. correntina)	AABB	7.90	13.3	1.45	0.27	
A. villosa × A. batizocoi	AABB	1.52	18.2	0.40	0.16	0.04
A. hypogaea × (A. villosa × A. batizocoi)	AABB	4.52	14.9	0.68	0.88	

Table 9. Agronomic and botanical features of cytogenetic entries currently in AICORPO Trials.

Identity	Pedigree	Duration (days)	Seed characteristics[1]					
			Disease score		100 seed mass (g)	Shelling (%)	Yield (kg ha⁻¹)	
			R	LLS			Pod	Haulm
1. CS-2	(A. batizocoi × A. sp 10038) × A. ogaea	120	2	8	33	76	3230	
2. CS-39	A. hypogaea × A. cardenasii	120	6	3	27	64	3200	6760
3. CS-52	A. hypogaea × A. cardenasii	120	3	5	40	60	3260	6320
Control Robut 33-1	Selection from Kadiri 3	100	9	9	36	70	1700	-
SE			±0.4	±0.4	±0.8			±186
CV(%)			14	13				12
4. CS-48	A. hypogaea × A. cardenasii	120	3	3	39	71	3250	6389
5. 943	A. hypogaea × A. cardenasii	120	6	2	30	53	3611	5611
Control Robut 33-1								
SE			±0.33	±0.25	±1.9		±283	±674
CV (%)			19	14	10		18	18

1. Rust = rust
   LLS = late leaf spot } in scale of 1 (low infection) to 9 (high infection).

Prospects

Many interspecific and intergeneric hybrids have been produced at ICRISAT, using hormone treatments and embryo rescue as necessary. Some of the hybrids have been fertile, and genes from the wild species have been transferred to derivatives, which are fertile and fully crossable with the cultivated species. Significant progress has been made in basic techniques of tissue culture, and in developing techniques to transfer genes from more distantly related taxa. The application of these techniques to selected hybrid combinations holds considerable promise for the production of new cultivars incorporating genes from wild species.

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