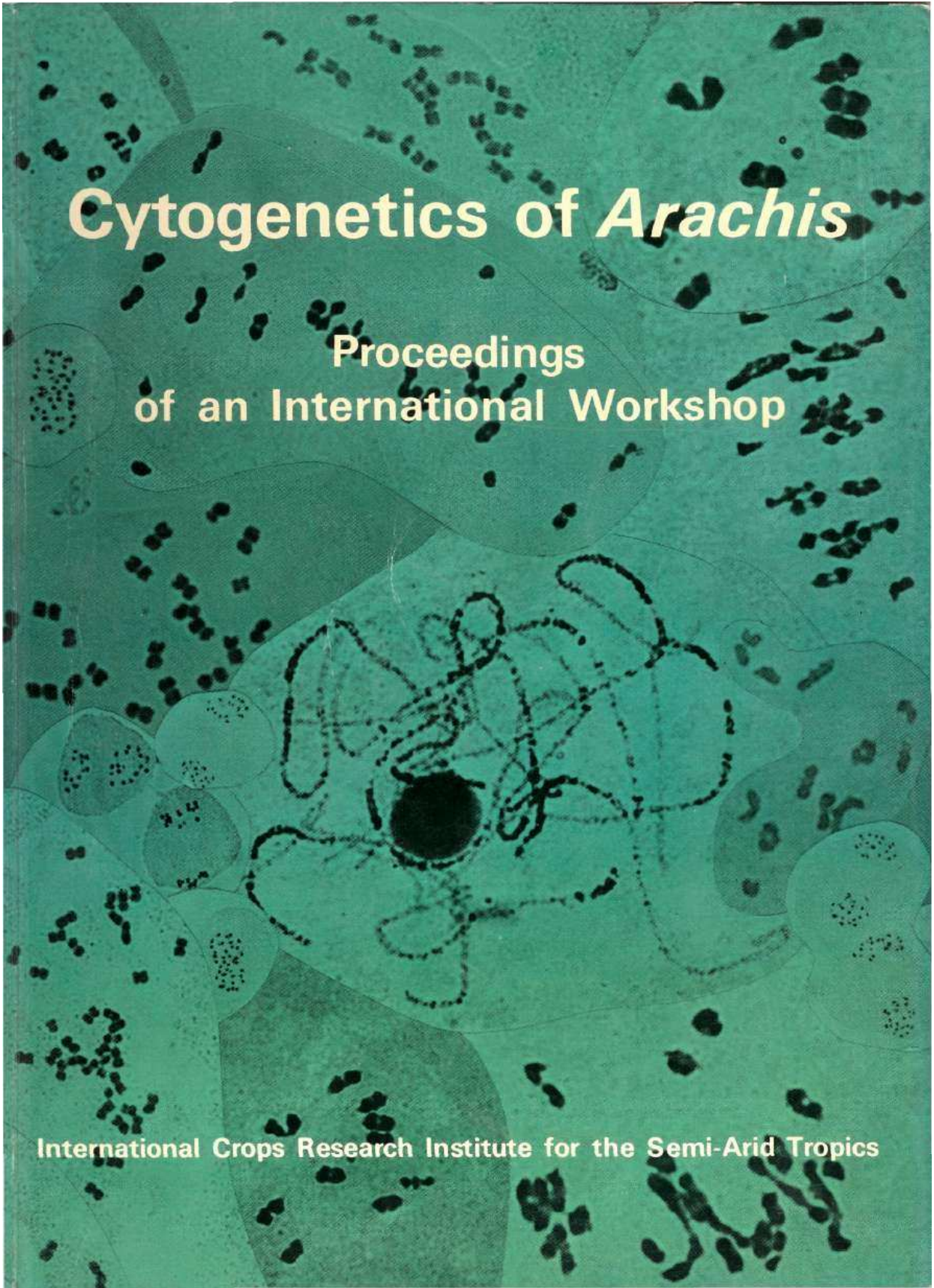
Cytogenetics of *Arachis*

Proceedings of an International Workshop



International Crops Research Institute for the Semi-Arid Tropics

Proceedings of an International Workshop on

Cytogenetics of *Arachis*

ICRISAT Center
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A Note on the Taxonomy of Arachis

Many species names in common use have not been validly published even though some have been used for 20 years. During this time *A. stenocarpa* (HLK 410) was changed to *A. stenosperma*, and it is likely that *A. chacoense* will change to *A. chacoensis* (Valls, pers. comm)

For consistency in the proceedings, I have used the forms most commonly used by the majority of authors, which are also the most common in the literature, ie., *A. chacoense* and *A. stenosperma*.

J.P. Moss

Contents

Foreword

An International Workshop on Groundnuts held at ICRISAT in 1980 covered a wide range of subjects, including genetics and breeding, cytogenetics and the utilization of wild species, crop nutrition and agronomy, entomology, and pathology. That general review of groundnut improvement will now be followed by more detailed examination of specific research aspects or disciplines. Such a workshop, in which a small group of cytogeneticists reviewed work on the utilization of wild species in the improvement of the cultivated groundnut, was held at ICRISAT Center in November 1983 and is reported in these proceedings.

ICRISAT is predominantly an applied research institute dedicated to improving the life of poor farmers in the semi-arid tropics, but undertaking some fundamental research when necessary. The use of wild species of Arachis as sources of genes for resistance to pests and diseases necessitates an understanding of the cytogenetics and incompatibility systems that exist, so that barriers to successful interspecific gene transfer can be overcome. Much fundamental work has already been done at institutions in the USA, facilitating our applied work. A great deal of help has also been given by the International Board of Plant Genetic Resources (IBPGR) who coordinated and funded the collection of wild material from threatened habitats in South America. In these endeavors we have seen excellent cooperation between institutions and scientists from North and South America, as well as between international bodies such as IBPGR and ICRISAT.

ICRISAT materials, developed by interspecific hybridization, have demonstrated their disease resistance, yield potential, and agronomic acceptability, and are now competing with conventional cultivars in national trials in India. We are encouraged by this success and hope that the work reported, and the new ideas discussed in this volume will promote further developments and forge stronger cooperative ties in this exciting field.

L.D. Swindale
Director General, ICRISAT

Opening Session

Introductory Address

Hugh Rees[1]

I am most grateful for this opportunity of saying a few words before the serious business of the day gets under way. Although this is an inaugural address, which sounds most formal and even presidential, I propose to be neither.

I am conscious that all of you present at this workshop have detailed knowledge of Arachis and expertise in applying this knowledge in various ways with a view to the improvement of the groundnut crop. I have neither the detailed knowledge nor the experience of working with *Arachis*. For these reasons it would be presumptious of me, indeed impertinent, if I were to offer quick and ready answers to the many problems that confront you, as breeders, pathologists, physiologists or cytogeneticists. What I propose to do is to consider a problem that, in general terms, faces all of us who are engaged in applied research work. The problem is this. To what extent, and in what measure do we place emphasis, on the one hand, on research which is strictly of an applied nature and, on the other hand, to work of a more basic kind? I shall offer, later, a comment on this same problem in relation to what I know of the work on *Arachis*.

I am a university man and you will know that for better or for worse the university scientist has the freedom to choose his field of research and, equally important, to choose which organism to work on. The scientist at an applied research station, in sharp contrast, is subject to certain and inescapable constraints. His research objective is defined, the organism specified. Those of you who are cytologists might with justice complain about the technical difficulties of interpreting the miniature chromosome configurations at meiosis in *Arachis* species. Your peers, however, would be less than enthusiastic if you were to propose instead to examine the large, fat chromosomes of *Lilium*. There is indeed a difference in approach but, as I shall indicate, it is often exaggerated. On the face of it another difference between the role of the university scientist and that of the research station scientist is that the former concentrates upon pure or basic research, the latter on the applied. To my mind, however, such statements are grossly misleading and, from many points of view, dangerously misleading. Consider, for example, some of the famous names who have contributed in recent times to basic science in the field of biology. R.A. Fisher worked at an agricultural research station. Watson and Crick worked at an unit of medical research. An eminent applied scientist in England, Professor Wain, was an university man. Equally misleading are the popular, dogmatic references to basic and applied research as if they were sharply distinct and disparate activities. Who indeed would care to define the borderline between them? We recognise the extremes well enough but the overlap is extensive and complex. Eminent cytologists such as C.D. Darlington in England; Marcus Rhodes, Barbara McClintock and Ernie Sears in the USA, could as well fit the description of applied scientists as of pure scientists. They contributed enormously to our knowledge of important horticultural and agricultural crops. Their contributions to basic science are equally celebrated.

I have said that it is dangerous to perpetuate the misleading compartmentalization of basic and applied research and dangerous also to overemphasize the different roles of university scientists, and of scientists at institutes of applied research. It is dangerous for this reason. Progress in both basic and applied research is, above all, a matter of interdependence and of interaction. For this reason the university man like his opposite number at a research station

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depends as much upon new knowledge from applied research as from basic research. There are, of course, exceptions but they are fewer than we generally acknowledge. If we fail to recognise this element of interdependence we are in danger not only of misunderstanding our science but of rendering it that much less effective.

Having said all this we now come to the difficult question. Where does one strike a balance? In terms of the applied research institute, how much basic research can one afford to do? Or better, how much basic research can one afford not to do to achieve one's objective? This to my mind is the most difficult question for a research director, at any level, to answer. It is also one of the most important. It is difficult because the criteria are never easy to define or to measure. With limited resources, and resources are always limited, the return on investment is never guaranteed. There is always an element of a gamble, of risk.

I shall now take a personal risk by venturing to refer, albeit very briefly, to the element of basic research in the context of the ICRISAT groundnut program. In the first place there can be no doubt that the elucidation of the chromosome organization and differentiation among the wild and cultivated species of Arachis provides a solid and immensely important base upon which to formulate and exploit the breeding potential within the genus. The basic science devoted to understanding the taxonomy and the cytology of the genus was not only fruitful but essential. A large part of this work was accomplised by participants at this workshop. Much of it was accomplished at this institute. But having said this I have little doubt but that there is every reason for extending this basic work. From a taxonomic standpoint there is much that needs doing, and urgently at that.

Scores of species have yet to be named. The names, though necessary and important in themselves, are no more important than the morphological, physiological and genetical characteristics which they reflect. More work also needs to be done on the cytology. While two genomes have been unambiguously identified within the genus there is ample evidence to show that others exist, to be manipulated to the profit of the breeder and the farmer. In this context I believe it would facilitate matters to apply new methods for the identification of chromosomes and of chromosome complements, not only in parental species, but in hybrids and in hybrid derivatives. Of these new methods estimates of nuclear DNA amount and the application of C-banding techniques are obvious possibilities. In the cytological field, also, an understanding of the genetics of the diploidisation mechanism in the polyploids of this genus could be of much value, since the introduction of useful genes from wild species will depend largely upon the synthesis of amphiploids to be used either in their own right, or as intermediates for crossing with cultivated varieties. There are other possibilities which no doubt will be considered in depth and detail during the next few days. In the meantime it is crucial to acknowledge and to appreciate the contribution of that element of basic research to groundnut improvement. In parenthesis let us not belittle, either, the contribution of this work to the basic and general understanding of the evolution of chromosome complements and of species.

Finally, I should like to return to the theme of interdependence, in the context of basic and of applied research, in the context of the scientist at the university and at the applied research institute. It seems to me that there is a case for involving, to an increasing degree, the university scientist in the work and objectives of the applied research institutes; of mixing, to an increasing degree, the work of the reputedly basic and applied scientists. I made allusions earlier to the need for exploring and developing new cytological techniques. Why not 'farm' them out to university departments? In the first place it would be economical. Certain university departments have the necessary equipment and expertise. Also, the commitments, from the standpoint of the research institute could be short term. If the problem cannot be solved in a few years, if the return on investment is unprofitable it can be dropped without embarrasment to person or to institution. In the second place I have no doubt it would be to the very great benefit of university scientists. It would do much, to those of us who need reminding, of the political realities of science. We would also learn a lot and, hopefully, be that much wiser and, as a community, that much less complacent in our traditional isolation.

Introduction to the ICRISAT Groundnut Improvement Program

J. P. Moss[1]

The average yield of dried groundnut pods in the world is 990 kg/ha. In the USA, the yield rose from 1000 kg/ha in 1920 to 3000 kg/ha in 1950. When compared to these figures, the average SAT yield of 800 kg/ha is quite low. The disparity between the figures can be attributed to the combination of improved cultivars, and high inputs, especially irrigation and weed, pest, and disease control by chemicals used by U.S. farmers. These inputs are not always available to the farmer of the SAT, where constraints have caused a reduction in yield, although in India total production has increased with an increase in the area cultivated (Table 1).

Once the constraints faced by the SAT farmer are overcome, the yield levels can be raised to match those in developed countries; in fact, the potential yields are as high as 10000 kg/ha. The highest yield achieved at ICRISAT, using improved genotypes and good cultural practices, is 7000 kg/ha.

Table 1. Area sown to groundnut and its yield and production in India from 1930-1981.

Year	Area (,000 ha)	Yield (kg/ha)	Production (,000 t)
1930-39	2901	1003	2910
1940-49	3876	899	3485
1951-56	4854	729	3539
1956-61	6150	722	4440
1961-66	7211	710	5120
1966-71	7278	715	5204
1971-76	7170	785	5628
1976-81	7142	803	5735

Constraints

Most constraints to production, such as uncertain rainfall, pests and diseases, can be overcome by using irrigation, fertilizers, and/or pesticides, including herbicides. These inputs are either not available, or too expensive for the SAT farmer who tends to invest all he can afford in cereals, his staple food crop.

The ICRISAT Groundnut Improvement Program started in 1976. Currently there are six subprograms: Breeding, Cytogenetics, Pathology, Entomology, Physiology, and Microbiology. The Genetic Resources Unit is responsible for germplasm collection, accession, maintenance, and description and we work in close collaboration with the groundnut germplasm botanist.

The groundnut program emphasises overcoming constraints using means available to farmers with low incomes. Foremost among these is the development of cultivars resistant to pests, diseases, and drought. All subprograms cooperate with the breeders to achieve this goal. It is also important to devise cultural methods to evade pests and diseases. For example, the combination of various practices such as correct sowing date, and plant spacing that leaves no bare soil at the time of peak incidence of the vector, can substantialy decrease the incidence of bud necrosis disease (BND) caused by tomato spotted wilt virus (TSWV) so that good yields can now be obtained from cultivars susceptible to TSWV.

Diseases

Foliar Diseases

The most important foliar diseases of groundnut caused by fungi are early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spots and rust (*Puccinia arachidis*). At ICRISAT Center rust and late leaf spot occur each year in epidemic proportions. Together they have been shown to cause yield losses of up to 70% in susceptible cultivars while each disease may separately cause up to 50% yield loss. All the released

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Indian cultivars are susceptible. Field screening of the world germplasm collection for resistance to these two diseases was started at ICRISAT Center in 1977 and over 9000 accessions have now been examined. Fourteen breeding lines with rust resistance have been jointly released by ICRISAT and the USDA.

Most of the rust and late leaf spot-resistant lines are low yielding and have undesirable pod and seed characters. Breeders have crossed them with high-yielding, but disease-susceptible cultivars and are making progress in breeding cultivars that are resistant to these two diseases but which have good agronomic characters.

At ICRISAT the early leaf spot disease caused by *C. arachidicola* does not normally become severe enough to permit reliable field resistance screening, but in the 1983 rainy season the attack by this disease was sufficiently severe to identity some genotypes with significant resistance.

Near-tetraploid derivatives have been developed from crosses between wild Arachis species that are immune or highly resistant to the leaf spots and rust diseases, and high-yielding groundnut cultivars. These derivatives have useful resistance to one or more of these important foliar diseases and are now in use in the resistance breeding programs.

Aspergillus flavus and Aflatoxins

Aflatoxins are toxic secondary metabolites produced by strains of fungi of the *Aspergillus flavus* group when growing on suitable substrates. Groundnut seed and groundnut products are very effective substrates for production of the toxins. Aflatoxin contamination can be minimized by adopting farming and produce-handling methods designed to avoid damage to pods and seeds, but few farmers in the SAT follow the recommended procedures. Breeding lines with testas resistant to fungal invasion in rehydrated dried seeds were reported from the USA. This resistance was confirmed at ICRISAT Center and several more dry seed-resistant genotypes identified. It is of interest that some of these genotypes were also found to resist invasion by the group of fungi that cause pod rot disease.

Virus Diseases

Virus diseases of groundnuts are common and can be serious but it has been difficult in many cases to estimate the losses caused by specific diseases because of confusion about their identification and distribution. Identification has too often been based only upon disease symptoms. At ICRISAT emphasis has been placed on the purification and precise characterization of groundnut viruses and on the production of antisera. This research has been accompanied by field and screenhouse evaluation of germplasm accessions for resistance or tolerance to such virus diseases as bud necrosis, peanut mottle, and peanut clump. Some 7000 genotypes have been screened for resistance to bud necrosis (caused by TSWV) but all were susceptible.

Wild *Arachis* species are now being screened. *Arachis* chacoense has been found resistant in mechanical and thrips inoculation tests. Almost 500 germplasm lines have been screened for resistance to peanut mottle using a field mechanical inoculation technique. All proved susceptible but 4 lines showed less than 5% yield loss which was in marked contrast to the 12 to 60% yield loss in infected plants of other lines. Two genotypes were found to have no seed transmission of peanut mottle virus from infected mother plants. Resistance breeding using the tolerant, and the 'no-seed-transmission' genotypes has just started. Screening for resistance to the soil-borne peanut clump virus disease has been in progress for several seasons but with conflicting results, probably due to the occurrence of virus strains with differing virulence on different host plant genotypes.

Groundnut rosette disease is the best known and most important virus disease of groundnuts in Africa south of the Sahara. ICRISAT is now involved in coordinated international research to resolve this problem.

Bacterial Disease

The only important bacterial disease of groundnut is the wilt caused by *Pseudomonas solanacearum.* This disease is common and serious on groundnuts in East Asia and has been reported from South Africa and the USA. It has not as yet been found in India, and therefore has not been investigated at ICRISAT Center.

Pests

Over 300 insect and mite species have been recorded from groundnut but most are of limited distribution. Yield losses worldwide have been

assessed at 17% from field pests and 6-10% from storage pests.

Virus Vectors

Insect pests may be of importance because of the direct damage they do or because of their role in transmission of virus diseases. At ICRISAT the emphasis of entomological research is to combine cultural practices and host plant resistance to develop integrated pest management systems.

Field Pests

The effects of cultural practices on the incidence of other important pests are being studied and particular attention is being given to effects of intercropping. The high-yielding and multiple pest-resistant genotype NC Ac 343 has been used to develop breeding lines with good resistance to thrips, jassids. and termites.

Breeding for Pest Resistance

Breeding for pest resistance was started in 1980 with the objective of combining resistance to jassids, thrips, and termites into high yielding genotypes. An extensive hybridization program was intiated and a large number of single and multiple crosses were made. Through repeated testing and selection, several high-yielding progenies have been developed which have good resistance to thrips and jassids. Some termite resistant progenies were also identified.

Drought

A method of parallel screening in the field has been developed and is being used to screen a large number of germplasm accessions and breeders lines. The other approach is to examine in detail the physiological responses of groundnut plants to drought stress, the factors that determine water use and water-use efficiency, and the physical and physiological basis for genetic differences in response to drought.

Drought Screening

Screening started in the 1980/81 postrainy season when a few treatments were applied to 80 genotypes. Drought stress was induced at different stages in crop development, and lines with 'tolerance' to drought were identified. We confirmed the hypothesis that there is an interaction between time of stress and genotype.

In 1982/83 line source irrigation was used to create 6 levels of water application in each of 4 drought timings, representing variations in early and mid-season drought environments, where rainfall is always less than potential evaporation.

In 1981 /82 an evaluation of 25 lines of known drought tolerance showed that early stress definitely provides adaptive advantages in the event of a second drought at a later stage. Long droughts with occasional short periods of good water relations do not change the nature of the basic response to that drought pattern. This series of trials has identified lines which have consistently given better than average yields under drought conditions.

Drought Physiology Studies

These have been conducted to investigate the effects of time and intensity of drought; the effect of plant population on water use and the development of drought conditions; and the effect of the time of stress on drought-recovery responses.

Nutrient Stress

Biological Nitrogen Fixation

Although groundnut is an efficient fixer of nitrogen, and most cultivated tropical soils contain large populations of *Rhizobium* bacteria capable of forming nodules with groundnut cultivars, there is scope for increasing nitrogen fixation by manipulating *Rhizobium* strains, host genotypes, environments, and their interactions.

Inoculation with *Rhizobium*

There are several reports of *Rhizobium* inoculation increasing groundnut yields in fields where the crop had not previously been grown. In trials at ICRISAT Center over the past seven years, it has been shown that inoculation of groundnut seed of several genotypes with an effective strain of *Rhizobium* can increase nitrogen fixation and pod yield, even when the crops are grown in fields well populated with effective strains of *Rhizobium* .

During the 1978 rainy season some F_2 progenies

in a rust-screening nursery were observed to have segregated for non-nodulation. Some of these have been purified to obtain non-nodulating lines.

Development of Genotypes with Specific Attributes

High Yield and Quality

Although breeding for stable production overyears and locations, and seeking resistance to various constraints have the highest priority, breeding for yield is itself important, particularly for areas where there are few constraints, or where progressive farmers can afford such inputs as insecticides and fungicides. High-yielding lines are also needed in the constraint-based breeding programs and to counteract the rising costs of cultivation.

Advanced breeding populations are evaluated in two different seasons at ICRISAT Center. In the rainy season they are evaluated under two production levels: high input (60 kg P_2O_5/ha with supplementary irrigation and insecticidal sprays when required) and low input (20 kg P_2O_5 /ha rainfed with no insecticidal sprays), and in the postrainy season under high input only. In the early generations there is very mild selection for yield. In the later generations, pod shape and seed size are used as selection criteria in addition to yield. Most of the material is bulked for further evaluation and selection by cooperators in national programs.

Several high-yielding lines with acceptable pod and seed characteristics and good shelling percentage have been developed. Based on consistently good performance, 62 lines have been entered in national trials in India. Lines ICGS 30 and 21 have given pod yields of over 6500 kg/ha which compare well with the 5500 kg/ha of the control cultivars, J 11, and Robut 33-1.

High-yielding lines suitable for rainy season use have also been developed. ICGS 50, 30, and 1 did well under both low input and high input conditions at ICRISAT Center and several are under test in Indian national trials.

Earliness and Dormancy

In the SAT, growing seasons can be very short because the rains do not last long. Earliness coupled with good seed size and yield would provide stable production in such years. Two early Spanish types (Chico and 91176) and a mid-early Virginia line (Robut 33-1) were crossed with other high-yielding bunch and runner types. Useful high-yielding, early-maturing material has been generated.

Utilization of wild *Arachis* Species

The groundnut program is the only ICRISAT program to include a cytogenetics unit. The unit's main aim is to tap the wealth of resistance genes in the wild species. The first, and still the major emphasis, is on resistance to the leaf spot diseases. Work began at Reading University in 1973, and still continues, as a collaborative program with ICRISAT. Since the cytogenetics program at ICRISAT got underway there has been a change of emphasis at Reading where more basic aspects are now studied. Many more types of resistance have been identified in wild species since 1976, and are being used in the crossing program at ICRISAT. However, desirable hybrids cannot be produced, and details of research in this field will be presented later in the workshop.

Cooperation with National Programs

In order to conduct trials under different climatic conditions, or where there is a different pattern of pests and diseases, the groundnut program conducts trials at many locations within India; on farmers' fields, on university farms, and at many national research stations. In some cases, these sites are the only possible location for the trial e.g., screening trials for pests or diseases which do not occur at ICRISAT. The program could not function adequately without this cooperation.

Advanced lines are submitted for entry to the All India Coordinated Research Program on Oilseeds (AICORPO) trials. ICRISAT provides one of the sites for AICORPO trials. Their results provide very valuable information on the comparative performance of ICRISAT and other material throughout India.

We have close links with the Directorate of Oilseeds Research and several universities. We have reciprocal visits with scientists from these institutions and we have provided training in specialist skills to many of them. We recently held a group discussion on 'Management of Pests and Diseases of Groundnut with Special Reference to Resistance Breeding', in which 50 scientists from India partici-

pated. Our research team in Malawi based at Chitedze Research Station, Lilongwe, has been mentioned earlier, and we are also in touch with other research institutions such as those in Brazil and the USA. This is the second major international workshop held by the groundnut improvement program; we had a general groundnut workshop in 1980, and plan to hold one on rust disease in 1984.

Conclusions

ICRISAT has an active groundnut improvement program, based on interdisciplinary research involving breeding, cytogenetics, physiology, pathology, entomology, and microbiology. There is close cooperation with the Genetic Resources Unit. An ICRISAT Regional Groundnut Program for Southern Africa was established in Malawi in 1982, and contact with other groundnut scientists has been maintained through visits and correspondence.

Many scientists have visited ICRISAT; some on short visits to concentrate on one aspect of research or to learn new techniques, others for longer periods to study a particular discipline in more depth. ICRISAT scientists publish journal articles on their work and visit other groundnut-producing countries whenever possible. ICRISAT publishes annual reports and miscellaneous bulletins, and organizes workshops, group discussions, training courses and breeders meets to stimulate the free flow of information.

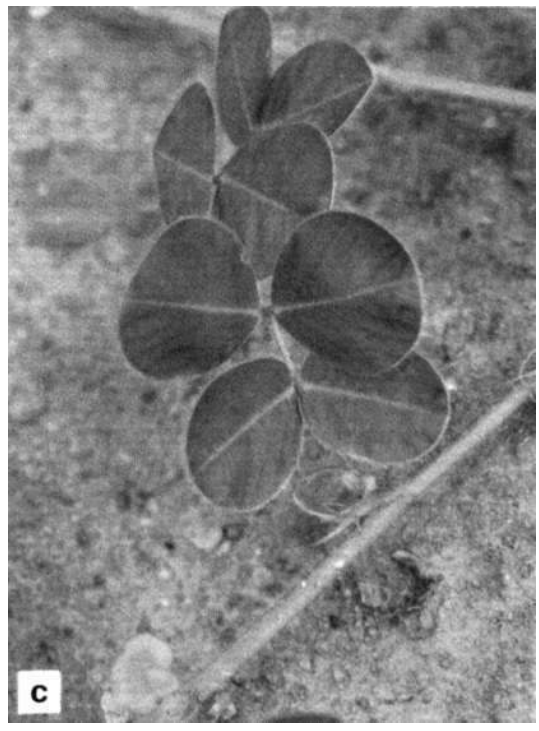
ICRISAT also provides material for research. This includes germplasm accessions with desirable characters, such as pest and disease resistance; early generation material produced by ICRISAT breeders, from which material adapted to local conditions can be developed, and advanced generation material. Information returned to ICRISAT on the performance of these lines helps us send material suited to local conditions in other recipient countries.

Wild Genetic Resources

Plate 1. a. *Arachis burkartii* VMoSi 7317, collected in Rio Grande do Sul, Brazil, (p. 21).
b. *Arachis stenosperma* VSMoGeSi 7379 collected at Ponta da Pinta, Parana Brazil, (p. 18).
c. Screening *A. batizocoi* for thrips resistance, (p. 121).


a


b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Current Status of Collection and Conservation of South American Groundnut Germplasm with Emphasis on Wild Species of *Arachis*

J.F.M. Vails[1], V. Ramanatha Rao[2], C. E. Simpson[3] and A. Krapovickas[4]

Abstract

The value of wild species of Arachis *in groundnut improvement is well recognized. Thus the* Arachis *germplasm available in South America needs to be collected, conserved, and evaluated. Information on the geographic distribution and frequency of collection of wild species germplasm is fairly well known. A number of collections have been made to increase available* Arachis *germplasm. Most of the collected material has been conserved and is being distributed. Evaluation is in progress at various research centers. Priority areas and target species for future collection have been established. For effective utilization of genetic resources for groundnut improvement, complete documentation, exchange of information, and full international cooperation are essential.*

Résumé

Situation actuelle de la collection et de la conservation du germplasme d'arachide, plus particulièrement des espèces sauvages d'*Arachis *d'Amérique du Sud : Etant donné la valeur reconnue des espèces sauvages d'*Arachis *pour l'amélioration de l'arachide, il paraît nécessaire de collecter, conserver et évaluer les ressources génétiques d'*Arachis *disponibles en Amérique du Sud. La répartition géographique ainsi que la fréquence de collecte du germplasme des espèces sauvages sont assez bien connues.

Un certain nombre d'expéditions de collecte ont été effectuées afin d'accroître l'accès au patrimoine génétique du genre* Arachis. *La plus grande partie du matériel collecté a été conservée et est en cours de distribution. Son évaluation est en cours dans plusieurs centres de recherche. Les régions prioritaires ainsi que les espèces-cibles pour les futures expéditions de collecte ont été déterminées. Une documentation complète, des échanges d'informations et une coopération internationale totale s'avèrent indispensables à une bonne utilisation des ressources génétiques pour l'amélioration de l'arachide.

Introduction

Crop improvement is based on the existing variability of the crop itself and, less frequently, on the use of induced mutations. The use of characters from wild relatives of crops is generally a rare event, particularly because of the amount of work needed to overcome interspecific barriers.

The use of wild species in groundnut improvement has been achieved by the release in 1970 of the cultivar Spancross, developed from a cross between *Arachis hypogaea* and *A. monticola* (hammons 1970). Many scientists have studied the crossability of *A. hypogaea* with wild species, and crossability between wild species. Restricted availability of germplasm has been a common

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problem in the use of wild *Arachis* species. Of an estimated 60-70 wild species, only about 20 have been generally available and entire sections of the genus have been sparsely studied due to lack of live material.

A comprehensive paper dealing with crosses between species of *Arachis* was published by Gregory and Gregory in 1979. All seven commonly-accepted sections of *Arachis* were among the 91 parents that included 82 wild species. Since then the number of wild accessions has significantly increased and many new species have been added, but some of the parents used by Gregory and Gregory, e.g., Arachis marginata and *A. prostrata*, could not be successfully maintained. *A.prostrata* was made available again in 1981 and *A.marginata* in 1982, (Plate 2b)(Valls 1983). It is important to point out that the names A. prostrata and *A. marginata* have been assigned to many different collections (Gregory et al. 1973) thus creating another serious problem in the incorporation of wild species in breeding programs. Not only is the germplasm scarce, it is often incorrectly identified. The deposition in herbaria of well-identified voucher specimens representing the accessions used for research may help to solve such problems. In *Arachis*, however, only a few names have been validly published and most users refer to their material by collector numbers.

This paper provides information on the geographic distribution and frequency of germplasm collection of species of *Arachis* in South America, the present conservation status of the accessions obtained, the evaluation of wild species for useful attributes, and priorities for future collections in terms of areas and species.

Geographic Distribution and Availability of Germplasm

Wild species of *Arachis* are naturally restricted to Argentina, Bolivia, Brazil, Paraguay and Uruguay,

Table 1. Geographic distribution and estimated numbers of available germplasm accessions of wild species of *Arachis*, section *Arachis*.

Section Series Species	Country[1]							
	Brazil[2]				ARG	BOL	PRY	URY
	NE	WC	SE	S				
Arachis Annuae								
A. batizocoi Krap. et Greg.						2		
A. duranensis Krap. et Greg. nom. nud.					8			
A. ipaensis Krap. et Greg. nom. nud.						1		
A. spegazzinii Krap. et Greg. nom. nud.					7			
Arachis spp (12-20 species)	1	10			7	24		
Arachis Perennes								
A. diogoi Hoehne		2						
A. heiodes Mart, ex Krap. et Rig.		10						
A. villosa Benth.[3]				2	4			11
A. correntina (Burkart) Krap. et Greg. nom. nud.						9		?
A. cardenasii Krap. et Greg. nom. nud.						10		
A. chacoense Krap. et Greg. nom. hud.							1	
A. stenosperma Krap. et Greg. nom. nud.			2	4				
Arachis spp (10-14 species)		14				26	3	
Arachis Amphiploides								
A. batizocoi Krap. et Greg.					1			
A. monticola Krap. et Rig.					6			

1. Countries: ARQ = Argentina; BOL = Bolivia; PRY = Paraguay; URY = Uruguay.
2. Regions of Brazil: NE = Northeast; WC = West central; SE = Southeast; S = South.
3. Seven additional germplasm collections of *A. villosa* were accessed from Uruguay but no information is available on their conservation.
? = Occurrence probable.

Table 2. Geographic distribution and estimated numbers of available germplasm accessions of wild species of *Arachis*, sections *Erectoides* and *Rhizomatosae*.

Section Series Species	Country[1]						
	Brazil[2]			ARG	BOL	PRY	URY
	WC	SE	S				
Erectoides Trifoliolatae							
A. guaranitica Chod. et Hassl.	1					P	
A. tuberosa Benth.	3						
Erectoides Tetrafoliolatae							
A. benthamii Handro	1	?					
A. martii Handro	P						
A. paraguariensis Chod. et Hassl.	2					6	
A. oteroi Krap. et Greg. nom. nud.	1						
Arachis spp (6-8 species)	33	?			8		
Erectoides Procumbensae							
A. rigonii Krap. et Greg.					1		
A. lignosa (Chod. et Hassl.) Krap. et Greg. nom. nud.						1	
A. appressipila Krap. et Greg. nom. nud.	6				?		
Arachis spp (2-3 species)	11				8		
Rhizomatosae Prorhizomatosae							
A. burkartii Handro			15	P			4
Rhizomatosae Eurhizomatosae							
A. glabrata Benth.	24	4	?	P		2	
A. hagenbeckii Harms.				2		1	
Arachis spp (2-3 species)	27	6	?	6		28	

1. Countries: ARG = Argentina; BOL = Bolivia; PRY = Paraguay; URY == Uruguay.
2. Regions of Brazil: WC = West central; SE = Southeast; S = South.
P = Occurrence confirmed but no viable accessions available.
? = Occurrence probable.

although some species such as *A. repens* and *A. glabrata* are now cultivated elsewhere.

Tables 1,2, and 3 indicate the known geographic distribution of wild species in the above countries. The information for Brazil is presented region-wise as it covers a larger area. Information on distribution is inferred from reports of previous collections. In some cases there is doubt about identity or location, but the presence is considered as probable. In others (P), the presence is established on the basis of previous collections either for herbaria, gene bank or both, even if viable germplasm is no longer available.

The highest number of species occurs in Brazil (Tables 1, 2, 3) and four sections of the genus *Arachis* are restricted to that country (Table 3). Bolivia has the second highest number of species, followed by Paraguay, Argentina and Uruguay. Most species occurring in Brazil are restricted to the west central region, but a group of endemic species occurs in the semi-arid northeast.

A wide range of environmental conditions are suitable for species of *Arachis*. The widely-accepted idea of well-drained sandy soils as the most appropriate environment for *Arachis* is clearly biased and refers to the ecological preferences of the cultivated groundnut. Wild *Arachis* species can grow on rock outcrops, layers of laterite pebble, heavy soils, ill-drained areas, and even in running water. They occur in both open and shady areas, ranging from near the equator to 34 °S and from sea level to almost 1600 m. Such wide ecological amplitude is obviously based on greater genetic variability than that presented by the cultivated species. The first step in the utilization of this variability for crop improvement is to assemble it in gene banks. Efforts to bring together the distinct species, each represented by several accessions

Table 3. Geographic distribution and estimated numbers of available germplasm accessions of wild *Arachis* species, sections restricted to Brazil.

Section Species	Regions of Brazil[1]				
	N	NE	WC	SE	S
Ambinervosae					
Arachis spp (2 species)	P	14		1	
Caulorhizae					
A. repens Mandro	C	C	C	5	C
A. pintoi Krap. et Greg. nom. nud.		2	5	5	
Extranervosae					
A. lutescens Krap. et Rig.			10		
A. marginata Gard.			2		
A. prostrata Benth.			10		
A. sylvestris (Chevalier) Chev.	?	17	3	1	
A. villosulicarpa Hoehne			2		
A. macedoi Krap. et Greg. nom. nud.				1	
Arachis spp (5-8 species)		10	19	1	
Triseminalae					
A. pusilla Benth.	9				

1. Regions of Brazil: N = North; NE = Northeast, WC = West central; SE - Southeast; S = South.
P = Occurrence confirmed but no viable accessions available.
C = Occurrence confirmed but only under cultivation.
? = Occurrence probable.

were started by Archer in 1936, followed by Stephens and Hartley in 1948, Krapovickas in 1950, 1953, 1957, and 1958, and many other collectors (Gregory and Gregory 1979; Simpson 1982).

Collections of *Arachis* Germplasm in South America

Table 4 summarizes the areas covered by expeditions undertaken since 1958, indicating the numbers of wild and cultivated groundnut accessions obtained. Figure 1 illustrates the location of species collected prior to 1959. Figures 2 to 8 detail the routes followed during the 1959 and subsequent expeditions, and the main achievements such as location of new species, first germplasm collection of a species, or recollections of lost germplasm, or germplasm where there is only one accession in collections (Plates 1 b,2c).

The expedition undertaken in 1959 (Fig. 2) resulted in the first germplasm collections of six previously-known species and five new species; four of these have already had their future name released in the specialized literature. A further species, as yet unnamed was also collected in the northernmost area reached by the expedition. From 1961 to 1967 (Fig. 3) the germplasm of at least eight species, five new and three already known, was collected for the first time. The future names of four of these new species are already in current use. Germplasm of a new species in section Ambinervosae was collected for the first time in 1967. The 1968 expedition (Fig. 4) resulted in the collection of germplasm of one previously-described and one still undescribed but already-named species, and the gathering of several undescribed and unnamed species from section Erectoides.

Many new wild species resulted from expeditions made from 1976 to 1979 (Fig. 5). Most of these new species are from previously-unexplored regions of Bolivia and some are from the edges of the Pantanal in Brazil. The 1976 expedition collected typical A. diogoi germplasm and recollected A. lutescens (Plate 2a). The main highlights of the expeditions in 1980 and 1981 (Fig. 6) were the first collections of germplasm of A. sylvestris, the discovery of a new species of the Ambinervosae in northeast Brazil and several new species of section Arachis series Annuae and Perennes and section *Erectoides* series *Trifolioiatae* and

Procumbensae, in west central Brazil. Germplasm of *A. tuberosa*, *A. lutescens*, and *A. prostrata*, which no longer existed in gene banks, was collected. In 1982 (Fig. 7), *A. marginata* was relocated and collected along with additional unnamed species from section Extranervosae and a new annual species from section *Arachis* which penetrates northeast Brazil representing a quite surprising extension of the area of natural occurrence for that section. The 1983 expeditions collected germplasm of two unidentified and possibly new species, from sections *Extranervosae* and *Erectoides* series *Tetrafoliolatae*. In addition to the collections illustrated in Figures 2 to 8, each expedition has provided a variable number of additional germplasm collections of previously-available species. The total collections of both wild and cultivated groundnuts are shown in Table 4.

As a direct consequence of this intensive collecting work in South America, the known areas of

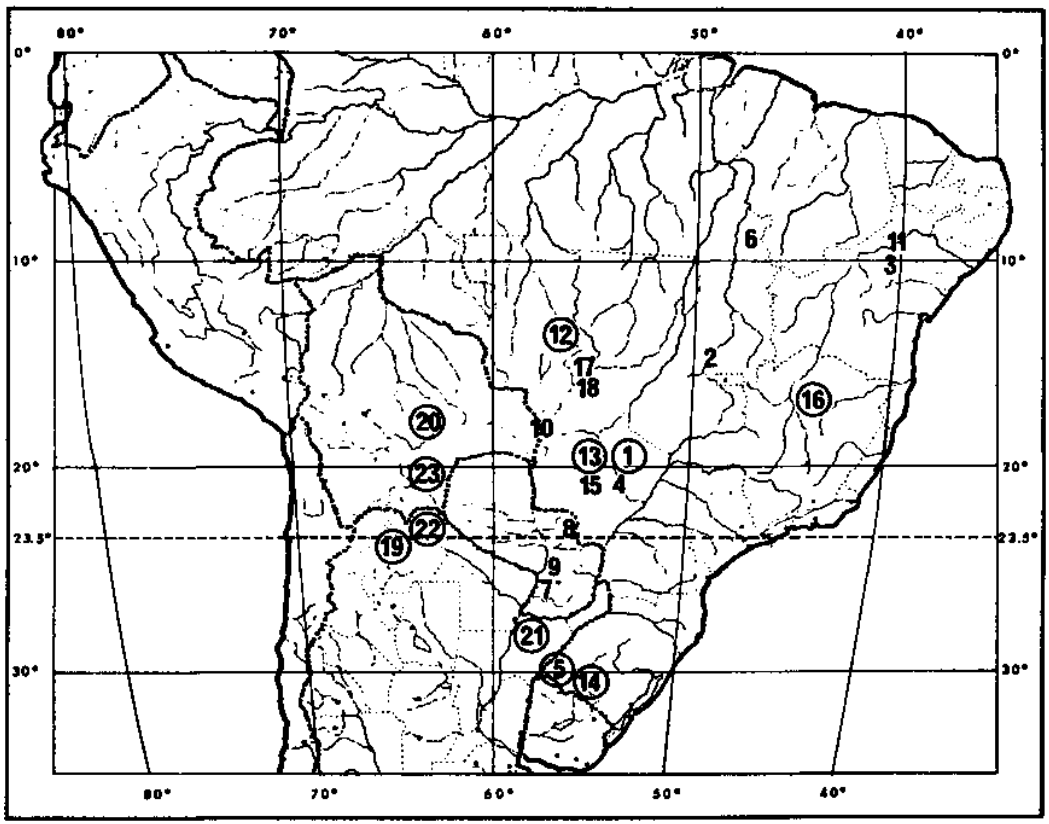

Figure 1. Parts of South America showing collections of germplasm of wild species of *Arachis* prior to 1959. Circles indicate availability of germplasm prior to 1959.

***Arachis* species collected:**

1. *glabrata*
2. *prostrata*
3. *pusilla*
4. *tuberosa*
5. *viliosa*
6. *marginata*
7. *hagenbeckii*
8. *guaranitica*
9. *paraguariensis*
10. *diogoi*
11. *sylvestris*
12. *villosulicarpa*
13. *benthamii*
14. *burkartii*
15. *martii*
16. *repens*
17. *heiodes*
18. *lutescens*
19. *monticola*
20. *rigonii*
21. *correntina*
22. *duranensis*
23. *batizocoi*

Table 4. Number of accessions of groundnut collected during expeditions in South America (1958-1983).

Year	Collectors[1]	Accessions of wild species/accessions of *A. hypogaea*					
		Argentina	Bolivia	Brazil	Peru	Paraguay	Uruguay
1958	K	1/22					
1959	GKP	4/23	3/30	127/64	0/9	24/90	
1961	GKP			35/56	0/6	12/0	
1964	KC					2/0	
1966	KCPa					8/0	
1967	QK			8/N			
1968	HLKPO	11/N		42/N		1/N	6/N
1976	GK	7/0		37/0			
1977	GKBSPScZ	9/7	34/111	20/0		31/8	
1979	GKSPScGb		7/24	0/1			
1980	SKBScCoZJA	8/0	30/102		0/27		
1980	MSv			0/22			
1980	V			2/17			
1981	SPZ				0/193		
1981	V			6/9			
1981	WeSV			7/21			
1981	VSGr		1/0	40/9			
1981	PZi		0/40				
1982	VKRSv			38/7			
1982	ScVn	10/0					
1982	VS			17/20			
1983	VKVeSv			27/1			
1983	SKSc	(6/55)					
1983[2]	VSMoGeSv			18/15			

1. Collectors: A = Arriola; B = Banks; C = Cristobal; Co = Coradin; G = Gregory; Gb = Gibbons, Ge = Gerin; Gr = Gripp; H = Hammons; He = Hemsy; J = Janicki; K = Krapovickas; L - Langford; M = Miranda; Mo = Moss; 0 == Ojeda; P = Pietrarelli; Pa = Palacios; R = Rao; S = Simpson; Sc = Schinini; Sv = Silva; V = Valls; ve = Veiga; Vn = Vanni; Z = Zurita; Zi = Zanini.
2. A collecting expedition to Ecuador was undertaken by Banks, Pietraelli and Zurita in 1983 during which 52 accessions of/. hypogaea were collected.

N = Information not available.

natural occurrence of the sections were modified from the map presented by Gregory et al. (1980). As shown in Figure 9 the known area of section *Arachis* now extends all the way to the edge of the semi-arid Brazilian northeast and to northern Bolivia, while the *Caulorhizae* are not restricted to the valley of the river Jequitinhonha but also occur in the valleys of the Sao Francisco and Tocantins rivers. However, the area of the *Triseminalae* still remains within the broad limits anticipated by Gregory and his associates.

The areas of sections *Erectoides* and *Extranervosae* (Fig. 10) were expanded; in the case of the *Extranervosae*, most of the expansion is due to the recently-revealed widespread distribution of A. sylvestris. The expansion of the *Erectoides* area is largely because new material of series *Procumbensae* was located in east Bolivia and north of the Pantanal in Brazil and also due to the discovery of a tetrafoliolate species further east of the previous limits.

Figure 11 shows the presently-known areas of natural distribution of sections *Ambinervosae* and *Rhizomatosae*. The area of the *Ambinervosae* shows a significant expansion to extend southward to the valley of the Jequitinhonha river overlapping with the *Caulorhizae* and *Triseminalae* (Fig. 9). The distribution of series *Eurhizomatosae* remains as presented by Gregory et al. (1980), while series *Prorhizomatosae* represented by *A. burkartii* (Plate 1 a) was found to extend eastward all the way to the Brazilian coast.

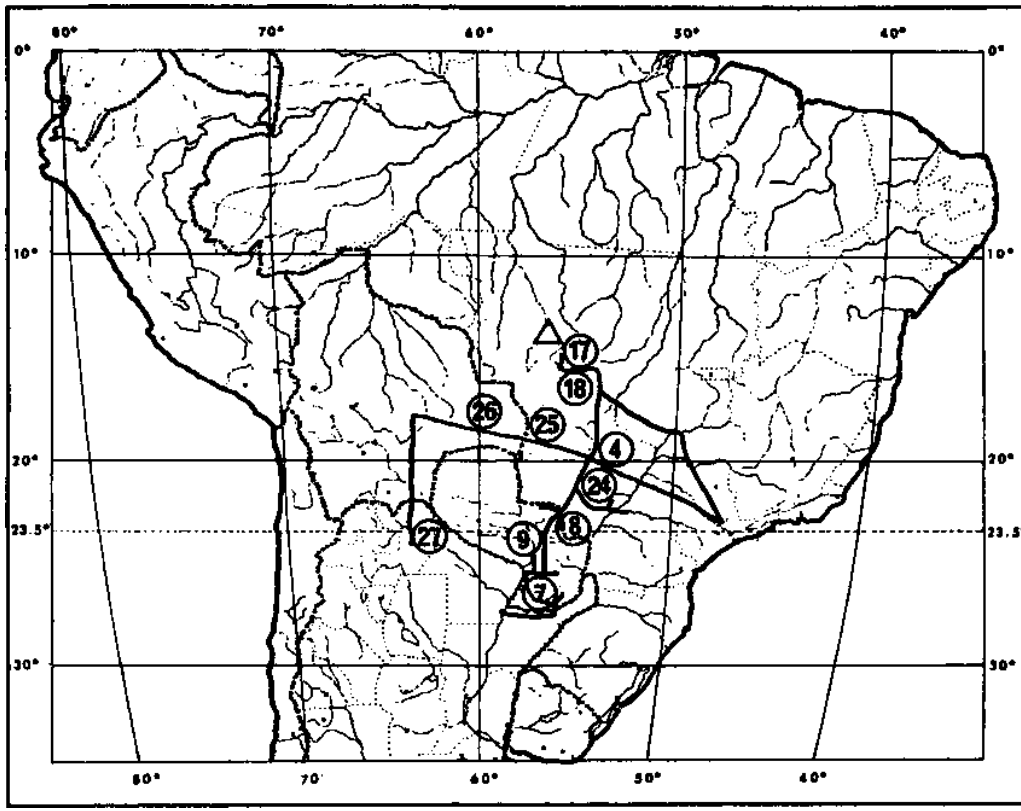

Figure 2. Parts of South America showing the route followed during the expedition in 1959.

***Arachis* species collected: New species; 24. *oterio*, 25. *appressipila*, 26. *cardenasii*, 27. *spegazzinii*, New germplasm: 4. *tuberosa*, 7. *hagenbeckii*, 8. *guaranitica*, 9. *paraguariensis*, 17. *helodes*, 18. *lutescens*, △ Unidentified.**

On the basis of present knowledge, no section can be considered geographically isolated.

Conservation

The main centers of conservation for wild Arachis species are in India, Brazil and USA. In India, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), maintains a collection of wild species as part of its world mandate to conserve groundnut germplasm. Partially supported by the International Board for Plant Genetic Resources (IBPGR), Centro Nacional de Recursos Geneticos (CENARGEN) of the Empresa Brasiliera de Pesquisa Agropecuaria (EMBRAPA) in Brazil holds a live collection of wild species, which is complemented by seed conservation of both wild and cultivated groundnut as a part of its national program, and includes accessions conserved at the Instituto Agronomico, Campinas (IAC), an associated institution. Wild species in the USA are primarily conserved at Texas A&M, North Carolina State Universities, and Oklahoma State (USDA), as a part of their groundnut improvement programs. A number of wild species are maintained in a live collection at Instituto Nacional de Tecnologfa Agropecuaria (INTA) at Manfredi in Argentina, following collection and subsequent taxonomic and cytological studies.

Table 5 shows the number of accessions held in

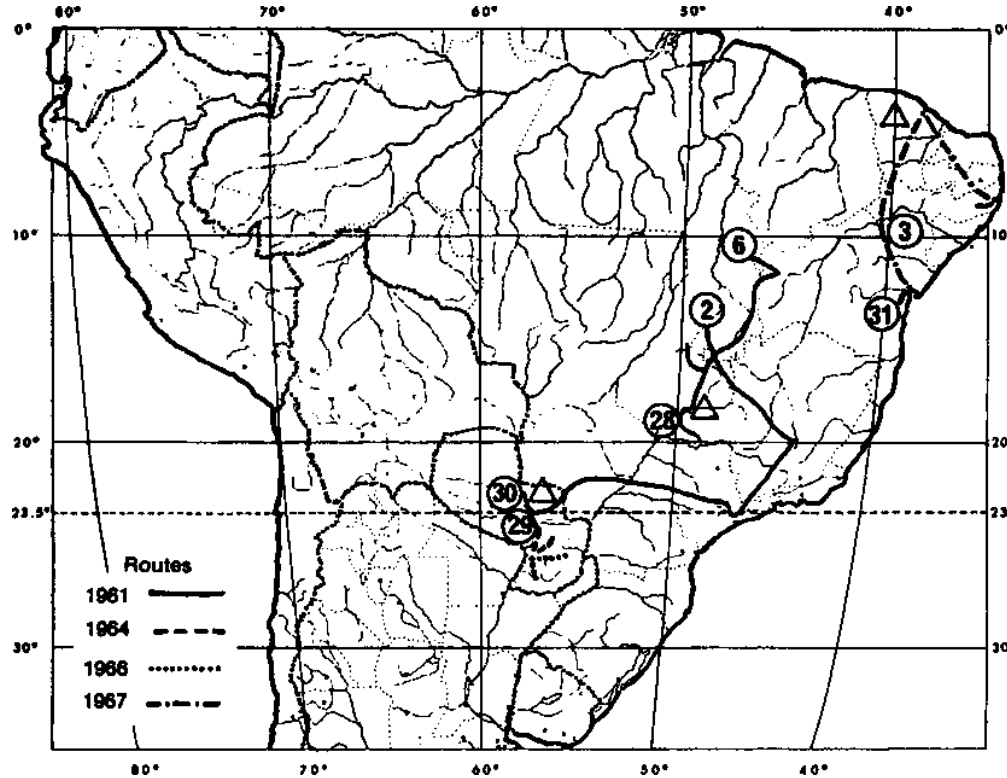

Figure 3. Parts of South America showing routes followed during the expeditions from 1961 to1967.

***Arachis* Species collected: New species; 28. *macedoi*, 29. *lignosa*, 30. *chacoense*, 31. *pintoi*, New germplasm; 2. *prostrata*, 3. *pusilla*, 6. *marginata*, △ Unidentified.**

the USA, Brazil and India, taking into consideration material available mainly at the major centers of conservation. From Table 5 it is clear that the rate of success in conservation of wild material varies with the intrageneric groups. Sections *Erectoides* and *Extranervosae* are apparently the most difficult to conserve and this may be related to their critical requirements for maintenance ex situ. Differences in the number of accessions available in each country are related to the fact that most accessions have only recently been collected and are being increased in South America and USA before further distribution, and also to strict quarantine regulations such as those in India, which require a longer time for introduction.

Proper maintenance of collected material to ensure minimum genetic drift is an essential genetic resource activity in conserving germplasm. The techniques presented here are those used by most of the centers which have wild *Arachis* collections. *Arachis* germplasm can be conserved either as seed, which needs to be rejuvenated from time to time and multiplied to meet the demand for supply of germplasm, or as live plants, which also need to be periodically rejuvenated. Conservation and exchange of *Arachis* as meristem culture can be visualized in the future (Sastri et al. 1982). Since *Arachis* is mostly self-pollinated, problems that may arise during rejuvenation due to out-crossing are limited (Hammons 1964; Gibbons and Tattersfield 1969). However, this may depend on the particular environment; bees are known pollen vectors

(Leuck and Hammons 1969). Seed-producing wild species can be maintained by growing at wide spacing in the field, to avoid mechanical mixtures and encroachment. The fruits are collected by digging around the plants and sifting the soil in large sieves. Seed can also be produced from plants grown in pots in a screenhouse. The non seed-producing wild species can be maintained in large pots or concrete rings, inside or outside a screenhouse. It is essential to change the soil periodically since continued watering may lead to salinity. Periodic pruning and replanting are necessary. Native soil conditions for each particular species must be taken into consideration when wild species of *Arachis* are planted for maintenance and multiplication.

Priority Areas for Collection of *Arachis* in South America

As shown in Figures 1 to 8 a significant area of South America has now been explored for the collection of wild and cultivated groundnut germplasm. However, rains, floods, drought, frosts, heterogeneous behavior of sympatric species, lack of adequate vehicles, unexpected delays, and insufficient assistance in the field have, from time to time, imposed restrictions on the field work, and even caused losses of already-collected material. A few losses during multiplication and conservation are always to be expected and future recollection is then necessary. Therefore, not only new

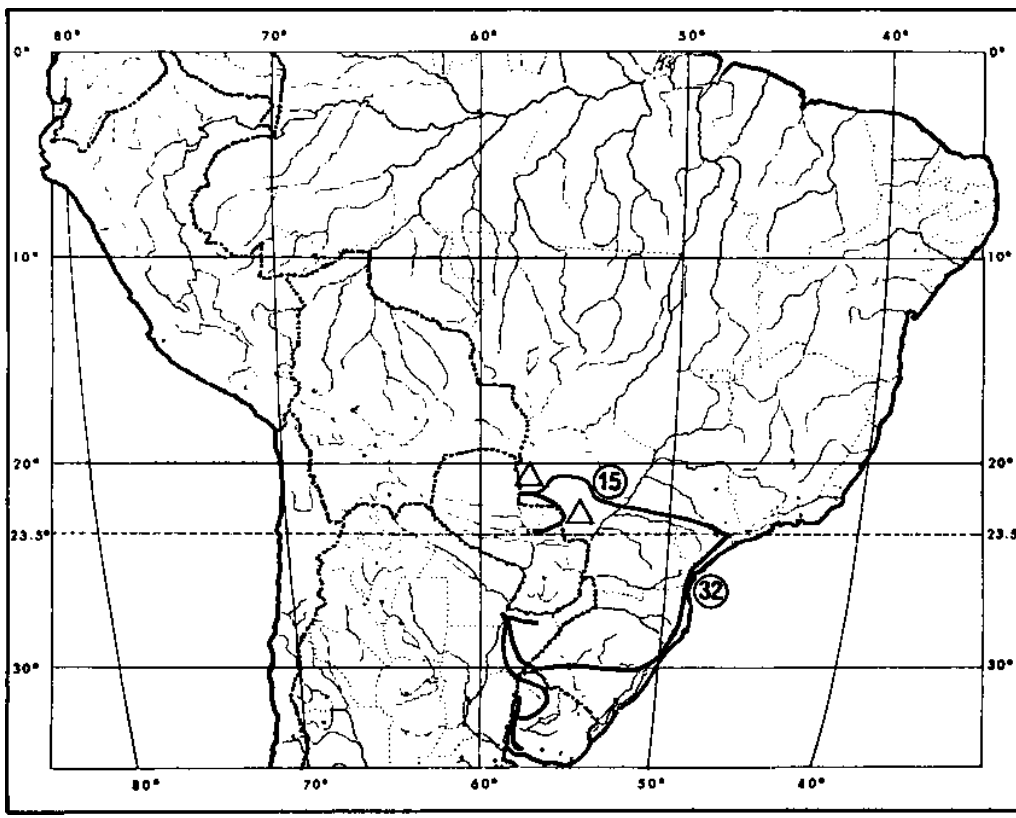

Figure 4. Parts of South America showing the route followed during the expedition in 1968.

***Arachis* species collected: New species; 32. *stenosperma*, New germplasm; 15. *martii*, △Unidentified.**

Table 5. Germplasm of wild *Arachis* collected between 1936 and 1983 and conserved at the main centers of conservation.

Section Series Species	Acc.[1] Coll.	Acc. Conserved[2] USA	BRA	IND	Total	Section Series Species	Acc. Coll.	Acc. Conserved USA	BRA	IND	Tot.
Arachis Annuae						*Erectoides Tetrafoliolatae*					
A. batizocoi	2	2	2	1	2	*A. benthamii*	10	1	1	0	1
A. duranensis	8	8	1	1	8	*A. martii*	1	0	0	0	0
A. ipaensis	2	1	1	1	1	*A. paraguariensis*	10	7	5	3	7
A. spegazzinii	7	2	2	7	7	*A. oteroi*	23	1	0	0	1
A. spp (12-20)	44	41	32	23	42	*A.* spp (6-8)	102	39	31	11	42
Arachis Perennes						*Erectoides Procumbensae*					
A. diogoi	2	2	2	0	2	*A. rigonii*	1	1	1	1	1
A. helodes	12	10	9	2	10	*A. lignosa*	1	1	1	0	1
A. villosa	18	7	5	3	10	*A. appressipila*	8	5	6	5	6
A. correntina	13	8	4	4	8	*A.* spp (2-3)	21	19	14	2	19
A. cardenasii	10	10	1	1	10						
A. chacoense	1	1	1	1	1	*Extranervosae*					
A. stenosperma	6	6	5	2	6	*A. lutescens*	24	9	8	0	9
A. spp (10-14)	46	46	27	8	46	*A. marginata*	3	2	2	0	2
						A. pro strata	14	7	9	0	9
Arachis Amphiploides						*A. sylvestris*	22	6	21	0	21
A. batizogaea	1	1	1	1	1	*A. villosulicarpa*	2	2	2	1	2
A. monticola	6	6	3	3	6	*A. macedoi*	1	1	1	0	1
						A. spp (5-8)	33	18	28	0	28
Ambinervosae											
A. spp (2)	20	8	15	0	15	*Rhizomatosae Prorhizomatosae*					
						A. burkartii	23	12	13	0	19
Cauiorhizae											
A. repens	5	4	5	1	5	*Rhizomatosae Eurhizomatosae*					
A. pintoi	12	7	12	0	12	*A. glabrata*	45	22	24	12	36
						A. hagenbeckii	7	4	2	7	7
Erectoides Trifoliolatae						*A.* spp (2-3)	134	67	17	39	67
A. guaranitica	4	1	0	0	1						
A. tuberosa	5	2	2	0	3	*Triseminalae*					
						A. pusilla	9	5	9	1	9

1. Acc. Coll. = Number of accessions collected.
2. Numbers ot accessions conserved in:USA = Texas A&M Univ., NCSU, and USDA; BRA = CENARGEN/EMBRAPA and IAC, Brazil; IND = ICRISAT, India.

areas, but also some previously-covered areas still need collecting.

The selection of priority areas aims at defining zones that can be suitably covered in a single field mission. The main reasons for assigning priorities are the belief that an area has one or more species under threat of genetic erosion, and that this germplasm is important for crop improvement. On the other hand, due to the scarcity of qualified collectors, some areas that have important species may have to be assigned a lower priority, If land development is not rapid and agricultural patterns tend to remain stable for many years.

Following these guidelines, a very high priority is assigned to the southern part of the state of Mato Grosso do Sul in Brazil, even though several previous expeditions have covered the area. Conservation of the germplasm obtained in that area was not very successful and, as a consequence, most of the collected germplasm of *A. benthamii*, *A. martii* and *A. oteroi* was lost. The area is rich in species from section *Erectoides*, but the drastic change in agricultural patterns in Mato Grosso do Sul will certainly lead to the extinction of many populations if not species of *Arachis*. Local landraces of the cultivated groundnut also are doomed to disappear

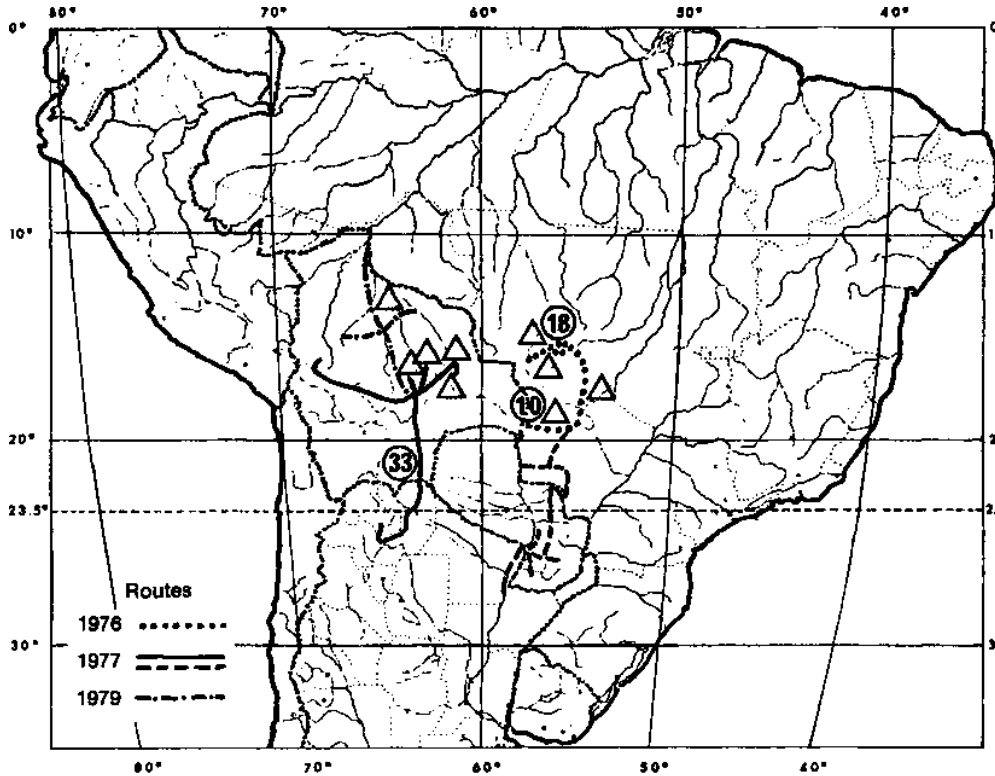

Figure 5. Parts of South America showing routes followed during the expeditions in 1976 to 1979.

***Arachis* species collected: New species; 33. *ipaensis*, New germplasm; 10. *diogoi*, 18. *lutescens*, △ Unidentified.**

in a few years, since mechanized crops like soybeans, now introduced to this area, are quickly replacing every local crop, even in backyards.

The following list of priority areas is based on the above considerations. However, it can be taken as only a general guide, always subject to modification as conditions change. The areas are shown in Figure 12.

Priority 1.

Area 1. Brazil: Eastern part Mato Grosso State and Ilha do Bananal (Goias State) - to cover the gap between the main area of occurrence of section *Arachis* series *Annuae* and the area of a recently-found annual species of section *Arachis* in north Goias and south west Maranhao.

Area 2. Brazil: Southern part of Mato Grosso do Sul State - to recollect germplasm of species of section *Erectoides* series *Trifoliolatae* and *Tetrafoliolatae*, which are under serious threat of genetic erosion.

Area 3. Brazil:North western Mato Grosso State- to collect additional germplasm and determine the conditions for natural occurrence of *A. villosulicarpa* (Plate 3a), so far unknown in the wild. Also to collect landraces of *A. hypogaea*.

Area 4. Paraguay: East of the Paraguay river - to recollect germplasm of many species under threat

of genetic erosion, though not so drastic as that in Mato Grosso do Sul, Brazil.

Area 5. Paraguay: West of the Paraguay river - to collect additional germplasm of *A. chacoense*, and of several additional annual and perennial species of section *Arachis*, and possibly also of *A. lignosa*.

Priority 2.

Area 6. Brazil: Rondonia and Acre States - to collect landraces of *A. hypogaea* which are quite variable in the area and are threatened by aggressive land development.

Area 7. Brazil: Western part of Minas Gerais State (Triangulo Mineiro) and valley of the Rio Grande in Mato Grosso do Sul and Sao Paulo States - to collect additional germplasm of *A. macedoi* and of several species of section Extranervosae and section *Erectoides* series *Tetrafoliatae* of which germplasm is not available.

Area 8. Brazil: Northwestern part of Goias, Maranhao, and northern Piaui States - to collect additional germplasm and define the limits of eastward distribution of an undescribed annual species of section *Arachis* recently found in the area. Also to collect additional germplasm of several species of sections *Ambinervosae* and *Extranervosae* and landraces of *A. hypogaea*. Special interest is assigned to *A. marginata*, of which only two populations are known to exist and are under serious threat of extinction due to agricultural

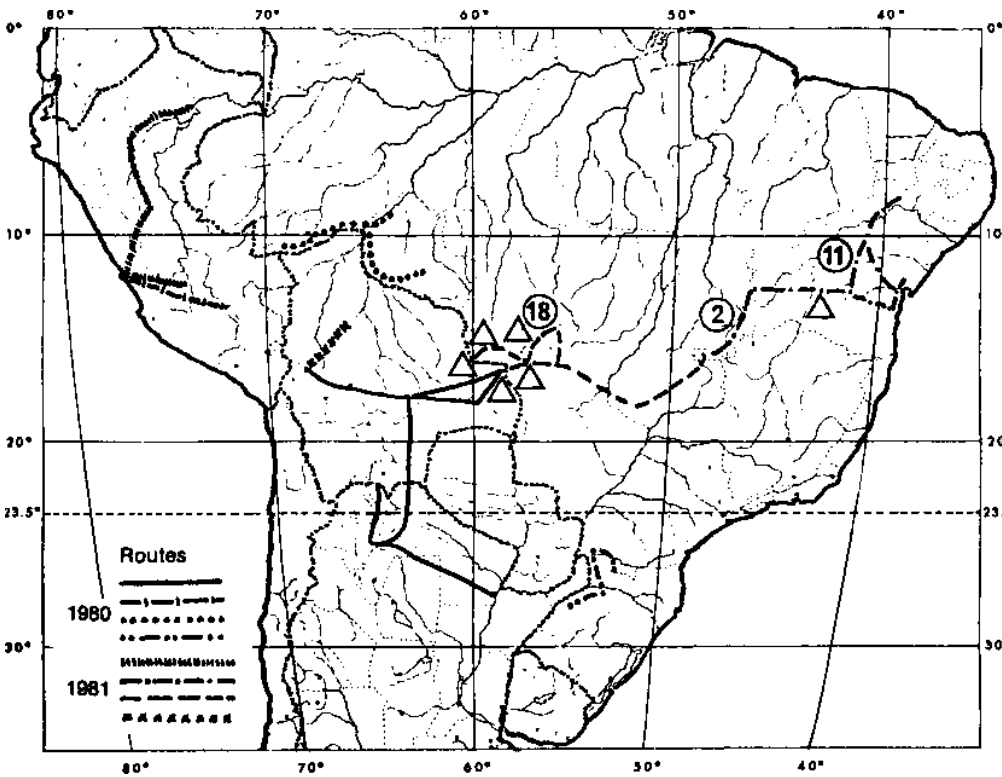

Figure 6. Parts of South America showing routes followed during the expeditions in 1980and 1981.

***Arachis* species collected: New germplasm; 2. *prostrata*, 11. *sylvestris*, 18. *lutescens*, △Unidentified.**

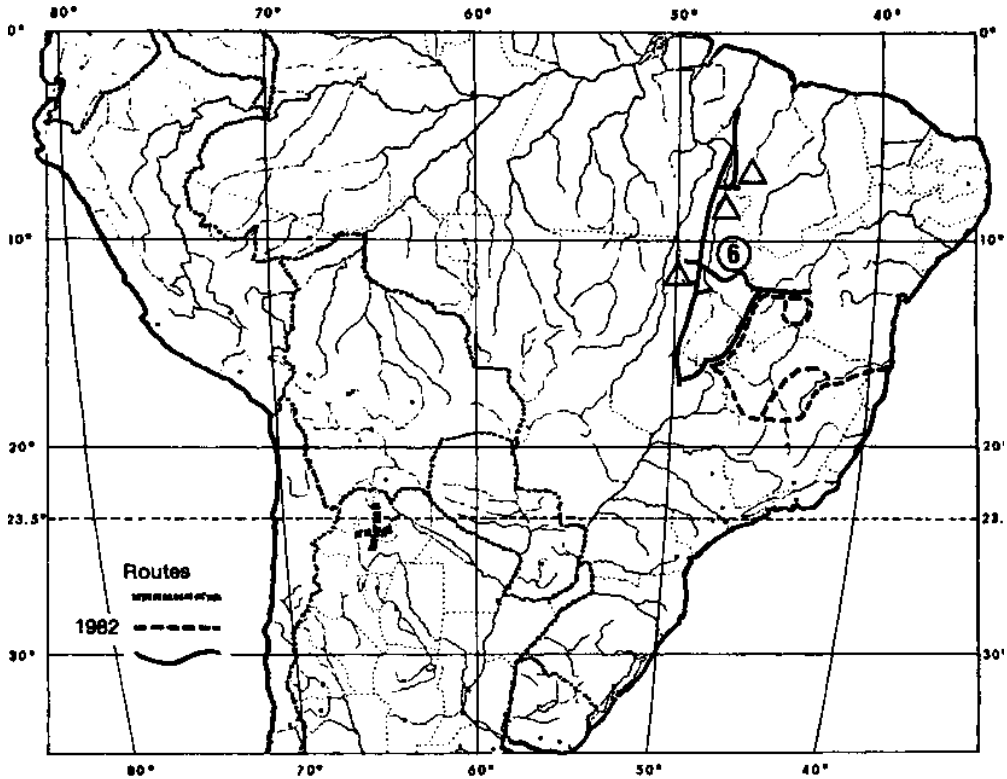

Figure 7. Parts of South America showing routes followed during the expeditions in 1982. *Arachis* species collected: New germplasm; 6. *marginata*, △Unidentified.

development.

Area 9. Brazil: Ceara, Rio Grande do Norte and Paraiba States - to collect landraces of *A. hypogaea* which are threatened by several years of extreme drought. Also to collect species of sections *Ambinervosae* and *Extranervosae*.

Priority 3.

Area 10. Brazil: Southeastern Amazon region - to collect landraces of *A. hypogaea* along the main tributaries of the Amazon.

Area 11. Uruguay and Brazil: Uruguay, especially along the Uruguay river and extending into west Rio Grande do Sul State in Brazil - to collect additional germplasm of *A. villosa* and of Uruguayan landraces of *A. hypogaea*.

Area 12. Bolivia and Brazil: Northeastern Bolivia along the valleys of the Mamore and Guapore rivers, extending into Brazil, south and west of the Guapore River - to collect additional germplasm of annual and perennial species of section *Arachis*.

Area 13. Bolivia: From the Brazilian frontier to longitude 65°W - to collect additional germplam and to define the limits of distribution of species such as *A. diogoi*, *A. appressipila*, and others in section *Arachis* series *Annuae* and *Perennes*, and section *Erectoides* series *Procumbensae* which are known only from the Brazilian side but probably extend into Bolivia.

Evaluation and Utilization

Thorough collection, maintenance, and conservation are necessary for a complete study of the taxonomic status and evolutionary relationships between and within the species. However, the main justification for such conservation is the utility of the germplasm in groundnut improvement (Rao 1980). A detailed morphological description of any accession is essential to maintain its purity, but the key to successful utilization of variability from broad genetic pools requires a knowledge of desirable attributes available in the germplasm. During the last two decades much emphasis has been placed on collection of wild species and exotic cultivars, though there is still the need to collect more material. But, as emphasis shifts from collection to evaluation and utilization of these genetic resources, progress in improving the productivity, pest resistance, and adaptability of groundnut can be expected (Wynne and Gregory 1981).

Fortunately, considerable variability is available in the cultivated groundnut (Rao 1980). However, resistance to certain diseases, nematodes, toxin-producing molds, and to drought is required (Norden 1980). Systematic evaluation of wild Arachis species to identify such useful attributes, has been in progress in ICRISAT, India; North Carolina State, Texas A&M Universities, and USDA, Stillwater, Oklahoma, USA, and elsewhere. The results indicate the significance of wild species in the improvement of cultivated groundnut. Attempts are already in progress to use these species as sources of resistance to various pests and diseases.

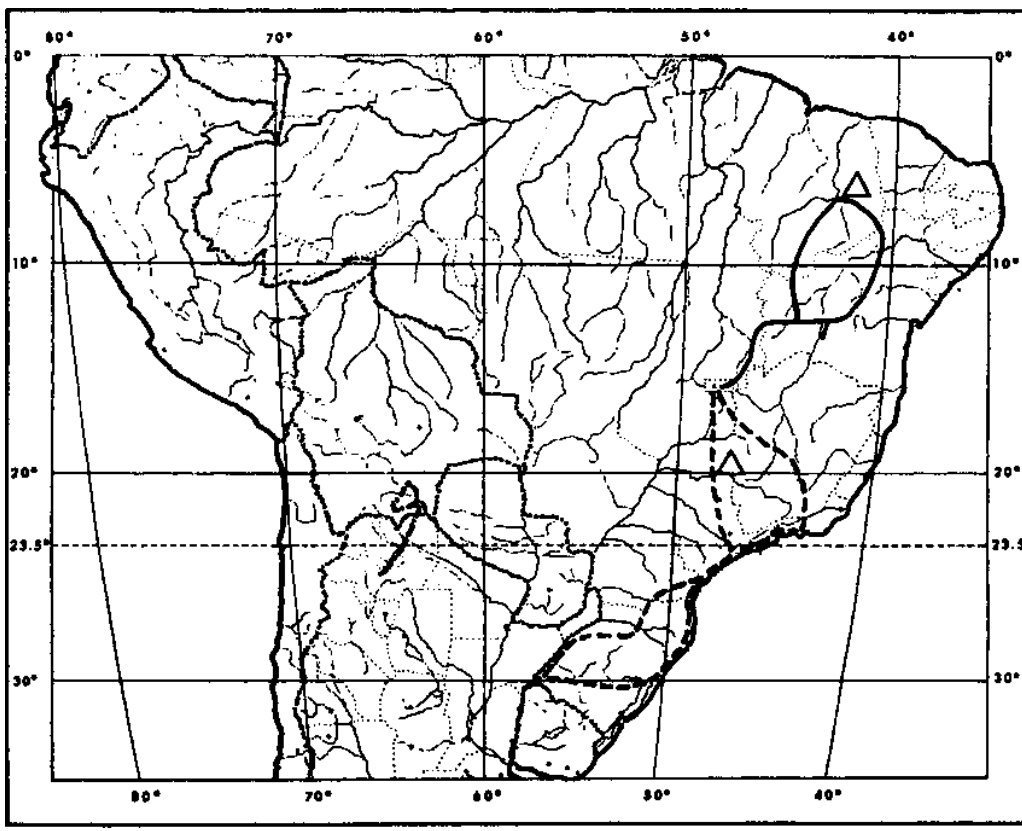

Figure 8. Parts of South America showing routes followed during expeditions in 1983.

△Unidentified *Arachis* species collected.

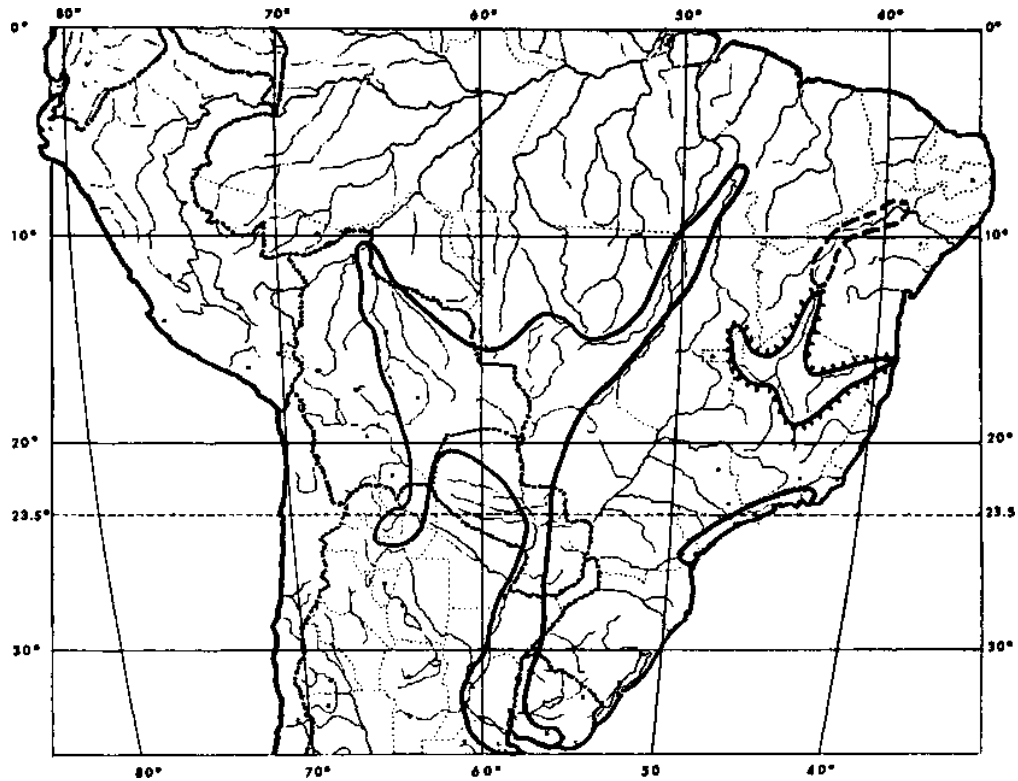

Figure 9. Parts of South America showing known distribution of sections *Arachis* (——), *Caulorhizae* (▪▪▪▪▪▪), and *Triseminale* (– – – –)

They may have mechanisms of resistance that are different from those in the cultivated groundnut. This may provide the possibility of combining the resistance of wild and cultivated species to give more effective and stable resistance in *A. hypogaea* (Subrahmanyam et al. 1983). There are still some gaps in our knowledge of useful attributes in wild *Arachis* species, and therefore systematic evaluation and information flow must be assured to enable full exploitation of the available genetic resources of *Arachis*.

Documentation

The extent of conservation of eroding genetic resources and utilization of the conserved germplasm are the yardsticks to measure progress in genetic resource activity. The progress depends on the evaluation of the conserved germplasm, and partly on the availability of information on the same. With increasingly large numbers of germplasm accessions at many centers, information exchange has assumed global importance (Rao 1980). No standard computer equipment or programs have been adopted across centers.

Though such uniformity is not essential, agreement on certain aspects, such as uniform descriptors, and assembly of existing information would go a long way to improve the efficiency of information retrieval and exchange. For this purpose IBPGR and ICRISAT (1981) have listed standardized groundnut descriptors. However, the existing descriptors need some modifications and additions to fully describe the wild species. This work is in pro-

gress. Publication of catalogs or special lists with notation on species having a combination of desirable attributes would be very useful.

International Cooperation

The collection, conservation, maintenance, and documentation of *Arachis* genetic resources calls for an international approach. This is already in existence as various national agencies in South America, universities in the USA, the USDA, International Agricultural Research Centers, and IBPGR are cooperating to collect and conserve the eroding genetic resources of the genus. Similarly the evaluation work needs international cooperation and this is exemplified in the work on rust and viruses (Ghanekar 1980; Subrahmnayam et al. 1980, 1983). The available genetic resources of *Arachis* at any center need to be freely available for worldwide exchange.

Conclusions

Prior to 1976 activities were based on the cooperative efforts of a few scientists, but international cooperation at institutional level with the support of IBPGR has lead to an acceleration of collecting activities and rapid development of the necessary infrastructure for conservation in well-managed gene banks. Material assembled at the main centers of conservation has a much better chance of survival and characterization. The use of computers is providing better documentation. Consequently, the variability made available to groundnut

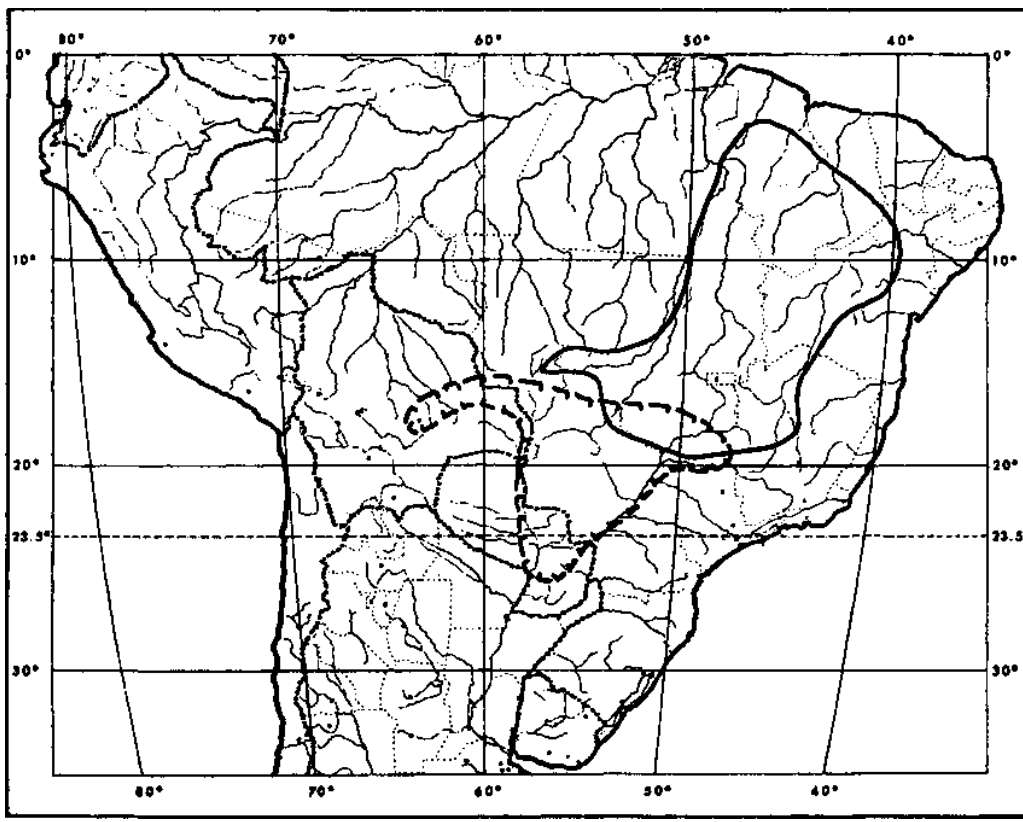

Figure 10. Parts of South America showing known distribution of sections *Erectoides* (▬▬▬) and *Extranervosae* (———).

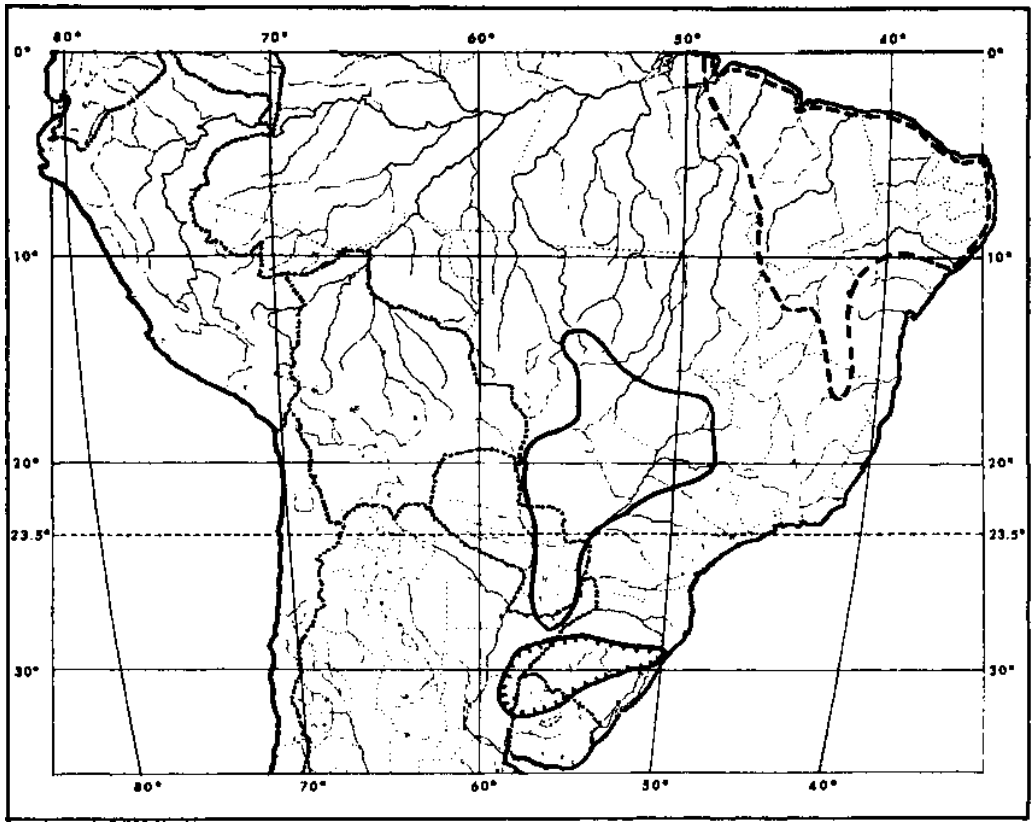

Figure 11. Parts of South America showing known distribution of sections *Ambinervosae* (– – –), *Rhizomatosae/Prorhizomatosae* (‥‥‥) and *Rhizomatosae/Eurhizomatosae* (———).

improvement programs is increasing significantly and is becoming better known. The continuing international cooperation in groundnut germplasm activities needs to be maintained to ensure better utilization of the genetic resources of *Arachis*.

Acknowledgement

The bulk of the collection information presented is drawn and interpreted from a number of published and unpublished informal reports. It is difficult to name all the persons whose reports have been used. Technical reports submitted to IBPGR by various collectors have been a great help. The routes followed in collection expeditions prior to 1976 have been inferred from Gregory et al. (1973).

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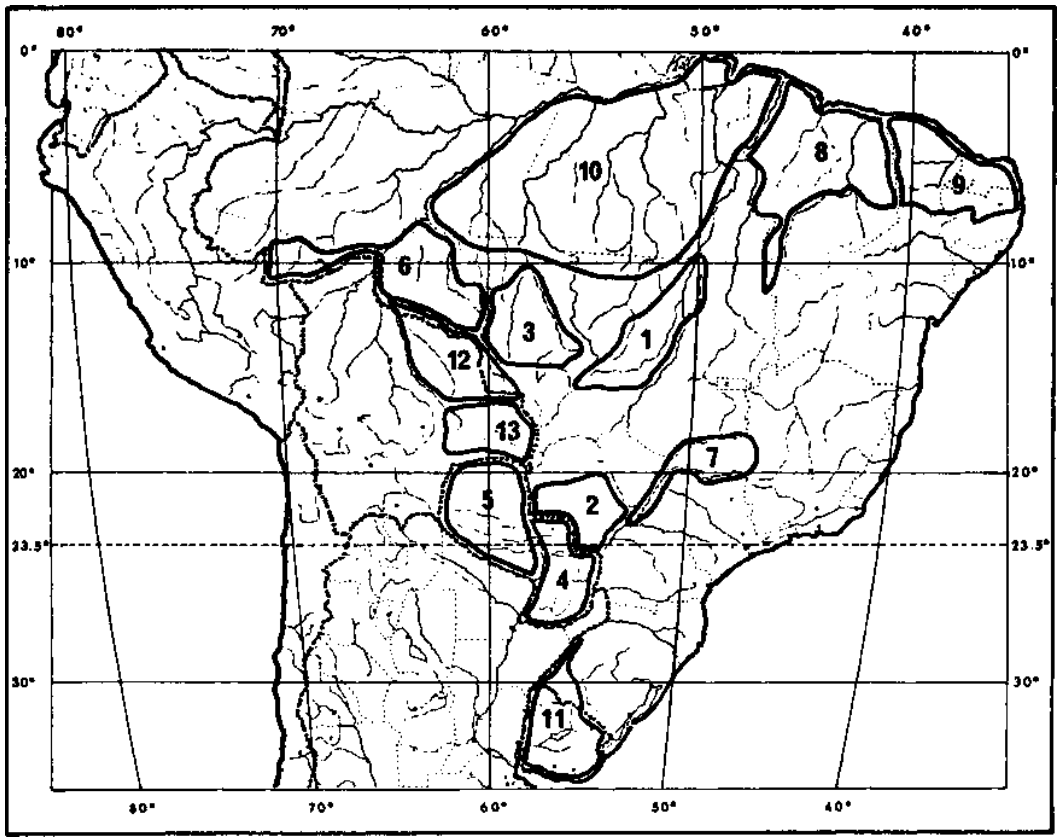

Figure 12. Parts of South America showing priority areas for collection of groundnut germplasm with emphasis on wild species.

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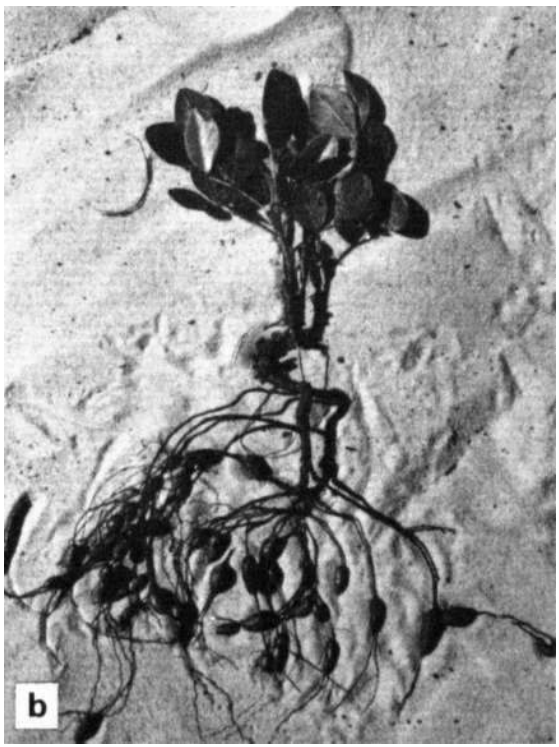
Plate 2. a. *Arachis lutescens* VSGr 6332 collected in Matto Grosso, Brazil, (p. 18).
b. *Arachis marginata* VKVeSi 6649 collected in Goais, Brazil, (p. 16).
c. Collecting *A. stenosperma* at Ponta da Pinta, Parana, Brazil, (p. 18).


a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Wild *Arachis* Genetic Resources at ICRISAT

V.Ramanatha Rao and A.K. Sadasivan[1]

Abstract

The conservation of Arachis germplasm is an urgent need. ICRISAT has been designated as a major repository of Arachis germplasm, with the objectives of collection, maintenance and evaluation of the genetic resources, and the documentation and distribution of material and information.

Wild species of Arachis are acquired through transfer from known genetic resource centers in India and abroad, and also by collecting expeditions. The accessions which reproduce by seed are multiplied by growing in the field. Currently the pods are stored at 4°C with 35% relative humidity. Long-term storage facilities are being built. The rhizomatous accessions are maintained by growing rooted cuttings in concrete containers. A series of descriptors suitable for the evaluation of wild species of Arachis is being developed; meanwhile the groundnut descriptors developed by IBPGR and ICRISAT are being used.

At ICRISAT, wild species are screened against diseases and pests. Those species with resistance are being utilized in the Groundnut Improvement Program. Arachis germplasm is available free of charge to all scientists who wish to use it. The current status of wild Arachis germplasm and the future program are presented.

Résumé

Ressources génétiques des espèces sauvages du genre* Arachis *à l'ICRISAT : La conservation du germplasme d'* Arachis *constitue un problème urgent. L'ICRISAT a été désigné comme le principal dépositaire de ce patrimoine avec pour objectif la collecte, la conservation et l'évaluation des ressources génétiques, la documentation, la distribution de matériel végétal et la diffusion d'informations.

Les espèces sauvages d'* Arachis *proviennent de transferts de centres de ressources génétiques connus en Inde et ailleurs et de missions de prospections. Les introductions qui se reproduisent par graines sont multipliées par culture au champ. Les gousses sont stockées à 4° C et 35% d'humidité relative. Des installations sont en cours de construction pour le stockage à long terme. Les introductions à reproduction végétative sont maintenues par culture de boutures racinées dans des conteneurs de béton. Une série de descripteurs pour l'évaluation des espèces sauvages d'* Arachis *est en cours d'élaboration; en attendant, ce sont les 'descripteurs pour l'arachide' du CIRPG et de l'ICRISAT qui sont utilisés.

A l'ICRISAT les espèces sauvages sont criblées pour la résistance aux ravageurs et aux maladies. Les espèces résistantes sont utilisées dans le Programme d'amélioration de l'arachide. Tous les chercheurs le désirant peuvent avoir accès gratuitement aux ressources génétiques du genre* Arachis*. La situation actuelle et le programme futur concernant le germplasme des espèces sauvages du genre* Arachis *sont présentés.

Introduction

Genetic resources of any crop are the base for crop improvement, and the significance of genetic resources of groundnut is very well understood. *Arachis* genetic resources include all the cultivars and related wild species. The latter are discussed in this paper. The genus *Arachis* L., which is native to South America, has presently 22 described species, including the cultivated groundnut, *A. hypo-*

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gaea L However, recent collecting expeditions in the center of diversity have indicated that there may be 40 or more undescribed annual or perennial species (Gregory et al. 1980). All these constitute a wealth of groundnut germplasm. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), designated as a major world repository of groundnut germplasm, started assembling wild Arachis species in 1976, an activity that has become more aggressive since the formation of the Genetic Resources Unit in 1979 (Rao 1980). The following account describes the present status of wild *Arachis* germplasm at ICRISAT.

Collection and Assembly

Arachis germplasm at ICRISAT is mainly being assembled, through correspondence and transfer from known genetic resource centers. Already a number of wild *Arachis* species have been obtained from Tamil Nadu Agricultural University, Coimbatore; and the University of Agricultural Sciences, Dharwad in India, and North Carolina State University, Raleigh; Texas A&M University, Stephenville; and the Agricultural Research Service-United States Department of Agriculture (ARS-USDA), Tifton in the USA. The assembly to date is presented in Table 1 by section and series

Table 1. *Arachis* species at ICRISAT, October 1983.

Section Series	Ploidy level	Species	No. of accessions
Arachis			
Annuae	2x	*A. batizocoi* Krap. et. Greg.	1
		A. duranensis Krap. et Greg. nom., nud.	1
Perennes	2x	*A. correntina* (Burkart) Krap. et Greg. nom. nud.	5
		A. chacoense Krap. et Greg. nom. nud.	1
		A. cardenasii Krap. et Greg. nom. nud.	1
		A. diogoi Hoehne	1
		A. helodes Mart, ex Krap. et Rig.	3
		A. stenosperma Krap. et Greg. nom. nud.	1
		A. villosa Benth.	1
		Unidentified	6
Amphiploides	4x	*A. monticola* Krap. et Rig.	3
Unidentified			6
Interspecific hybrids			2
Caulorhizae	2x	*A. repens* Handro	2
Erectoides			
Tetrafoliolatae	2x	*A. paraguariensis* Chod. et Hassl.	1
		A. apressipila Krap. et Greg. nom. nud.	1
Unidentified			1
Procumbensae	2x	*A rigonii* Krap. et Greg.	2
Unidentified			15
Extranervosae	2x	*A. villosulicarpa* Hoehne	2
Rhizomatosae			
Prorhizomatosae	2x	*A. burkartii* Handro	1
Eurhizomatosae	4x	*A. glabrata* Benth.	13
		A. hagenbeckii Harms.	4
		Unidentified	28
Unidentified			32
Triseminalae	2x	*A. pusilla* Benth.	1
Unidentified			46

Table 2. *Arachis* germplasm collected in expeditions involving ICRISAT scientists during 1982-83.

State	Section	Species	No. of accessions: As seeds or plants	No. of accessions: Herbarium only	Remarks
Wild	*Erectoides*	*Arachis* species	1		*A. benthamii* Handro?
Wild	*Caulorhizae*	*A. repens* Handro		1	
Wild	*Rhizomatosae*	*A. glabrata* Benth.		1	
Wild	*Rhizomatosae*	*A. burkartii* Handro	12	2	
Wild	*Extranervosae*	*A. lutescens* Krap. et Rig.	1		
Wild	*Extranervosae*	*A. prostrata* Benth.	2	2	
Wild	*Extranervosae*	*A. burchellii* Krap. et Greg. nom. nud.	18	3	
Wild	*Extranervosae*	*A. sylvestris* Krap. et Greg. nom. nud.	2	1	
Wild	*Extranervosae*	*A. marginata* Gard.	2		
Wild	*Extranervosae*	*Arachis* species	10		Close to *A. prostrata* Benth. and *A. burchellii* Krap. et Greg. nom. nud.
Wild	*Ambinervosae*	*Arachis* species	1		New species?
Wild	*Arachis*	*Arachis* species	2		New species?
Wild	*Arachis*	*A. stenosperma* Krap. et Greg. nom. nud	4		
Cultivated	*Arachis*	*A. hypogaea* L	22		Includes ten market samples

following Gregory et al. (1980). It must be noted that most of the names used are nomina nuda, since these have yet to be validly published (Stalker 1985).

ICRISAT scientists have participated in two collection expeditions which were jointly organized by ICRISAT and Centro Nacional de Recursos Geneticos (CENARGEN) of the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), Brazil in collaboration with the International Board for Plant Genetic Resources (IBPGR). The first expedition, in February-March, 1982, followed the Brasilia-Belem road, with lateral diversions . The second, during May 1983, was from Curitiba to the east of Parana, Sao Paulo and Rio de Janeiro States. Details of the material collected are given in Table 2. These expeditions gave us first-hand information on the natural habitats of *Arachis* species, an estimate of the variability in the populations of some species, and an indication of the pest and disease situation at the center of origin of the genus. Collected material will come to ICRISAT via Texas A&M University.

All the material introduced to ICRISAT undergoes strict quarantine inspection by the Central Plant Protection Training Institute (CPPTI), Rajendranagar on behalf of the Government of India. Material that is exchanged as vegetative cuttings passes through the University of Reading, UK. Seed material is first grown at CPPTI and then at ICRISAT and the resulting plants are examined through maturity jointly by a team of CPPTI and ICRISAT scientists to make sure that no pests and diseases are introduced.

Maintenance and Storage

The seed-producing wild species are space-planted in sandy soil on the ICRISAT farm. Seeds are germinated in small paper cups and then transferred to the field. The plants are protected from pests as and when necessary. Harvesting involves digging and sifting soil from around the plants in large sieves to collect the small pods. The pods are then dried and stored. Presently the wild species accessions are stored as unshelled pods in air-tight plastic containers in our medium-term cold storage facility at 4°C and 35% relative humidity. They will be transferred to long-term storage (-18°C) when the facility becomes available.

The rhizomatous and non seed-producing accessions are maintained live in concrete rings to prevent contamination. Rejuvenation is carried out by rooting stem cuttings and rhizomes. Efforts are underway to prepare herbarium specimens, which will serve as voucher specimens, of all the available accessions.

Evaluation and Utilization

Wild *Arachis* species are considered important sources of many economically-important characters. A large number of new accessions and species have been collected in recent years, and are now becoming available at ICRISAT. This material has yet to be properly identified, described, and evaluated. Descriptors which were developed mainly for the cultivated groundnut (IBPGR and ICRISAT 1981), are presently used for morphological evaluation of wild *Arachis* species. However, we feel that these are inadequate to describe the wild species and efforts are in progress to develop and incorporate additional descriptors which will be more diagnostic.

Screening for various desirable attributes in the cultivated and wild groundnut germplasm accessions has been carried out at ICRISAT. ICRISAT pathologists and entomologists have screened wild species against important diseases and pests (Subrahmanyam et al. 1985; Amin 1985) and a number of species have shown immunity or high degrees of resistance to various diseases and pests.

Utilization of this valuable germplasm has already begun in our Groundnut Improvement Program. Resistances to diseases and pests, available in the species belonging to section *Arachis* are being exploited by ICRISAT cytogeneticists. It is possible that wild species may have different resistance mechanisms from those of *A. hypogaea*. This may help to broaden the genetic base and to develop stable resistance in the cultivated groundnut.

Documentation

Most of the passport and preliminary evaluation data on *Arachis* species have been computerized. Some of the storage information is also available on computer.

Distribution

Worldwide distribution of germplasm accessions to interested scientists is an important objective of the ICRISAT Genetic Resources Unit. At present the demand for wild species is not high; 62 samples have been distributed within India, and 16 abroad. However, as more information on the desirable attributes of wild species and the techniques for successful interspecific hybridization, and transfer of desirable traits become available, we expect the demands to increase.

Future outlook

Accession of new material to the ICRISAT gene bank will continue. More material is to be collected in South America, since it is estimated that only about 60% of the wild species germplasm available there has so far been collected (Simpson 1982). We plan to participate in collection expeditions in collaboration with CENARGEN/EMBRAPA and IBPGR.

Rejuvenation and seed multiplication will be more streamlined. More information on dormancy and viability of wild species seed will be obtained. When the long-term storage facility becomes available, material presently in medium-term storage will be transferred.

Emphasis will be given to evaluation of wild species for various desirable attributes. We have been concentrating solely on resistance to major diseases and pests, but other characters such as oil content and quality, drought tolerance, and yield will also be evaluated.

Additional descriptors for wild species will be developed and incorporated in the Groundnut Descriptors, and documentation will be improved to facilitate exchange of information, and distribution of germplasm to interested scientists will increase.

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Groundnut Germplasm Management in Brazil

J. F. M. Valls[1]

Abstract

The paper updates the status of germplasm since Pompeu's review in 1980. EMBRAPA has a strong program of collection and conservation, partially supported by IBPGR; collections are maintained at CENARGEN, Brasilia and at Instituto Agronbmico, Campinas (IAC). Voucher specimens are deposited in the CENARGEN herbarium.

Accessions are being characterized according to selected descriptors. Documentation of field collections and material in the living collection is maintained on computer at CENARGEN, and information is available on request.

Some species may best be conserved in situ. Many natural populations of wild Arachis accessible from CENARGEN, are visited regularly, and their seeds collected when necessary. At CENARGEN, 326 accessions of wild species are presently conserved, along with 12 hybrid progenies. The collection of new accessions will continue.

Résumé

Politique relative au germplasme d'arachide au Brésil : Ce document est une mise à jour de la situation du germplasme depuis la synthèse de Pompeu en 1980. L'EMBRAPA poursuit un programme actif de collecte et de conservation, financé en partie par le CIRPG; des collections sont maintenues à CENARGEN, à Brasilia, et à l'Instituto Agronômico, Campinas (IAC). Des duplicata sont déposés à l'herbier de CENARGEN.

Les introductions sont actuellement caractérisées suivant des descripteurs sélectionnés. Toute la documentation concernant les échantillons de prospection et le matériel en collection vivante est stockée sur ordinateur à CENARGEN, et toutes les informations sont disponibles sur demande.

Certaines espèces peuvent être mieux conservées in situ. De nombreuses populations naturelles d'Arachis sauvages, accessibles de CENARGEN, sont visitées régulièrement et leurs graines récoltées quand cela est nécessaire. A CENARGEN, 326 introductions d'espèces sauvages sont actuellement conservées avec 12 descendances hybrides. La prospection de nouvelles introductions doit se poursuivre.

Introduction

A general review of groundnut production, utilization, research problems and future research needs in Brazil was presented by Pompeu in 1980.

Groundnuts are an important crop in Brazil, and there is valuable wild and cultivated germplasm in the country, which urgently needs to be conserved. Empresa Brasiliera de Pesquisa Agropecuaria, (EMBRAPA) decided to implement an intensive program to collect and conserve groundnut germplasm through its Centro Nacional de Recursos Geneticos, (CENARGEN) in cooperation with the Instituto Agronomico, Campinas (IAC), Sao Paulo State.

Conservation

CENARGEN has been granted support by the International Board for Plant Genetic Resources (IBPGR) to coordinate a series of field missions to collect germplasm of both wild and cultivated spe-

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cies of *Arachis* (Valls 1983) and to establish a living collection of vegetatively-propagated wild species. Seed-producing species are conserved at CENARGEN, according to EMBRAPA's national objectives. The wild species germplasm available at CENARGEN includes some of the old collections assembled by W.C. Gregory, A. Krapovickas, and their associates (Gregory et al. 1973). and new accessions since 1981 from expeditions coordinated by CENARGEN. All sections and series of the genus *Arachis* are represented.

Another set of accessions is presently maintained at IAC, under the care of A.S. Pompeu. This collection includes materials obtained by Gregory and his associates in Argentina, Bolivia, Brazil, and Paraguay in 1976-77 (the 30000 series of collector numbers). When appropriate, materials from this collection will be incorporated into the base collection located at CENARGEN in Brasilia.

Supported by EMBRAPA's National Program on Genetic Resources, an active gene bank of cultivated groundnut is also located at IAC. This gene bank has 1300 accessions of *A. hypogaea* L, 600 obtained since 1980, mostly as a result of expeditions to different regions of Brazil, or as subsamples of materials collected in Argentina, Bolivia and Peru, and made available to CENARGEN through Texas A&M University.

Table 1. Number of accessions of *Arachis* species and hybrids presently conserved in Brazil (October 1983).

Section Series Species	CEN[1]	IAC[2]	Section Series Species	CEN	IAC
Arachis Annuae			*Erectoides Tetrafoliolatae*		
A. batizocoi	2	-	*A. benthami*	-	1
	1	-	*A. paraguariensis*	5	1
A. ipaensis	-	1	*A.* spp	11	20
A. spegazzini	2	-			
A. spp	6	26	*Erectoides Procumbensae*		
			A. rigonii	1	-
Arachis Perennes			*A. appressipila*	4	2
A. diogoi	-	2	*A.* spp	9	5
A. helodes	5	4			
A. villosa	5	-	*Extranervosae*		
A. correntina	4	-	*A. lutescens*	8	-
A. cardenasii	1	-	*A. marginata*	2	-
A. chacoense	1	-	*A. prostrata*	9	-
A. stenosperma	5	-	*A. sylvestris*	21	-
A. spp	12	15	*A. villosulicarpa*	1	2
			A. macedoi	1	1
Arachis Amphiploides			*A.* spp	28	-
A. batizogaea	1	-			
A. monticola	1	2	*Rhizomatosae Prorhizomatosae*		
			A. burkartii	12	1
Ambinervosae					
A. spp	15	-	*Rhizomatosae Eurhizomatosae*		
			A. glabrata	21	3
Caulorhizae			*A. hagenbeckii*	-	2
A. repens	5	1	*A.* spp	-	17
A. pintoi	12	-			
			Triseminalae		
Erectoides Triloliolatae			*A. pusilla*	9	-
A. tuberosa	2	-			
			TOTAL		328

1. CEN = CENARGEN, Brasilia.
2. IAC = Institute Agronomico, Campinas, Sao Paulo State.

Documentation

Voucher specimens of all the recent collections are deposited in the CENARGEN herbarium and duplicates will be distributed to the main herbaria of the world, and to institutions involved with groundnut germplasm. Field collection data and information on material in the living collection is computerized at CENARGEN. Information on germplasm accessions is available to the scientific community on request. Characterization according to selected descriptors is in progress both at the active gene bank in Campinas and at the living collection of wild species in Brasilia. Crossing experiments involving *A. hypogaea* and *A. diogoi* Hoehne and two unnamed species are conducted at IAC (Pompeu 1983).

Distribution

Groundnut germplasm maintained in Brazil is generally available for distribution to interested scientists and institutions, once its long-term conservation in guaranteed by successful multiplication. CENARGEN is ideally located in the center of the area of natural occurrence of wild *Arachis* species. This allows frequent visits to well-known sites of occurrence of many populations representing several species. Some of these sites are in government-controlled areas, such as national parks, others are on private land. The populations are revisited whenever it is necessary to collect additional seeds. To guarantee the future availability of species which are very difficult to maintain such as *A. marginata* Gardner, and *A. tuberosa* Bentham, the best alternative may be to conserve them in situ but official agreement has not yet been reached. In vitro conservation is also envisaged for critical species and for international germplasm exchange. Wild species with potential for use as forage plants are made available to EMBRAPA's network of active forage crop gene banks, thus achieving some degree of duplication in gene banks, and reducing the chances of losing accessions, as happened so often in the past.

The groundnut germplasm conserved in Brazil at CENARGEN and IAC is detailed in Valls (1985). A total of 328 accessions of wild species are presently conserved (Table 1), along with 12 hybrid progenies derived from crossing experiments (Gregory and Gregory 1979). As yet only *A. repens* Handro and *A. villosulicarpa* Hoehne are duplicated in both collections.

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Wild Genetic Resources - Discussion

Stalker:
What is the status of *A. angustifolia*?

Valls:
So far as the name *A. angustifolia* is concerned, it seems to be a validly-published name but is perhaps not applicable to any of the material presently available as germplasm. A similar situation occurred with the name *A. sylvestris* which had been left aside but now is in use, since 21 populations of this species have been accessed as germplasm in Brazil during the past 3 years.

Stalker:
In the slide shown by V.R. Rao, the plants were grown close together. How do you maintain seed purity in your quarantine nurseries?

V.R. Rao:
In the ICRISAT Post-Entry Quarantine Isolation Area (PEQUIA), the wild species plants are spaced 1.5 m apart with extra space between different accessions. Cross-pollination by bees is very low in the PEQIA plants. When we harvest, we dig the area around the plant deeply and we usually only collect pods attached to plants. In this way we avoid any mechanical mixtures and maintain purity.

Sastri:
Are there any wild species with pegs that have very good strength of attachment?

Valls:
The most distinctive characteristic of the cultivated groundnut is the absence of a well-defined abcission layer in the peg, so that the pod remains attached to the plant after maturation. This trait is the typical result of a long process of domestication. The pegs of the presently-known wild species have well-defined abcission layers and collapse after pod maturation. The pegs of wild species may vary from very thin to very thick, but do not retain the pods after maturation.

Potentials of Wild Genetic Resources

Resistance to Groundnut Diseases in Wild *Arachis* Species

P. Subrahmanyam, A.M. Ghanekar, B.L. Noit, D.V.R. Reddy, and D. McDonald[1]

Abstract

Diseases are major constraints to groundnut production. The most economically-important fungal diseases on a worldwide scale are leaf spots (Cercospora arachidicola, Cercosporidium personaturn), *and rust* (Puccinia arachidis). *Sources of resistance to these diseases have been identified within the cultivated groundnut and are being utilized in resistance breeding programs. High levels of resistance, and/or immunity to the diseases have been identified among wild* Arachis *species and cytogeneticists have been successful in incorporating some of these resistances into the cultivated groundnut.*

Groundnuts are also subject to several damaging virus diseases and few sources of resistance to these have been found in the cultivated groundnut. However, high resistances to groundnut rosette, peanut mottle, peanut stunt, and tomato spotted wilt viruses have been found in some wild Arachis *species, and it is important that these resistances should also be incorporated into the cultivated groundnut. Similarly, resistance to some nematode diseases has also been found in wild* Arachis *species and efforts should be made to incorporate this into the cultivated groundnut.*

Résumé

Résistance aux maladies de l'arachide dans les espèces sauvages d' **Arachis** ***L. : Les maladies constituent l'un des principaux facteurs limitants de la production arachidière. Au niveau mondial les maladies cryptogamiques économiquement les plus importantes sont les cercosporioses*** (Cercospora arachidicola, Cercosporidium personatum) **et la rouille** (Puccinia arachidis). ***Des sources de résistance à ces maladies identifiées dans l'arachide cultivée sont actuellement utilisées dans les programmes de sélection pour la résistance. Des niveaux de résistance et/ou d'immunité élevés aux maladies ont été identifiées parmi des espèces sauvages d'*** **Arachis** ***et des cytogénéticiens ont réussi à introduire certaines de ces résistances dans les arachides cultivées.***

Les arachides sont également sensibles à plusieurs maladies à virus, et les sources de résistance suffisantes découvertes jusqu'ici dans les arachides cultivées sont peu nombreuses. Cependant, certaines espèces d' **Arachis** ***sauvages se sont révélées présenter une bonne résistance aux virus de la rosette, de la marbrure foliaire, du nanisme, et de la maladie bronzée de la tomate.***

Il est donc important que ces résistances soient également introduites dans les arachides cultivées. De même, une résistance à certains nématodes a été trouvée parmi les espèces d' **Arachis** ***sauvages, des efforts devront être mis en oeuvre pour introduire cette résistance dans l'arachide cultivée.***

Introduction

A large number of fungal, virus, and nematode diseases of groundnut have been reported, and with few exceptions, they are commonly present in all groundnut-growing regions of the world. The most important fungal diseases causing severe yield losses on a worldwide basis are the leaf spots (*Cercospora arachidicola* Hori and *Cercosporidium personatum* [Berk, et Curt.] Deighton) and

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rust (*Puccinia arachidis* Speg.). Losses in yields due to leaf spots of around 10% have been estimated in the USA despite the widespread application of fungicides (Jackson and Bell 1969). In the semi-arid tropics, where chemical control is rarely used, losses in excess of 50% are commonplace (Gibbons 1980). Loss in yields of around 70% was estimated in India due to a combined attack of leaf spots and rust (Subrahmanyam et al. 1984). Although these diseases can be controlled by certain chemicals, this approach is not at present feasible in many less developed countries. Research on identification of resistance to these diseases has received much attention over the last decade, not only in the developing countries, where chemical control is rarely practised, but also in developed countries where costs of chemical control have become very high (Gibbons 1982). There has been intensive research on screening groundnut germplasm for resistance to various fungal diseases, and several lines with high levels of resistance to these diseases have been identified (Subrahmanyam et al. 1980, 1982, 1983; Porter et al. 1982).

Among the virus diseases of groundnut, peanut mottle virus (PMV) is the most widespread (Reddy et al. 1978) and causes yield losses up to 30% (Kuhn and Demski 1975). Other economically-important virus diseases have more restricted distributions. For instance, groundnut rosette virus (GRV) is important in Africa south of the Sahara; peanut clump virus (PCV) in West Africa and in India; bud necrosis disease (BND) caused by tomato spotted wilt virus (TSWV) in India; and witches' broom in Southeast Asia (Reddy 1980; Ghanekar 1980; Porter et al. 1982). The control strategy for many of the virus diseases has traditionally been a manipulation of cultural methods, either to evade the peak populations of the vector, or to avoid infection at the susceptible seedling stage of crop growth. Although these alternative methods of control do help in reducing the disease, they are usually location-specific and are not therefore universally acceptable. In addition, farmers in the developing countries, where the majority of the world's groundnut crop is grown, are reluctant to modify their age-old cultural practices. The use of insecticidal sprays to control vectors of these viruses is not a practical proposition for most farmers in developing countries. Therefore, use of host plant resistance is, where possible, the most practical, effective, and hence the best way to control virus diseases.

Diseases caused by nematodes are economically important in some parts of the world. The principal species involved are in the genera *Meloidogyne*, *Pratylenchus*, *Belonolaimus*, and *Macroposthonia* (Porter et al. 1982). In recent years, germplasm screening for resistance to various nematode diseases has been carried out in the USA, and several sources of resistance have been reported (Porter et al. 1982).

The sources of resistance to various fungal, virus, and nematode diseases in cultivated groundnut germplasm reported so far represent a narrow range of variability that could be improved by the discovery of additional genes for resistance to these diseases. Wild *Arachis* species are potentially useful for broadening the genetic base of the cultivated groundnut. In recent years, there has been considerable emphasis on screening wild *Arachis* species for resistance to various diseases and some species have been reported to have high levels of resistance to diseases caused by fungi, viruses, and nematodes. Cytogenetic research aimed at incorporating disease resistance and other useful traits from wild *Arachis* species into cultivated groundnut is in progress at several research institutions (Moss 1980; Singh et al. 1980; Stalker 1980; Wynne and Gregory 1981).

In this paper, the literature on identification of sources of resistance to various fungal, virus, and nematode diseases of groundnut in wild *Arachis* species is reviewed.

Disease Resistance in Wild *Arachis* Species

Fungal Diseases

Leaf spots

Gibbons and Bailey (1967) reported that three *Arachis* species, *A. hagenbeckii* Harms, *A. glabrata* Benth and *A. repens* Handro did not develop any *C. arachidicola* lesions when grown in plastic pots in the open under natural disease pressure in Malawi. Abdou et al. (1974) screened 94 accessions of *Arachis* species for resistance to *C. arachidicola* and *C. personatum* under laboratory conditions. Resistance was evaluated by measuring the number of lesions per leaflet, lesion diameter, percentage leaf area damaged, percentage defoliation, and sporulation index. They found several immune and highly resistant species in the sections *Arachis* Krap. et Greg. nom. nud., *Erectoides* Krap. et Greg. nom. nud., *Rhizomatosae* Krap. et Greg. nom. nud., and *Extranervosae* Krap.

et Greg. nom. nud. Kolawole (1976) reported an unnamed diploid species as resistant to both leaf spot pathogens in Nigeria. Sharief et al. (1978) believed that this species was probably A. stenosperma (HLK 410). Foster et al. (1981) evaluated nine Arachis species for resistance to *C. arachidicola* by measuring various disease parameters and concluded that the number of lesions per leaf, and percentage defoliation were most useful for evaluation of resistance to *C. arachidicola*. *A. chacoense* and *A. stenosperma* were found to be highly resistant. Abdou et al. (1974) reported that *A. chacoense* Krap. et Greg. nom. nud. was highly resistant to *C. arachidicola* but susceptible to *C. personatum*. However, Subrahmanyam et al. (1980) found only a few, tiny, non-sporulating lesions of both leaf spot pathogens on *A. chacoense* under both field and laboratory conditions. Melouk and Banks (1978) and Sharief et al. (1978) observed no lesion development on *A. chacoense* when inoculated with *C. arachidicola* under artificial inoculation conditions. *A. cardenasii* Krap. et Greg. nom. nud. was susceptible to *C. arachidicola* but immune to *C. personatum* (Abdou et al. 1974, Sharief et al. 1978, Subrahmanyam et al. 1980). Nevill (1979) did not observe any lesions on *A. cardenasii* and *A. stenosperma* when inoculated with *C. personatum* in Nigeria. Company et al. (1982) evaluated *A. chacoense* and *A. cardenasii* for their reaction to *C. arachidicola* during an investigation on cytology and leaf spot resistance in interspecific hybrid derivatives. Both species showed the presence of *C. arachidicola* lesions in field trials but did not produce any lesions in laboratory tests. Abdou et al. (1974) reported that three accessions of *A. villosulicarpa* Hoehne were immune to both leaf spot pathogens in the USA. However, Gibbons and Bailey (1967) observed considerable damage to the foliage of this species due to *C. arachidicola* infection in Malawi. Subrahmanyam et al. (unpublished) observed lesions of *C. personatum* on *A. villosulicarpa* but the lesions were small and non-sporulating. An unidentified species of *Arachis* (GKP 10596, PI 276233) in section Rhizomatosae was reported immune to both leaf spot pathogens in the USA and India (Abdou et al. 1974, Subrahmanyam et al. 1980). However, Melouk and Banks (1978) in the USA observed small, non-sporulating lesions on this species when inoculated with *C. arachidicola* (Table 1).

Some of these differences in disease reactions could be due to variation in the pathogen; interaction between host, pathogen, and environment; or confusion in identification of, or variation within, the host species.

Rust

Subrahmanyam et al. (1983) screened 61 accessions of wild species, representing five sections of the genus *Arachis*, under field and laboratory conditions for reaction to groundnut rust. Most were immune, six were highly resistant, and two were susceptible to the pathogen. Some of the immune and highly resistant accessions are listed in Table 2. Several accessions of *A. glabrata* were found immune when tested in the USA and India (Bromfield and Cevario 1970, Subrahmanyam et al. 1980, 1983). However, rust was observed on an accession of the same species collected in Brazil (Bromfield 1971, V.Ramanatha Rao and J.F. Hennen, personal communication). *A. glabrata* is a very variable species and many need to be reclassified. It is not surprising that different accessions of a species can vary in disease reaction, and more attention should be given to recording diseases present on wild *Arachis* spp when collecting.

Attempts are being made to use species that are resistant and immune to *P. arachidis* as practical sources of rust resistance. They may have genes for resistance to rust different from those in *A. hypogaea*, thus providing the possibility of combining the rust resistance of wild and cultivated species to give more effective and stable resistance in the cultivated groundnut (Subrahmanyam et al. 1983). Even if the genes are identical, they may be linked to different desirable characters or may produce more effective allelic combinations.

Singh et al. (1984) evaluated the first generation hybrid progenies of two rust-susceptible groundnut cultivars crossed with rust-immune *A. batizocoi* Krap. et Greg. nom. nud. diploid and autotetraploids, and its amphiploid with two other immune diploid wild species for reaction against groundnut rust. They concluded that rust resistance in diploid wild species is of a partially dominant nature, unlike in *A. hypogaea*, where it is recessive. The transfer of rust resistance from wild species should be straightforward because of the dominant nature of the genes.

The tetraploid or near-tetraploid lines derived from crosses between *A. hypogaea* and wild species immune and highly resistant to rust, were systematically evaluated for their rust reaction during the 1981 and 1982 rainy seasons at ICRISAT Center. A very high degree of resistance to rust was

Table 1. Sources of resistance to leaf spots in wild *Arachis* species.

Species	Section	Collector initial and number or other identity	Disease reaction[1]		Investigators
			C.arachidicola	*C.personatum*	
A.chacoense	*Arachis*	GKP 10602, PI 276325	HR	S	Abdou et al. (1974)
A.chacoense	*Arachis*	GKP 10602, PI 276325	HR	HR	Subrahmanyam et al. (1980)
A.chacoense	*Arachis*	GKP 10602, PI 276325	I		Melouk and Banks (1978)
A.cardenasii	*Arachis*	GKP 10017, PI 262141	S	I	Abdou et al. (1974) Subrahmanyam et al. (1980)
A.stenosperma	*Arachis*	HLK 410, PI 338280	HR	HR	Subrahmanyam et al. (1980)
A.stenosperma	*Arachis*	HLK 410, PI 338280	HR		Melouk and Banks (1978)
A.repens	*Caulorhizae*		I		Gibbons and Bailey (1967)
A.repens	*Caulorhizae*			HR	Subrahmanyam et al (unpub.)
Arachis species	*Erectoides*	GK 10573, PI 276225	HR	HR	Abdou et al. (1974)
A.appressipila	*Erectoides*	GKP 10002		HR	Subrahmanyam et al. (unpub.)
A.paraguariensis	*Erectoides*	KCF 11462		HR	Subrahmanyam et al. (unpub.)
A.villosulicarpa	*Extranervosae*		I		Abdou et al. (1974)
A.villosulicarpa	*Extranervosae*	ICG 8142		HR	Subrahmanyam et al. (unpub.)
A.hagenbeckii	*Rhizomatosae*		I		Gibbons and Bailey (1967)
A.hagenbeckii	*Rhizomatosae*	HL 486, PI 338267		HR	Subrahmanyam (unpub.)
A.glabrata	*Rhizomatosae*		I		Gibbons and Bailey (1967)
A.glabrata	*Rhizomatosae*	GKP 9830, PI 262797	HR	HR	Abdou et al. (1974)
A.glabrata	*Rhizomatosae*	GKP 9830, PI 262797		HR	Subrahmanyam (unpub.)
Arachis species	*Erectoides*	GKP 10574	HR	HR	Abdou et al. (1974)
Arachis species	*Rhizomatosae*	GKP 10596, PI 276233	I	I	Abdou et al. (1974) Subrahmanyam et al. (1980)
Arachis species	*Rhizomatosae*	GKP 10596, PI 276233	HR		Melouk and Banks (1978)

1. I = Immune; HR = Highly Resistant; S = Susceptible.

Table 2. Sources of resistance to rust in wild *Arachis* species.

Species	Section	Collector initial and number, or other identity	Rust reaction[1]
A.batizocoi	*Arachis*	K9484, PI 298639; PI 338312	I
A.duranensis nom. nud.	*Arachis*	K7988, PI 219823	I
A.spegazzinii nom. nud.	*Arachis*	GKP 10038, PI 263133	I
A.correntina nom. nud.	*Arachis*	HL176, PI 331194, GKP 9548	I
A.stenosperma nom. nud.	*Arachis*	HLK 410, PI 338280	HR
A.cardenasii nom. nud.	*Arachis*	GKP 10017, PI 262141	I
A.chacoense nom. nud.	*Arachis*	GKP 10602, PI 276235	I
A.villosa	*Arachis*	PI 210554	I
A.apressipila nom. nud.	*Erectoides*	GKP 10002, PI 262140	I
A.paraguariensis	*Erectoides*	KCF 11462	I
A.pusilla	*Triseminalae*	GK 12922, PI 338449	I
A.villosulicarpa	*Extranervosae*	ICG 8142 ex. Coimbatore	I
A.hagenbeckii	*Rhizomatosae*	HLK0 349, PI 338305	I
A.glabrata	*Rhizomatosae*	HLKHe 552, PI 338261	I

1. I = Immune; no rust disease symptoms.
HR = Highly resistant; very small necrotic lesions formed but no production of pustules or urediniospores.

observed in most of the interspecific hybrid derivatives. On resistant lines, the uredosori were slightly depressed, small, and did not rupture to release the comparatively few urediniospores produced. The affected leaflets showed only limited necrosis.

Virus Diseases

Peanut mottle virus (PMV)

Demski and Sowed (1981) reported that six wild rhizomatous groundnut introductions, (most were probably *A. glabrata*) were not infected by mechanical or aphid (*Aphis craccivora*) inoculation, or in the field under high disease pressure (Table 3).

Fifty wild *Arachis* species accessions have been screened for PMV resistance at ICRISAT Center under greenhouse conditions using mechanical leaf rub, and air brush inoculations. All were infected except *A. pusilla* Benth.(12922), *A. cardenasii* (10017), *A. chacoense* (10602), and A. correntina (Burk.) Krap. et. Greg. nom. nud. (9530). Two of these species, *A. chacoense* and *A. pusilla*, after repeated graft inoculations remained free from infection as determined by assays on *Phaseolus vulgaris* (cv Topcrop) and by enzyme-linked immunosorbent assay (ELISA).

Table 3. Wild *Arachis* species resistant to peanut mottle virus[1].

Identity	Species
PI 262794	*A. glabrata*
PI 421707	*A.glabrata*
AM 3867	*A.glabrata* (?)
PI 262818	*Arachis* sp
PI 262817	*Arachis* sp
PI 262839	*Arachis* sp

1. After Demski and Sowell (1981).

Groundnut rosette virus (GRV)

Very little published information is available about the identification of sources of resistance to groundnut rosette virus in wild *Arachis* species. Gibbons (1969) in Malawi tested eleven wild *Arachis* species including four annuals and seven perennials, by aphid (*Aphis craccivora*) and graft inoculation. He observed that *A. repens*, diploid and tetraploid, and *A. glabrata* remained free of rosette virus infection. However, Klesser (1967) using similar experimental methods in South Africa, reported that *A. glabrata* was a symptomless carrier of groundnut rosette. Immune lines which do not show rosette virus symptoms should be confirmed as virus-free using ELISA.

Tomato spotted wilt virus (TSWV)

At ICRISAT Center, a total of 42 wild Arachis species accessions were tested in the greenhouse by mechanical and thrips (*Frankliniella schultzei*) inoculation. Three species, *A. pusilla* (12922), *A.*

correntina (9530), and *A. cardenasii* (10017), although infected by mechanical and thrips inoculation in the laboratory, showed no infection under field conditions, based on observations over many seasons. Only *A. chacoense* remained free from TSWV infection after mechanical and thrips inoculation as determined by indexing on Vigna unguiculata (cv C 152), and by ELISA. However, TSWV could be detected in *A. chacoense* following graft inoculation. Additionally, *A. chacoense* always remained free from infection under field conditions. Therefore, *A. chacoense* can be considered highly resistant to TSWV and a potential source of resistance genes in interspecific crosses with the cultivated groundnut.

Peanut stunt virus (PSV)

Hebert and Stalker (1981) tested 90 collections of wild *Arachis* species by mechanical inoculation, and those that were not infected were further tested by graft inoculation. Forty-eight collections from four sections were highly resistant and several of these are presented in Table 4. The resistance of these selected lines was confirmed by ELISA and by assays on *V. unguiculata*. The selected lines were also tested for susceptibility to PSV in a field where the disease pressure was adequate and they still remained free from PSV infection.

Nematode Diseases

Banks (1969) evaluated some 33 accessions of wild *Arachis* species for resistance to the northern root knot nematode (*Meloidogyne hapta* Chitwood). Only one species from section *Rhizomatosae*, PI 262286, had a moderate level of resistance. Castillo et al. (1973) tested 12 accessions for resistance to northern root knot nematode. Four accessions of section *Rhizomatosae*, PI 262286, PI 262841, PI 262814, and PI 262844, had fewer galls than the control *A. hypogaea* cv Spantex. The number of egg-laying females was also reduced. At present no information is available on utilization of these species in breeding for resistance to *M. hapla*.

Table 4. Wild *Arachis* species resistant to peanut stunt virus[1].

Species or collector number	Section	PI number
9571	*Rhizomatosae*	262818
9806	*Rhizomatosae*	262792
9921	*Rhizomatosae*	262296
A.glabrata B1	*Rhizomatosae*	—
10596	*Rhizomatosae*	276233
7988	*Arachis*	—
10598	*Arachis*	276234
9764	*Erectoides*	262859
10573	*Erectoides*	276225
A.repens	*Caulorhizae*	—

1. After Hebert and Stalker (1981).

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Resistance of Wild Species of Groundnut to Insect and Mite Pests

P.W. Amin[1]

Abstract

While sources of resistance are available in cultivated species of groundnut to some pests such as, thrips, (Scirtothrips dorsalis Hood., Frankliniella schultzei *Trybom., and* F. fusca *Hinds.), jassids* (Empoasca fabae Harris and E. kerri *Pruthi), termites* (*Odontotermes spp*), *and southern corn root worm (Diabrotica undecimpunctata howardi Barber), a high level of resistance has yet to be identified for several important pests such as the groundnut aphid (Aphis craccivora Koch.),* Spodoptera *spp,* Heliothis *spp, and mites (Tetranychus spp). Available reports indicate that some wild* Arachis *species have very high levels of resistance to these pests. Species within the section* Arachis *offer the highest potential for rapid utilization of wild germplasm. Future hybridization programs should utilize* A. chacoense *as a source of resistance to aphids, thrips, jassids and tomato spotted wilt virus;* A. batizocoi *and* A. correntina *for jassid resistance;* A. chacoense *and* A. stenosperma *for pod-boring insect resistance;* A. villosulicarpa, A. correntina, *and* Arachis *sp PI 263996 for mite resistance; and* A. correntina *for* Heliothis *resistance.*

Résumé

Résistance des espèces d'arachide sauvages aux insectes et acariens nuisibles : Les espèces sauvages présentent des niveaux de résistance élevés à divers insectes et acariens et peuvent être utilisées pour l'amélioration des arachides cultivées.

***Alors que l'on dispose chez les espèces d'arachide cultivées de sources de résistance à certains ennemis tels que les thrips* (Scirtothrips dorsalis *Hood.,* Frankliniella schultzei *Trybom., et* F. fusca *Hinds.), les jassidae* (Empoasca fabae *Harris et* E. kerri *Pruthi*), *les termites* (Odontotermes *spp*) *et* Diabrotica undecimpunctata howardi *Barber, il reste encore à identifier chez celles-ci un degré de résistance élevé à plusieurs ravageurs importants comme le puceron de l'arachide* (Aphis craccivora *Koch.*), Spodoptera *spp,* Heliothis *spp et* Tetranychus *spp. Des rapports révèlent que quelques espèces sauvages d'* Arachis *présentent des niveaux de résistance très élevés à ces ravageurs. Les espèces de la section* Arachis *offrent le potentiel le plus élevé pour une utilisation rapide du germplasme sauvage. On devra donc utiliser comme source de résistance dans les futurs programmes d'hybridation,* A. chacoense *pour la résistance aux pucerons, thrips et jassidae, ainsi qu'au virus de la maladie des taches bronzées de la tomate, maladie transmise par le thrips;* A. batizocoi *et* A. correntina *pour la résistance aux jassidae;* A. chacoense *et* A. stenosperma *pour la résistance au borer des gousses;* A. villosulicarpa, A. correntina *et* Arachis *sp PI 263996 pour la résistance aux acariens et* A. correntina *pour la résistance à* Heliothis.

Introduction

Groundnut is attacked by more than 360 species of insects and mites (Stalker and Campbell 1983). In India the annual losses from five major insect pests have been estimated at Rs. 1600 million (US $ 160 million) (Amin 1983).

The same pests do not cause damage every year on every farm but in the SAT a number of species are always prominent. These are the groundnut aphid *Aphis craccivora* Koch., thrips *Scirtothrips dorsalis* Hood., *Caliothrips indicus* Bagnall, *Frankliniella schultzei* (Trybom)., *F. fusca* Hinds, *Enneothrips flavens* Moulton, jassids *Empoasca* spp, armyworm *Spodoptera* spp, and termites *Microtermes* spp, *Odontotermes* spp.

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Aphids, and thrips are usually important as vectors of viruses. *Aphis craccivora* is best known as the vector of rosette virus in Africa but is also the vector of peanut mottle virus (PMV), which is a problem wherever groundnuts are grown. *F. schultzei* is of major economic importance in India because it transmits tomato spotted wilt virus (TSWV), the cause of bud necrosis disease (BND) (Amin et al., 1981).

Until recently, pest control in groundnuts was based on pesticide application. However, the concept of reducing losses by breeding pest-resistant plants is now receiving attention. Wild species are potentially highly resistant to a range of insect pests but in most cases investigations have been limited to the identification of sources of resistance. This is because: pest-resistant varieties of cultivated groundnut are available; there is a limited supply of wild species at research centers; the special breeding techniques required to utilize wild species (Johnson et al., 1977); and the long breeding periods required to remove undesirable characters which have been transferred from the wild species. Dahms (1972) stated that wild species should only be screened for resistance after a thorough search of the cultivated germplasm. However, the rapidly-changing situation created by recent developments in cytogenetic techniques invalidates this view(Sastri et al., 1982).

Resistance of Wild *Arachis* Species to Sucking Pests

Thrips

Of the several species of thrips that attack groundnut only a few are pests. These include *S. dorsalis*, and *C. indicus* in India, *C. indicus* in Sudan (Clinton 1962), *F. fusca* in the USA and *E. flavens* in Brazil.

Frankliniella spp

Stalker and Campbell (1983) screened several wild *Arachis* germplasm collections against *F. fusca* and found 17 accessions to be totally free from injury symptoms. These included; *A. batizocoi*, *A.pusilla*, *A. paraguariensis*, *A. repens*, *A. villosa*, and 12 others.

At ICRISAT, preliminary studies were conducted on the survival and fecundity of *S. dorsalis* and *F. schultzei* by caging five females of each species on individual detached leaflets of wild *Arachis* under controlled conditions of temperature and light (28 °C day-time temperature at 700 lux artificial light for 12 h and 21 °C night-time temperature). The survival and fecundity of both thrips species on wild *Arachis* was considerably reduced when compared to those living on *A. hypogaea* (cv TMV 2), indicating a high level of resistance in most wild species tested. *F. schultzei* females survived for 2.7 to 5.7 days on the *Arachis* species compared to 8.7 days on TMV 2 and *Arachis* sp PI 10596. Less than 4.0 nymphs per female were obtained from individual females on wild species compared to 12.2 on *A. hypogaea* (cv TMV 2) and 5.0 on Arachis sp PI 10596.

High levels of resistance to *F. schultzei* have been identified in cultivated groundnut. They are being utilized in the breeding program at ICRISAT Center and at North Carolina State University, USA. *A. chacoense* has been found to be resistant to TSWV, a trait that has not been located in cultivated groundnuts. This was discovered by exposing seedlings to viruliferous thrips. None of the 20 *A. chacoense* seedlings developed symptoms after 60 days, whereas all the other lines of wild species, the check cultivar TMV 2, and the susceptible host, urd bean, *Vigna mungo* (cv UPU 2) produced symptoms within 10 to 30 days. No viral antigens could be detected in young and old leaves from the *A. chacoense* plants after they had been exposed to viruliferous thrips. The leaves were assayed by the enzyme-linked immunosorbent assay (ELISA) technique.

The mechanism of resistance to TSWV in *A. chacoense* is not known. The reduced survival of *F. schultzei* on *A. chacoense* plants is not responsible for non-transmission of TSWV, because *F. schultzei* adults survived for 2 to 3 days which is long enough to inoculate the plants; the minimum inoculation access period is 5 minutes (Amin, personal observation).

A. chacoense has since been crossed with *A. hypogaea* in the hope of transferring resistance. Several near tetraploid progenies are being evaluated in open field screening.

S. dorsalis

The longevity and fecundity of *S. dorsalis* adults were also lower on the wild species and their derivatives than on *A. hypogaea*. Five females of *S. dorsalis* produced 103 nymphs on *A. hypogaea* (cv TMV 2) while no nymphs were obtained from the same number of females caged on *A. chacoense*, *A. duranensis*, and on a hybrid between *A. chacoense* x *A. cardenasii*.

Aphid, *A. craccivora*

It has not been possible to screen wild *Arachis* species for resistance to *A. craccivora* in field conditions at ICRISAT Center. Screenhouse tests showed that *A. chacoense*, *A. villosa*, *A. correntina*, and *A. glabrata*, all exhibited high levels of resistance. Forty females caged on four plants produced 1050 nymphs on TMV 2 while the same number of females produced no nymphs on *A. villosa* and *A. glabrata*, 2 on *A. chacoense*, and 43 on *A. duranensis*. Progenies of interspecific hybrids involving *A. chacoense* and *A. villosa* also showed high resistance.

Gibbons (1969) reported high resistance to rosette virus in *A. repens* and *A. glabrata* tested under laboratory conditions in Malawi. However, no attempts were made to screen these species for aphid resistance.

A. craccivora is responsible for the spread of rosette virus between and within crops. Therefore, resistance to *A. craccivora* in groundnut cultivars selected for African conditions should have characters that combine both nonpreference (to lessen the attractiveness of crop to immigrant alatae), and the reduction of fecundity (to reduce aphid spread within a crop). The latter characteristic has been identified in wild *Arachis* species tested with the Indian biotype of *A. craccivora*. The former must await the results of field evaluations of wild *Arachis* species, and crosses incorporating wild *Arachis* genes under African conditions.

Groundnut jassid, *Empoasca* spp

Several species of the genus *Empoasca* are pests of groundnut in various parts of the world. They cause similar damage symptoms i.e., stunting, vein clearing, and a wedge-shaped yellowing (hopper burn) at the tip of leaflets. On very young plants the leaflets wither and die. Stalker and Campbell (1983) reported 21 collections free from jassid injury. Four of these, *A. correntina*, *A. cardenasii*, *A. duranensis*, and *A. villosa* belong to section *Arachis*, three to Erectoides, one to Ambinervosae and 13 to Rhizomatosae. The F_1 hybrid of *A. villosa* x *A. hypogaea*, cv NC Ac 18000-2 was susceptible to jassids while the reciprocal hybrid expressed a high level of resistance.

Preliminary experiments at ICRISAT (unpublished) demonstrate that some wild *Arachis* accessions decrease jassid fecundity and were tolerant to jassid attack.

In view of the high level of jassid resistance present in *A. hypogaea* there is little need to consider wild *Arachis* spp unless an alternative mechanism of resistance is needed in the future.

Mites, *Tetranychus* spp

Mites are important pests of groundnut in the USA. They suck sap from the foliage which initially results in leaf stippling, and ultimately in the foliage drying. Screening for mite resistance is difficult under field conditions because the mites are unevenly distributed. Screening in greenhouses is simpler. Leuck and Hammons (1968) reported that *Arachis* sp PI 262841 was highly resistant to Tetranychus *tumidellus* Prichard et Baker, with less than 10% foliar damage; *A. villosulicarpa*, *Arachis* sp PI 262841, and *A. repens* showed 10 to 25% damage. The resistance to mites was attributed to nonpreference, because they failed to establish on resistant plants.

Johnson et al. (1977) initiated greenhouse tests of several accessions from seven sections of *Arachis* for resistance to *Tetraychus urticae* Koch. Most species in section *Rhizomatosae* were highly resistant. One accession, *A. correntina* PI 331194 in section *Arachis* also had low damage. Johnson et al. (1980) observed considerable variation in the relative feeding preference on wild species. Two species in section *Rhizomatosae*, PI 262286, and PI 262840 were non-preferred by *T. urticae* with relative preference ratings of 1.8 and 13.3 respectively when compared to *A. hypogaea* cv Nc Ac 5 that had a preference rating of 100. For other wild species, PI 262142 (*Erectoides*) and PI 331194 (*Arachis*) the preference rating was 31.9 and 40.6 respectively. Fecundity was considerably reduced on two wild species of *Rhizomatosae* but not on single species from both sections *Erectoides* and *Arachis*.

It appears that high levels of resistance are only found in section *Rhizomatosae*, but the use of these as resistant sources appears to be restricted unless techniques are developed to hybridize the *Rhizomatosae* species with *Arachis hypogaea*.

Resistance to Chewing Insects

Armyworm, Spodoptera spp

Lynch et al. (1981) evaluated 14 *Arachis* species for resistance to *S. frugiperda* by calculating a host suitability index (HSI).

$$HSI = \frac{\text{Pupal wt. (or fecundity)/ Development time}}{\text{Leaf consumption}} \times \% \text{ survival}$$

They found that *A. villosa* and *A. burkartii* were totally unsuitable hosts because armyworm larvae did not develop on them at all. Other *Arachis* species with low HSIs were *A. cardenasii* (HSI = 0.09), *A. lignosa* (HSI = 1.3), *A. correntina* (HSI = 1.4), and *A. chacoense* (HSI = 1.6). The remainder had HSIs in the range of 4.6 to 6.5. It is also interesting to note that on *A. villosulicarpa* the survival was low (15%), but the mean pupal weight was high (209 mg) as compared to *A. hypogaea* on which survival was high (75%) and pupal weight low (162 mg).

Heliothis spp

Though various Heliothis species attack groundnut in different parts of the world, screening has only been carried out against H. zea Boddie in the USA. Stalker and Campbell (1983) evaluated 53 collections and most of them were damaged less than A. hypogaea. In section *Arachis*, *A. correntina*, *A. villosa*, *A. chacoense*, and *A. stenosperma* leaf feeding damage ranged from 0.5 to 1.6% compared to 37% in *A. hypogaea* cv Florigiant. An F_1 progeny of *A. villosa* x NC Ac 18000-2 had 38% damaged leaves although the reciprocal hybrid displayed only 4.4% damage. Under laboratory conditions *A. batizocoi* proved to be highly resistant as Heliothis larvae failed to survive on this species. When segregates from the interspecific hybrid derivative populations were evaluated, they had a significantly higher level of resistance than their cultivated parent. For example, when *A. hypogaea* PI 261942-3 (with 38.3% damaged leaves) was crossed with *A. cardenasii* (with 2.7% damaged leaves), the progeny had only 4.6% leaves damaged. Similar results were obtained with other crosses involving PI 261942-3 and *A. duranensis*, or with cv NC 2 x (*A. batizocoi* x *A. spegazzini*).

Conclusion

There is clear evidence that wild species in section *Arachis* have a high degree of resistance to several insect pests. These species are being used in the groundnut breeding program at ICRISAT Center to transfer this resistance to *A. hypogaea*.

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Potentials of Wild Genetic Resources—Discussion

Rees:
What are the mechanisms of resistance in wild *Arachis* species?

P.Subrahmanyam:
The resistance to rust is dominant when transferred from *Arachis* species to cultivated varieties whereas resistance so far transferred from cultivated *A. hypogaea* is recessive.

Stalker:
The inheritance of genes for resistance has been reported in several cases. For example, Sharief reported that *Cercospora arachidicola* resistance is a multigenic recessive trait. However, in triploid *A. hypogaea* x *A. chacoense* or *A. hypogaea* x *A. cardenasii*, *C.arachidicola* resistance acted as a dominant trait. Further reciprocal differences have been reported for insect resistance where high levels were observed in F_1 interspecific hybrids when *A. hypogaea* was the female parent, but not when the cultivated species was the male parent.

Rees:
These are examples of inheritance of resistance. It is necessary to make efforts to understand mechanisms of resistance.

Amin:
The mechanisms of resistance to insects are only understood in a few cases, e.g., resistance to jassids is ascribed to the density and length of hairs on the leaflets.

Stalker:
Campbell's results show that not only density, or angle but also type and location of hairs at insect feeding, or oviposition sites is very important. Hairiness does not always contribute resistance to pests. In fact, some pests such as *Heliothis* prefer hairy leaves for oviposition e.g. in cotton.

Singh:
Should defoliation in cultivars due to foliar diseases be considered as the most important criterion for susceptibility?

P.Subrahmanyam :
Yes, it is one of the criteria for susceptibility, but other parameters such as smaller and fewer lesions on the leaflets should be also considered important for resistance to the fungal pathogens.

Singh:
Has hairiness any correlation with resistance to jassids in all germplasm lines?

Amin:
In several lines there appears to be a strong correlation between hairiness and jassid resistance.

Sastri:
Does staining hair with Sudan IV have any correlation with resistance?

Amin:
The staining procedure only helps facilitate counting of hairs.

M.V.Reddi:
In your presentation on the sources of rust and leaf spot resistance in wild species, you stated that no morphological characteristic could be attributed as a mechanism of resistance. May the resistance be enzymatic in nature?

P. Subrahmanyam:
Probably, yes. At present we are not investigating these aspects.

Murty:
From your long experience, do you think that the genetic mechanism of resistance to rust in wild species may be different from that in the cultivars of groundnut?

P. Subrahmanyam:
Yes, there is evidence that different genes or alleles are involved.

Taxonomy and Means of Utilization

Cytotaxonomy of Arachis[1]

H. T. Stalker[2]

Abstract

The genus Arachis *comprises a large number of species native to South America. Members of sections* Erectoides, Extranervosae, *and* Rhizomatosae *are among the oldest species in the genus and their center of origin is Brazil. The seven morphologically-described sections of* Arachis *correspond closely to cross-compatible groups. Interspecific hybrids are difficult to make and most are sterile.*

Although polyploidy has evolved independently in sections Arachis *and* Rhizomatosae, *most species of the genus are diploid (2n = 20). Chromosomes are small and mostly median. However, several species in section* Arachis *have an asymmetrical genome suggesting a recent origin. Chromosome homologies have been reported within section* Arachis *and between sections* Arachis *and* Erectoides, *and* Erectoides *and* Rhizomatosae. *At least nine genomic groups exist among diploid species of the genus, with three of these genomes in section* Arachis.

Arachis hypogaea *originated in the southern Bolivian region, probably from a wild allotetraploid. The cultivated species probably has an A-B genome, but the diploid progenitors have not been identified. There is variation in karyotype among varieties.*

Arachis hypogaea *has been crossed with 13 species of section* Arachis, *and one of section* Rhizomatosae *with the aid of in vitro culture. A gene pool system has been proposed where* A. hypogaea *and* A. monticola *belong to the primary gene pool, diploid members of section* Arachis *belong to the secondary gene pool, and species in other sections of the genus are in the tertiary gene pool.*

Résumé

Cytotaxonomie d'*Arachis : *Le genre* Arachis *comprend un grand nombre d'espèces originaires d'Amérique du Sud. Les membres des sections* Erectoïdes, Extranervosae, *et* Rhizomatosae *sont parmi les plus anciennes espèces du genre et leur centre d'origine est le Brésil. Les sept sections d'*Arachis *décrites morphologiquement correspondent étroitement aux groupes à croisement compatible. La création d'hybrides interspécifiques est difficile à réaliser et la plupart d'entre eux sont stériles.

***En dépit d'une évolution indépendante de la polyploïdie dans les sections* Arachis *et* Rhizomatosae, *la plupart des espèces du genre sont diploïdes (2n = 20). Les chromosomes sont petits et pour la plupart à centromère médian. Cependant, plusieurs espèces de la section* Arachis *possèdent un génome asymétrique, laissant supposer une origine récente. On a rapporté des homologies chromosomiques à l'intérieur de la section* Arachis *et entre les sections* Arachis *et* Erectoïdes, *ainsi que* Erectoïdes *et* Rhizomatosae. *Il existe au moins neuf groupes génomiques parmi les espèces diploïdes du genre, dont trois de ces génomes dans la section* Arachis.

Ayant son origine dans la région méridionale de la Bolivie, l'*Arachis hypogaea *est probablement dérivé d'un allotétraploïde sauvage. Il est probable que l'espèce cultivée comprend un génome A-B, mais on n'a pas identifié les descendants diploïdes. On a constaté une variation de karyotype parmi les variétés.

On a réalisé les croisements de l'*Arachis hypogaea *avec 13 espèces de la section* Arachis, *ainsi qu'avec l'une de la section* Rhizomatosae *à l'aide de la culture in vitro. On propose un système de regroupement ('pool') de gènes : selon ce système,* A. hypogaea *et* A. monticola *appartiennent au pool primaire, les membres diploïdes de la section* Arachis *appartiennent au pool secondaire, et les espèces des autres sections du genre sont regroupées dans le pool tertiaire.

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Introduction

The cultivated groundnut (*Arachis hypogaea* L) is widely distributed in the tropical and subtropical areas of the world. The seeds are rich in oil and protein and make a major contribution to human nutrition. Brooks (1966) lists groundnut together with banana, barley, common bean, cassava, coconut, maize, potato, rice, sorghum, soybean, sugar beet, sugarcane, sweet potato, and wheat as man's principal food crops. In addition to cultivated groundnut, the genus *Arachis* is composed of a large number of diploid and tetraploid species native to South America. This paper will attempt to review taxonomic, cytogenetic, and biosystematic information related to *Arachis* species. It summarizes current views concerning the origin and evolution of the genus and possibilities for gene transfer among species.

Taxonomy

Arachis is a large genus with 50 or more species belonging to the family Leguminosae. It is placed in the tribe Aeschynomeneae, subtribe Stylosanthinae because members of the genus have alternately attached basal and dorsal anthers, flowers in terminal or axillary spikes or small heads, pinnate leaves and leaflets few without stipules (Taubert 1894). The genus has been further subdivided into sections by Krapovickas (1969,1973) and by Gregory et al. (1973). Resslar (1980) reviewed the validity of sectional classifications and concluded that epithets were invalidly published according to the International Code of Botanical Nomenclature and that an author could use any of the three systems (Table 1). However, the infrageneric divisions serve a useful purpose in dividing the genus into general cross-compatible groups. The system of sectional classification derived from Gregory et al. (1973) and Resslar (1980), which was published by Smartt and Stalker (1982), will be followed in this paper (Table 2).

The first species described was *Arachis hypogaea* L in 1753 by Linnaeus. Almost 100 years later the following wild species were described: *A. villosa* Benth., *A. prostrata* Benth., *A. tuberosa* Benth., *A. glabrata* Benth., and *A. pusilla* Benth. (Bentham 1841). Monographs have since been published by Chevalier (1933,1934, 1936), Hoehne (1940), and Hermann (1954). Because of the deficiencies in the herbarium collections which were observed by

Table 1. A comparision of sectional classifications of *Arachis*[1].

Krapovickas (1969)	Gregory et al. (1973)	Krapovickas (1973)
Section *Axonomorphae*	Section *Axonomorphae*	Section *Arachis*
	series *Annuae*	
	series *Perennes*	
	series *Amphiploides*	
Section *Erectoides*	Section *Erectoides*	
	series *Trifolioiatae*	Section *Trierectoides*
	series *Tetrafoliolatae*	Section *Tetraerectoides*[2]
	series *Procumbensae*	
Section *Caulorhizae*	Section *Caulorhizae*	Section *Caulorhizae*
Section *Rhizomatosae*	Section *Rhizomatosae*	Section *Rhizomatosae*
	series *Prorhizomatosae*	
	series *Eurhizomatosae*	
Section *Extranervosae*	Section *Extranervosae*	Section *Extranervosae*
Section *Ambinervosae*	Section *Pseudoaxonomorphae*	Section *Ambinervosae*
	Section *Triseminalae*	
Section *Goniorhiza*[3]		

1. From Resslar (1980).
2. Includes series Procumbensae of Gregory et al. 1973.
3. Included in section Erectoides sensu Gregory et al. 1973 and section Tetraerectoides sensu Krapovickas 1973.

Table 2. Key to the sections[1] of *Arachis* L[2].

1 Plants with rhizomes; solid stems, large flowers.	Section *Rhizomatosae*
2 Plants delicate; flowers large; red veins on both sides of standard; 2n = 2x = 20.	series *Prorhizomatosae*
2 Plants usually robust; flowers large; no red veins on back of standard; 2n = 4x = 40.	series *Eurhizomatosae*
1 Plants without rhizomes.	
3 Plants tap rooted.	
4 Pegs vertical.	
5 Rooting at nodes; plants with hollow stems; standard yellow; 2n = 2x = 20.	Section *Caulorhizae*
5 No rooting at nodes; standard yellow or orange.	
6 Prominent red veins on front and back of standard; flowers very small; standard yellow 6-8 mm wide x 5-6 mm high; 2n = 2x = 20.	Section *Ambinervosae*
6 No prominent red veins on back of standard: flowers small to large; standard yellow or orange.	Section *Arachis*
7 2n = 2x = 20.	
8 Flowers small to medium; standard 9-14 mm wide x 7-12 mm high; short-lived, usually annual.	series *Annuae*
8 Flowers medium to large; standard 14-22 mm wide x 12-18 mm high, orange or yellow; perennial.	series *Perennes*
7 2n = 4x = 40; flowers small to large.	series *Amphiploides*
4 Pegs horizontal.	
9 Purple mark inside orange standard; flowers small; standard 10-12 mm wide x 8-10 mm high; fruits often 3-segmented; 2n = 2x = 20.	Section *Triseminalae*
9 No purple mark on inside standard; flowers medium to large; standard 16-24 mm wide x 12-20 mm high; usually 2-segmented fruits; 2n = 2x = 20.	Section *Erectoides*
10 Standard yellow; plants prostrate.	series *Procumbensae*
10 Standard orange; plants erect or prostrate.	series *Tetrafoliolatae*
3 Plants with thickened lomentiform tuberoid roots or with tuberiform hypocotyl.	
11 Pegs horizontal; flowers large.	Section *Erectoides*
12 Hypocotyl tuberiform, leaves trifoliate.	series *Trifoliolatae*
12 Hypocotyl not tuberiform, leaves tetrafoliate.	series *Tetrafoliolatae*
11 Pegs vertical, sometimes with adventitious roots; flowers small to medium, standard orange or yellow with prominent red veins on back; 2n = 2x = 20.	Section *Extranervosae*

1. All section and series names are nomina nuda as used by Krapovickas and Gregory.
2. After Gregory et al. 1973, Ressler 1980, and Smartt and Stalker 1982.

these investigators, Gregory et al. (1980) considered the works to be unsatisfactory representations of the variability in *Arachis*. Twenty-two species of the genus have been described and diagnoses published (excluding *A. nambyquarae* Hoehne, which is a form of *A. hypogaea*, and *A. batizogaea* Krap. et Fern., which originated from a man-made hybrid) (Table 3). In addition, 11 species names have been reported in the literature. The number of species eventually to be published has been estimated to be between 40 and 70 (Gregory and Gregory 1976); but the number will probably be nearer the higher estimation of 70 species. The taxonomic work needed to describe the known variation in the genus is immense.

In addition to taxonomic treatments of wild *Arachis* species, the cultivated groundnut, *A. hypogaea*, has been divided into two subspecies, each with two botanical varieties (Krapovickas 1968, 1973). Subspecies *hypogaea* does not have flowers on the plant main stem and has alternating pairs of vegetative and reproductive axes along the lateral braches. Subspecies *fastigiata* has flowers on the plant main stem and sequential reproductive

Table 3. Species of Arachis and their sectional designations.

	Species	Section[1]	Series	Year of diagnosis
1.	*A.batizocoi* Krap. et Greg.	*Arachis*	*Annuae*	1974
2.	*A.duranensis* Krap. et Greg. nom. nud.	*Arachis*	*Annuae*	
3.	*A.spegazzinii* Krap. et Greg. nom. nud.	*Arachis*	*Annuae*	
4.	*A.stenosperma* Krap. et Greg.	*Arachis*	*Annuae*	
5.	*A.ipaensis* Krap. et Greg. nom. nud.	*Arachis*	*Annuae*	
6.	*A.helodes* Martius ex Krap. et Rig.	*Arachis*	*Perennes*	1957
7a.	*A.villosa* Benth. var. *villosa*	*Arachis*	*Perennes*	1841
7b.	*A.villosa* var. correntina Burkart [*A.correntina* (Burk) Krap. et Greg. nom. nud.]	*Arachis*	*Perennes*	
8.	*A.diogoi* Hoehne	*Arachis*	*Perennes*	1919
9.	*Acardenasii* Krap. et Greg. nom. nud.	*Arachis*	*Perennes*	
10	*A.chacoense* Krap. et Greg. nom. nud.	*Arachis*	*Perennes*	
11.	*A.hypogaea* L.	*Arachis*	*Amphiploides*	1753
12.	*A.monticola* Krap. et Rig.	*Arachis*	*Amphiploides*	1957
13.	*A.guaranitica* Chod. et Hassl.	*Erectoides*	*Trifoliofatae*	1904
14.	*A.tuberosa* Benth.	*Erectoides*	*Trifoliolatae*	1841
15.	*A.benthamii* Handro	*Erectoides*	*Tetrafoliolatae*	1958
16.	*A.martii* Handro	*Erectoides*	*Tetrafoliolatae*	1958
17.	*A.paraguariensis* Chod. et Hassl.	*Erectoides*	*Tetrafoliolatae*	1904
18.	*A.oteroi* Krap. et Greg. nom. nud.	*Erectoides*	*Tetrafoliolatae*	
19.	*A.rigonii* Krap. et Greg.	*Erectoides*	*Procumbensae*	1960
20.	*A.lignosa* (Chod. et Hassl.) Krap. et Greg. nom. nud.	*Erectoides*	*Procumbensae*	
21.	*A.repens* Handro	*Caulorhizae*		1958
22.	*A.pintoi* Krap. et Greg. nom. nud.	*Caulorhizae*		
23.	*A.burkartii* Handro	*Rhizomatosae*	*Prorhizomatosae*	1958
24.	*A.giabrata* Benth.	*Rhizomatosae*	*Eurhizomatosae*	1841
25.	*A.hagenbeckii* Harms	*Rhizomatosae*	*Eurhizomatosae*	1898
26.	*A.marginata* Gard.	*Extranervosae*		1842
27.	*A.lutescens* Krap. et Rig.	*Extranervosae*		1957
28.	*A.villosulicarpa* Hoehne	*Extranervosae*		1944
29.	*A.macedoi* Krap. et Greg. nom. nud.	*Extranervosae*		
30.	*A.prostrata* Benth.	*Extranervosae*		1841
31.	*A.pusilla* Benth.	*Triseminalae*		1841
32.	*A.angustifolia* (Chod. et Hassl.) Killip.	?		1940

1. No species names have been given for species in section *Ambinervosae.*

axes along the lateral branches. Evidence exists for genetic differentiation between subspecies (Krapovickas 1973; Wynne 1974).

Origins and Dispersals

The present known distribution of *Arachis* species in their native habitat is geographically from the mouth of the Amazon near the equator to approximately 34°S in southern Uruguay. The species range is an east-west pattern from the Atlantic Ocean to the foothills of the Andes (Figure 1). The known distribution is continuing to expand as plant explorations are made (Simpson 1982; Valls 1983). Gregory et al. (1980) reported species growing at various elevations from sea level to approximately 1450 m. Arachis can be found in open grasslands, broken forest, submerged in water, or in flood plains. Some species are found in semi-arid regions while others grow in areas with more than 2000 mm of rainfall annually.

Because of the geocarpic nature of the fruit, species distributions generally follow major river valleys. Infrageneric groups may be closely associated with specific drainage basins. For example, members of section *Triseminalae* are found in the Sao Francisco. Section *Arachis* species are most commonly found in the drainage basin of the River Paraguay, but also in the Amazon drainage basin. Section *Cauiorhizae* is found in the Jequitinhonha river valley, and several other drainage basins in Brazil. Section *Ambinervosae* occurs predominently on the northeast coast of Brazil. Some overlaps in distributions of major subgeneric groups do occur, especially for sections *Arachis*, *Erectoides*, *Rhizomatosae*, and *Extranervosae* (Gregory et al. 1973; Valls 1983), but gene exchange among species of different sections in nature is probably minimal or nonexistent.

Figure 1. Geographic distribution of *Arachis* in South America. Sectional abbreviations: A = *Arachis*, Am = *Ambinervosae*, C = *Caulorhizae*, E = *Erectoides*, Ex = *Extranervosae*, R = *Rhizomatosae*, T = *Triseminalae*.

Arachis Species

The center of origin for the genus *Arachis* is probably in the 'planaltine ellipse' demarked by the species distribution above 550 m in the Brazilian shield (Gregory et al. 1980). This conclusion was based upon general distribution patterns, differentiation confined in drainage basins, and the geocarpic habit of groundnuts. Gregory et al. (1973) hypothesized that the adaptive features specifically directed toward highland adaptation include tuberiform hypocotyls and roots (section *Erectoides*), adventitious and tuberoid roots plus shoots on pegs (section *Extranervosae*), and rhizomes (section *Rhizomatosae*). By inference, members of sections *Erectoides*, *Rhizomatosae*, or *Extranervosae* are the most primitive species in the genus. Another primitive trait found only in two species of section *Erectoides* is trifoliate leaves. Because of distribution patterns and cross-compatibility relationships (to be discussed later in this paper), the most ancient members of the genus *Arachis* may belong to section *Erectoides*.

Arachis hypogaea

The center of origin of cultivated groundnuts is somewhat obscured by time and man's dispersal of seeds. The first archaeological record, and conclu-

sive evidence of a New World origin of cultivated groundnuts was at a cemetery near Lima, Peru (Squier 1877). Towle (1961) dated the site at approximately 3000-2500 BC. However, *A. hypogaea* most likely originated further east in the northwestern Argentine—southern Bolivia region of South America (Krapovickas 1968; Gregory and Gregory 1979). *Arachis monticola* Krap. et Rig., the only known tetraploid species which is cross-compatible with *A. hypogaea*, is also found in this area. Hammons (1982) also concluded that the southern Bolivia region is an important center of variability of *A. hypogaea* subspecies *hypogaea*. Members of this subspecies are believed to be the most closely related to wild *Arachis* species because they have a runner habit, similar branching pattern to other *Arachis* species, and lack compound spikes (Krapovickas 1969). However, based on karyological data of chromosome symmetry, Stalker and Dalmacio (in review) proposed that members of *A. hypogaea* subsp fastigiata var. vulgaris comprise the most ancient cultivated group. Furthermore, members of var. vulgaris have karyotypes very similar to *A. monticola*. Gregory and Gregory (1976) described six secondary centers of variation in South America. Africa is an important tertiary center of diversity for the cultivated species (Gibbons et al. 1972).

Although Husted (1936) proposed that *A. hypogaea* was of direct amphidiploid origin, Krapovickas and Rigoni (1957), and Smartt and Gregory (1967), suggested that the cultivated groundnut evolved from a pre-existing wild allotetraploid. Furthermore, an annual x perennial interspecific hybrid within section *Arachis* was considered important in the evolution of *A. hypogaea* (Gregory and Gregory 1976). Several diploid species of section *Arachis* have been suggested as possible progenitors, including *A. villosa* (Varisai Muhammad 1973b), *A. duranensis* Krap. et Greg. nom. nud. (Seetharam et al. 1973), *A. batizocoi* Krap. et Greg. (Krapovickas et al. 1974), *A. duranensis* and *A. cardenasii* Krap. et Greg. nom. nud. (Gregory and Gregory 1976), *A. cardenasii* and *A. batizocoi* (Smartt et al. 1978a,b), and *A. cardenasii* (Singh and Moss 1982). Smartt and Stalker (1982) concluded the most likely candidates were *A cardenasii* and *A. batizocoi* among the species currently in herbarium collections. However, many species are only represented by one or a few collections and intraspecific variability in the genus is poorly understood. Presently, the diploid progenitor species of *A. hypogaea* have not been verified.

Cytogenetics of *Arachis* Species

The first comprehensive reports of *Arachis* cytogenetics was by Husted (1933,1936) for *A. hypogaea*. He confirmed the somatic chromosome complement of six cultivars and *A. nambyquarae* (synonymous with *A. hypogaea*) as 2n = 4x = 40 (Husted 1931). In most metaphase I pollen mother cells, chromosome pairing was 20 II, but a few multivalents were also observed (Husted 1933, 1936). Hybrids among the varieties of *A. hypogaea* showed the same patterns as parental materials, with mostly bivalents and a few multivalents. Stalker (1980) observed cultivated lines of Spanish (subsp *fastigiata* var. *vulgaris*), Valencia (subsp *fastigiata* var. *fastigiata*), and Virginia (subsp *hypogaea* var. *hypogaea*) and found similar patterns of chromosome pairing as Husted (1936). Intersubspecific hybrids among the Spanish, Valencia, and Virginia varieties also had mostly bivalent chromosome pairing, but univalents and multivalents were also observed (Stalker 1980). Valencia x Virginia hybrids had the most irregular meiosis with an average of 0.51 I + 19.59 II + 0.01 III + 0.08 IV per pollen mother cell. Meiotic analyses suggested chromosome structural differences may exist among subspecies (Husted 1936; Raman 1976; Stalker 1980). Furthermore, Gregory et al. (1980) reported reduced fertility in F_1 hybrids between alternately and sequentially branching genotypes, which correspond to the subspecies *hypogaea* and fastigiata of *A. hypogaea*, respectively.

The chromosomes of *A. hypogaea* are small (approximately 1-4 μ m) and difficult to karyotype. Husted (1933, 1936) was able to distinguish a chromosome pair with a secondary constriction (B chromosome) and one pair which was distinctively smaller than the others (A chromosome). The terminology of A and B chromosomes has persisted in the literature; however, they should be discarded as the term B chromosome is universally used to indicate supernumerary heterochromatic chromosomes found in some plant cells, and not as an indication of chromosomes with a secondary constriction. The A chromosome pair can just as easily, and more correctly, be called a distinctively small chromosome number 20. The letters, A and B can then be used as genomic designations which will be discussed later.

Several types of secondary constriction chromosomes have been reported in *A. hypogaea* by Babu (1955). Groundnut cultivars can be distinguished based on differences among chromosomes

(D'Cruz and Tankasale 1961; Stalker and Dalmacio 1981b). Singh and Moss (1982) further reported karyotypes of *A. monticola* and two *A. hypogaea* cultivars. Stalker and Dalmacio,(in press) grouped cultivars into their respective botanical variety designations based on a principal component analysis of chromosome arm ratios. At least 15 of the 20 chromosome pairs can be distinguished cytologically in somatic cells (Stalker and Dalmacio, in press).

The first chromosome report for a wild species was 2n = 40 for *A. glabrata* (Gregory 1946). Mendes (1947) reported 2n = 20 for A. diogoi Hoehne, *A. marginata* Gard., *A. prostrata*, and *A. villosulicarpa* Hoehne. Smartt and Stalker (1982) summarized reports of chromosome numbers in the genus and found only 26 species with associated cytological analyses. Since 33 species have been named, and eventually the number may reach 70, the inadequacy of cytological analyses in the genus is evident.

At least two chromosome series, 2n = 2x = 20 and 2n = 4x = 40, are present in the genus. Polyploidy arose twice, once in section *Arachis* and a second time in section *Rhizomatosae* (Smartt and Stalker 1982). However, most species in the genus are diploids.

Meiotic studies of *Arachis* species have revealed that chromosome pairing in the diploids is normally 10 II (Raman 1976; Resslar and Gregory 1979; Smartt et al. 1978a,b; Stalker and Wynne 1979; Singh and Moss 1982). However, quadrivalents have been reported at a low frequency in the two diploid species *A. villosa* and *A. spegazzinii* Greg, et Greg. nom. nud. (Singh and Moss 1982); but because of the prevalence of 'sticky chromosomes', the observed multivalents may have been artifacts of chromosome preparations.

Meiosis in *A. monticola* (2n = 40) is generally normal with 20 II and occasional multivalents (Raman 1976). Meiosis in tetraploid rhizomatous taxa is less regular than in *A. monticola* or *A. hypogaea*, with up to four quadrivalents per pollen mother cell (Raman 1976). In the section *Rhizomatosae* species collection GKP 9570 (PI 262817), an average chromosome association of 19.92 II + 0.04 IV was also observed (Table 4). The less regular meiosis observed in tetraploids of section *Rhizomatosae* (based on a very narrow range of genotypes) may be due to the vegetative reproduction

Table 4. Cytological analyses of species and interspecific and intersecttonal hybrids involving sections *Erectoides*, *Rhizomatosae*, and *Arachis*.

Species/hybrids	Section	2n	Pollen stain (%)	Cells observed (no.)	Chromosome association			
					I	II	III	IV
A.paraguariensis, GKP 9646 (PI 262842)	E	20	98.3					
Arachis sp, GKP 9841 (PI 262278)	E	20	93.8	25	0.08	9.96	0	0
A.rigonii, GKP 10034 (PI 262142)	E	20	99 9	25	0	10.00	0	0
Arachis sp, GKP 9570 (PI 262817)	R	40	97.6	25	0	19.92	0	0.04
A.glabrata, GKP 9830 (PI 262797)	R	40	84.6					
Arachis sp, HLKHe 569 (PI 338301)	R	40	95.5					
Erectoides x *Erectoides*								
10034x9841-F_1	ExE	20	12.2					
10034 x 9841-C_1	ExE	40	87.8	50	2.12	18.32	0.04	0.28
10034x9646-F_1	ExE	20	1.0	25	1.20	9.40	0	0
10034x9646-C_1	ExE	40	81.9	51	1.23	19.20	0.04	0.02
Erectoides x *Rhizomatosae*								
9841x9570-F_1	ExR	40	93.9	29	0.48	19.55	0	0.10
4x(10034x9646)x9830-1	4x(ExE)xR	40	70.8	25	0.04	19.68	0.08	0.08
4x(10034x9646)x9830-2	4x(ExE)xR	40	68.0	25	0.20	19.60	0.12	0.04
4x(10034x9646)x9830-3	4x(ExE)xR	40	56.3	25	0.50	19.40	0.12	0.08
4x(10034x9841)x(9841 x9570)-1	4x(ExE)x4x(ExR)	40	70.6	25	2.68	17.72	0.28	0.20
4x(10034x9841)x(9841 x9570)-2	4x(ExE)x4x(ExR)	40	81.8	28	2.19	18.88	0.36	0.12
4x(10034x9646)x569-1	4x(ExE)xR	40	68.5	25	0.64	19.48	0	0.08
4x(10034x9646)x569-2	4x(ExE)xR	40	70.8	25	1.16	19.00	0.12	0.12
4x(10034x9646)x569-3	4x(ExE)xR	40	63.6	25	0	19.60	0.04	0.12

prevalent in the group, and thus selection for fertility and regular meiosis is not a major factor in the survival of rhizomatous species.

The somatic chromosomes of *Arachis* species are generally small and metacentric. Reports of *Arachis* species karyotypes have almost exclusively been restricted to members of section *Arachis*, largely because of interest generated by being closely related to the cultivated groundnut. Raman (1959) and Smartt (1964) reported a distinctively small chromosome in most diploid species of section *Arachis*. *Arachis batizocoi* is the only species of section *Arachis* without the small chromosome (Smartt et al. 1978a,b). The distinctive pair was also absent in *A. paraguariensis* Chod. et Hassl. (Coll. GKP 9646) of section *Erectoides* (Smartt 1964).

Although the chromosomes of *Arachis* species are small, they can be accurately karyotyped (Stalker and Dalmacio 1981a; Singh and Moss 1982). Although the karyotypes of eight species presented by the two investigators differed slightly (apparent differences in published tables were largely due to methodology used to determine chromosome symmetry), several trends are apparent in the section *Arachis*. First, most chromosomes of the *Arachis* species genomes are relatively symmetrical. The genomes ranged in symmetry from two submedian or nearly submedian chromosomes in *A. spegazzinii* to seven submedian or nearly submedian chromosomes in *A. batizocoi* and *A. cardenasii* (Stalker and Dalmacio 1981 b). Although a distinctively small chromosome was earlier reported for most section *Arachis* species, except *A. batizocoi*, Stalker and Dalmacio (1981a) and Singh and Moss (1982) reported the smallest chromosome found in *A. cardenasii* was not so short as the other species of the group. Each investigator also concluded that the genome of *A. batizocoi* was significantly different from the other species of the section. Furthermore, the remaining cluster of seven analyzed species could be further subdivided into less distinct karyological groups.

A third distinct karyological group in section *Arachis* was reported by Stalker and Cross (1983) for *Arachis* sp Coll. GKSSc 30091 and 30099. The karyotype of this species was highly asymmetrical, with a subtelocentric chromosome which has not been observed in other species.

Interspecific Hybridization

To realize the potentials for germplasm introgression to *A. hypogaea*, biosystematic relationships among a broad spectrum of species must be known. Although a recent taxonomic monograph has not been published, the number of reported attempts to obtain interspecific hybrids of *Arachis* is very great. As the following discussion will illustrate, general cross-compatibility relationships within and among sections of the genus are now known.

Hybrids with *A. hypogaea*

The first report of an unsuccessful attempt to produce interspecific hybrids in the genus was between *A. hypogaea* and *A. glabrata* (Hull and Carver 1938). Krapovickas and Rigoni (1951) were the first to hybridize *A. hypogaea* with another species, *A. villosa* var. *correntina*. The same hybrid has since been reported by several other investigators (Kumar et al. 1957; Raman 1959; Smartt and Gregory 1967 and others). *Arachis villosa* is a member of section *Arachis* and, as Gregory and Gregory (1979) reported, the cultivated groundnut will only hybridize with members of this section. Twelve diploid species have been successfully hybridized with *A. hypogaea*, including: *A. villosa*, *A. villosa* var. *correntina*, *A. duranensis*, *A. cardenasii*, *A. chacoense* Krap. et Greg. nom. nud., *A. helodes* Martius ex. Krap. et Rig., *Arachis* sp GKP 9901 (Smartt and Gregory 1967); *A. batizocoi*(Krapovickas etal. 1974); *A. stenosperma*, *A. spegazzinii* and *A. ipaensis* Greg, et Greg. nom. nud. (Gregory and Gregory 1979); and *A. diogoi* (Stalker unpublished). However, not all hybrids have been successful in reciprocal; for example, *A. spegazzinii* and *A. ipaensis* succeed only as female parents and *A. helodes* and *A. diogoi* are most successful as male parents in crossing programs.

Reports of cytological analyses of triploid hybrids involving *A. hypogaea* have been infrequent. Smartt (1964) reported an average of 0.95 III for an *A. hypogaea* x *A. villosa* var. correntina hybrid, 2.15 III for *A. hypogaea* x *A. duranensis*, and 3.40 III for *A. hypogaea* x *A. helodes*. Because a common *A. hypogaea* parent was used for all three hybrid combinations, the chromosomes of *A. villosa* can be inferred to be more similar to the cultivated parent than the other two species. Meiosis in other triploid hybrids between *A. hypogaea* and species *A. chacoense* (Company et al. 1982; Bharathi et al. 1982) and *A. cardenasii* (Company et al. 1982) was observed and few multivalents were present. To date, a complete set of hybrids has not been cytologically analyzed. To draw conclusions regarding species relationships before a single cultivated

parent is hybridized with the diploid *Arachis* species and cytologically observed would be premature.

To restore fertility in sterile interspecific hybrids with *A. hypogaea*, plants have been obtained at the hexaploid (2n = 60) level by obtaining naturally-occurring polyploids or by colchicine treating vegetative tissues. Hexaploids have been obtained for crosses of *A. hypogaea* and the following species: *A. villosa*, *A. villosa* var. *correntina*, and *A. duranensis* (Smartt and Gregory 1967); *A. chacoense* and *A. cardenasii* (Spielman and Moss 1976; Company et al. 1982); *A. stenosperma* (Spielman and Moss 1976); *A. spegazzinii* (Peters et al. 1982) and *A. batizocoi* (Stalker unpublished). Although meiosis would be expected to be relatively normal with a minimum chromosome association of 30 II, 60-chromosome hybrids between *A. hypogaea* and *A. chacoense* or *A. hypogaea* and *A. cardenasii* are highly irregular with up to 20 I per pollen mother cell (Spielman et al. 1979; Company et al. 1982). In *A. hypogaea* x *A. villosa* var. correntina hybrids, D'Cruz and Chakravarty (1960) reported average chromosome associations of 0.18 I + 25.91 II + 2.0 IV and 9.0 I + 23.85 II + 0.3 III + 0.6 IV in different plants. Raman (1976) reported *A hypogaea* x *A. villosa* hybrids with 0.861 + 26.55 II + 0.18 III + 1.21 IV + 0.11 VI. Although meiosis is highly irregular in hexaploid hybrids with *A. cardenasii*, all selfed progenies in the following generation remained at the 60-chromosome level (Company et al. 1982). However, in the fifth generation after selfing hexaploid (*A. hypogaea* x *A. cardenasii*) hybrids produced by Smartt (1964), Davis and Simpson (1976) reported variable chromosome numbers, Spielman et al. (1979) and Stalker et al. (1979) reported 40-chromosome hybrid derivatives in the hybrid population. Chromosome loss had evidently occurred in the allohexaploid plants and many progenies at the lower chromosome number had wild species traits. Reducing the chromosome number from 2n = 60 in interspecific hybrids to 2n = 40 of cultivated groundnuts via backcross programs is difficult. Hexaploid x diploid crosses are apparently incompatible. Stalker (unpublished) made pollinations of 16 different hybrid combinations and only obtained pods with aborted embryos. Hexaploid x tetraploid crosses, which result in pentaploid progenies, are more successful at least in certain cross-combinations (Peters et al. 1982). Many pentaploids do not flower profusely and Stalker (unpublished) observed meiosis in three pentaploid [*A. hypogaea* x (*A. hypogaea* x *A. cardenasii*)] hybrids, that had been selfed one generation, and found an average of 1.09 I + 24.52 II + 0.04 III + 0.08 IV. A similar pentaploid [*A. hypogaea* x (*A. hypogaea* x *A. chacoense*)] F2 averaged 0.50 I + 22.30 II + 0.28 III + 0.01 IV. Considering the abnormal meiosis in their hexaploid parents and the nature of the genomes from different species, there was greater chromosome homology and a more regular meiosis in these pentaploid plants than expected.

Direct hybridization of *A. hypogaea* x diploid section *Arachis* species usually results in sterile triploid hybrids. However, Simpson and Davis (1983) reported an *A. hypogaea* x 2x (*A. cardenasii* x *A. chacoense*) hybrid which was male-fertile. When backcrossed to *A. hypogaea*, 50-chromosome progenies resulted. Krapovickas et al. (1974) reported 40-chromosome progeny obtained from a triploid plant which was self-fertilized. The resulting hybrid was fertile. D'Cruz and Chakravarty (1961) found hexaploid progenies from triploid (*A. hypogaea* x *A villosa*) hybrids. Singh (1984) has obtained 40-chromosome plants directly from triploid hybrids. Stalker (1981) found unreduced male gametes in intersectional *Erectoides* x *Arachis* hybrids. In non-hybrid materials, a plant of cultivar Florigiant with 60 chromosomes has been observed (Stalker unpublished). Although the hexaploid plant flowers, it is sterile. Natural production of unreduced gametes in the genus appears to be rather common and offers a simplified method to alter ploidy levels if population sizes are of sufficient numbers.

In addition to hybridizing *A. hypogaea* with diploid members of section *Arachis*, the cultivated groundnut hybridizes with *A. monticola* (2n = 4x = 40) (Krapovickas and Rigoni 1957). The F1 is fertile and chromosome pairing is generally 21 + 11 to 1511+2 to 4 IV (Raman 1958). Kirti et al. (1982) observed pachytene in *A. hypogaea* x *A. monticola* hybrids and found perfect homologies among chromosomes of the two species. Amphidiploids of diploid species can also be obtained naturally (Raman and Manimekalai 1973) or following colchicine treatments (Singh et al. 1980, Gardner and Stalker 1983). When the amphidiploids of section Arachis species containing only an A genome are hybridized with *A. hypogaea*, hybrids are mostly sterile; but fertile progenies have been obtained for a few hybrid combinations (Gardner and Stalker 1983). When *A. batizocoi* is used as one of the parents in the amphiploid, *A. hypogaea* x 40-chromosome plants are semifertile.

Hybrids between *A. hypogaea* and species of

other sections of the genus have been reported, including *A. glabrata* var. *hagenbeckii* (Nair et al. 1964), '*A. diogoi* (this material was not authentic A. diogoi, vide Gregory and Gregory 1979), A. glabrata and *A. villosulicarpa* (Raman 1976; Varisai Muhammad 1973a, b, c, d). However, Pompeu (1977) was unable to duplicate the hybrids using the same materials. Gregory and Gregory (1979), Smartt (1979) and Smartt and Stalker (1982) concluded that all hybrids to date involving *A. hypogaea* are within section *Arachis*. Several mechanisms of hybrid breakdown have been observed including delayed fertilization (Murty et al. 1981) and hypertrophy of integuments (Johansen and Smith 1956, Murty et al. 1981). Advances in tissue culture techniques may circumvent sterility barriers among the many species of the genus and *A. hypogaea*. Shastri and Moss (1982) obtained the following intersectional *Arachis* x *Rhizomatosae* hybrids: *A. monticola* x *Arachis* sp PI 276233, and *A. hypogaea* x PI 276233 after applying growth-regulator treatments.

Arachis Species Hybrids

The first hybrid between diploid Arachis species was between *A. duranensis* and *A. villosa* var. correntina (Raman and Kesavan 1962). Meiosis was regular in the hybrids. Most F_1 hybrids (excluding ones with *A. batizocoi*) in section *Arachis* have 10II and regular meiosis (Resslar and Gregory 1979; Smartt et al. 1978a,b, Stalker and Wynne 1979). However, male fertility of F_1 hybrids in this section ranges from 20 to 85%. Most hybrid combinations will produce seeds, but several combinations (i.e., *A. chacoense* x *A. cardenasii* and reciprocals) produce few or no seeds.

Hybrids in section *Arachis* involving *A. batizocoi* have irregular meiosis and are sterile (Gibbons and Turley 1967; Smartt et al. 1978a,b; Stalker and Wynne 1979). Stalker and Cross (1983) reported F_1 hybrids between *A. duranensis* x *Arachis* sp GKSSc 30099 and *A. batizocoi* x *Arachis* sp GKSSc 30099. F_1 hybrids of both crosses were sterile and had irregular meiosis.

As the result of an extensive hybridization program using 91 *Arachis* collections, including all seven sections of the genus, Gregory and Gregory (1979) reported general cross-compatibility relationships among *Arachis* species. Evident from their work is the success in obtaining intrasectional hybrids and the difficulty in obtaining intersectional hybrids. The mean pollen stainability of intrasectional hybrids (2x x 2x or 4x x 4x) was *Arachis* x *Arachis*, 30.2%; *Erectoides* x *Erectoides*, 12.9%; *Caulorhizae* x *Cauiorhizae*, 86.8%; *Rhizomatosae* x *Rhizomatosae*, 68.1%; *Extranervosae* x *Extranervosae*, 0.2%; *Triseminale* x *Triseminale*, 59.5%, *Ambinervosae* x *Ambinervosae*, 20.6%. Most frequent successes among the attempted intersectional hybrids were sections *Arachis* x *Rhizomatosae* and sections *Erectoides* x *Rhizomatosae* (Gregory and Gregory 1967,1979). They also were able to hybridize sections *Arachis* x *Erectoides*, *Erectoides* x *Ambinervosae*, *Erectoides* x *Caulorhizae*, and *Extranervosae* x *Ambinervosae*. The mean pollen stainability of the intersectional hybrids was 1.9%. Meiotic observations have not been made for this group of hybrid plants.

Complex hybrids have also been reported in an effort to circumvent hybridization barriers among species of different sections and to obtain hybrid derivatives which are fertile. Stalker (1981) reported a triploid F_1 hybrid between a 40-chromosome section *Erectoides* amphiploid (derived from an F_1 hybrid between *A. rigonii* (GKP 10034, PI 262142) x *Arachis* sp (GKP 9841, PI 262278] and *A. duranensis* or *A. stenosperma* of section *Arachis*. Thirty-, 31-, and 32-chromosome plants were observed with mostly bivalents and a low frequency of trivalents. He concluded chromosome homology existed between the sections *Erectoides* and *Arachis*. Other complex intersectional hybrids and their corresponding chromosome configurations are presented in Table 4. The diploid section *Erectoides* F_1 hybrids were sterile, but amphidiploids had regular meiosis and produced seeds. When intersectional hybrids were made between a 40-chromosome amphiploid (*Erectoides* x *Erectoides*) x *Rhizomatosae*, chromosome associations were mostly bivalents, but univalents were also observed (Table 4). Because of the complexities of most intersectional hybrids, conclusions concerning intersectional chromosome associations are difficult to make. However, chromosome homologies are evident in at least one hybrid 4x [*A. rigonii* 10034 (E) x *Arachis* sp GKP 9841 (E)] x 4x [*Arachis* sp GKP 9841 (E) x *Arachis* sp GKP 9570 (R)]. No seeds were produced from the complex intersectional hybrids even though all plants were at the tetraploid level. One tetraploid intersectional E x R F_1 hybrid combination, *Arachis* sp GKP 9841, PI 262278, x *Arachis* sp GKP 9570, PI 262817, apparently arose from an unreduced gamete. This hybrid was male-fertile, had regular meiosis, and produced seeds.

When the four intersectional hybrids listed in Table 4 were used as male parents in a crossing program, with members of section *Arachis*, including *A. duranensis*, *A. spegazzinii*, *A. monticola*, and *A. hypogaea*, a total of 2231 pollinations resulted in three hybrids, as follows: *A. spegazzinii* x [4x(GKP 10034 x GKP 9646) x HLKHe 569], *A. spegazzinnii* x [4x (GKP 10034 x GKP 9646) x GKP 9570], and *A. duranensis* x [4x(GKP 10034 x GKP 9841) x 4x(GKP 9841 x GKP 9570)] (Stalker unpublished). After two years of propagation, no flowers have been produced on the plants and efforts to restore fertility at a higher ploidy level have all failed.

Genomic Evolution and Germplasm Pools

Interspecific hybridization in the genus *Arachis* is difficult and, even when hybrids are obtained, many have only been produced after several hundred pollinations. Most interspecific hybrid combinations are sterile or only semifertile. Although cytological analyses have not been completed in the genus, a series of genomes for diploid species was proposed by Smartt and Stalker (1982) based on the extensive hybridization programs of Gregory and Gregory, as follows:

A = section Arachis, perennials and most annuals
B = section *Arachis* (*A. batizocoi*)
Am = section *Ambinervosae*
C = section *Caulorhizae*
E = section *Erectoides* (subgenomes E_1, E_2, E_3, corresponding to series ?)
Ex = section *Extranervosae*
T = section *Triseminalae*
R_1 = section *Rhizomatosae*, series *Prorhizomatosae*

Another genome for section *Arachis* was proposed by Stalker and Cross (1983) for taxa *Arachis* sp GKSSc 30091 and *Arachis* sp GKSSc 30099. Karyotypes of *Arachis* sp GKSSc 30091 and 30099 were significantly different and more asymmetrical than other members of the section. The karyological data indicate that the species is of recent origin. The D genomic designation is proposed for the genome. As cytological analyses are performed on interspecific hybrids and species relationships are better understood, the numbers of genomic groups will probably increase. For example, cytological analysis of [*A. rigonii*, GKP 10034 (E3) x *A. paraguariensis*, GKP 9646 (E2)] indicated that the chromosomes in different species of section *Erectoides* series are nonhomologous and that series designations probably represent distinctive genomes.

Determining genomic relationships among the tetraploid members of the genus is more difficult than for the diploid species. For example, at least some of the tetraploid members of section *Rhizomatosae* are cross-compatible with species in sections *Arachis* and *Erectoides* and may contain a similar genome to each of the two groups. However, cytological confirmation of chromosome homologies is difficult. Gregory and Gregory (1979) concluded that diploid ancestors of section *Rhizomatosae* probably hybridized with a diploid member of section *Erectoides* and, after chromosome doubling, gave rise to the tetraploid rhizomatous species.

Section *Arachis* also has two polyploid members, *A. hypogaea* and *A. monticola,* but the origin of polyploidy in this group was independent from section *Rhizomatosae*. *Arachis hypogaea* is an allotetraploid, probably with A and B genomes. Even though diploid members of section Arachis will hybridize with *A. hypogaea*, isolation due to polyploidy restricts germplasm introgression. The polyploids of section *Arachis* are apparently completely isolated from other members of the genus. Based on cross-compatibility relationships of *A. hypogaea* and other species, a germplasm pool system can be hypothesized. *Arachis hypogaea* and the closely related wild species *A. monticola* are in the primary gene pool, diploid members of section *Arachis* are in the secondary gene pool, and other members of the genus are in a tertiary gene pool. One can only speculate as to when all the potential resources of the genus will be readily available for cultivar improvement.

Conclusions

Extensive efforts have been made to determine biosystematic relationships among taxa in the genus *Arachis*. General cross-compatibility relationships among sections are known, but cytogenetic analyses of many hybrids produced in the genus have not been completed. The biosystematic area requiring the most immediate attention is the taxonomic treatment of the numerous species in the genus. Although the number of distinct taxa now known is greatly increasing, no species have been described during the past two decades.

The origin of groundnuts in South America was most likely in the 'planaltine ellipse' in Brazil. Members of sections *Erectoides*, *Extranervosae*, and *Rhizomatosae* are among the oldest species in the genus. Genomic differentiation has apparently followed sectional designations based on plant morphology. However, section *Erectoides* may have been important in the evolution of polyploid members of section *Rhizomatosae*. Interspecific hybridization in the genus is difficult and intersectional hybrids are all sterile. Because of genomic differentiation in the genus, introgression of the cultivated genomes may be especially difficult even after viable hybrids are produced among *A. hypogaea* and species outside section *Arachis*.

Most cytological analyses in the genus have been with diploid and polyploid members of section *Arachis*. Chromosomes can be identified and at least three genomes have evolved in the group. Based on the asymmetry of some species chromosomes, several members of the group are apparently of relatively recent origin. *Arachis hypogaea* will hybridize with other members of section *Arachis* and evidence exists for genetic recombination among species. Polyploidy apparently does not restrict gene transfer in the genus. A gene pool system has been proposed where *A. hypogaea* and *A. monticola* belong to the primary gene pool, diploid members of section Arachis belong to the secondary gene pool, and species in other sections of the genus are in the tertiary gene pool. Utilization of species in the tertiary gene pool will be very difficult.

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Chromosome Morphology and Gregory's Sectional Delimitation in the Genus *Arachis* L.

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Abstract

The value of studying pachytene chromosome morphology in problems of species interrelationships and chromosome aberrations has been realized since the pioneering work of McLintock in 1929, but the difficulties associated with such studies have discouraged several cytogenetic attempts. In a genus such as Arachis *where the mitotic chromosomes are small and where several interspecific crosses are unsuccessful, pachytene chromosome studies should be helpful in evaluating species relationships. Eight wild species, one tetraploid and two triploid hybrids have been studied so far. These studies are reviewed and their taxonomic utility discussed in this report.*

Résumé

Morphologie des chromosomes et regroupement en section par Grégory dans le genre* Arachis *L. : C'est depuis les travaux de pionnier de Mc Lintock en 1929 que l'on a compris l'importance des études sur la morphologie des chromosomes au stade pachytene pour ce qui concerne les problèmes de relations entre espèces et d'anomalies chromosomiques, mais les difficultés liées à ce type d'étude ont découragé plusieurs tentatives dans le domaine de la cytogénétique. Pour un genre tel qu'* Arachis *où les chromosomes mitotiques sont petits et où plusieurs croisements interspécifiques n'ont pas réussi, les études sur les chromosomes au stade pachytene devraient permettre une évaluation des rapports entre espèces. Huit espèces sauvages, un hybride tétraploïde et 2 hybrides triploïdes ont été étudiés jusqu'ici. Ce rapport présente une synthèse de ces études et une discussion de leur utilité taxonomique.

General Account of Arachis Pachytene Chromosomes

The staining and spreading characteristics of pachytene chromosomes of Arachis species are very poor and it is difficult to get full complements of the chromosomes from single nuclei (Murty et al. 1982). There are no distinct differences in length between chromosomes; they range in length from 5 to 7µm. The chromosomes are differentiated, each chromosome consisting of a centromere with proximal heterochromatin and distal euchromatin. Metacentric, submetacentric, and subtelocentric chromosomes occur.

The diploid species contain a single chromosome associated with the nucleolus while the tetraploid species have two nucleolar chromosomes. The characters that were used to identify chromosomes include; total length, arm ratio, nucleolus attachment, proportion and distribution of heterochromatin, and number and position of heterochromatic blocks. The karyotype consists of a mixture of the differentiated chromosomes, and a few specialized chromosomes.

Differentiated Chromosomes

This category includes those chromosomes which can be identified only on the basis of total length and arm ratio. They are generally distinguished into three classes on the basis of length; 1. long chromosomes—longer than 40 µm. 2. medium chromosomes—length from 25 to 40 µm and 3.

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short chromosomes—length less than 25 µm. Arm ratio can be used to further distinguish them although these differentiated chromosomes are generally either metacentric or submetacentric.

Specialized Chromosomes

Some chromosomes can be readily identified from their morphological appearance without the necessity of measuring their arm ratios or total lengths. Such chromosomes should readily provide cytological identification of trisomics. There are generally four such types; 1, chromosomes staining lightly but uniformly throughout their length, referred to as the 'euchromosomes', 2, chromosomes staining heavily throughout their length, referred to as the A chromosomes, 3, chromosomes with a completely heterochromatic short arm, and 4, chromosomes associated with the nucleolus.

Species Studied

The eight wild species whose pachytene chromosomes were studied along with two cultivated varieties belong to five sections of *Arachis* (Gregory et al 1973):

Section Arachis

A. hypogaea subsp *hypogaea* var. *hypogaea* (M 13)
A. hypogaea subsp *fastigiata* var. *vulgaris* (TMV 2)
A. chacoense
A. villosa (Coimbatore)
A. batizocoi PI 338312
A. correntina PI 331194

Section *Erectoides*
Arachis sp GKP 9990 PI 261877

Section *Rhizomatosae*
A. glabrata (Coimbatore)

Section *Extranervosae*
A. villosulicarpa (Coimbatore)

Section *Ambinervosae*
A. pusilla PI 338449

Generalized Karyotype

An examination of the idiograms of the various diploid and tetraploid taxa gave evidence for the existence of a basic karyotype with 10 chromosomes. Out of these 10 chromosomes, six are of the differentiated type, divisible into, and identifiable as long, medium and short, meta- and submetacentric chromosomes. The rest of the genome is made up of a nucleolar organizer and three specialized chromosomes. Marked variation exists in the specialized chromosomes of various species.

Karyotype Variation in Different Sectional Species

Detailed karyotypic descriptions are given in Murty et al. (1982), Kirti et al. (1982,1983), Bharathi et al. (1983), Jahnavi (1983), and Jahnavi and Murty (in press).

Section *Arachis* Species

In all four diploid species the karyotype consisted of six differentiated chromosomes, a nucleolar organizer, a chromosome with a completely heterochromatic short arm, and a euchromosome. In the three species, *A. villosa*, *A. chacoense*, and *A. correntina*, there was a euchromosome, an *A chromosome*, and a chromosome with a completely heterochromatic short arm. There were two types of nucleolar organizing chromosomes, one having a median centromere and a nucleolar-organizer region constriction near the centromere which was the site of nucleolus attachment. The second pair had a sub-median centromere and the nucleolus attachment was at the end of the short arm. Both types of nucleolar organizers are present in the so-called A genome species. In *A batizocoi*, there was a chromosome that is similar to the A chromosome except that it has a small, distal euchromatic segment.

The tetraploid species, *A. hypogaea* and *A. monticola* have almost identical karyotypes. Their genomes appeared to have two subgenomes. These two species, however, did not correspond in toto to a combination of the A and B genomes. Chromosomes of similar length were morphologically distinct and could be distinguished. Although basically the two constituent genomes appeared alike, differentiation and repatterning appeared quite marked.

Section *Erectoides* Species

The single *Erectoides* species investigated (Jahnavi 1983) has six basic differentiated chromo-

somes, a nucleolar organizer, and three specialized chromosomes, all with completely heterochromatic short arms. The karyotype shows resemblance to both the A and B genome species of *Arachis*.

Section *Triseminalae* Species

A. pusilla from this section has the basic karyotype present in section *Arachis*. The A chromosome is similar to that of *A. batizocoi*. However, the karyotype is unique in that chromosomes stain very poorly and the heterochromatin content is low.

Section *Extranervosae* Species

A. villosulicarpa has a karyotype similar to other species. Six differentiated chromosomes are present. It has the usual euchromosome and a nucleolar organizer. The two specialized chromosomes that distinguish this species have completely heterochromatic short arms.

Section *Rhizomatosae* Species

The tetraploid *A. glabrata* has highly specialized chromosomes. The karyotype has two genomes, each with the usual differentiated chromosomes, and all 20 chromosomes can be distinguished. It has the A chromosome characteristic of the A genome species in section *Arachis*.

Conclusions

The various species of section *Arachis* appear to have evolved from a basic karyotype. During the course of evolution, six differentiated chromosomes appear to have undergone very little structural change. The nucleolar organizer appears to be of either median or submedian type. Only three other chromosomes appear to have undergone structural changes. All species except those in section *Erectoides* seem to have retained the euchromosome.

Section *Arachis* seems to be a natural group with remarkable uniformity in the karyotype of its constituent species, with the exception of *A. batizocoi*. *A. batizocoi* is genetically distant from the other species of section *Arachis*. The hybrids formed are not fully fertile though in few pollen mother cells 8 to 9 chromosomes out of 10 pair.

The Triseminalae species, *A. pusilla* appears to be not very close to other sections. It has, as yet, not been possible to cross members of this section with species of any other section. The section seems to be geographically-isolated from other sections of the genus (Gregory et al. 1973).

Section *Erectoides* species seem similar to A or B genome species as well as to *A. glabrata*. This indicates that karyotypic similarity runs parallel to the cross-compatibility studies of these sections (Gregory and Gregory 1979).

Sections *Extranervosae*, *Erectoides*, and *Rhizomatosae* have been considered as the most ancient. The karyotype of *A. villosulicarpa* deviates very little from the basic *Arachis* karyotype. However, the tetraploid *A. glabrata* seems to be very highly specialized. It is most likely an allotetraploid, possibly derived from two *Rhizomatosae* species or from species from two different sections.

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Molecular Screening of Varietal / Specific Variation in Groundnuts - Peroxidase, a Model Enzyme

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Peroxidase (EC 1.11.1.7) isoenzymes have been used by geneticists and evolutionary biologists as markers of specific/varietal differences. However, detailed investigations in our laboratory showed that the four anionic peroxidase isoenzymes released into the medium of groundnut cells grown in cell suspension culture were formed from a single isoenzyme by interaction with phenolics. The large number of peroxidase isoenzymes reported in the literature had already induced workers to question their significance and authenticity. These reports coupled with our own observations questioned the use of peroxidase for studies in plant systems as such.

To overcome the problem of artifact formation, studies were initiated to purify peroxidase isoenzyme to homogeneity. The most abundant isoenzyme of peroxidase (cationic) which accounted for three-quarters of the peroxidase activity in the medium was purified to apparent homogeneity. Antibodies were raised in rabbits against the purified protein and these antibodies reacted with the peroxidase extracted from the groundnut leaves. These antibodies did not, however, react with the anionic peroxidase or with peroxidase from horse-radish (*Trametes versicolor*) or mung beans (*Phaseolus aureus*). Thus, these immunoglobins (IgGs) may be used as the first step in the identification of specific/varietal differences. However, with the available technology, we are in the process of isolating the mRNA for this peroxidase. This mRNA will then be used for the production of cDNA, which will be our molecular probe for specific/varietal variation. Such molecular probes are already in use in maize and some other crops.

Criblage moléculaire pour la variation intervariétale ou interspécifique des arachides - la peroxydase, un enzyme type : Les isoenzymes de la peroxydase (EC 1.11.1.7) ont été utilisés par les généticiens et les biologistes de l'évolution comme marqueurs des différences spécifiques/variétales. Cependant, des recherches précises réalisées dans nos laboratoires ont montré que les 4 isoenzymes de la peroxydase anioniques libérés dans le milieu par des cellules d'arachide cultivées en suspension se sont formés à partir d'un seul isoenzyme par intéraction avec des produits phénoliques. Le nombre élevé d'isoenzymes de la peroxydase rapportés dans la littérature a déjà conduit les chercheurs à s'interroger sur leur importance et leur authenticité. Ces rapports, associés à nos propres observations, remettent en question l'utilisation de la peroxydase dans les études sur les systèmes des plantes en tant que tels.

Des études sur la purification de l'isoenzyme de la peroxydase jusqu'à homogénéité ont été entreprises pour résoudre le problème de la formation d'artefacts. Le plus abondant isoenzyme de peroxydase (cationique), représentant les trois quarts de l'activité de la peroxydase dans le milieu, a été purifié jusqu'à une homogénéité apparente.

Face à cette protéine purifiée, des lapins ont fabriqué des anticorps réagissant à la peroxydase extraite des feuilles d'arachide. Cependant ces anticorps n'ont pas réagi à la peroxydase anionique ou à la peroxydase extraite du raifort (*Trametes versicolor*) ou des haricots mungo (*Phaseolus aureus*). Ces immunoglobines peuvent donc être utilisés comme premlère étape de l'identification des différences spécifiques/variétales. Cependant, grâce aux moyens technologiques dont nous disposons, nous sommes en train d'isoler le mARN pour cette peroxydase. Ce mARN sera ensuite utilisé pour la production de cADN qui pourra nous servir de sonde moléculaire pour l'étude de la variation spécifique/variétale. Ces sondes moléculaires sont déjà utilisées pour le maïs et d'autres cultures.

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Molecular Approaches to Genome Analysis in Arachis Species

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Abstract

Genomes of A. hypogaea *(cvs TG 1 and Florigiant),* A. rigonii, A. glabrata, A. hagenbeckii, *and* A. villosulicarpa *were assessed in terms of their DNA buoyant densities in cesium chloride density gradients, melting temperature (Tm), and base composition. The buoyant densities of the different species were within a narrow range (1.6954* g/cm^3*-1.6962 g/cm^3) except for* A. villosulicarpa *(1.6923 g/cm^3) and their Tms ranged from 83.13°C to 85.29° C. The guanine cytosine (GC) content ranged from 34.1% to 39.4% indicating heterogeneity among the species studied, and depicts the generally adenine thymine (A T)-rich nature of their DNAs. A comparison of DNA derivative melting curves revealed four to six components depending on the species. Some components appear to be common between species suggesting the possibility of conservation, while others are section or species specific. The buoyant densities of different species were different from the predicted values based on their Tms, indicating the presence of rare or modified bases.*

Résumé

Approches moléculaires de l'analyse du génome chez les espèces du genre* Arachis *: Des génomes de* A. hypogaea *(cvs TG 1 et Florigiant),* A. rigonii, A. glabrata, A. hagenbeckii *et* A. villosulicarpa *ont été évalués en fonction de la densité de flottaison de l'ADN dans un gradient de densité au chlorure de césium, de la température de fusion (Tf) et de la composition en bases. Les densités de flottaison des différentes espèces ont peu varié (1,6954 g/cm³-1,6962 g/cm³) sauf pour* A. villosulicarpa *(1,6923 g/cm³), leurs Tfs étant comprises entre 83,13° C et 85,29° C; la teneur en guanine cytosine a varié de 34,1% à 39,4%, indiquant une hétérogénéité parmi les espèces étudiées et une nature généralement riche en adénine thymine de leur ADN. La comparaison des courbes de fusion des dérivés de l'ADN a permis de relever 4 à 6 composants selon l'espèce. Certains composants apparaissent communs aux espèces laissant supposer des possibilités de conservation, d'autres sont spécifiques de sections ou d'espèces. Les densités de flottaison de différentes espèces ont été différentes de celles prévues sur la base de leurs Tfs, indiquant la présence de bases rares ou modifiées.

Introduction

Karyotypic analyses and studies on genome relationships between species of Arachis have been restricted to chromosome morphology and meiotic pairing in interspecific hybrids (Gregory and Gregory 1979; Singh et al. 1980; Stalker 1980; Raman 1981; Stalker and Dalmacio 1981; Kirti et al. 1982). Considering the difficulties in identifying different chromosomes within each genome and in distinguishing them from chromosomes of other genomes, there can be genuine doubts in assessing the type of pairing (autosyndetic or allosyndetic) in experimental hybrids. Furthermore, production of experimental hybrids between species belonging to different sections were reported

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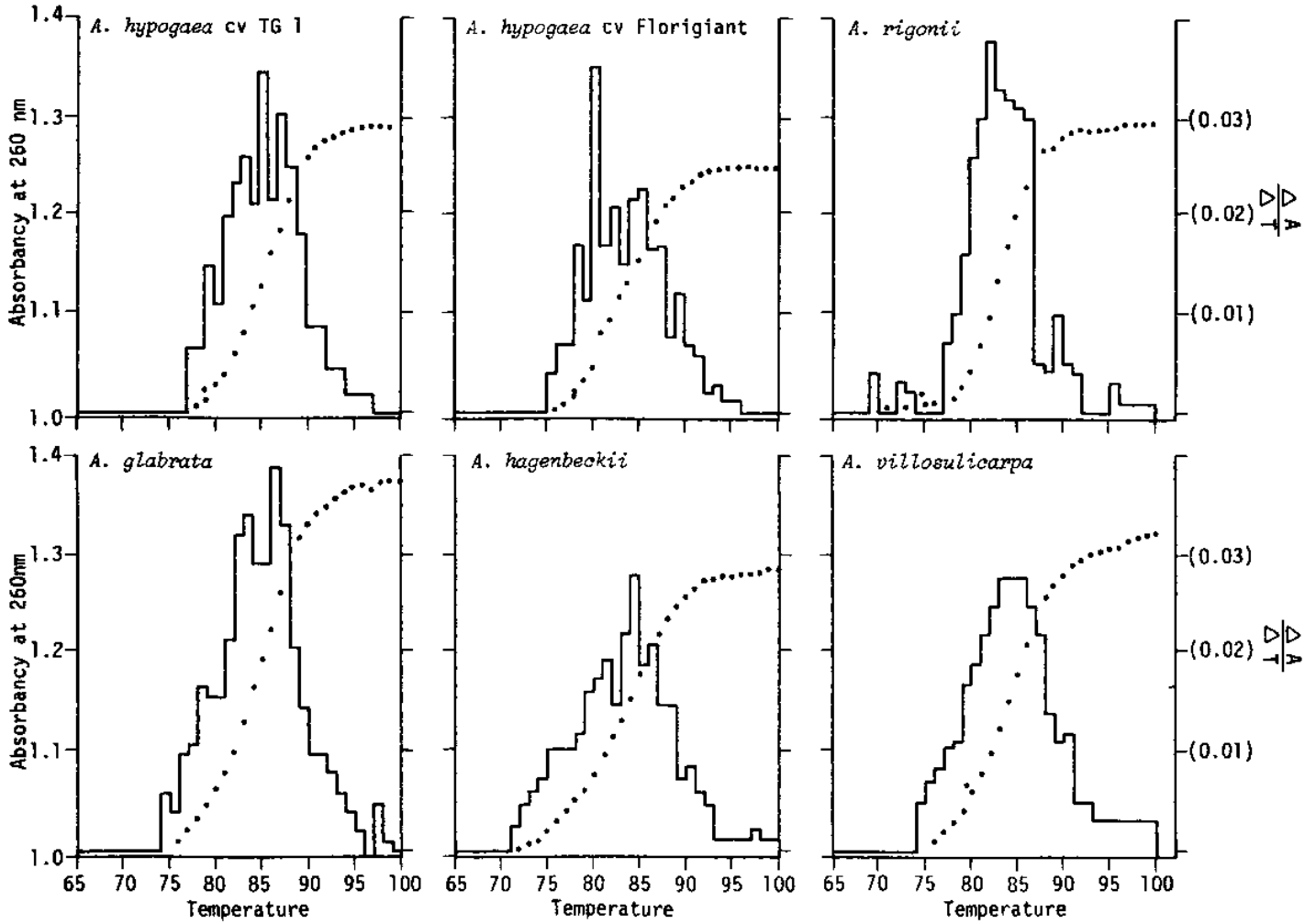

Figure 1. Thermal dissociation profiles of DNAs from different species of *Arachis*. .... melting curve - absorbance; —— differential plot $\Delta A / \Delta T$.

to be unfeasible (Gregory and Gregory 1979; Sastri and Moss 1982). With the advent of recent techniques in plant molecular biology, it should be possible to determine the phylogenetic relationships between species through the analysis of their DNA sequences, since their phenotypic differences reflect the expression of their genetic material.

Ressler et al. (1981) estimated the amount of DNA per cell in section Arachis to trace the ancestry of *A. hypogaea*. Dhillon et al. (1980) compared the base composition, buoyant densities, and denaturation-renaturation kinetics of DNA from two cultivars of *A. hypogaea*. Our studies aim to characterize DNA from different *Arachis* species in terms of melting temperature, buoyant density, base composition, reassociation kinetics, and restriction enzyme analysis. We report here a comparative study of five species of *Arachis* from four different sections based on the thermal dissociation kinetics and buoyant densities of their DNAs.

Methods and Materials

DNAs from leaves of five different species (Table 1) were isolated and purified according to Subrahmanyam and Azad (1978). Repeated RNase treatments and deproteinizations were done until an absorbance ratio (A260/280) of 1.9 for each DNA sample was achieved.

Table 1. *Arachis* species used.

Section[1]	Species	Source
Arachis	*A. hypogaea* (TG 1)	M.V.R. Prasad IARI Regional Res. Stn. Rajendranagar Hyderabad
	A. hypogaea (Florigiant)	M.V.R. Prasad IARI Regional Res. Stn. Rajendranagar Hyderabad
Erectoides	*A. rigonii* (8186)	J.P. Moss ICRISAT
Extranervosae	*A. villosulicarpa* (8142)	J.P. Moss ICRISAT
Rhizomatosae	*A. glabrata*	V.S. Raman TNAU, Coimbatore
	A. hagenbeckii	V.S. Raman TNAU, Coimbatore

1. According to Gregory and Gregory (1979).

Thermal dissociation of DNAs was carried out in 0.12 M phosphate buffer (pH 6.8) and monitored using a Gilford Spectrophotometer 250 equipped with thermo-programmer 2527 and chart recorder 6051. Melting temperatures and GC contents were calculated according to Mandel and Marmur (1968). First derivatives of the melting profiles were plotted as $\Delta A/\Delta T$ against temperature using an Apple II Computer. Each sample of DNA along with the marker DNA (Micrococcus lysodeikticus) was run in cesium chloride density gradients (initial RI = 1.4000) in an analytical ultracentrifuge (Beckman Model E) at 3.6×10^4 rpm for 24 h at 25°C. The buoyant densities of different samples were determined according to Schildkraut et al. (1962).

Results and Discussion

Thermal denaturation data, GC contents and buoyant densities of DNA from different Arachis species are presented in Table 2. Thermal denaturation profiles (Fig. la to f) indicated that the Tm in different species varied from 83.13°C (*A. rigonii*) to 85.29°C (*A. hypogaea*, cv Florigiant) corresponding to GC contents of 34.14% and 39.41% respectively. The two cultivars of *A. hypogaea* showed very little variation in their Tms and GC contents. Differential analysis of denaturation data based on the first derivatives of increase in absorbancy ($\Delta A/\Delta T$) revealed five common thermal components in the two cultivars of *A. hypogaea*, an additional component at 78°C in TG 1 which brings down the average Tm to 84.42°C compared to that of the other cultivar. The melting temperature and GC cpntent in *A. rigonii* were the lowest among the species studied and showed four thermal components, three common with other species and a species-specific thermal component at 74°C. This species was placed in a separate section, *Erectoides* by Gregory and Gregory (1979). The DNAs of *A glabrata* and *A. hagenbeckii* known as the 'glabrata-hagenbeckii complex of section *Rhizomatosae* (Gregory and Gregory 1979) showed similar Tms and thermal components. However, *A. villosulicarpa* belonging to section Extranervosae showed an average Tm similar to that of *A. hypogaea* cvTG 1, it possessed five thermal components in common with TG 1 but lacked one component (83 to 84°C).

An overall comparison of the derivative melting curves of each species revealed two components/ component complexes ('87-88', and '90-91') common to all the five species studied, indicating the

Table 2. Melting temperatures, GC contents, and buoyant densities of different *Arachis* species.

Species	Tm°C[1]	Thermal components[2]							% GC[3]	Buoyant density g/cm³	
		74	76-77-78	80	83-84	85-86	87-88-89	90-91		Experimental[4]	Predicted[5]
A.hypogaea (TG 1)	84.42+0.31		+	+	+	+	+	+	37.29	1.6956	1.6965
A. hypogaea (Florigiant)	85.29+0.39			+	+	+	+	+	39.41	1.6962	1.6987
A. rigonii	83.13+0.18	+			+		+	+	34.14	1.6954	1.6933
A. glabrata	83.83+0.65		+	+	+		+ +	+	35.85	1.6961	1.6954
A. hagenbeckii	83.62+0.44		+	+		+	+ +	+	35.34	1.6955	1.6945
A. villosulicarpa	84.58+0.12		+	+		+	+	+	37.68	1.6923	1.6969

1,3. Calculated according to Mandel and Marmur (1968).
2. First derivative melting curves plotted as $\triangle A/\triangle T$ against temperature.
4. Calculated according to Schildkraut et al. (1962).
5. Calculated from the GC content of *E. coli* with 50.5% as equivalent to a buoyant density of 1.710 g/cm³ and a change of 0.98% GC as equivalent to a buoyant density of 1 mg/cm³.

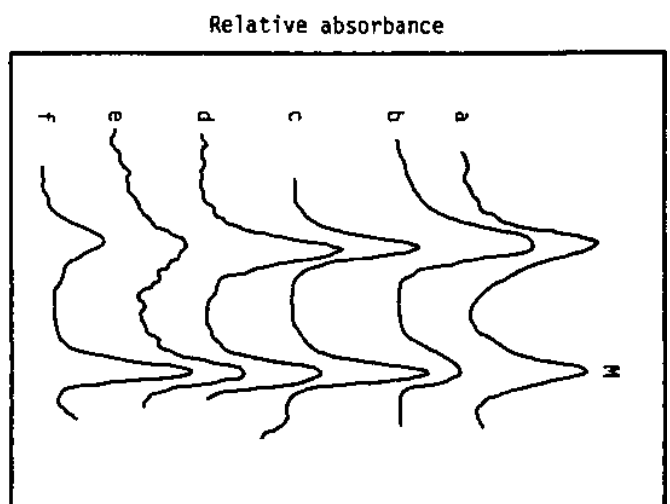

Figure 2. Buoyant density profiles of DNAs of different *Arachis* species:
a. *A. hypogaea* cv TG 1
b. *A. hypogaea* cv Florigiant
c. *A. rigonii*
d. *A. glabrata*
e. *A. hagenbeckii*
f. *A. villosulicarpa*
M = Marker DNA bd 1.731 g/cm³ (*Micrococcus lysodeikticus*)

conservation of those DNA components during evolution. This feature supports the inclusion of these species in one genus, *Arachis*. Variation in GC content (34.14%-39.41%) is indicative of the heterogeneity among the species studied, which can be interpreted as species-specific within the genus, and in general depicts the AT-rich nature of *Arachis* DNAs. The EcoRI digestion pattern of DNA from *A. hypogaea* TG 1 (Atreya et al. in press) is also consistent with these observations.

The buoyant densities (Fig.2a to f) of DNAs from different Arachis species fall within a narrow range (1.6954 g/cm³-1.6962 g/cm³) except for *A. villosulicarpa* (1.6923 g/cm³), which is believed to be distantly related to the cultivated species, based on

its cross-incompatibility with *A. hypogaea*. Furthermore, the predicted buoyant densities based on Tms for different species differed from the experimental values. It is likely that these differences are due to the presence of rare or modified bases. Most of the higher plant DNAs possess 5-methyl cytosine (m^5C) in 3.6 to 7.1 % of their bases. This plays a role in restriction-modification, spontaneous mutation, higher order chromosomes structure, DNA template activity, and differentiation (Ehrlich and Wang 1981). The presence of m^5C lowers the buoyant density without altering the Tm (Kemp and Sutton 1976) while the presence of 5-hydroxymethyluracil (Rae 1976) increases the buoyant density. In the light of these assumptions, the lower experimental values of buoyant density in TG 1, Florigiant and *A. villosulicarpa*, and higher values in other species as compared to the predicted buoyant density values can be attributed to the presence of such modified bases.

Our results, in general, indicate:

1. The heterogeneity and relatively AT-rich nature of *Arachis* DNA,
2. The presence of species-specific thermal components in different species of *Arachis*,
3. The occurrence of modified or rare bases in the DNAs of the different species, and
4. The consistency of the classification of these species suggested by Gregory and Gregory (1979) at the molecular level.

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Breeding Strategies for Utilization of Wild Species of Arachis in Groundnut Improvement

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Abstract

Utilization of wild species entails bringing genomes together and producing progenies which can be subjected to selection. The degree of similarity between species and between genomes dictates the techniques to be used. Studies on cytology, genetics, morphology, and evolution lead to a knowledge of species relationships, ploidy levels, and the taxonomic limits to hybridization. Closely-related species at the same ploidy level present few problems, but genes controlling chromosome pairing, and variation in number and position of chiasmata, may reduce or enhance the production of desirable segregants.

Differences in ploidy level between wild and cultivated species present problems in interspecific breeding, but ploidy manipulation has been successfully used, not only to achieve fertility in sterile hybrids, but also to adjust ploidy levels prior to hybridization. Induction of haploidy also has a role to play.

Aneuploidy, and substitution and addition lines can be useful tools in the identification and manipulation either of chromosomes carrying the genes that are to be transferred, or of genes controlling pairing or crossability that can be used to facilitate gene transfer.

Using these techniques, a wide range of interspecific Arachis *hybrids have been produced at ICRISAT, and derivatives with disease resistance and good yield have been introduced into the trials of the All India Coordinated Project for Oilseeds.*

Résumé

Stratégies de sélection pour l'utilisation des espèces sauvages : L'utilisation des espèces sauvages implique de rassembler des génomes et de produire des descendances pouvant être soumises à une sélection. Le degré de similitude entre les espèces et entre les génomes impose les techniques à utiliser. Les études de cytologie, génétique, morphologie et évolution, conduisent à une connaissance des relations des espèces, des niveaux de ploïdie, et des limites taxonomiques à l'hybridation. Les espèces voisines de même niveau de ploïdie posent peu de problèmes, mais les gènes contrôlant l'appariement des chromosomes et les différences de nombre et de position des chiasmas peuvent réduire ou accroître la production des ségrégants intéressants.

Les différences du niveau de ploïdie entre les espèces sauvages et cultivées posent des problèmes pour la sélection interspécifique, mais la manipulation de la ploïdie s'est révélée être un instrument puissant, utilisé non seulement pour rendre fertiles les hybrides stériles, mais également pour ajuster les niveaux de ploïdie avant l'hybridation. L'induction de l'haploïdie a également un rôle à jouer.

L'aneuploïdie, et les lignées de substitution et d'addition, peuvent se révéler très utiles pour l'identification et la manipulation soit des chromosomes portant les gènes à transférer, soit des gènes contrôlant l'appariement ou l'aptitude au croisement, qui peuvent être utilisés pour permettre le transfert de gènes.

Grâce à ces techniques, une gamme assez large d'hybrides interspécifiques d'* Arachis *ont été produits à l'ICRISAT. Les dérivés de ceux-ci présentant les caractères de résistance aux maladies et de bon rendement font l'objet actuellement des essais de l'All India Coordinated Project for Oilseeds (Projet Coordonné Indien sur les Oléagineux).

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Introduction

Wild relatives of crop plants contain many characters, which may be valuable to the breeder in crop improvement. Foremost among these are disease and pest resistance, but wild species have also been shown to contribute other desirable characters, such as increased yield (Takeda and Frey 1976). The wild species of *Arachis* have been of interest primarily as a source of disease and pest resistance (Subrahmanyam et al. 1985; Amin 1985). The pioneering work on crossability (Gregory and Gregory 1979) made the utilization of wild *Arachis* species an attainable goal, and the early screening for disease resistahce (Abdou et al. 1974; Sharief 1972) indicated which species were to be used.

When the desired character is not available in the cultivated germplasm, wild species and induced mutations present alternative sources of variability to the plant breeder. In the cultivated groundnut mutagens have produced some interesting novel plant forms but few, if any, widely adopted new cultivars have been released for commercial production (Gregory 1966; Anon 1967; Patil 1977). The use of tissue culture for the release of somaclonal variation (Larkin and Scowcroft 1981) or for selection of cells in culture has yet to be attempted in *Arachis*.

Wild species can be of value even when good resistance is available in the cultivated germplasm, as they may have different resistance genes which can be utilized to give more stable resistance (Singh et al. 1985). The choice of cultivated parent is important, and there are many genotypes of cultivated germplasm from which to choose. Although there is no evidence of crossability genes in *Arachis* as effective as those in wheat, cultivars differ in their crossability with wild species.

This paper discusses the stategies used to obtain interspecific derivatives which can be used in crop improvement programs.

Wild Species

In any genus, the wild species available to the plant breeder consist of a number of accessions, whose correct taxonomy may or may not be known. Accessions of the same species may differ from one another, and all accessions should be screened for desirable characters. Where more than one species or accession is resistant to a pathogen, the type of resistance may differ, and it may be possible to transfer different components of resistance (Subrahmanyam et al. 1983). The wild species with the desired character may be very similar to, and freely crossable with, the cultivated species, and the progeny of the interspecific hybrids may be fully fertile. Hammons (1970) crossed *A. hypogaea* with *A.monticola* and developed 'Spancross' without the application of special cytogenetic techniques. This successful production of a cultivar indicated that pairing and recombination between wild and cultivated chromosomes had occurred.

A number of intrasectional hybrids have been produced within section *Arachis* (Raman and Kesavan 1962; Gregory and Gregory 1979; Moss et al. 1981). However, only a few of these had been used in attempts to transfer characters from wild species into the cultivated groundnut (Moss and Spielman 1976; Stalker et al. 1979; Moss 1980) until ICRISAT groundnut cytogeneticists began an extensive program of interspecific hybridization (ICRISAT 1981). Despite many attempts, very few hybrids have been produced between *A.hypogaea* and species of section Rhizomatosae (Gregory and Gregory 1979; Moss and Sastri 1982) and none of these have been used in a breeding program. Attempts to tap this valuable source of resistance genes by bridge crossing, using a third species compatible with both parents, have not been fruitful (Stalker 1981).

There are seven sections with a wide range of morphology and two ploidy levels in the genus. Some intersectional hybrids have been produced, but some sections are isolated, and all sections other than *Arachis* are isolated from *A.hypogaea*. There are, therefore, two major priorities in gene transfer from wild *Arachis* species. One is to transfer resistance to the two major leaf spot diseases, Cercospora *arachidicoia* Hori and Cercos*poridium personatum* Berk, et Curt, from compatible wild species in section *Arachis* (Abdou et al. 1974; Subrahmanyam et al. 1985). The second is to tap the virus resistance of section *Rhizomatosae* (Subrahmanyam et al. 1985; in press). The former involves ploidy manipulations among hybrids that are comparatively easy to obtain. The latter involves a major input to overcome barriers to hybridization between sections *Arachis* and *Rhizomatosae*, with the possibility that the techniques developed will be of value in producing other intersectional hybrids.

The production of an interspecific hybrid brings genomes together in the cytoplasm of one of the species. Often that is a considerable achievement,

but the desired end product is usually the cytoplasm and genome of the cultivated species with only a small part of the wild species genes. It is often more difficult to produce the agronomically-acceptable line than to produce the original interspecific hybrid. Production of interspecific derivatives involves the disciplines of cytogenetics and plant breeding. Where resistance genes are to be transferred, the assistance of pathologists and entomologists will be needed. The success of utilization of wild species of *Arachis* at ICRISAT owes much to the strong interdisciplinary cooperation in the Groundnut Improvement Program.

Intrasectional Hybrids

Intrasectional hybrids have been produced in most sections (Gregory and Gregory 1979) but their possible use in the improvement of *A.hypogaea* has not been explored. The possibility of using induced auto- or allotetraploids from intrasectional hybrids has not been researched. Emphasis has been placed on hybrids within section *Arachis* to utilize resistance to leaf spots and rust (*Puccinia arachidis* Speg.) diseases because of the crossability of most section *Arachis* species with *Arachis hypogaea*.

The cultivated groundnut is a successful allotetraploid, probably originating from two section *Arachis* diploid species. There is some controversy as to the donor of the A genome but *A. batizocoi* which is always cited as the B genome donor (Smartt et al. 1978) is the only known B genome species. Little is known of the genomes in other sections, as detailed karyomorphological studies have only been completed in section *Arachis* (Stalker and Dalmacio 1981; Singh and Moss 1982) and few intersectional hybrids have been produced or studied (Stalker 1981).

There are several routes to transfer genes from a diploid wild species to a cultivated tetraploid. Amphiploidy and autotetraploidy have been used extensively at ICRISAT (Singh 1985). Haploidy could be a valuable tool, not only to transfer genes from wild species, but also in breeding programs using cultivated germplasm. Haploidy cannot be induced in *Arachis* at present, though some progress has been made in anther culture techniques (Mroginski and Fernandas 1979,1980). Aneuploidy has been reported in the genus *Arachis* (Spielman et al. 1979). and aneuploids could be used in future to transfer whole chromosomes by substitution or addition. Aneuploidy is also useful in the manipulation of genes controlling chromosome pairing or crossability.

All available means of interspecific gene transfer by sexual hybridization involve the production of a hybrid between the wild species and a cultivar or germplasm line of the cultivated species. This may be the product of the first hybridization in the crossing program, or the cultivated species may not be used as a parent until after a series of crosses and/or ploidy manipulations. The primary wild x cultivated hybrids may be sterile, or only partially fertile, (e.g., most triploids), or may be fully fertile and vigorous, (e.g., some amphiploidx *A.hypogaea* hybrids). Some of the latter hybrids must be repeatedly backcrossed to *A.hypogaea* to produce genetically - and cytologically-stable tetraploid lines compatible with *A.hypogaea*, and with the desired genes from the wild species. The ideal introgressed line will have a genetic and cytoplasmic background in which the desired gene can be expressed. Such lines must also have suitable agronomic characters, such as yield, duration, and plant habit, that make them more useful to conventional plant breeders as sources of resistance. When backcrossing wild species hybrids to the cultivated species, the parental cultivar is not always used as recurrent parent. The introduction of another cultivar can bring in other useful characters, as well as inducing variation in the genetic background for the best expression of the desired gene. It is the best use of resources for the cytogeneticist to produce *A.hypogaea*-like derivatives that can be used by many breeders, rather than for individual breeders to use the primary hybrids. The breeder can then cross these lines to locally adapted cultivars without undesirable wild species characters occurring in the progenies.

The fertility, genomic constitution, and degree of recombination of the hybrids produced after the first crossing of the wild and cultivated species are the major factors that determine the time taken to achieve productive *A. hypogaea*-like derivatives of value to the breeder as sources of wild species genes. However, a Knowledge of the genomes of the species does not imply that one can successfully transfer genes. Often that knowledge comes from the pairing behaviour at meiosis in hybrids. Thus, the attempts to transfer genes, and the cytogenetic analysis of species and interspecific hybrids have been a concurrent process at ICRISAT. Where cytogenetic studies have provided a sound base for planning future strategies for inter-

specific gene transfer (ICRISAT 1981; Singh and Moss 1982,1984). The primary hybrid provides the first opportunity for wild and cultivated chromosomes to pair and recombine, and the progeny produced provides the first opportunity to select recombinants. This generation is the equivalent of an F_2 in a conventional breeding program, and for successful gene transfer this F_2 population must satisfy a number of requirements:

Size. In practice, the population should be as large as can be handled, to increase the opportunity of selecting fertile, productive plants which have the desired character combinations. Vegetative propagation of the primary wild x cultivated hybrid may be necessary to produce large segregating generations.

Range of genetic and cytoplasmic backgrounds. The cytogeneticist must produce more than one primary hybrid, produce reciprocals, use different accessions of wild species, and use different genotypes of the cultivated parent as these may differ in genes controlling pairing (Singh and Moss 1984). In one population, Company et al. (1982) reported that disease resistance was closely linked with undesirable pod shape, but disease-resistant lines with good agronomic characters.including pod shape have been selected from another population (Moss 1984).

Effective screening. Facilities to screen large populations are essential. The frequency of desirable segregants may be low; for example, the number of disease-resistant plants may be adequate, but many of these may be sterile or may have undesirable wild species characters (ICRISAT 1980; Company et al. 1982).

Working with large populations obviates the need to count chromosomes or check meiosis in all plants. Chromosome counts and meiosis checks are only made on key hybrids and plants with abnormal characters or reduced fertility. A number of plants from each selection are checked for chromosome number and regular meiosis (Table 1).

Table 1. Program for development of wild species derivatives[1]

Season	Activity	Selection criteria
1979 rainy	Seed increase, preliminary observations	Disease resistance, Yield, Season length, Chromosome number, Growth habit
1979-80 postrainy	Seed increase	Yield, Uniformity, Season length
1980 rainy	Progeny rows of selected plants	Disease resistance, Yield, Pod characters, Uniformity, Cytological stability
1980-81 postrainy	Seed increase	Yield, Uniformity
1981 rainy	Selection of lines	Disease resistance, Yield
1981-82 postrainy	Seed increase	Yield, Uniformity
1982 rainy	a. Line selection	Disease resistance, Yield, Haulm yield, Oil yield
	b. Seed increase for conservation	
	c. Testing at different locations	
1982-83 postrainy	Seed increase	Yield

1. Lines with sufficient seed tested in randomized block or triple lattice designs.

Results

A number of lines with resistance to late leaf spot and/or rust have been selected. The material which is most advanced was received as progenies of interspecific hybrids among the germplasm transferred to ICRISAT during 1978 and 1979. The species hybrids from which selections were made are listed in Table 2. Whilst this germplasm was being grown in the ICRISAT Post-Entry Quarantine Isolation Area, it was observed that these progenies were still segregating and that there were some desirable plants. A program of single plant selection and progeny row testing was started. Disease resistance was assessed in the rainy season when natural levels of inoculum were high, and the postrainy season was used for generation advance and assessment of yield, crop duration, uniformity,

and other agronomic characters (Table 1). Early generation yield selections were based on number of pods per plant, and in later generations on pod weight per plot. Selections were grown in replicated trials as soon as possible, usually in the second or third generation after single-plant selections were made (Plate 4a). Trials were also grown at two sites in Tamil Nadu, South India, where there was a heavy incidence of leaf spot but little rust infection and so minimal interaction between pathogens.

A number of interspecific derivatives have been entered in varietal trials of the All India Coordinated Research Project on Oilseeds (AICORPO). One derivative, ICGS 50, [*A.hypogaea* x *A.cardenasii* (115 M), Selection H3/4E] was entered in the initial evaluation trial for Virginia bunch types, in 1983 rainy season. It was retained in Zone IV (Southeastern) and Zone V (Peninsular) for further testing in the 1984 rainy season.

Two derivatives were entered in the foliar disease resistant varieties trial in 1983. Twenty entries and 3 controls were successfully grown at 6 locations. The entries were ICG FDRS 17 [*A.hypogaea* x *A.cardenasii* (9B) Selection 1 /IV/3/11] and ICG FDRS 18 [*A.hypogaea* x *A. cardenasii* (115 M) Selection H3/4L B4]. At Aliyarnagar, both entries were the most resistant to rust and late leaf spot. At Dharwad, ICG FDRS 18 was resistant to early and late leaf spots. At ICRISAT, both entries had higher pod yield than all three controls. ICG FDRS 17 yielded 3535 kg/ha, ICG FDRS 18, 3360 and the controls 2250, 1843, and 1715 kg/ha. ICG FDRS 18 was one of three entries with the highest shelling percentage (64%), and also had the highest hundred kernel weight of 37 g. ICG FDRS 17 was the most resistant to late leaf spot. At Jalgoam, ICG FDRS 18 recorded the highest pod yield, 2055 kg/ha. At Kadiri, ICG FDRS 17 and 18 were resistant to late leaf spot, and at Vriddhachalam FDRS 17 was the most resistant to late leaf spot and rust (AICORPO 1984).

These and other selections from wild species derivatives also have good haulm yield, producing up to 6500 kg/ha of field-dried hay at harvest (Moss 1984). Many tetraploid wild species derivatives with disease resistance and good agronomic potential have been distributed to breeders.

Table 2. Pedigrees of hybrids from which wild species derivatives with resistance to disease, and with good agronomic characters have been selected.

Pedigree	Interspecific hybrid
82[1] x 34 - 9B	*A.hypogaea* x *A cardenasii*
82 x34 - 115M	*A.hypogaea* x *A.cardenasii*
82 x 19	*A.hypogaea* x *A.batizocoi*
82 x 19 x 8	*A.hypogaea* x (*A.batizocoi* x *A.spegazzinii*)
HP12-8B	(*A.batizocoi* x *A.hypogaea* x *A.spegazzinii*)

1. 82, 34, 19, 8, are Gregorys parental numbers, (Gregory and Gregory 1979). HP12 designates a hybrid combination, and 9B. 115M and 8B are the original hybrid numbers.

Intersectional Hybrids

Very few intersectional hybrids have been produced (Gregory and Gregory 1979). Of the 42 possible intersectional combinations, including reciprocals, only eight have been produced; six of these involve section *Erectoides*. Although there has been some interest in bridge crosses, attempting to use the crossability of species of section *Erectoides* with species of other sections to transfer genes to *A. hypogaea*, there has been no success.

The major problem in intersectional crossing has been that few pegs are produced, and most hybrid pegs stop growing before they reach the soil. Pods are occasionally formed, but they contain very small seeds. Embryo rescue by tissue culture has been suggested, but has not been successful enough to be used as a routine technique. Ovule culture has been attempted as a means of rescuing embryos which are too small to excise and culture (Martin 1970).

Therefore emphasis has recently been placed on this technique at ICRISAT (Nalini and Sastri 1985). We all await with interest the production of a range of hybrids which can be studied in detail to further understand the genomic constitution of the genus, and to see whether whole genomes, or only parts of genomes are being transferred, and whether genes from other sections can be used in genetic improvement of *A. hypogaea*.

Protoplast culture in *Arachis* is a recent development, some success in culture, and in regeneration of callus, has been achieved (Oelck et al. 1982; Rugman and Cocking 1985). Hopefully the use of protoplasts to transfer whole or parts, of genomes will become a reality in legumes in the near future.

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Taxonomy and Means of Utilization — Discussion

Rees:
I am struck by the lack of references to single gene "markers" or to linkage data, either from the cultivated or wild species. Are such data difficult to obtain? They might well be useful to mark and follow the transmission of chromosomes in hybrids, hybrid derivatives, and in aneuploids?

Moss:
There is very little information on gene markers in *Arachis*, and very few linkage groups are known (see Wynne, J.C., and Coffelt, T.A. 1982. Genetics of *Arachis hypogaea* L. Pages 50-94 *in* Peanut science and technology (Pattee, H. E., and Young, C.T., eds.). Yoakum, Texas, USA: American Peanut Research and Education Society, Inc.).

Appa Rao:
Is there any information on the assignment of linkage groups to the chromosomes?

Stalker:
Only a few linkage groups are known, I recollect three being reported. As aneuploids are being developed the association of genes to specific chromosomes will be possible. Some work is in progress at several universities to characterize linkage groups, but the work is long term and information is slow to accumulate. Presently no genes have been associated with any particular chromosome.

Rees:
I think it may well be a difficult task to construct linkage maps in *Arachis* species. The reason is that the chiasmata at meiosis are highly localised towards the distal regions of the chromosomes. Unless master genes are located within these distal segments they cannot, by normal means, be mapped. Genes in interstitial segments will remain inseparable by crossing over.

Stalker:
Only three linkage groups have been described in *Arachis* and the majority of genetic work associated with morphological variants has been with seed coat color. Approximately 20 other genes have been identified. As a result of the few genetic studies, as compared to maize or tomato, linkage of genes with specific wild species traits are not known, and extensive work will be required before these association can be made.

Singh:
Dr. Murty, your inference, based on pachytene analysis, of putting *A. batizocoi* out of section *Arachis* or assigning it a different status within the section does not conform with other results. In biosystematic studies the conclusions are made based on collective evidence from different sources, such as morphology, geographical distribution, mitotic karyotype affinities, crossability, and pairing of chromosomes in F_1 hybrids, nearly all of which indicate that *A. batizocoi* is in section *Arachis*.

Murty:
F_1 hybrids of *A. hypogaea* with A genome species have higher pollen fertilities than those between *A. hypogaea* x *A. batizocoi*. All section Arachis hybrids have reasonable levels of fertility except those involving *A. batizocoi*. *A. batizocoi* has entirely different leaf anatomy from other section Arachis species. Amphidipioids among A genome species look more like *A. monticola* or *A. hypogaea*, but this is not so for those between A genome species and *A. batizocoi*.

Stalker:
Why do you consider *A. batizocoi* as belonging to a different section from *Arachis*?

Murty:
We do not consider *A. batizocoi* as belonging to another section. However, we do consider *A. batizocoi* as very diferent from other section *Arachis* species for the following reasons; it forms mostly sterile hybrids with other section *Arachis* species including the cultivated groundnut and it does not have the completely heterochromatic chromosome (A chromosome) characteristic of all other section *Arachis* species.

We do not at this stage recommend that *A. batizocoi* be separated from section *Arachis* but we emphasize its dissimilarity with the other section *Arachis* species.

N.C.Subrahmanyam:
How many genotypes from the species did you use for the measurements, how many cells were scored for each chromosome measurement and what is the coefficient of variation for each chromosome?

Murty:
One genotype of each species was used, and more than 30 pollen mother cells were scored. Coefficients of variation were very low.

Stalker:
How do you account for the fact that the somatic chromosome data of Stalker and Dalmacio, or Singh and Moss (which are very similar) is so greatly different from your data on meiotic chromosomes? This is especially evident for chromosomes with the nucleolar organizer, which is most easily observed?

Murty:
Differential condensation of eu- and heterochromatin during mitosis and meiosis, and also the pretreatment agents used by mitotic cytologists, may perhaps be responsible for miotitic karyotypes differing from pachytene karyotypes.

Sastri:
Based on pachytene analysis of representatives of different sections, will it be possible to predict the cytological performance of intersection hybrids?

Murty:
It is possible. It is likely that essentially true pairing resulting in chiasma formation and exchange of chromosome segments may occur to a certain extent in intersectional hybrids, since the basic karyotype in several sections appears to be the same. There should be homoeologies in the case of the six differentiated chromosomes.

Moss:
How much do your results correlate with mitotic results? ICRISAT's and NCSU's results on mitosis agree in broad principle, variations in numbering being consistent with stastitical variation due to fixing, staining, etc. Can you identify which meiotic chromosomes in your study correspond to the accepted numbering of the karyotype?

Murty:
The classification of chromosomes at pachytene in *Arachis* species has been based to a large extent on morphology. Slight length and arm ratio differences were shown to be adjusted at pairing in hybrids as has been found in other species. That the morphological criteria are accurate was confirmed by observing chromosome pairing in interspecific hybrids such as *A. hypogaea* x *A. monticola*, *A. hypogaea* x *A. villosa* and *A. hypogaea* x *A. chacoense*.

Singh:
In our study of somatic complements *A. glabrata* does not have the distinct A chromosome. However, it was shown to have a similar type of secondary construction to that present in *A. batizocoi*, which has also been found to cross with *A. glabrata* by Gregory and Gregory.

Murty:
The A chromosome was distinguished, by size only, by Husted and subsequent workers as a very small chromosome. At pachytene, we characterized it as the completely heterochromatic chromosome. The nucleolar organizers in *A. glabrata* are of two types: metacentric and submetacentric. *A. batizocoi* has centric nucleolar organizers.

Amin:
A. monticola is very similar to *A. hypogaea* and they also produce fertile hybrids. The pest reaction of *A. monticola* and *A. hypogaea* to a large number of sucking and defoliating insects is similar. Based on the available evidence, can we really call them separate species?

Stalker:
A. hypogaea and *A. monticola* are the same biological species, but different morphological species. Whether *A. monticola* should be made a subspecies of *A. hypogaea* is a taxonomic question whose answer depends on whether you are a "lumper" or "splitter". Because they are morphologically different, and the literature is full of references where they are two species, *A. hypogaea* and *A. monticola*, I believe the taxa should be referred to as two species.

Murty:
If you assume *A. hypogaea* or *A. monticola* to have been derived from an A genome species x *A batizocoi*, don't you also expect that *A. hypogaea* x A genome hybrids should have similar cytology and fertility to *A. hypogaea* x *A. batizocoi* hybrids?

Stalker:
With the present accumulation of information, I believe *A. hypogaea* and *A. monticola* were derived sometime in ancient history, from a hybrid between an A genome and a B genome species. However, generalizations concerning meiotic behaviour of

triploid, interspecific hybrids should not be made using the presently-available cytological reports. To do a proper job, one should hybridize a common *A. hypogaea* with all the section *Arachis* species and then observe meiosis under uniform environments. The question of triploid fertility has to do with the frequency of unreduced gametes in specific hybrid combinations, not the closeness or relationship of the two species which were hybridized.

Rees:
Atreya has provided sophisticated information about the composition of the nuclear DNA in *Arachis* species. I would have thought that estimates of nuclear DNA amounts in themselves would be useful. Are there such estimates?

Atreya:
Yes. Resslar et al, 1981. [American Journal of Botany 68(2): 149-153] reported DNA amounts, and according to them the values are such that they cannot be used as a character in determining the ancestry of the cultivated groundnut.

Sastri:
Can we use haploid tissue for these studies? Resslar showed differences between the two subspecies of *A. hypogaea*.

Atreya:
Theoretically this is a good suggestion, but the amount of haploid tissue that is required to isolate pure DNA is impractical, especially when genomes of different species are to be analysed. Ressler's work is quantitative, it shows the amount of DNA per cell in different species in section *Arachis*. Our work is in analysing genomes (DNA) in terms of the DNA quality, so the work cannot be compared; each aspect gives different information. However, DNA content per cell together with qualitative analysis of DNA in different species, would help in establishing the extent of genome relationships or divergence between species.

Rees:
Peroxidase may be a 'model enzyme', but whatever the advantages or disadvantages of peroxidases as isozymes for distinguishing between varieties or species, I am sure that the comparisons would be that much more effective if five or six different groups of isozymes were used.

P.Subrahmanyam:
Utilization of high-yielding, rust-resistant *A. hypogaea* genotypes in backcrossing with hexaploids or pentaploids to produce near tetraploids may be useful in accumulation of rust-resistant genes from both wild species and cultivated groundnut. What are the problems involved in utilizing rust-resistant *A. hypogaea* in backcrossing?

Moss:
The combination of genes from wild species and from *A. hypogaea* may give more stable resistance, because there is evidence that they are different genes. However, it is difficult to detect the presence of both genes. At the time of backcrossing hexaploids, when we are losing chromosomes, is not the best time to introduce the other gene. This should be done by the breeder at the tetraploid level. The genes can then be detected either by their phenotype, if they affect different components of resistance, or by genotype, by crossing with a susceptible tester variety, and examining F_1 populations. In fact we have used NC107090 in some of our backcrosses, but will not test for the presence of both genes until the tetraploid level.

N.C.Subrahmanyam:
On the problem of achieving recombination between chromosomes from wild relatives and those of the cultivated species it may be worthwhile using autotetraploids of wild diploid relatives which show useful characters, crossing them with the cultivated species, and then looking for recombinants.

Moss:
This is already being done (ICRISAT Annual Report 1983, pp 212-214) and is a valuable method.

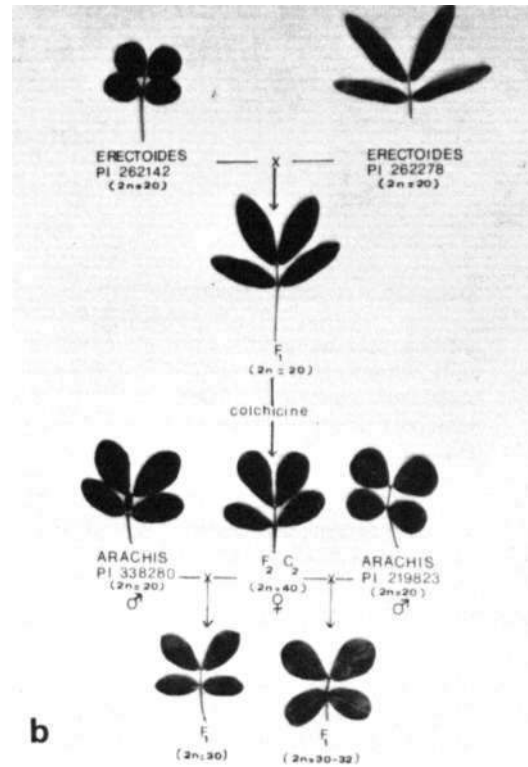
Rao:
When we harvest groundnuts we observe many immature pods. Can we reduce the length of the flowering period to get more filling (i.e., make groundnut determinate with synchronous maturity)? Are there any such types in wild germplasm? Can we transfer the trait to cultivated species?

Moss:
We found a line with the opposite effect in one of our populations; it produced so many pegs that few pods matured. We have also found many other variants, but not the one you describe. The physiologists are now looking more closely at our lines and we hope something will materialize.

Use of Compatible Species

Plate 3. a. *Arachis villosulicarpa* Plot 71, Sandhills 77, USA, (p. 27)
b. Pedigree of intersectional hybrids, (p. 120). [Reproduced from Crop Science 21: 359-362, 1981 by permission of the Crop Science Society of America.]
c. (E x E) x a triploid hybrid LT 92, (p. 120).


a


ERECTOIDES
PI 262142
(2n=20)
ERECTOIDES
PI 262278
(2n=20)
F1
(2n=20)
colchicine
ARACHIS
PI 338280
(2n=20)
♂
F2 C2
(2n=40)
♀
ARACHIS
PI 219823
(2n=20)
♂
F1
(2n=30)
F1
(2n=30-32)
b


c

Genetic Introgression from Compatible Wild Species into Cultivated Groundnut

A. K. Singh[1]

Abstract

The diploid wild species (2n=20) compatible with the cultivated tetraploid groundnut A, hypogaea *(2n=40), are good sources of resistance to many diseases of groundnut.*

The fertility observed in triploid hybrids of A. hypogaea *and eight diploid species, and the recovery of progenies with diploid to hexaploid chromosome numbers have led to the identification of a rapid method for the production of tetraploid interspecific derivatives. The hexaploids raised from the triploid hybrids show intra- and intergenomic pairing in the form of bivalents and multivalents. Hexaploids and the progenies of the triploids were back crossed with* A. hypogaea *and produced* A. hypogaea-*like derivatives.*

Autotetraploids were crossed with A. hypogaea *and some fertile F_1 progenies obtained.*

Forty-six of the 56 possible interspecific crosses among eight diploid wild species produced seeds. Amphiploids were established in 31 combinations, 23 have been crossed with A. hypogaea.

Résumé

Introgression génétique à partir d'espèces sauvages compatibles dans l'arachide cultivée : Les espèces sauvages diploïdes (2n=20) compatibles avec l'arachide tétraploïde cultivée* A. hypogaea *(2n=40) se révèlent constituées des sources de résistance très appréciables à de nombreuses maladies de l'arachide.

***La fertilité observée chez des hybrides triploïdes entre* A. hypogaea *et 8 espèces diploïdes, et la récupération de descendants à nombres chromosomiques diploïdes à hexaploïdes ont conduit à l'identification d'une méthode rapide permettant la production de tétraploïdes interspécifiques. Les hexaploïdes issus des hybrides triploïdes présentent un appariement intra- et inter-génomique sous forme de bivalents et de multivalents. Rétrocroisés avec* A. hypogaea, *les hexaploïdes et les descendances des triploïdes ont donné des descendants semblables à* A. hypogaea.

Des autotétraploïdes ont été croisés avec* A. hypogaea *avec obtention de certains descendants F_1 fertiles.

***Quarante-six des 56 croisements interspécifiques possibles entre les 8 espèces sauvages diploïdes ont produit des graines. Des amphiploïdes ont été obtenus dans 31 combinaisons; 23 ont été croisés avec* A. hypogaea.**

Introduction

Groundnut (*Arachis hypogaea* L) suffers from many diseases and pests that cause serious yield losses. Wild relatives of crop species have been found to be potential sources of a number of desirable characters, especially resistance to diseases and pests (Watson 1970; Knott and Dvorak 1976). The genus *Arachis* contains a number of such wild species. Gregory et at. (1973) divided the genus into seven sections based on morphological affinities and cross compatibility. The section *Arachis* Krap. et Greg. nom. nud. comprises the cultivated tetraploid species, *A. hypogaea*, and a number of compatible diploid wild species. The diploid species are good sources of resistance to many groundnut diseases, such as rust (Puccinia arachidis) and leaf spots (*Cercospora arachidicola* Hori) and (*Cercosporidium personatum* (Berk, et Curt.) Deighton), and to insect pests, such as thrips (Sci-

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rotothrips dorsalis Wood), and aphids (Aphis craccivora Koch). (Abdou et al. 1974; Herbert and Stalker 1981; Subrahmanyam et al. 1980, 1983, 1985; Amin 1985). Thus they have immediate potential as sources for the genetic improvement of groundnut.

Recent investigations on genome relationships in section *Arachis* have led to the identification of two genomes, A and B, in the diploid species. There is evidence that the cultivated tetraploid species *A. hypogaea* is an amphiploid (AABB), involving these two genomes from the diploid species (Singh and Moss 1982, 1984a) (Fig. 1). These observations have led to the identification of certain routes for the introgression of desirable genes from the compatible wild diploid species of section *Arachis* into cultivated groundnut. This paper discusses the efficacy of different cytogenetic manipulations for transfer of desirable characters from the eight available diploid wild species of section *Arachis* into *A. hypogaea*.

Materials and Methods

The source and identity of eight diploid species (2n=20), namely *A. batizocoi* Krap. et Greg. nom. nud., *A. duranensis* Krap. et Greg. nom. nud, *Arachis* species GKP 10038 (PI 263133), *A. correntina* (Burk.) Krap. et Greg. nom. nud., *A. chacoense* Krap. et Greg. nom. nud., *A. villosa* Benth., *A. cardenasii* Krap. et Greg. nom. nud., *Arachis* sp HLK 410 (PI 338280) and the two subspecies of *A. hypogaea* (2n=40), *A. hypogaea* L. subspecies *hypogaea* Krap. et Rig. and *A. hypogaea* L subspecies fastigiata Waldron, all in section *Arachis*, have been given earlier (Singh and Moss 1982, 1984a).

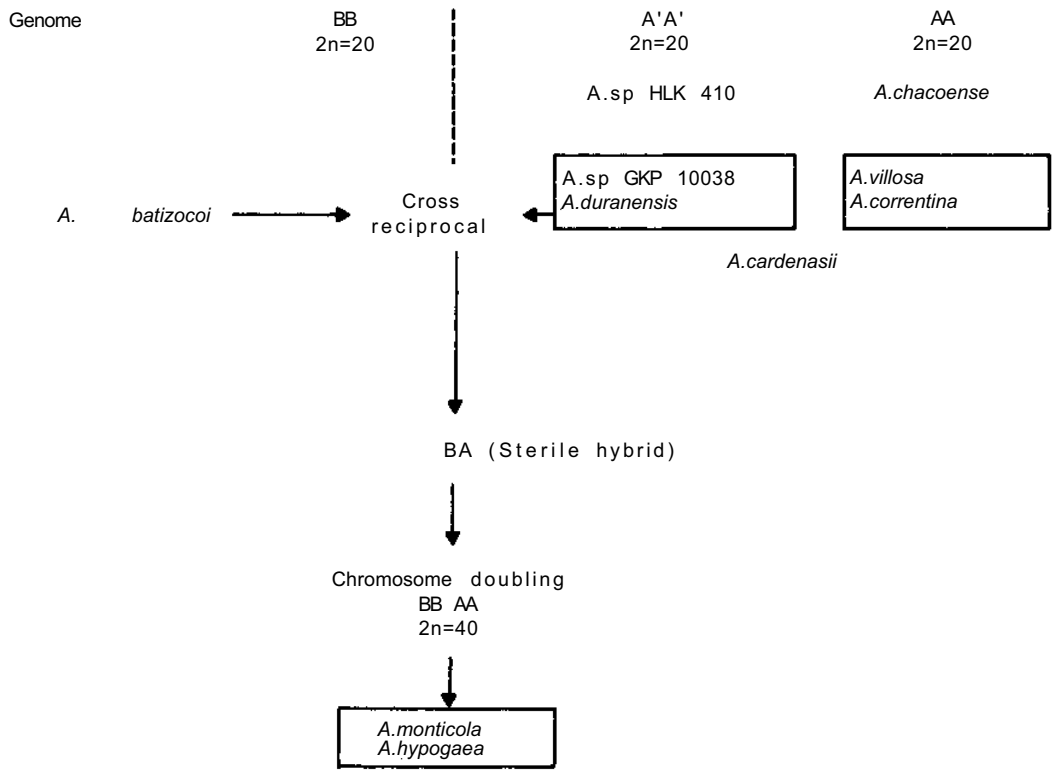

Figure 1. Genomic relationships in section *Arachis*, 'A' and 'B' genome/species have the same basic complement. Species have been arranged to indicate relative affinities based on geographical, morphological and cytogenetical evidence.

All the experiments were done in the screenhouse, or in the field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru, near Hyderabad, India. The techniques followed for hybridization, cytological analysis, polyploid induction, and screening interspecific derivatives have been previously discussed (Subrahmanyam et al. 1980; Singh et al. 1983; Singh and Moss 1984a).

Results and Discussion

Based on the ploidy differences and genomic relationships between wild *Arachis* species and cultivated *A. hypogaea* (Fig. 1) the following cytogenetic manipulations were chosen for genetic introgression from the wild diploid species into *A. hypogae.*

Introgression through Amphiploids (Hexapioids)

In the section *Arachis* the most common way to incorporate desired characters from the diploid species into tetraploid *A. hypogaea* has been to cross the diploid species with tetraploid *A. hypogaea* to produce triploid hybrids, and then to double their chromosome number by colchicine treatment to produce hexapioids and regain fertility (Smartt and Gregory 1967; Raman 1976; Moss 1980; Singh et al. 1980; Moss et al. 1981; Company et al. 1982). At ICRISAT, the triploid and hexaploid hybrids from crossing all eight available diploid species with *A. hypogaea* have been raised (ICRISAT 1982), Cytological analysis of hexapioids has revealed 10 to 30 bivalents, with means ranging from 20.3 to 23.9, and 0 to 8 multivalents, with means ranging from 1.1 to 3.2 per pollen mother cell (PMC) (Table 1; Fig. 2d). The multivalent associations in these hexapioids indicate that intergenomic (A-B) pairing occurs between the chromosomes of wild species and those of the cultivated species in all combinations, besides intragenomic or intraspecific (A-A or B-B) pairing of chromosomes. This suggests that the desired recombinants combining wild and cultivated species characters can be obtained through natural meiotic pairing and crossing over. However, the frequency of such recombinations is very low, as evidenced by low multivalent frequency due to preferential autosyndetic pairing between the homologous chromosomes of one species, giving a high bivalent frequency (Table 1). Hence, a very

Table 1. Chromosome associations, and pollen and pod fertilities in hexaploids from *A. hypogaea* (4x) x *Arachis* (2x) hybrids.

A. hypogaea cross	Cells analysed (no.)	Chromosome associations						Pollen stainability (%)	Pods produced (range)
		I	II	III	IV	V	VI		
A. villosa	7	8.3 ±1.83	23.9 ±0.63	0.2 ±0.14	0.9 ±0.34	0	0	60	0-200
A. correntina	43	6.7 ±0.44	22.2 ±0.47	1.1 ±0.16	1.1 ±0.19	0	0.5 ±0.02	26[1]	0-110
A. chacoense	216	6.6 ±0.33	20.9 ±0.26	1.2 ±0.17	1.4 ±0.10	0.3 ±0.01	0.1 ±0.02	88	0-53
Arachis sp HLK 410	88	7.1 ±0.52	20.6 ±0.45	1.1 ±0.26	1.5 ±0.17	0.5 ±0.01	0.1 ±0.04	61	0-180
A. cardenasii	160	6.9 ±0.35	20.3 ±0.29	1.3 ±0.20	1.5 ±0.10	0.1 ±0.01	0.1 ±0.02	62	0-113
A. duranensis	10	6.6 ±0.64	21.8 ±1.29	1.1 ±0.38	1.4 ±0.48	0.1 ±0.1	0	73	0-99
A. batizocoi	7	5.6 ±1.13	23.7 ±0.68	0.7 ±0.42	1.1 ±0.34	0	0	93	5-92

1. From two screenhouse plants.

Table 2. Pod production in backcrossing of *A. hypogaea* (4x) × wild species (2x), amphiploids (6x) with *A. hypogaea*, and number of fertile derivatives obtained by postrainy season 1982-83.

A. hypogaea × *Arachis* sp (6x)	BC_1			BC_2			BC_3			BC_4			BC_5			
	Poll. (no.)	Pod (no.)	Poll./ pod (%)	Poll. (no.)	Pod (no.)	Poll./ pod (%)	Poll. (no.)	Pod (no.)	Poll./ pod (%)	Poll. (no.)	Pod (no.)	Pod./ pod (%)	Poll. (no.)	Pod (no.)	Poll./ pod (%)	Fertile tetraploids (no.)
A. cardenasii	1277	109 (2[1]+10)[2]	9	2516	464 (5[1]+1)	18	709	80 (5[1])	11	432	95	22				23
A. chacoense	302	19 (1)	6	462	40 (2)	9	303	51 (4)	17	220	14 (1)	6	94	15	16	8
Arachis sp HLK 410	254	36 (1)	14	169	27 (2)	16	863	81	9	28	1	4				3
A. correntina	56	14	25	252	12	5										
A. villosa	224	16	7													
A. balizocoi	179	17	10													

1 From progenies of selfed triploids. 2. Number of fertile tetraploid derivatives obtained.

large population of interspecific derivatives is essential for the selection of desired recombinants. Backcrossing these hexaploids with *A. hypogaea*, either using the same, or different cultivars produced *A. hypogaea*-like derivatives in different backcross generations (Table 2) as a result of increased autosyndetic preferential pairing between chromosomes of *A. hypogaea* in backcross progenies. However, this pairing behaviour restricts further genetic exchange between cultivated and wild species chromosomes in subsequent generations, but has helped in the rapid production of tetraploid derivatives similar to *A. hypogaea*. At ICRISAT 22 *A. hypogaea*-like tetraploid lines involving three diploid species, *A. cardenasii*, *A. chacoense*, and *Arachis* sp HLK 410, resistant to rust and leaf spot diseases were obtained using this method in the postrainy season 1982 (Table 2). From these interspecific derivatives a large number of segregates resistant to late leaf spot and rust have been selected (Fig. 3) (ICRISAT 1982; and Singh unpublished).

Introgression through Triploids

Recently the triploid hybrids between *A. hypogaea* and diploid species, which were hitherto reported sterile except by Smartt and Gregory (1967) for three combinations, and Simpson and Davis (1983) for a single plant from a complex triploid, have been found fertile across all combinations (Singh and Moss 1984b). Cytological analysis of these triploid hybrids revealed interspecific, and intragenomic pairing between the chromosomes of wild and cultivated species in the form of up to 10 bivalents with means ranging from 8.0 to 9.9/cell. Intergenomic pairing also occurred in cells with more than 10 bivalents or with multivalents (Table 3; Fig. 2a). The frequency of cells with multivalents and/or more than 10 bivalents indicates a high degree of genetic exchange (crossing over) between chromosomes of wild and cultivated species. At anaphase I (AI) and anaphase II (AII) unequal chromosome segregation results in the formation of haploid to hyperdiploid gametes, and spindle breakdown results in the formation of restitution nuclei and unreduced gametes. Fertilization between such viable gametes results in the fertility of triploids previously considered sterile. As this process was never observed in the triploids produced and maintained at Reading University, UK during the period 1973 to 78, but was observed at ICRISAT in plants grown

Table 3. Chromosome associations, pollen, and pod fertility in F_1 triploids of *A. hypogaea* (4x) *x Arachis* species (2x).

A. hypogaea cross	Cells analysed (no.)	Chromosome association				Pollen stainability (%)	Pods produced (range)
		I	II	III	IV		
A. villosa	25	9.1 ±0.52	8.6 ±0.37	1.0 ±0.22	0.2 ±0.1	19	0-7
A. correntina	21	8.3 ±0.43	9.9 ±0.31	0.6 ±0.16	0.1 ±0.05	20	0-25
A. chacoense	40	9.7 ±0.4	8.7 ±0.25	0.8 ±0.16	0.1 ±0.49	17	0-19
Arachis sp HLK 410	30	9.2 ±0.43	9.6 ±0.28	0.5 ±0.13	0.1 ±0.03	13	0-16
A. cardenasii	25	8.3 ±0.52	9.7 ±0.27	0.5 ±0.17	0.2 ±0.08	9	0-10
Arachis sp GKP 10038	25	10.0 ±0.44	8.0 ±0.26	1.2 ±0.18	0.1 ±0.06	11	2[1]
A. duranensis	20	8.3 ±0.45	9.4 ±0.24	1.0 ±0.17	0.1 ±0.05	18	4[1]
A. batizocoi	21	6.2 ±0.42	8.7 ±0.49	2.0 ±0.29	0.1 ±0.07	7	3-18

1. Single plant.

from rooted cuttings from the same tripioids, it is probable that the phenomenon is affected by environment. The occurrence of 82% hexaploids in progenies of tripioids suggests the greater success of unreduced gametes than other types of gametes in fertilization. These progenies are the product of fertilization between gametes resulting from pairing and crossing over between the chromosomes of wild and cultivated species at metaphase (MI), and therefore have a greater degree of recombination between wild and cultivated species characters than artificially-induced hexaploids. Many triploid progenies have fewer chromosomes than hexaploid, or even a few tetraploids, and have reduced the number of backcross cycles required for the production of tetraploid derivatives. At ICRISAT, following this method, 13 tetraploid *A. hypogaea*-like derivatives involving *A. cardenasii*, *A. chacoense*, and other species have been obtained and segregates resistant to insect pests, such as leaf miner (*Aproaerema modicella* Dev.), jassids (*Empoasca kerii* Pruthi) and thrips have been selected (Singh unpublished).

The gametic fertility of tripioids suggests that they can also be used directly in backcrossing to recipient cultivars to obtain pentaploids (Simpson and Davis 1983) and also tetraploid *A. hypogaea*-like progenies as in wheat (Kerber and Dyck 1973).

Introgression through Amphiploids (Tetraploids)

Identification of A and B genomes in wild diploid species of section *Arachis*, and the amphiploid origin of tetraploid *A. hypogaea* involving A and B genome species, suggest that maximum genetic exchange between wild and cultivated species chromosomes can be achieved when two wild species, with AA and BB genomes, are crossed, the chromosome number doubled, and the AABB amphiploid so produced crossed with *A. hypogaea* (Singh and Moss 1984a). The resultant hybrids are fertile. The evidence of chromosome pairing in the hybrids between *A. hypogaea* and these species at different ploidy levels suggests that both auto- and allosyndetic pairing will occur.

Amphiploids were raised from sterile or semi-sterile hybrids in 34 diploid species hybrid combinations (ICRISAT 1982). Cytological analysis

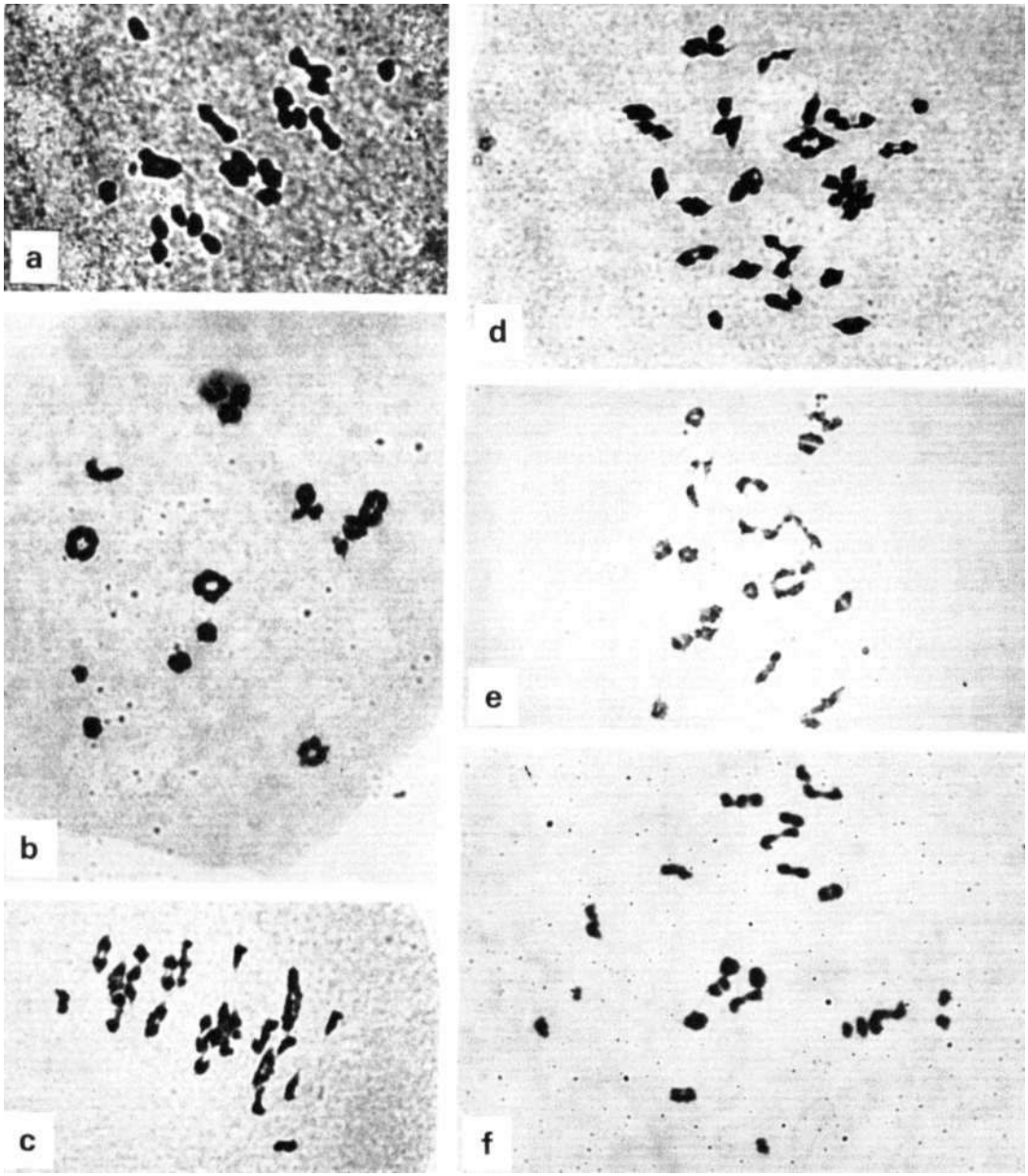

Figure 2. Pollen mother cells at metaphase I showing:

a. 8I + 9II + 1IV in a triploid hybrid, *A. hypogaea* subsp *hypogaea* x *A. correntina*
b. in an autotetraploid, *A. correntina*, 2I + 9II + 5 IV;
c. in an amphiploid, *A. batizocoi* x *A. villosa*, 2I + 17II + 1IV;
d. in an amphiploid, *A. hypogaea* x *A. cardenasii*, 4I + 18II + 5 IV;
e. in a hybrid *A. hypogaea* subsp *fastigiata* x (*A. batizocoi* x *A. duranensis*) amphiploid, 1I + 14II + 1III + 2IV;
f. in a hybrid, *A. hypogaea* subsp *fastigiata* x *A. batizocoi* autotetraploid, 8I + 14II + 1IV.

Table 6. Chromosome associations, pollen and pod fertility in *A. hypogaea x* diploid species autotetraploids(4x).

A. hypogaea X autotetraploid (4x)	Crossability with *A. hypogaea* (%)	Cells analysed (no.)	Chromosome associations				Pollen stainability (%, range)	Pod produced (no., range)
			I	II	III	IV		
A. batizocoi	14	23	5.8 ±0.52	14.1 ±0.56	0.9 ±023	0.9 ±0.19	43-64	5-26
Arachis sp HLK 410	11	21	10.1 ±0.78	11.1 ±0.52	1.9 ±0.28	0.5 ±0.18		2-5
A. villosa	22	9	7.2 ±0.68	11.8 ±0.94	2.2 ±0.4	0.3 ±0.17	44-75	2-4

genome. The autotetraploids of these species do not differ significantly between each other in chromosome associations but the annual species autotetraploids have higher pollen and pod fertility (Singh unpublished). Crossabilities between *A. hypogaea* and the autotetraploids of section *Arachis* diploid species do not differ, but the fertility of the resulting first generation hybrids does differ. The hybrids with two annual species autotetraploids, *A. hypogaea* x *A. batizocoi* (4x) and *A. hypogaea* x *Arachis* sp 10038 (4x) produced most pods when backcrossed, *A. hypogaea* x *A. batizocoi* (Ax) hybrid showed the highest mean bivalent associations (14.1) (Fig. 2f), and moderate pollen fertility (43 to 64%), and pod production (range 3 to 21) (Table 6). It produced five *A. hypogaea*-like tetraploid progenies within two backcross generations and enabled incorporation of resistance to the rust pathogen from this species into *A hypogaea* (Table 7; ICRISAT1983).

The autotetraploids of other species in section Arachis with the A genome can also be of great value, as more than 10 bivalents and a few multivalent associations in PMCs of the hybrids between *A. hypogaea* and A genome species autotetraploids, were recorded (Table 6). Their first generation hybrids do not produce pods on selfing but this has been overcome by backcrossing to *A. hypogaea*. The genomic constitution of these hybrids (AAAB) is conducive to intergenomic A = B pairing, altering

Table 7. Pod production in backcrossing of *A. hypogaea x* diploid species autotetraploids (4x) hybrids with *A.hypogaea,* and number fertile derivatives obtained by postrainy season 1983-84.

A. hypogaea X amphiploid (4x)	BC_1			BC2			BC3			BC_4			Fertile tetraploids (no.)
	Poll, (no.)	Pod (no.)	Poll./ pod (%)	Poll. (no.)	Pod (no.)	Poll./ pod (%)	Poll (no.)	Pod (no.)	Poll./ pod (%)	Poll, (no.)	Pod (no.)	Poll./ pod (%)	
A. batizocoi	1012	59 (1)[1]	6	1563	110 (4)	7	346	47	14	182	19	10	5
A. villosa	368	20	5	31	2	6							0
A. correntina	50	1 0)	2	209	61	29	7	1	14				1
Arachis sp HLK 410	301	9 (1)	3	103	13	14	51	2	4				1
Arachis sp GKP 10038	75	8	11										

1. Number of fertile tetraploid derivatives obtained.

Figure 3. A field view of some late leaf spot-resistant segregates from *A. cardenasii*.

the genetic constitution of chromosomes, such as those of the B genome carrying susceptibility to late leafspot.

Conclusions

The degree of genomic affinity between *A. hypogaea* and diploid species of section *Arachis* permits intergenomic and intragenomic pairing between chromosomes of wild and cultivated species. The production of hybrids at different ploidy levels through conventional interspecific hybridization and cytogenetic manipulations leads to incorporation of desired traits from wild species into *A. hypogaea*. The different pathways for gene transfer from wild into cultivated species can be adopted based on; phylogenetic relationships between species, an understanding of the cytogenetic behaviour of the two genomes involved at the different ploidy levels, and the nature of the gene(s) and their expression. With such an understanding, genes can be introgressed from the wild diploid species into *A. hypogaea*.

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Groundnut Cytogenetics at North Carolina State University

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Abstract

Research is directed toward answering basic questions concerning groundnut genetics, characterizing Arachis *species, and developing populations for utilization by breeders. Approximately 300* Arachis *collections, and several hundred interspecific hybrids are maintained. Species are being cytologically characterized. An aneuploid series is also being developed for cultivated groundnuts. Species of the genus are evaluated for reaction to diseases and insect pests. Genomic studies are being conducted in section* Arachis, *by analyzing fertility levels and meiotic behaviour of* F_1 *hybrids. The mechanism of incompatibility is studied, and an embryo rescue project has been initiated.*

A primary goal is to introgress germplasm from Arachis *species to* A. hypogaea. *Forty-chromosome amphidiploids are hybridized with* A. hypogaea *in reciprocal, and fertile progenies selected. Direct interspecific hybrids with* A. hypogaea *and all available section* Arachis *species have been made, colchicine treated, and hexaploid interspecific hybrids backcrossed. Fertile 40-chromosome hybrids are evaluated for reaction to diseases and insect pests. Selections with high levels of resistance to* Cercospora arachidicola *and a complex of insects have been made.*

Résumé

Cytogénétique de l'arachide à la North Carolina State University : Les recherches poursuivies ont plusieurs objectifs : la réponse aux questions fondamentales relatives à la génétique de l'arachide, la caractérisation de l'espèce* Arachis *et la création de populations à l'usage des sélectionneurs. Environ 300 origines d'*Arachis *et plusieurs centaines d'hybrides interspécifiques sont maintenus. Les espèces sont caractérisées du point de vue cytologique. Une série aneuploïde est également actuellement créée pour les arachides cultivées. Les espèces du genre sont évaluées pour leur réponse aux maladies et aux insectes nuisibles. Des études génomiques sont en cours concernant la section* Arachis, *avec l'analyse des niveaux de fertilité et du comportement méiotique des hybrides* F_1. *Le mécanisme d'incompatibilité est étudié et un projet de culture de l'embryon a été entrepris.

L'objet premier est d'intégrer le germplasme de l'espèce* Arachis *dans* A. hypogaea. *Des amphiploïdes à 40 chromosomes sont hybridés d'une façon réciproque avec* A. hypogaea, *et les descendances fertiles sont sélectionnées. Des hybrides interspécifiques directs entre* A. hypogaea *et toutes les espèces disponibles de la section* Arachis *ont été obtenus, traités à la colchicine et rétrocroisés avec des hybrides hexaploïdes interspécifiques. Les hybrides fertiles à 40 chromosomes sont évalués pour leur réponse aux maladies et aux insectes nuisibles. Des sélections possédant un niveau élevé de résistance à* Cercospora arachidicola *et à un complexe d'insectes ont été réalisées.

Introduction

The groundnut cytogenetics program is a component of the overall breeding effort in North Carolina. Research is directed toward answering basic questions on groundnut genetics, characterizing *Arachis* species, and developing populations for use in the groundnut breeding program. The specific objectives of the groundnut cytogenetics program are: 1. to acquire and maintain *Arachis*

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species germplasm, 2. to cytogenetically and botanically characterize the groundnut species, 3. to develop methods to introgress desirable germplasm from Arachis species to *A. hypogaea*, and 4. to develop and evaluate 40-chromosome interspecific hybrid populations. Research is in progress in several areas such as cytogenetics, genetics, tissue culture, plant pathology, entomology, and plant breeding.

Germplasm at North Carolina State University

A working collection of Arachis species is maintained at NCSU either as living plants or seeds. Approximately 300 plant collections are propagated annually. These represent 25 of the 32 named species and possibly 50 additional species which have not been described and named. Species collections, including all members of section *Rhizomatosae*, which do not produce seeds in North Carolina, are maintained in greenhouses as living plants. Seeds of the other species collections are initially grown in the greenhouse and then transplanted into seed-increase nurseries. All seed collections are propagated annually in pots, 1 m in diameter, in the greenhouse and in seed-increase plots in the field. Limited quantities of seeds are distributed to other research institutions on request. Herbarium specimens are prepared and maintained for all species collections.

In addition to the *Arachis* species collection, a group of *A. hypogaea* mutants are maintained by the cytogenetics program. Investigations of the genetics and linkages among mutants are in progress.

Interspecific hybrids within and between sections of the genus are in the NCSU collection. Included in this group are hybrids between *A. hypogaea* and all members of section *Arachis*; intrasectional *Erectoides*, *Rhizomatosae*, *Caulorhizae*, and *Arachis* hybrids; and intersectional *Erectoides* x *Rhizomatosae*, *Arachis* x *Erectoides*, *Arachis* x *Rhizomatosae*, and *Arachis* x (*Erectoides* x *Rhizomatosae*) hybrids (Plate 3b,c). Crosses are being made with several of these hybrid combinations at different ploidy levels in attempts to introgress genes to *A. hypogaea* and to investigate genomic relationships. Because of severe sterility barriers, direct hybridization between the cultivated groundnut and most species of the genus is not possible at the present time.

Species Characterization

Taxonomy

The genus *Arachis* probably contains 75-100 species, but only 21 have been botanically described. A monograph of the genus by W.C. Gregory (NCSU) and A. Krapovickas (Argentina) has been in preparation for several years. In addition to their work, data have been collected to describe patterns of variation by numerical taxonomic procedures, especially for section *Rhizomatosae*. As additional plant collections are obtained they are classified to botanical groups and to species level whenever possible.

Biosystematics

The general patterns of cross-compatibility in the genus are now understood. Species within a section will usually hybridize, but hybrids between species of different sections are difficult to obtain and are sterile. Programs are being designed to overcome crossability barriers between species. Many hybrid combinations used in crossing programs have two to five species in their pedigree and are at various ploidy levels. Although *A. hypogaea* will not hybridize with species outside section *Arachis*, several diploid species of this group will cross with more distantly-related taxa. Attempts are being made to utilize some diploid species such as *A. duranensis*, *A. spegazzinii*, and *A. batizocoi* as bridges for germplasm introgression.

Most biosystematic work at NCSU is concentrated in section *Arachis*. The species *A. duranensis* (A genome) and *A. batizocoi* (B genome) have been hybridized with other members of the section. Analyses to date indicate that most species have an A genome, only the species *A. batizocoi* has thus far been identified as having a B genome and the genome of an unnamed species (GKSSc 30091,30099) has recently been designated as the D genome. Amphidiploids of section *Arachis* species hybridized with *A. hypogaea* indicate that the cultivated species has an A and B genome. However, the diploid progenitor species have not been defined.

Causes of failures to obtain many interspecific hybrids in the genus are also being investigated. Pollination and embryo studies indicate that both pre- and post-fertilization barriers prevent species hybridization. The timing of such failures is being investigated with the intention of overcoming these

barriers. Hormones have been applied to flowers to induce fertilization and a project is currently in progress to rescue hybrid embryos in vitro.

Diseases

Species collections in all seven sections of the genus have been evaluated for disease resistances in the greenhouse and field. The objective of these studies is to document the relative levels of resistance for the most important diseases of groundnut. While most work has been with early leaf spot (*Cercospora arachidicola*), species have also been evaluated for resistance to rust (*Puccinia arachidis*), late leaf spot (*Cercosporidiumpersonatum*), cylindrocladium black rot (*Cylindrocladium crotalariae*), stem rot (*Sclerotium rolfsii*), and stunt virus. High levels of resistance or immunity have been found for early and late leaf spots, rust, and stunt virus. Preliminary evaluations for *C. crotalariae* and *S. rolfsii* indicate high levels of resistance to these pathogens may also be present. Attempts are made to evaluate plant material in the field whenever possible and then to study the mechanisms of resistance in the greenhouse. For example, detached-leaf studies of section *Arachis* species indicate that several genotypes have resistance to initial infection and a long latent period of sporulation. The inheritance of leaf spot resistance in diploid species is being investigated. As new accessions are added to the *Arachis* species collection, they are evaluated for disease reaction.

Insects

Species in all *Arachis* sections have been evaluated for insect resistance in the field and screenhouse (Plate 1c). Very high levels of resistance or immunity have been found to tobacco thrips, (*Frankliniella fusca*); potato leafhopper, (*Empoasca fabae*); and corn earworm, (*Heliothis zea*). Moderate levels of resistance to lesser cornstalk borer, (*Elasmopalpus lignosellus*), have been found, but the resistance is not sufficiently high to be useful in an interspecific breeding program. Attempts are currently being made to introgress the genes conferring resistances to insects. Several advanced-generation hybrid populations have shown promise as new sources of insect resistances for cultivar improvement.

In addition to taxonomic, biosystematic, disease, and insect studies, *Arachis* species are being evaluated for other traits. Species of section *Arachis* have been tested for responses to long- and short-day photoperiods. This work has potential for interpreting crossing program results, increasing seed supplies, and possibly changing the reproductive efficiency of groundnuts. Species of section *Rhizomatosae* have been planted with forage grasses to evaluate their vegetative yield potential. Other traits such as oil percentage and fatty acid contents are being evaluated.

Cytogenetics

Many *Arachis* species and hybrids have been cytologically analyzed. *Arachis hypogaea* is an allotetraploid, probably with both A and B genomes. Hybrids among subspecies often result in unexpected variation and/or lethals. Meiotic analysis of pollen mother cells revealed that most chromosomes of diverse genotypes pair, but univalents are observed in several hybrid combinations. The chromosomes of NC 4 were karyotyped and ordered from largest to smallest. Fifteen of the 20 chromosome pairs are distinct and can be accurately classified. Nine other cultivars and *A. monticola* have also been karyotyped and botanical varieties can be cytologically distinguished. *Arachis monticola* is cytologically most closely related to spanish-type groundnuts.

An aneuploid series is now being developed for the *A. hypogaea* genome, using NC 4 as the source cultivar. Trisomics (2n + 1) or double trisomics (2n + 1 + 1), with eight different extra chromosomes have been isolated and attempts are being made to obtain stable primary trisomic plants for each extra chromosome. The chromosome numbers of plants grown from small seeds are being determined in an effort to isolate additional aneuploids.

Because several chromosomes are difficult to distinguish, chromosomes of NC 4 and *Arachis* species are being banded with Giemsa stain to further differentiate the genome. Initially, diploid species were studied and satisfactory bands were identified in *A. spegazzinii*. Banding *A. hypogaea* chromosomes is more difficult, but techniques are now being developed to characterize the cultivated genome.

Cytological analyses of the mitotic chromosomes of other species have been exclusively performed with diploids of section *Arachis*. Fourteen taxa have been karyotyped, representing 10 species. The A genome has been karyologically subdi-

vided into three groups. *Arachis batizocoi* has a karyotype distinct from other species and was confirmed as having the B genome. A third genome has recently been identified in a newly-collected species.

Interspecific hybrids have been produced among diploid species of section *Arachis*, between diploid and tetraploid species and among different sections of the genus. Pollen mother cells of these interspecific hybrids are being analyzed to determine species relationships, reasons for sterility and chromosome homologies. The most intensive evaluations have been with hybrids between sections *Arachis* and *Erectoides*, sections *Erectoides* and *Rhizomatosae*, *A. hypogaea* x diploid species at the triploid, tetraploid, pentaploid, and hexaploid chromosome levels, and among diploid species of section *Arachis*.

The current investigations are mostly within section *Arachis*. *Arachis duranensis* (A genome) and *A. batizocoi* (B genome) have been hybridized with 15 taxa of the section. Fertility levels and chromosome homologies are being evaluated to characterize genomes in the group. A series of hybrids between *A. hypogaea* and the other section *Arachis* species are also being evaluated. Many amphidiploids of section *Arachis* have been identified, and their hybrids with *A. hypogaea* are currently being analyzed.

In vitro Culture

The cytogenetics program has started work on tissue culture. Preliminary work centered around producing callus from groundnut tissues and regenerating plants from hypocotyls. Although both phases of the research were successful, regenerating plants from callus or single cells was not possible.

Emphasis of the in vitro research is currently on rescuing embryos of potential hybrid plants. The endosperm and embryo aborts in many interspecific hybrids, and embryo culture will facilitate desired hybrid combinations. To date, research is concentrating on determining when embryos abort and rescuing embryos at a very early age.

Germplasm Introgression to *A. hypogaea*

A primary aim of the groundnut cytogenetics program is to broaden the germplasm base of *A. hypogaea*. The *Arachis* species have many genes with great potential for crop improvement, especially for disease and insect resistances. However, introgressing genes to cultivated groundnut is difficult because of genomic and ploidy differences among species. Hybrids are usually made using spanish- and virginia-type groundnuts to test differences within *A. hypogaea* as well as between the *Arachis* species. Reciprocal hybrids are made in attempts to introduce the cultivated genome to foreign cytoplasms.

Triploid-hexaploid Pathway

To introgress genes into *A. hypogaea*, NC 4 (Virginia type) and Argentine (Spanish type) are being crossed, reciprocally, to all known members of section *Arachis*. Vegetative cuttings of triploid F_1 plants have been colchicine treated, and hexaploids obtained with six species. About 3000 colchicine treatments are made annually and other hexaploid combinations should be recovered. Progress in reducing the chromosome number from hexaploid to tetraploid has been slow. Pentaploids of *A. hypogaea* with *A. cardenasii*, *A. chacoense*, *A. correntina*, and *A. batizocoi* have been obtained, but most plants are sterile, and backcrossing with *A. hypogaea* has been unsuccessful. However, a few pentaploids produce seeds and several appear to be losing chromosomes.

Hexaploid hybrids have been backcrossed with diploid species to rapidly lower the chromosome number from hexaploid to tetraploid. However, the several thousand pollinations all resulted in aborted embryos. The exact causes of hybrid failure and methods to directly obtain 40-chromosome hybrid derivatives are now being investigated.

Amphidiploid Pathway

An alternative method to introgressing germplasm from diploid species to *A. hypogaea* is to first double the chromosome number of the diploids by producing amphidiploids. Thirty-two interspecific hybrid combinations of diploid section *Arachis* species (with A genome) have been raised to tetraploid level. The plants are generally weak, but hybrids between *A. hypogaea* and four amphidiploid combinations have been obtained. Although the *A. hypogaea* x amphidiploid hybrids were initially sterile, a few seeds have been produced and fertility restored in at least one combination—NC 6 x (*A. stenosperma* x *A. chacoense*). Progenies from this cross are being analyzed and fertile segregates

identified. Crossing programs are attempting to recover additional fertile hybrid combinations.

When *A. batizocoi* (B genome) F_1 is used as a parent with other diploid species, all F_1 hybrids are sterile. Amphidiploids, however, are partially fertile and will hybridize with *A. hypogaea*. The *A. hypogaea* x (A-B genome amphidiploids) are again partially fertile. To date, advanced generation *A. hypogaea* hybrids have been obtained for two combinations and additional *A. batizocoi* amphidiploids are being produced and crossed with cultivated groundnuts.

Evaluating Advanced Generation Interspecific Hybrids

Advanced generation 40-chromosome interspecific hybrid combinations with *A. hypogaea* and four species (*A. cardenasii*, *A. batizocoi*, *A. duranensis*, and *A. spegazzinii*) have been produced. Selections from these populations are being evaluated for resistance to rust, early leaf spot, late leaf spot, tobacco thrips, corn earworm, potato leafhopper and southern corn earworm (*Diabrotica undecimpunctata howardii*) with the emphasis to date on selection for early leaf spot resistance.

Advanced generation *A. hypogaea* x *A. cardenasii* hybrid derivatives were selected for early leaf spot resistance in 1979. Hybrid selections have been tested for three years in the field and compared to leaf spot-resistant cultivated lines. Several hybrid selections have very high levels of resistance compared to the best cultivars. Greenhouse evaluations indicate that several mechanisms of resistance are present in hybrid selections. However, these lines have small seeds and low yields compared to commercial cultivars.

The hybrid selections with the highest levels of disease resistance are being backcrossed with several commercially-acceptable cultivars to increase yields and seed quality. The hybrid selections have also been hybridized to leaf spot-resistant cultivated lines in attempts to create breeding lines with multiple mechanisms of resistance.

Breeding Potential of Interspecific Tetraploids in *Arachis* L.

U.R. Murty and M.R. Jahnavi[1]

Abstract

Ten popular varieties of groundnut belonging to the three botanical types were crossed as females to three diploid, section Arachis *wild species (*A. correntina *PI 331194,* A. villosa *from Coimbatore and* A. chacoense *PI 276235). These triploids were for the most part sterile, but occasionally set some seed. Thirty-four of the progenies of such triploids were analyzed, 28 were hexaploids, 5 were tetraploid, and one was pentaploid. The tetraploids obtained in this manner were advanced for three generations. The progenies were fertile and stable with a constant chromosome number of 2n=40. The three generations were cytologically analyzed. The advanced generation plants were evaluated in replicated trials for vegetative and yield attributes with cultivated varieties as controls. The differences between the cultivated varieties and the derived tetraploids were not significant in respect of yield components. Differences in the vegetative characters and in disease resistance, however, suggested that the wild species characters had been incorporated.*

Résumé

Potentiel de sélection de tétraploïdes interspécifiques chez Arachis. *L. : Dix variétés connues d'arachide appartenant aux trois types botaniques ont été croisées en tant que parents femelles à trois espèces sauvages diploïdes de la section* Arachis (A. correntina *PI 331194,* A. villosa *de Coimbatore et* A. chacoense *PI 276235). Ces triploïdes ont été en grandepartie stériles, avec quelques mises à graine occasionnelles. Trente-quatre des descendances de ces triploïdes ont été analysées, 28 étaient hexaploïdes, 5 tétraploïdes et une pentaploïde. On a continué avec les tétraploïdes obtenus ainsi jusqu'à 3 générations. Les descendances se sont révélées fertiles et stables avec un nombre chromosomique constant de 2n = 40. Les trois générations ont été analysées cytologiquement. Les caractères végétatifs et les facteurs du rendement des générations avancées ont été évalués dans des essais avec répétitions et avec pour témoin des variétés cultivées. En ce qui concerne les facteurs d'élaboration du rendement, les différences observées entre les variétés cultivées et les tétraploïdes dérivés n'ont pas été significatives. Toutefois, les différences constatées concernant les caractères végétatifs et la résistance aux maladies laissent supposer qu'il y a eu introduction des caractères des espèces sauvages.*

Isolation of Tetraploids in Interspecific Crosses

Triploid hybrids resulting from crosses of *A. hypogaea* with diploid section *Arachis* species are generally sterile. However, triploids occasionally set a few seeds. Generally, such seed results in pentaploids and hexaploids or aneuploids (Smartt and Gregory 1967; Raman 1977; Spielman et al. 1979). At the Indian Agricultural Research Institute, several triploid hybrids were produced using several groundnut varieties and three wild diploid species; *A. chacoense* PI 276235, *A. villosa* (from Tamil Nadu Agricultural University, Coimbatore), and *A. correntina* PI 331194.

The triploid hybrids were grown under optimum field conditions. The triploids were for the most part sterile, but occasionally seeds were produced. Seed obtained from the various triploids was saved and the next generation grown (Table 1). The pro-

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Table 1. Seed production by *Arachis* triploids and ploidy level of progeny produced.

Pedigree	Seeds produced (no.)	F_2 plants reaching maturity	F_2 plants analysed (no.)	Tetra-ploids (no.)	Penta-ploids	Hexa-ploids
TMV 2 x *A. chacoense*	25	20	9	2	0	7
Gaug 1 x *A. chacoense*	10	3	0	0	0	0
GDM x *A. chacoense*	15	12	7	0	1	6
Acholi white x *A. chacoense*	10	8	3	0	0	3
Gangapuri x *A. chacoense*	3	0	0	0	0	0
MK 374 x *A. chacoense*	0	0	0	0	0	0
Florunner x *A. chacoense*	20	19	7	1	0	6
Robut 33-1 x *A. chacoense*	5	3	0	0	0	0
M 13 x *A. chacoense*	5	4	0	0	0	0
TMV 2 x *A. correntina*	3	2	1	1	0	0
M 13 x *A. villosa*	10	2	1	1	0	0
GDM x *A. villosa*	400	50	6	0	0	6
Total	506	123	34	5	1	28

geny consisted of five tetraploids, one pentaploid and twenty eight hexaploids.

The tetraploids set seed, and produced only tetraploid progenies (Table 2). These thirteen tetraploid F_2 plants yielded varying numbers of seeds. From their progenies thirty-one plants were analysed. These were all tetraploids.

Evaluation of Derived Tetraploids

An experiment with two replications was conducted with 12 progenies derived from the F_3 tetraploid plants from the following four crosses: TMV 2 x *A. chacoense*, TMV 2 x *A . correntina*, Florunner x *A. chacoense*, and M 13 x *A. villosa*. These 12 plants were selected because they yielded adequate seed to obtain satisfactory stands for the two replications. The three cultivated parents, TMV 2, M 13, and Florunner were also included in the experiment.

The experiment was conducted during the post-rainy season of 1982-83. Four vegetative characters: length of the primary axis, length of n + 1 branches, number of n + 1 branches, and number of n + 2 branches: five yield components; pod weight, pod number, kernel number, kernel weight, and shelling percentage; and rust incidence were studied. Rust incidence was assessed using the method of Russel and Hudson (1973) where rust incidence is recorded on a 0 to 5 scale based on infection, reaction, and size of pustules (Table 3).

After scoring the plants the rust index was calculated using the formula:

$$\frac{(ax0)+(bx1)+(cx2)+(dx3)+(ex4)+(fx5)x100}{n \times 5}$$

Where a to f = number of plants with each score

n = total number of plants scored

The experiment was harvested 120 days after sowing; all the plants had some mature pods, but some

Table 2. Number of seeds produced and plant survival in tetraploid F_2 and F_3 progenies.

Pedigree	Seeds (no.)	Plants reaching maturity (no.)
F_2 generation (single plants)		
TMV 2 x *A. chacoense*	25	5
TMV 2 x *A. chacoense*	-[1]	-
TMV 2 x *A. correntina*	20	2
Florunner x *A. chacoense*	30	2
M 13 x *A. villosa*	200	4
F_3 generation		
TMV 2 x *A. chacoense* (5 plants)	50	30
TMV 2 x *A. correntina* (2 plants)	40	15
Florunner x *A. chacoense* (2 plants)	30	17
M 13 x *A. villosa* (4 plants)	300	50

1. Plant died.

Table 3. Mean values for vegetative and reproductive characters in cultivated varieties and interspecific derivatives.

Pedigree	Length of primary axis(cm)	Length of branches (cm)	n + 1 branches (no.)	n+2 branches (no.)	Pod (no.)	Pod weight (g)	Kernel (no.)	Kernel weight (g)	Shelling %	Rust[1] index
TMV 2 x *A. correntina*	13.0	21.6	15.3	27.3	16.4	15.5	19.2	9.4	60.6	15.0
M 13 x *A. villosa*	15.1	25.0	15.8	26.5	14.8	16.8	12.4	7.8	46.4	42.5
M 13 x *A. villosa*	14.7	26.5	16.0	29.0	14.8	15.9	6.8	4.9	38.1	55.0
Florunner x *A. chacoense*	18.7	42.3	21.3	38.3	8.5	10.9	12.0	4.5	41.2	35.0
TMV 2 x *A. correntina*	16.0	22.5	17.5	39.2	9.6	14.5	11.2	4.6	31.7	45.0
M 13 x *A. villosa*	18.1	29.2	17.7	36.5	11.5	14.8	13.8	3.8	25.6	43.3
M 13 x *A. villosa*	14.5	18.8	18.9	26.8	9.2	12.9	11.5	4.3	33.3	45.0
TMV 2 x *A. chacoense*	12.1	18.5	9.8	29.3	7.5	12.3	11.3	4.2	34.4	0
TMV 2 x *A. chacoense*	13.7	20.5	15.6	30.3	7.7	12.7	12.6	3.6	28.3	3.0
TMV 2 x *A. chacoense*	15.6	24.1	15.7	35.3	6.5	12.4	10.2	3.0	24.1	5.0
TMV 2 x *A. chacoense*	14.3	18.3	10.0	29.8	8.5	11.9	10.0	3.8	31.9	5.0
TMV 2 x *A. chacoense*	14.4	11.6	13.0	27.7	21.1	10.0	11.6	4.2	42.0	15.0
Mean	15.1	23.2	15.6	31.1	11.7	13.4	11.9	4.8	33.0	25.7
Florunner	17.3	12.8	12.4	25.0	19.6	15.8	17.1	6.3	39.8	20.0
M 13	24.5	19.3	30.3	48.6	18.6	10.0	20.3	6.3	63.0	30.0
TMV 2	13.3	13.3	7.3	3.3	16.3	10.7	11.5	4.0	37.3	55.0
Mean	18.4	15.1	19.1	26.7	18.2	12.2	167	49	46.7	35.0

1. Rust index calculated according to Russel and Hudson (1973).

interspecific tetraploids still had a number of immature pods. The interspecific tetraploids exhibited a strong tendency for perennation. The Virginia runner parents, M 13 and Florunner exhibited a similar tendency although it was less pronounced.

The interspecific tetraploids were similar to the cultivated varieties. They grew as vigorously; the general appearance of the plant, color and size of the foliage, branching and flowering pattern, frequency of flower, peg, and pod production, size, shape, and appearance of the pod, and size, shape and appearance of kernels were all similar in the various entries. With the exception for TMV 2 a Spanish type with small pods and kernels, the remaining entries had similar pods.

The mean values for the different vegetative and reproductive characters, and for rust incidence for the 15 entries are given in Table 3. The length of the main axis varied from 13 to 24.5 cm. As expected, the secondary branches were longer in the Virginia types than in the Spanish types. M 13 had the maximum number of secondary and tertiary branches. All plants set seed. No sterile, abnormal, or stunted plants were encountered. The various genotypes differed significantly from each other only in four vegetative characters and shelling percentage (Table 4). There were no significant differences between treatments in pod and kernel characters, except for shelling percentage. The interspecific derivatives also did not differ significantly for most

Table 4. Mean sum of squares for 4 vegetative and 5 yield components in interspecific derivatives and cultivated varieties.

Character Source	Length of primary axis (cm)	Length of branches (cm)	n+1 branches (no.)	n+1 branches (no.)	Pod (no.)	Pod weight (g)	Kernel (no.)	Kernel weight (g)	Shelling %
Treatments	31.03**	185.91**	90.56**	180.06**	1.49	13.44	48.54	5.87	0.313*
Parents	63.8**	70.1	291.3	1028.9	5.7	21.6	40.24	5.02	0.002
Derivatives	15.7	62.4	23.9	153.0**	39.3	8.90	20.9	1.83**	0.64**
Parents vs derivatives	71.05*	221.4**	354.36**	1222.9**	224.68	9.22	45.382	0.168	3.01**

* Significant at 5% level. ** Significant at 1% level.

of the characters. The only differences were in respect of number of n + 2 branches, kernel weight and shelling percentage. A comparison between the parents and derivatives showed that they differed from each other only in vegetative characters and shelling percentage.

The rust index ranged from 0 to 5 between the derivatives and from 1 to 3 between the parents. None of the parents was immune to rust. Almost 50% of the derivatives, however, had plants with no rust incidence. Rust incidence was recorded after harvest during the rainy season. By this time, the number of plants, both parents and derivatives had decreased due to losses from rodent attack and other mechanical damage. The frequency distribution of the rust incidence in the plants is shown in Figure 1, but this data could not be statistically analysed since the number of plants was low.

Evidence for the Occurrence of Alien Incorporation

The cross TMV 2 x A. chacoense and TMV 2 x *A correntina*, yielded some with alternately branched tetraploids plants. Since TMV 2 is a sequentially-branched type, the isolation of such plants confirmed that alien incorporation had taken place. The derived tetraploids were mostly spreading types and produced more vegetative matter, longer secondary branches, and more secondary and tertiary branches. These features are mostly ascribable to the wild species. The presence of nearly 50% plants immune or resistant to rust among the derivatives and their absence among the cultivated varieties lends additional support to the conclusion that alien incorporation had occurred.

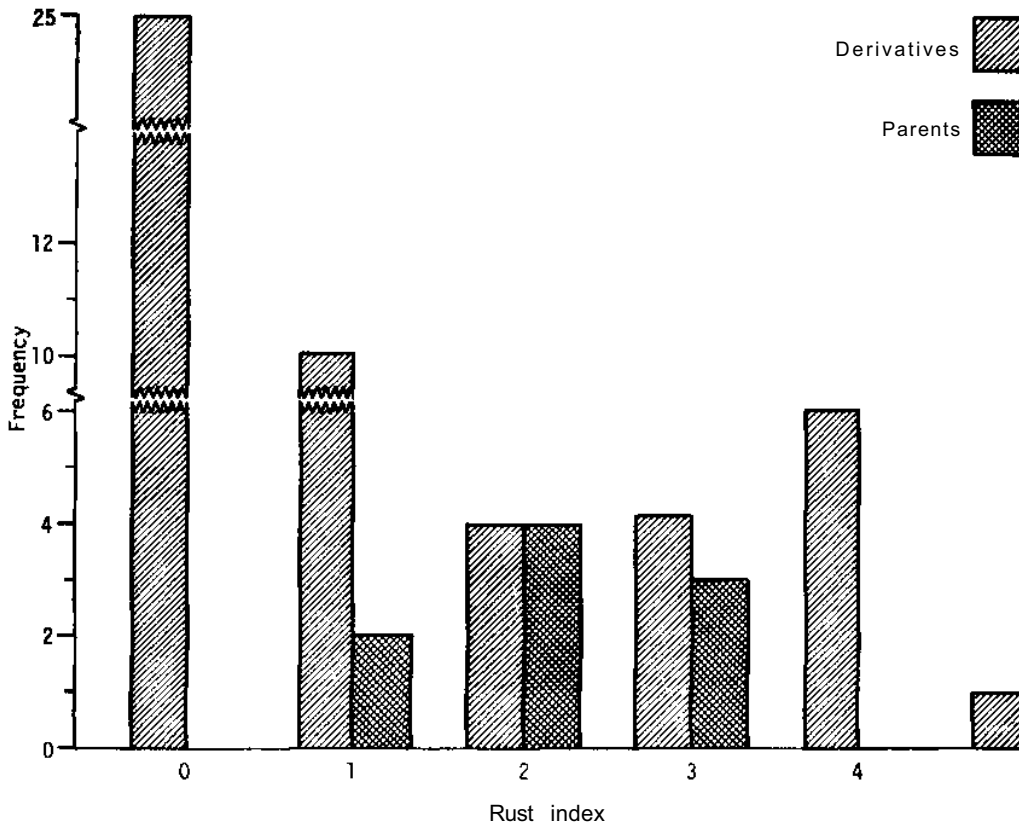

Figure 1. Frequency distribution of plants with different degrees of rust incidence. Rust index calculated according to Russel and Hudson (1973).

Table 5. Frequencies of chromosome associations, chiasmata, and meiotic abnormalities in tetraploids (F_2, F_3, and F_4 generations).

Pedigree	Mean number respective chromosome associations/cell												Mean number chiasmata/cell			Mean number meiotic abnormalities[1]/cell		
	I			II			III			IV								
	F_2	F_3	F_4	F_2	F_3	F_4	F_2	F_3	F_4	F_2	F_3	F_4	F_2	F_3	F_4	F_2	F_3	F_4
TMV 2 × *A. chacoense*	3.5	0.6	0.5	15.8	17.0	17.6	0.3	0.2	0.1	1.0	0.8	0.7	31.1	33.2	34.2	12.5	15.1	8.0
TMV 2 × *A. correntina*	1.1	4.8	0.5	17.0	17.5	17.2	0.3	0.2	0.2	0.8	1.05	0.9	33.5	34.5	34.2	5.4	7.0	5.0
Florunner × *A. chacoense*	0.8	0.2	0.6	17.3	17.2	17.2	0.2	0.4	1.0	1.0	1.1	0.8	34.0	34.0	34.2	1.5	7.3	5.5
M 13 × *A. villosa*	0.3	0.9	0.3	17.3	16.6	17.7	0.2	0.4	0.2	1.0	1.1	0.8	35.0	33.5	35.2	18.1	12.2	6.2
Mean	1.4	1.6	0.4	16.9	17.0	17.4	0.25	0.3	0.37	0.9	1.0	0.8	33.4	33.8	34.4	9.3	10.3	6.1

1. Mean number of laggards at AI and TI and disjunction bridges at AI.

The tetraploids exhibited regular meiosis, comparable with that of cultivated varieties of groundnut. This may lead to the conclusion that these tetraploids originated by a fusion of diploid gametes carrying the genomes of *A. hypogaea*. However, the occurrence of trivalents and quadrivalents in triploids provides additional cytological evidence for the occurrence of recombination between the wild and cultivated species genomes. The frequency of chromosome associations, chiasmata, and meiotic abnormalities were recorded in the F_2,F_3, and F_4 generations (Table 5). They showed little change from F_2 to F_4, and were similar to reported figures for *A. hypogaea*, indicating that alien incorporation, if any, did not significantly affect meiosis.

Conclusions

The successful production of derivatives from interspecific crosses, with immunity to rust, and at the same time a plant type analogous to cultivated varieties (especially in reproductive features), indicates that the methods followed in the present study are quite efficient and practicable for alien incorporation.

The evidences enumerated in this report indicate that gene transfer occurred in the triploids. The wild species genome was transferred to the triploid, and alien genes were transferred to the tetraploid.

Comparative performance of parents and interspecific derivatives leads to the conclusion that these two categories differ from each other only in vegetative characters and shelling percentage. This means that the interspecific derivatives differ slightly in vegetative from the cultivated varieties, but are reproductively more or less the same. Therefore the tetraploid progeny of triploids are useful for gene transfer.

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Utilization of Wild Species of *Arachis*

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Abstract

Several wild species belonging to sections Arachis, Triseminalae, *and* Rhizomatosae *along with hybrids between wild species of section* Arachis *and cultivated groundnut are being maintained, evaluated, and utilized for the transfer of desirable characters. The work is confined to utilization of triploid and hexaploid hybrids obtained from different centers. Advanced-stage wild species derivatives obtained from Tamil Nadu Agricultural University, Coimbatore, India, were evaluated in a replicated trial for two seasons. Five derivatives had good yield and tolerance to rust* (Puccinia arachidis), *and late leaf spot diseases* (Cercosporidium personatum). Rhizobium *isolates from wild species are being tested for cross-infectivity and their efficiency in nitrogen fixation in cultivated groundnut especially under stress conditions. The rhizomatous species* A. hagenbackii *and A.* glabrata *were found to possess a very high percentage of leaf protein. Wild species in general, and a high percentage of those specifically reported to be drought resistant, were found to possess a higher proportion of palisade parenchyma in their leaves than is found in cultivars.*

Résumé

Utilisation des espèces sauvages d'Arachis **:** ***Plusieurs espèces sauvages appartenant aux sections*** Arachis, Triseminalae ***et*** Rhizomatosae, ***ainsi que des hybrides d'espèces sauvages de la section*** Arachis x ***arachide cultivée, sont actuellement maintenus, évalués et utilisés pour le transfert des caractères recherchés. Les travaux sont limités à l'utilisation d'hybrides triploïdes et hexaploïdes de différents centres. Des espèces dérivées d'espèces sauvages en générations avancées obtenues auprès de la Tamil Nadu Agricultural University, à Coimbatore, en Inde, ont été évaluées dans un essai avec répétitions pendant deux saisons. Cinq espèces dérivées ont présenté un bon rendement et une bonne tolérance à la rouille*** (Puccinia arachidis) ***et à la cercosporiose***(Cercosporidium personata). ***Des isolats de rhizobium d'espèces sauvages sont actuellement testés pour leur pouvoir d'installation et pour leur capacité de fixation de l'azote sur des arachides cultivées, plus particulièrement en conditions d'agression ('stress'). Les espèces rhizomateuses*** A. hagenbackii ***et*** A. glabrata ***ont présenté une quantité très élevée de protéine foliaire. On a constaté chez les espèces sauvages en général, et chez la plupart de celles considérées, en particulier, résistantes à la sécheresse, une proportion supérieure de parenchyme palissadique dans les feuilles par rapport à celle observée chez les cultivars.***

Introduction

Several wild diploid species, and tetraploid *A. monticola* belonging to section *Arachis*, along with *A. hagenbeckii* and *A. glabrata* of section *Rhizomatosae*, and *A. pusilla* of section *Triseminalae* are being maintained and evaluated at the National Research Centre for Groundnut of the Indian Council of Agricultural Research (ICAR), Junagadh, India.

Gene Transfer from Compatible Wild Species

A. duranensis and its tetraploid, *A. chacoense*, and *A. monticola* are being utilized for the transfer of desirable characters especially resistance to rust and late leaf spot diseases. The triploid route for gene transfer from wild species (Fig. 1 a and b) has been extensively utilized (Singh et al. 1980; ICRISAT 1983). A triploid genotype (2x = 30) obtained

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as a result of the cross *A. hypogaea* cv TMV 2 (4x = 40) x *A. chacoense* (2x = 20) and its hexaploid were hybridized with TMV 2 to produce pentaploids that are being backcrossed to cultivars to get desirable tetraploid segregants. Since 3x x 4x and its reciprocal of this material were observed to yield pentaploid seeds, owing to the formation of restitution nuclei, triploids form a bypass in the conventional triploid-hexaploid route of ploidy manipulation (Fig. 1 c). This would eliminate the necessity of chromosome doubling of triploids in order to get hexaploids and would result in the early recovery of pentaploids from 3x x 4x crosses. The segregants thus obtained are being backcrossed and evaluated.

Evaluation of Wild Species Derivatives

Wild species derivatives involving *A. monticola* and *A. villosulicarpa* obtained from Tamil Nadu Agricultural University.Coimbatore were evaluated in a replicated trial for two seasons. Derivatives 8020 and 8002 were found to be comparable in yield to cultivar JL 24 (Table 1) but possessed only a degree of tolerance to rust (score 3.6) and late leaf spot (3.4) as compared to JL 24 (5.0, highly susceptible). However, it should be pointed out that these derivatives were the result of outdoor crosses attempted at Coimbatore (Raman 1976). Moss (1980) had questioned the origin of these derivatives. Our results showed these derivatives to be similar to the cultivars in yield and with a low level of disease resistance (Table 1).

Table 1. Average performance of wild Arachis species derivatives.

Derivatives	Rust score[1]	Late leaf spot score[1]	Yield (kg/ha)	Oil content (%)
8002	3.6	3.4	1552	50.4
8020	3.6	3.4	1372	50.2
7905	3.4	3.6	1292	49.2
791 OB	3.6	3.4	1069	48.5
7910A	3.0	3.6	899	49.1
Control cv JL 24	5.0	5.0	1401	47.4

1. Determined on a 1 to 5 scale where 1 is resistant, and 5 is highly susceptible.

Miscellaneous Utilization of Wild Species

The rhizomatous species *A. hagenbeckii* and *A. glabrata* were found to possess high percentages of leaf protein (Table 2). These species have a fast rate of multiplication and could be a good source of leaf protein. Studies pertaining to their utility as fodder crops, especially in marginal areas, are also warranted. The rhizomatous species are capable of producing about 30 t/ha green fodder and even higher yields have been obtained in some cases (Chandrasekhar 1980).

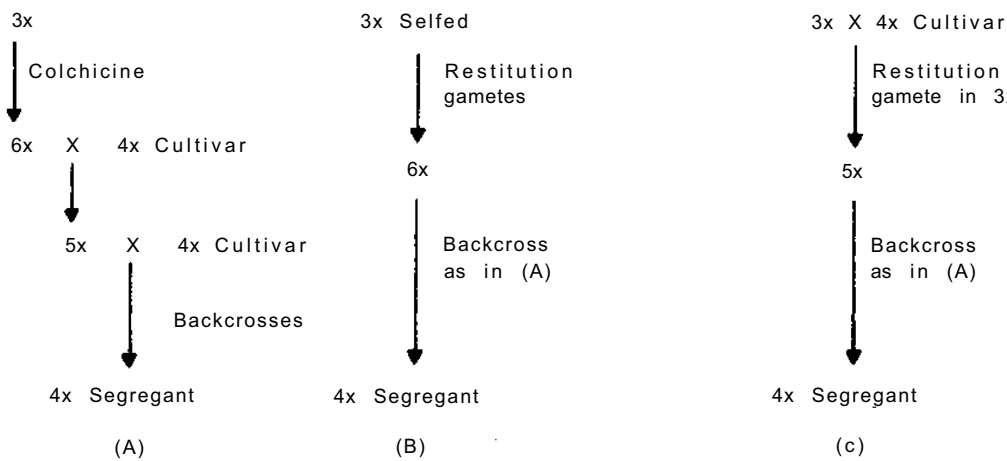

Figure 1. Alternatives in triploid route of gene transfer.

Table 2. Percentage leaf protein content in different *Arachis* genotypes

Species/hybrid	Leaf protein (%)
A. hagenbeckii	36.6
A. glabrata	24.1
A. pusilla	20.8
Triploid (TMV 2 x *A. chacoense*)	20.8
Control	
cv J 11	15.6

Wild species are also being used as model plants in understanding stress resistance. *Rhizobium* isolates obtained from wild species are being tested, after establishing their cross-infectivity, for efficiency of nitrogen fixation in cultivated groundnut especially under stress conditions.

Leaf anatomical characterisitics in wild species and cultivars were studied by the authors (Tiwari 1981) and by Suryakumari et al. (1983). At the National Research Centre for Groundnut, thickness of epidermis, palisade cell length and total leaf thickness were studied in cultivated tetraploids, interspecific triploid hybrids, and diploid and tetraploid wild species of *Arachis*. No significant difference was observed in epidermis thickness. Leaf thickness and palisade thickness were found to vary considerably (Table 3). Some of the known drought-resistant forms such as *A. hagenbeckii* and *A. pusilla* were found to possess more palisade than others. The differences in this xeromorphic characteristic were quite obvious when the proportion of palisade parenchyma expressed as a percentage in the total leaf thickness, was compared. The lowest proportion of palisade parenchyma was obtained from leaves of JL 24 and Robut 33-1 and the highest (around 75%) from *A. pusilla*, *A. hagenbeckii*, and a triploid. The observed differences in leaf anatomical characters could not be ascribed to differences in ploidy status. Extending this relationship to cultivars, in some drought-resistant strains like Krapovickas, Nc Ac 17090, and others, the proportion of palisade in the leaf thickness was more than 60%.

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Table 3. Leaf and palisade parenchyma thickness in different *Arachis* genotypes.

	Leaf thickness		Palisade thickness		Proportion of palisade parenchyma in leaf thickness %	
Material	Mean(mm)	S.D.	Mean(mm)	S.D.	Mean	S.D.
I Wild species						
A. hagenbeckii	212.4	4.9	154.8	9.9	72.88	9.7
A. duranensis	188.0	2.2	124.3	2.2	66.09	0.4
A. pusilla	295.2	9.9	221.4	8.2	75.00	1.0
A. glabrata	178.5	2.2	98.8	2.2	55.34	0.5
A. sp 10038 (LL)	207.0	9.9	117.0	9.9	56.52	2.1
A. sp 10038 (SL)	195.2	4.4	113.6	2.9	58.20	6.1
II Interspecific hybrid, triploid						
(*A. hypogaea* x *A. chacoense*)	219.9	2.1	156.2	2.2	71.01	0.1
III Cultivated forms						
JL 24	282.0	0.0	162.0	0.0	57.45	0.0
Robut 33-1	255.0	4.6	150.0	9.3	58.82	3.7
M 13	252.0	0.0	162.0	0.0	64.28	0.0
GAUG I	273.3	5.6	175.3	8.1	64.15	1.7
Krapovickas	245.4	1.7	156.2	2.2	63.64	0.6
Nc Ac 17090	261.3	1.7	157.8	1.7	60.36	0.3

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Preliminary Cytogenetical Studies on Wild Species of *Arachis* at Bangalore University

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Abstract

Substantial cytogenetical studies have been carried out in the genus Arachis *since the first report of its chromosome number by Kawakami in 1930, although the existence of diploids was revealed in 1947 by Mendes. The earlier investigations have shown the potential for the transference of desirable characters from various sources for the improvement of cultivated* Arachis. *Our studies have concentrated on the wild species of section* Arachis. *Six hybrids have been examined for their cytological behaviour in pollen mother cells (PMCs). They apparently indicate that one genome is common to both diploid and tetraploid species in section* Arachis. *The resistance observed in diploid hybrids appears to be partial in triploids. The genetic mechanism of disease resistance needs more critical studies.*

Résumé

Etudes cytogénétiques préliminaires sur des espèces sauvages d' Arachis *à l'Université de Bangalore : Des études cytogénétiques importantes ont été poursuivies sur le genre* Arachis *depuis le premier rapport de Kawakami en 1930 sur son nombre de chromosomes, alors que l'existence des diploïdes n'a été découverte par Mendes qu'en 1947. Les premières recherches ont révélé le potentiel de transfert des caractères recherchés de diverses sources pour l'amélioration d'* Arachis *cultivé. Nos études ont surtout porté sur les espèces sauvages de la section* Arachis; *six hybrides ont été examinés pour leur comportement cytologique dans les cellules mères de pollen (CMP). Elles montrent qu'un seul génome est commun aux espèces diploïdes et tétraploïdes de la section* Arachis. *La résistance constatée chez les diploïdes semble être partielle chez les triploïdes. Enfin, des études critiques doivent être poursuivies davantage sur le mécanisme génétique de la résistance.*

Results

The diploid hybrid, *Arachis* sp HLK 410 x Arachis sp HLK 408 is found to be resistant to both leaf spot (*Cercosporidium personatum*, *Cercospora arachidicola*), and rust (*Puccinia arachidis*) diseases. Meiotic behaviour is mostly normal with 10 bivalents at diakinesis and metaphase I. However, one bivalent appears to be loosely paired, and its homologues seperate early during late M-I. The distribution of chromosomes in anaphase I and II is also regular.

The hybrids of *A. duranensis* x *Arachis* sp 408 and its reciprocal crosses (diploids) are also resistant to leaf spot and rust diseases. Meiotic configurations in diakinesis and M-I show 10 bivalents. One bivalent is associated with the nucleolus. Anaphases I and II are mostly regular.

The triploid hybrids, A. stenosperma (2n = 20) x *A. hypogaea* cv TMV 2 (2n = 40), *A. monticola* (2n = 40) x *A. duranensis* (2n =20), and *A. cardenasii*(2n = 20) x *A. hypogaea* cv Robut 33-1 are susceptible to leaf spot disease, but showed no symptoms of rust. The hybrid, *A. duranensis* x *A. hypogaea* is highly susceptible to leaf spot, rust, and peanut mottle virus (PMV) diseases; pycnidia have also been observed on the rust pustules. Meiotic behaviour in all the triploids is highly irregular. The presence of 6-14 univalents in M-I, laggards and chromatin bridges at anaphase I and II lead to about 90% to 95% pollen sterility.

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Discussion

Several plant breeders have established the possibility of incorporating resistance into diploid and triploid hybrids, and amphiploids have been shown to be highly useful in plant breeding (Abdou et al. 1974; Gibbons and Bailey 1967; Moss 1977). In the present work the resistant characters for leaf spot and rust diseases from one of the respective parents appear to have been transferred to the diploid hybrids evolved here; but the resistant character for leaf spot disease from the diploid parent is not expressed in triploid hybrids.

Meiotic configurations showing the presence of 10 bivalents in diploid hybrids and an average of nearly 10 bivalents in triploid hybrids give an indication of the probable existence of a common genome in both diploid and tetraploid taxa. This view has also been expressed by earlier investigators (Raman 1973; Resslar and Gregory 1979; Smartt et al. 1978a,b; Moss 1980; Stalker 1980). The frequent occurrence of 10 bivalents and 10 univalents in the hybrid of *A. duranensis* x *A. monticola* also suggests a close homology between the genome of *A. duranensis* and a genome of *A. monticola*. On the other hand, the sporadic occurrence of trivalents, tetravalents, and chromatin bridges in hybrids may be attributed to structural changes among the chromosomes. The variation in number of bivalents from 6 to 12 in triploids is also evidence for partial homology. The semisterility noticed in the diploid interspecific hybrids may be due to the existence of genetic differences between species. However, detailed analysis of pairing behaviour coupled with critical karyomorphological studies are necessary to throw more light on the genomic relationships among the species of section *Arachis*.

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Studies on Cytogenetics of Arachis at Regional Research Station, Vriddhachalam, Tamil Nadu, India.

N. Sundaram[1]

The wild species *Arachis villosa* (2n = 20), autotetraploid *Arachis villosa* (2n = 40), and autotetraploid *Arachis villosulicarpa* (2n=40) were utilized as pollen parents while TMV 9, Co 1, Ah 8188, Ah 8524, Pol 2, TMV 7, Mutant 1, Mutant 3, and small-podded mutant (all of *Arachis hypogaea* - 2n =40) were the ovule parents.

The primary triploids between *A. hypogaea* x *A. villosa* (2n=20) were found to be profusely branching, vigorous, and profusely flowering. The hybrids were sterile, with pollen fertility ranging from 3.8 to 11.4 percent. The frequent association of 10 11-10 I noticed indicated that the haploid complement of *A. villosa* is more akin to one of the genomes of *A. hypogaea*. The distributions observed at anaphase I were 16/14, 15/15, 17/13, and 20/10. Bridges and laggards were noticed in about 50% of cells both in I and II division of meiosis.

These tripioids occasionally produced pods which on analysis were found to be hexaploids. These exhibited 30 bivalents in 28 percent of the cells; the mean association was 0.11 VI, 1.2 IV, 0.18 III, 26.55 II, 0.961 I per cell. These hexaploids were in general less vigorous, and less productive.

Backcrossing the triploid to *A. hypogaea* resulted in plants of all three growth forms i.e., bunch, semispreading, and spreading. They showed varying degree of associations of characters for leaf size, number of branches, thickness of main stems, hairiness, depth of burial of pods, constriction and beak of pods, weight of pods and kernels, and size of flower.

On crossing the natural tetraploid *A. hypogaea* with the autotetraploid of *A. villosa*, a synthetic allotetraploid was produced. In 24 PMCs analyzed at metaphase 1,1-3 IV, 1-5 111,4-1011 and 12 to 291 were observed. The average association per cell was 0.16 IV, 3.16 III, 6.33 II, 16.2 I. One third of the PMC showed 5III + 5II + 15I and one cell showed 1011 + 201. The pollen fertility was 68.4% and pollen grain size was variable.

Hexaploid progeny from the primary hexaploids exhibited all three forms, i.e., bunch, semispreading, and spreading. There were both flowering and non-flowering forms. They showed variation for all the quantitative characters assessed. Cytological analysis showed a maximum chromosome association of 2 VI + 5 IV + 3 III + 7 II + 5 I.

The mean chromosome association was 0.28 VI, 3.20 IV, 1.04 III, 20.28 II, 1.28 I. The pollen fertility was 65 %. Diads, triads, and supernumary spores were seen besides tetrads.

The primary hexaploids also gave rise to tetraploids and triploids. The synthetic allotetraploids on open pollination also gave rise to triploids and tetraploids. The secondary triploids showed chromosome associations of 1-5 III, 4-10 II, 5-10 I, the average association being 3.28 III, 6.05 II, 8.071. Laggards ranged from 5 to 11 at anaphase I. Aberrations occurred in both first and second divisions of meiosis.

The hybrids between *A. hypogaea* + *A. villosulicarpa* were vigorous, short in stature with reduced internodal length and more pods per leaf axil. The number of pods per plant showed transgressive segregation. In the subsequent generations, there was reduction in number and size of pods. Variation has been observed in subsequent generations.

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Interspecific hybridization has been initiated and hybrids between *A. hypogaea* + *A. chacoense* (which showed resistance to leaf spot and rust) has been established. A study of these hybrids showed that they were highly vigorous and profusely flowering but susceptible to leaf spot diseases. The pollen fertility ranged from 8 to 11%. Study of PMC at metaphase 1 indicated 8-11 II and 7-11 I, with a mean chromosome association of 0.3 III, 9.9 II, 9.3 I. Higher association was infrequent. Further studies are in progress. Interspecific hybridization using *A. hypogaea* as ovule parent and *A. pusilla*, *A. stenosperma*, and *A. cardenasii* as pollen parents is in progress.

Etudes sur la cytogénétique d'*Arachis* à la Station de recherche régionale de Vriddhachalam, Tamil Nadu, en Inde: On a pris comme parents m+les l'espèce sauvage *Arachis villosa* (2n = 20), l'autotétraploïde *Arachis villosa* (2n = 40) et l'autotétraploïde *Arachis villosulicarpa* (2n = 40) et comme parents femelles TMV 9, Co 1, Ah 8188, Ah 8524, Pol 2, TMV 7, Mutant 1, Mutant 3 et un mutant à petites gousses (tous d' *Arachis hypogaea* 2n = 40).

Les premiers triploïdes entre *A. hypogaea x A. villosa* (2n = 20) ont présenté des ramifications importantes, une floraison abondante et une bonne vigueur. Les hybrides ont été stériles, avec une fertilité pollinique variant de 3,8 à 11,4%. L'association de 10 II + 10 I souvent observée montre que le complément haploïde de *A. villosa* est plus proche de l'un des génomes de *A. hypogaea* . Les distributions observées à l'anaphase I ont été de 16/14, 15/15, 17/13 et 20/10. On a noté des ponts et des chromosomes trainards dans 50% environ des cellules tant à la première division méiotique qu'à la seconde.

Ces triploïdes ont parfois produit des gousses se révélant hexaploïdes à l'analyse. On a observé 30 bivalents dans 28% des cellules, l'association moyenne étant de 0,11 VI, 1,2 IV, 0,18 III, 26,55 II, 0,961 I par cellule. Ces hexaploïdes ont été en général moins vigoureux et moins productifs.

Recroisé avec *A. hypogaea* , le triploïde a produit des plants des trois types de port, érigé, semi-rampant et rampant. Leur degré d'association des caractères pour la taille des feuilles, le nombre de ramifications, l'épaisseur des tiges principales, la pilosité, la profondeur d'enfouissement des gousses, les constrictions et le bec des gousses, le poids des gousses et des graines, la taille des fleurs, a été variable.

En croisant le tétraploïde naturel *A. hypogaea* avec l'autotétraploïde de *A. villosa* , on a obtenu un allotétraploïde synthétique. Dans les 24 CMP analysés à la métaphase I, les associations I, 1-3 IV, 1-5 III, 4-10 II et 12 à 29 I ont été observées, l'association moyenne par cellule étant de : 0,16 IV, 3,16 III, 6,33 II, 16,2 I. On a constaté chez un tiers des CMP l'association 5 III + 5 II + 15 I et dans une cellule 10 II + 20 I. La fertilité pollinique a été de 68,4% et la dimension des grains de pollen a été variable.

La descendance hexaploïde des premiers hexaploïdes a présenté les trois formes, érigée, semi-rampante, rampante, et avec ou sans floraison. Tous les caractères quantitatifs évalués ont été variables. L'analyse cytologique a révélé une association chromosomique maximum de 2 VI + 5 IV + 3 III + 7 II + 5 I.

L'association chromosomique moyenne a été de 0,28 VI, 3,20 IV, 1,04 III, 20,28 II, 1,28 I et la fertilité pollinique de 65%. On a trouvé des groupes de deux, des triades et des spores en surnombre à côté de tétrades.

Les premiers hexaploïdes ont également donné naissance à des tétraploïdes et des triploïdes. Les allotétraploïdes synthétiques ont également donné en pollinisation libre des triploïdes et des tétraploïdes. Les seconds triploïdes ont présenté des associations chromosomiques de 1-5 III, 4-10 II, 5-10 I, l'association moyenne étant de 3,28 III, 6,05 II et 8,07 I. Les chromosomes trainards ont varié de 5 à 11 à l'anaphase I. On a constaté des anomalies dans les première et deuxième divisions méiotiques.

Les hybrides *A. hypogaea* x *A. villosulicarpa* ont été vigoureux, courts, avec des entrenoeuds de longueur réduite et un nombre accru de gousses par aisselle. On a constaté une ségrégation transgressive dans le nombre de gousses par plant. Dans les générations suivantes est apparue une réduction du nombre et de la taille des gousses. On a observé des variations dans les générations suivantes.

Une hybridation interspécifique a été entreprise et des hybrides de *A. hypogaea* x *A. chacoense* (résistants à la cercosporiose et à la rouille) ont été obtenus. Une étude de ces hybrides a montré qu'ils étaient très vigoureux, florissaient abondamment mais étaient sensibles à la cercosporiose. La fertilité pollinique a varié de 8 à 11%. L'étude de CMP à M1 a donné 8-11 II et 7-11 I, avec une association chromosomique moyenne de 0,3 III, 9,9 II et 9,3 I. Une association supérieure a été rare. D'autres études sont actuellement poursuivies. Une hybridation interspécifique avec *A. hypogaea* comme parent femelle (ovule) et *A. pusilla, A. stenosperma* et *A. cardenasii* comme parents mâles (pollen) est en cours de réalisation.

Use of Compatible Species - Discussion

Murty:
Did you produce any genetic male steriles in your mutagenesis experiments? If so, what is their morphological appearance?

Stalker:
We have not yet found genetic or cytoplasmic sterility in groundnuts. We treated seeds of NC 4 with ethyl methane sulfonate and streptomycin and then selected "abnormal" plants in the field, especially ones with fewer seeds. We will plant progenies next year and look for sterile segregates. We do not know the probability of success nor whether NC 4 is an appropriate genotype for inducing cytoplasmic male sterility.

N.C.Subrahmanyam:
With reference to the identification of aneuploids in barley, the chromosomes are large but the numbers assigned recently had to be changed because of the use of genetic markers. It may be worthwhile using linkage groups to identify different aneuploids in *Arachis* since the chromosomes are small.

Stalker:
We are sure we have a number of different aneuploids. However, only about 15 of the 20 chromosomes can be identified; by chromosome lengths, centromere positions, nucleolar organizers, and, in a few cases, heterochromatic regions. Before assigning numbers we will carefully identify the specific chromosome wherever possible (and be more tentative about identification of the median chromosomes). While identification by linkage groups is desirable, we cannot do so at this time because only a few linkage groups are known. We must begin somewhere, and our intention is to obtain as many aneuploids as possible, and as marker and linkage groups become available, we will begin associating genes with specific chromosomes.

P.Subrahmanyam:
I would like to know more about resistance to Cercospora arachidicola in the interspecific hybrid derivatives. How do they perform in comparison with NC 3033 and PI 270806?

Stalker:
A few tetraploid interspecific hybrid derivatives are significantly more resistant than either PI 270806 or NC 3033. The mechanism of resistance of several of the interspecific hybrids is believed to be due to a long latent period.

L.J. Reddy
As Dr. Murty pointed out, the use of wild species in crop improvement is limited to unit transfers such as disease resistance. However, introgression in nature is not limited to such unit transfers alone and has resulted in altogether new species. Recently, Frey and Lawrence at Iowa State University have obtained transgressive segregants for yield. As Dr Stalker pointed out the problem of recombination is very important in the interspecific crosses. Plant breeders working to combine high yield and other desirable characters in cotton were very frustrated. This is because we know so little about the dynamics of introgression. Now that sophisticated statistical techniques are available to study experimental populations, we should make efforts to understand introgression.

Satish Kumar:
Are the cultivar differences in triploid fertility cytologically manifested?

Murty:
There are differences in the frequency of chromosome associations and chiasma frequencies in different triploids. However, these differences were taken to indicate a differentiation in the genomes of the different groundnut genotypes. No attempts were made to quantify cytological parameters and correlate them with seed fertility.

L.J.Reddy:
You mentioned that you were able to get some immune derivatives from interspecific crosses. Did you study the F_1's for their disease reaction? How were the derivatives studied for disease reaction, under artificial laboratory, or natural field conditions? If under field conditions how intensive was the disease pressure?

Murty:
We screened only the F_4 derivatives under field conditions during the rainy season 1983. Rust innoculum was repeatedly sprayed on the field-grown plants from emergence. The disease pres-

sure was very intense. Most groundnut cultivars were susceptible.

Moss:
Were there unfertilized ovules in pegs, where the other ovule was fertilized?

Murty:
Yes.

Moss:
Had pollen tubes entered the micropyle and what was the condition of the synergids?

Murty:
It is difficult to say,but it is likely that pollen tubes had reached the nucellus, since pollen tubes were seen in some of the nucelli. Both synergrids remained intact in unfertilized embryosacs in contrast to fertilized embryosacs where at least one synergid degenerated.

N.C.Subrahmanyam:
Your figure on chromosome stability in the F_2, F_3, F_4 generation shows single points - do they represent the populations at each generation? They are very misleading if they represent means for each generation. Family-wise data would be more realistic.

Murty:
In general, similar trends were obseved in all the families. The data on minimum, maximum, and modal chromosome associations were given family-wise in the text and these are more or less the same in different families.

Rao:
There was an increase in bivalent frequency from F_2 to F_4, which indicates cytological stability; although the univalent and quadrivalent frequencies increased in F_3, there was a decrease in F_4. Are the increase and decrease significant or not, and what may be the reasons for these changes?

Murty:
The increase and decrease are consistent. This trend could have resulted from selection for fertility in these tetraploids. Another reason could be that any chromosomal heterozygosities might have gradually become homozygous as the generations advanced.

Rasheedunisa:
What parameters have you selected for morphological studies and what are the differences found in wild species? Did you study any anatomical features, and are there differences between species?

Murty:
We studied several leaf characters from epidermal peelings, and sections (Suryakumari, D., Seshavatharam, V., and Murty U.R., 1983. Oleagineux 38:27-40). There are several differences.

Stalker:
During backcross generations, there is the possibility of recombination, chromosome elimination, or perhaps chromosome substitution. The decision whether to self pollinate for a number of generations to develop uniform hybrids, or to backcross the primary hybrid, depends on the genetics of the trait. For monogenic, digenic traits we probably do not want to self pollinate for many generations, but to select specific genotypes with desired traits immediately. However, for multigenetic, low heretability characters, (for example, yield) we probably want to induce maximum possible recombination and select in later generations.

Moss:
Our primary emphasis has been on disease and pest resistance, and evidence so far points to few genes being involved, as reported by Sharief. So we backcross the primary hybrids. Using the hexaploid route, the pentaploids produced after the first backcross can be selfed, and will eventually revert to tetraploid. In fact, we select for yield as well as disease resistance in all generations, but with less emphasis than we put on disease or pest resistance.

We have obtained high-yielding disease-resistant lines from NCSU hybrids which were maintained for 6 or 7 generations without any selection, though there may have been some effect of season length at NCSU.

Murty:
Your table shows that pollen fertility of *A. hypogaea* x *A. batizocoi* is only 5%, although pairing is very high. This is not the case with A genomes. Which criterion has more weight, hybrid fertility or chromosome pairing?

Singh:
All the triploids had abnormal meiotic cycles, and the *A. batizocoi* triploid differs from the others in univalent and trivalent association, both of which contribute to abnormal segregation. The pollen fertility was low in *A. batizocoi* triploids, but most of the pollen grains were of uniform large size unlike that of other triploids, suggesting that in this combination, restitution nuclei mainly produced the viable stainable gametes. Trivalent frequency should not therefore be expected to affect higher pollen fertility.

Murty:
What is the morphological and cytological status of the amphiploid between any A genome species and *A. batizocoi*?

Singh:
Amphiploids between A x B genome species are runners, more like *A. batizocoi* with yellow flowers and hairy leaves, although their leaves are dark green and thick unlike those of *A. batizocoi*.

M.V.Reddi:
It may be worthwhile to bring the two A and B genomes together against the background of *A. hypogaea* by crossing derivatives of *A. hypogaea* x A genome and *A. hypogaea* x B genome in reciprocal combination. It may be possible to successfully obtain recombination between A and B genomes by this method.

Singh:
It is a viable suggestion,but long term compared to the one used in our program.

Moss:
The production of *A. hypogaea*-like lines with leaf spct resistance shows that there is some recombination, but it may be at a very low frequency.

M.V.Reddi:
Are there discernable morphological differences among the stable cross derivatives of *A. chacoense*, *A. cardenasii*, and *A. batizocoi* with *A. hypogaea*?

Singh:
Yes, there are different marker characters in these species which are expressed in different frequencies in populations of selected segregants. *A. chacoense* populations have lanceolate leaves, *A. batizocoi* populations have round hairy leaves. However, in all these derivatives the catenate pod character of the wild species is invariably expressed.

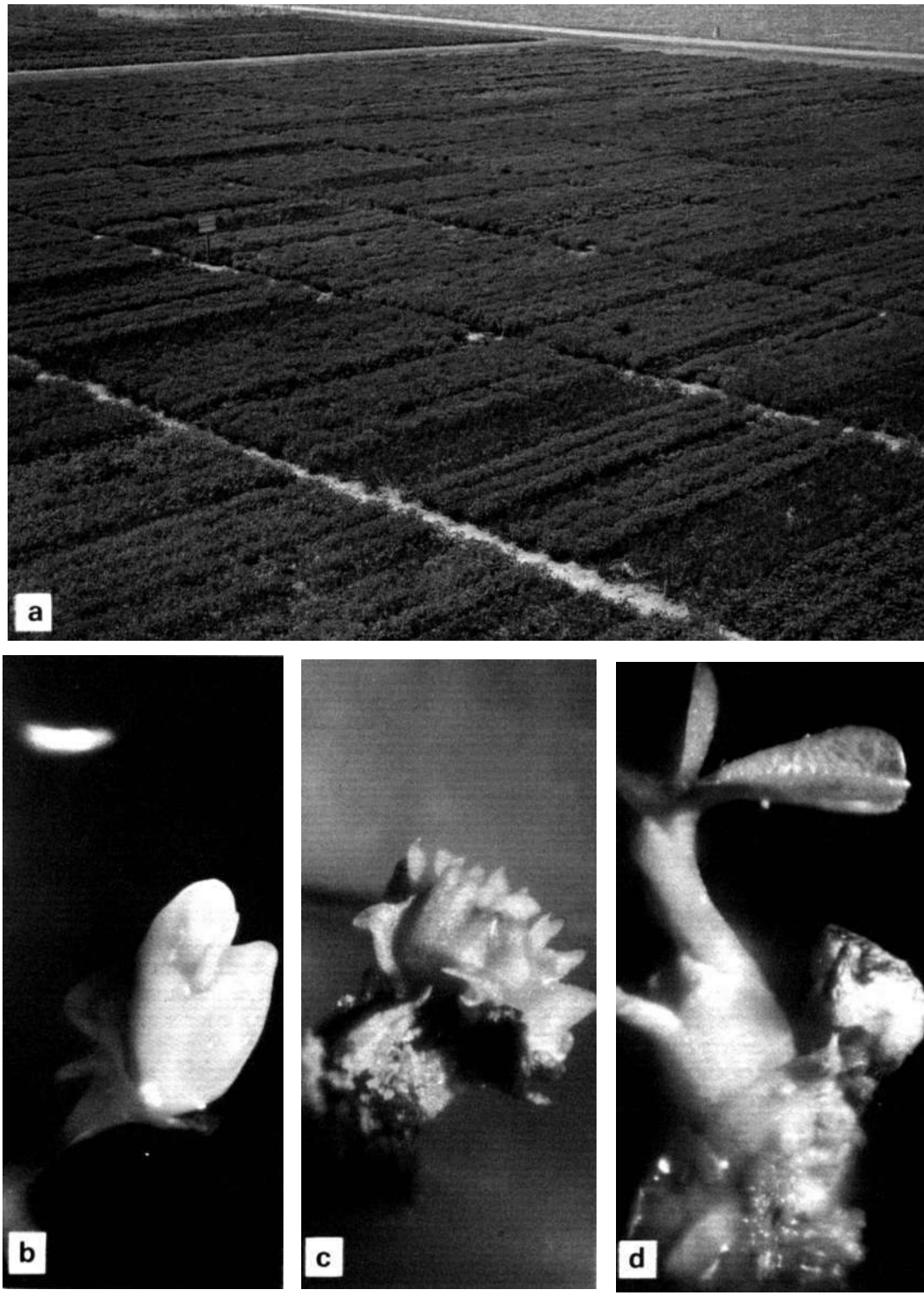
Use of Incompatible Species and Tissue Culture

Plate 4. a. Screening wild species derivatives for resistance to rust and leaf spot diseases at ICRISAT Center, (p. 97).

b. A freshly dissected embryo from a cultured ovule *A. hypogaea* cv Chico x *Arachis* sp Coll.9649, (p. 154).

c. Multiple buds from a cultured embryo *A. hypogaea* cv Robut 33-1 x *Arachis* sp PI 276233, (p. 154).

d. Germinating embryo *A. hypogaea* cv Robut 33-1 x *Arachis* sp PI 276233, (p. 154).


a
b
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d

Utilization of Incompatible Species in *Arachis:* Sequential Hormone Applications

Nalini Mallikarjuna and D.C. Sastri[1]

Abstract

Barriers to interspecific crossability pose serious constraints to gene transfer by sexual means, and several methods are available to overcome these barriers.

Based on knowledge of the reproductive biology and interspecific incompatibility in the genus Arachis, *a range of techniques were tested. Experiments were also initiated to explore the utility of in vitro methods. In vitro pollinations, young ovule cultures, and young ovary cultures have been used to create hybrids from different incompatible crosses in different taxa.*

Applications of hormones to flowers induced peg and pod formation in incompatible interspecific crosses in the genus. Ovules in hormone-induced pods did not develop beyond a certain stage, so ovules and/or embryos from these pods were cultured to raise hybrids.

Résumé

Utilisation des espèces incompatibles de l'*Arachis *: applications d'hormones séquentielles : Les barrières à l'aptitude aux croisements interspécifiques posent de sérieuses contraintes au transfert des gènes par voie sexuée. Or, plusieurs méthodes existent qui permettent de les surmonter.

Tout un éventail de techniques, basées sur les connaissances de la biologie de la reproduction et de l'incompatibilité interspécifique dans le genre* Arachis *ont été testées. Des essais ont également été entrepris pour étudier l'utilité des méthodes in vitro. Des pollinisations in vitro, des cultures de jeunes ovules et des cultures de jeunes ovaires ont été utilisées pour l'obtention d'hybrides à partir de différents croisements incompatibles dans des taxa différents.

Des applications d'hormones aux fleurs induisent la formation de gynophores et de gousses dans des croisements interspécifiques incompatibles dans le genre. Les ovules dans les gousses induites par les hormones ne se développant pas au-delà d'un certain stade, il est nécessaire d'avoir recours à des cultures des ovules ou des embryons de ces gousses pour obtenir des hybrides.

Introduction

Interspecific incompatibility in angiosperms has been the subject of several investigations. The topic has recently grown in importance because of greater interest in utilization of germplasm with attributes desirable in the cultivated species.

Transfer of desirable characters between species is often difficult. The most common reason is the failure of either fertilization between the two gametes, or the development of the zygote. During the last three or four decades a number of ways have been found to tackle such problems in different taxa (Sastri 1984).

Many of the wild relatives of Arachis hypogaea have been identified as good sources of resistance to several diseases and pests which seriously reduce groundnut yields (Moss 1980, Subrahmanyam et al. 1985, Amin 1985). A few of these wild species are crossable with A. hypogaea, but most are not (Gregory and Gregory 1979). Although failure to obtain hybrids from interspecific crosses in the genus Arachis was known as early as 1938 (Hull and Carver 1938, Gregory 1946), no concerted attempts have been made to investigate the reasons for this, or to produce hybrids. In the single

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detailed report on embryology in the crosses *Arachis hypogaea* x *A. diogoi* and *A. hypogaea* x *A. glabrata*, Johansen and Smith (1956) found retardation and cessation of embryo and endosperm growth accompanied by hypertrophy of seedcoats and eventual embryo death without differentiation. Murty.et al. (1981) observed that fertilization was delayed up to 48 hours after pollination in the incompatible cross, *A. hypogaea* x *A. glabrata* and the seed aborted very early.

There are difficulties in following the known methods of overcoming barriers for production of hybrids in the genus Arachis.

The geocarpic habit of the genus is one constraint in any manipulation for sexual hybrid production of wide hybrids by sexual means. Unlike other taxa with aerial fruits, in this genus seed development has two phases. In the aerial phase, dominated by geotropic elongation of the gynophore, the proembryo formed after the first few divisions ceases to grow, and development is resumed only after the peg has entered the subterranean phase.

In such crosses so far attempted at ICRISAT and elsewhere, the peg aborted early in its aerial phase when ovules were very small and immature, and rescue by culture as suggested by Martin (1970) was difficult. Success with culture of such immature ovules has been limited to a very few taxa (Sastri et al. 1980); generally the culture requirements for younger ovules are more complex. Culture of very young embryos is more difficult because their dissection is difficult and time consuming. Hybridization by somatic methods is not warranted until sufficient information about isolation, culture, and fusion of protoplasts of groundnut and *Arachis* wild species is obtained. Such investigations have been recently initiated (Oelck et al. 1982, Rugman and Cocking 1985).

Sastri et al. (1982) and Sastri and Moss (1982) reported the production of hybrids by culture of embryos from some incompatible crosses with single hormone treatments. The hormone application was intended to delay peg degeneration but hormones not only prolonged peg survival, they also increased the numbers of pegs formed. However, the assumption that subsequent development would be normal was not realised. A few pods were formed, but these contained very immature ovules, from which very few embryos could be dissected. This report presents results on research to increase pod numbers and ovule sizes, to facilitate in vitro culture.

Materials and Methods

Five cultivars of *Arachis hypogaea* were grown in the screenhouse at ICRISAT Center. Emasculations and pollinations were performed as described by Sastri and Moss (1982). *A. hypogaea* cultivars were always used as female parents while the wild species were used as pollen donors (Tables 1 and 2).

Table 1. Production of pegs and pods in crosses of five cultivars of *A. hypogaea* with *Arachis* sp PI 276233 following gibberellic acid application (87.5 mg/l).

Cultivar	Pollinations (no.)	Pegs/pollination (%)	Pods/pollination (%)	Pods/peg (%)
Robut 33-1	491	81.9	14.6	17.9
MK 374	707	77.4	32.7	42.2
M 13	54	87.3	1.9	2.1
TMV 2	423	76.6	20.6	26.9
Chico	103	81.7	11.6	14.1

To effect peg initiation and elongation, bases of incompatibly pollinated flowers were treated with 87.5 ppm gibberellic acid (GA) as described by Sastri and Moss (1982). In some crosses the gibberellin-induced pegs were subsequently treated with different concentrations of indole acetic acid (IAA) and/or kinetin 10, 15, 20 or 25 days after pollination to examine the effects, if any, of an auxin and/or kinetin on pod set, ovule size, and embryo development. Auxin and kinetin at various concentrations were separately incorporated into lanolin and the lanolin-hormone mixture was applied to the peg bases (proximal to the node).

About 25 to 30 flowers were pollinated on a plant during a period of three to four weeks. All the pods formed were harvested at least 30 days after the last treatment to the pegs obtained from the last pollination on each plant. Details on culture of immature ovules from these pods and culture of embryos are described by Nalini and Sastri (1985).

Results and Discussion

Effect of Gibberellin

Application of GA to the bases of incompatibly pollinated flowers was found to induce peg initiation and growth in a few intersectional crosses which were not earlier successful (Table 2). In

Table 2. Peg and pod production after gibberellin treatment in some intersectionsl crosses.

Cross	Polli-nations (no.)	Pegs/polli-nation (%)	Pods/peg (%)
Section *Arachis* x Section *Triseminaiae*			
A. duranensis (2n=20) x *A. pusilla* (2n=20)	33	79	39
A. hypogaea cv Robut 33-1 x *A. pusilla* (2n=20)	78	46	0
Section *Arachis* x Section *Erectoides*			
A. hypogaea cv Robut 33-1 x *A. rigonii* (2n=20)	45	64	13
A. hypogaea cv M 13 x *A. rigonii*	18	94	22
A. hypogaea cv TMV 2 x *A. rigonii*	43	86	26
Section *Arachis* x Section *Extranervosae*			
A. hypogaea cv Robut 33-1 x *A. villosulicarpa* (2n=20)	39	59	3
A. hypogaea cv MK 374 x *A. villosulicarpa* (2n=20)	9	89	11
Section *Extranervosae* x Section *Triseminaiae*			
A. villosulicarpa (2n=20) x *A. pusilla* (2n=20)	24	54	46
Section *Arachis* x Section *Rhizomatosae*			
A. hypogaea cv Robut 33-1 x *Arachis* sp Coll. 9649	82	44	6
A. hypogaea cv Robut 33-1 x *Arachis* sp Coll. 9797	46	57	2
A. hypogaea cv Robut 33-1 x *Arachis* sp Coll. 9806	26	62	0
A. hypogaea cv TMV 2 x *Arachis* sp PI 276233	408	76	20
A. hypogaea cv TMV 2 x *Arachis* sp Coll.9649	11	73	0
A. hypogaea cv MK 374 x *Arachis* sp PI 276233	648	68	32
A. hypogaea cv MK 374 x *Arachis* sp Coll. 9649	26	42	15
A. hypogaea cv M 13 x *Arachis* sp PI 276233	75	56	5
A. hypogaea cv Chico x *Arachis* sp PI 276233	58	66	9
A. hypogaea cv Chico x *Arachis* sp Coll. 9649	26	73	19

some crosses pods were also formed, notable among these were crosses involving *A. pusilla*, which belongs to a monotypic section and has not so far been crossed with any other species of the genus (Gregory and Gregory 1979).

Cultivar differences in gibberellin-aided crosses of *A. hypogaea* with *Arachis* sp PI 276233 of section *Rhizomatosae*

Among the five cultivars used for crosses with three members of section *Rhizomatosae*, MK 374 produced most pods, although peg production did not differ between cultivars (Table 2).

Effect of IAA and kinetin on pod set, and ovule size in gibberellin-induced pegs

Pod set on gibberellin-treated incompatibly pollinated flowers in the cross *A. hypogaea* cv Robut 33-1 x Arachis sp PI 276233 ranged from 0 to 36%. It varied from plant to plant, and from season to season. On average about 15% of the flowers set pods. When IAA, (four concentrations) was applied to the GA-induced pegs on different days after pollination, there was a varying response which appeared to be dependent more upon the day of application than on the concentration (Table 3). All concentrations applied on the 20th day after pollination increased pod set. Most IAA treatments increased ovule sizes; the largest ovules were from 100 ppm IAA on the 20th day (Table 4). Some of these treatments resulted in more, larger ovules for culture.

Kinetin on the 10th day, and at concentrations of 25 ppm, or more, reduced pod production. Some concentrations of kinetin did improve pod set to a maximum of 37.5% of the pegs formed (Table 3). Ovule sizes were also increased (Table 4). There were similar effects on ovule sizes in other cultivars of *A. hypogaea* crossed with the same male parent (Table 5).

The combinations of hormone treatments

Table 3. Pod production (% pods/peg) by *A. hypogaea* cv Robut 33-1 x Arachis sp PI 276233 after hormone treatments.

Hormones applied[1] (ppm)	Days after pollination			
	10	15	20	25
Control				
GA 17.9				
IAA 10	13.3	12.5	25.8	9.1
25	22.2	16.0	20.0	13.9
50	12.1	18.9	23.9	33.3
100	30.8	18.0	20.0	40.0
Kn 1	16.7	18.4	7.0	7.1
5	0.0	28.6	25.0	8.3
10	0.0		14.7	37.5
25	0.0	4.3	0.0	
50	8.8		10.6	

1. GA = Gibberellic acid (87.5 ppm; aqueous) applied to bases of flowers soon after incompatible pollinations, followed by IAA or kinetin (Kn) at different concentrations in lanolin on different days after pollination.

increased pod production and ovule size, but the treatments which are the best for maximum pod production may not be the best for obtaining the largest ovules (Tables 3 and 4). These ovules, however, have to be cultured for subsequent growth (Nalini and Sastri 1985).

Table 4. Ovule lengths (mm) in A. hypogaea cv Robut 33-1 x Arachis sp PI 276233 after hormone treatments.

Hormones applied[1]	Days after pollination			
	10.	15	20	25
Control				
GA 2.1 mm				
IAA 10 ppm	2.8	2.1	2.9	2.0
25	2.4	2.2	3.0	2.4
50	2.5	2.6	2.3	2.5
100	1.7	2.1	4.8	1.5
Kn 1 ppm		2.3	2.7	2.3
5		2.1	2.8	2.0
10			3.8	2.6
50			3.0	

1. GA = Gibberellic acid (87.5 ppm; aqueous) applied to bases of flowers soon after incompatible pollinations, followed by IAA or kinetin (Kn) at different concentrations in lanolin on different days after pollination.

Table 5. Ovule length (mm) from pods obtained in three *A. hypogaea* cultivars crossed with *Arachis* sp PI 276233 with subsequent hormone treatments.

Hormone treatment[1] (ppm)	dap[2]	MK 374	TMV2	M 13
Nil		1.6		
GA		2.6	2.3	2.8
GA;IAA 10	10		3.8	
GA; IAA 10	15	3.1		2.8
GA; IAA 25	10		2.5	
GA; IAA 25	15	2.0		
GA; IAA 50	15	2.4		
GA; IAA 100	15	2.8		

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

Suggestions by Gregory (1946) for embryo culture and by Martin (1970) for ovule culture for hybrid production from incompatible crosses have not been taken up until the present study. In unaided pollinations a few pegs were obtained but the pegs, ovules, and embryos degenerated, and ovules and embryos were too immature to be successfully cultured before degeneration. Martin's (1970) success in culturing very young ovules from aerial gynophores could neither be repeated with the ovules obtained from these crosses, nor even with ovules from selfed pegs (Sastri et al. 1980). Peg production in crosses was insufficient to initiate in vitro experiments to culture ovules or embryos. Gibberellin treatment increased the number of pegs and delayed, if not prevented, their degeneration. The application of gibberellin followed by a further application of IAA or kinetin resulted in increased pod set and larger ovules.

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In vitro Culture of Ovules and Embryos from some Incompatible Interspecific Crosses in the Genus *Arachis* L.

Nalini Mallikarjuna and D.C. Sastri[1]

Abstract

In vitro culture of ovules and embyros is commonly used to produce hybrids from incompatible crosses. In several interspecific crosses in Arachis, *ovule and/or embryo culture is necessary because the ovules do not develop fully in vivo.*

Ovule sizes from different crosses and different hormone treatments range from less than 0.3 mm to 5.0 mm, rarely 7.0 mm. Ovules smaller than 3.0 mm had to be cultured entire as the proembryos they contained were too small for dissection and culture. Ovules larger than 3.0 mm were dissected and their embryos cultured.

All the ovules were cultured on MS media with or without agar and with different concentrations of various hormones.

Depending on the hormones used and their concentrations, different responses were observed. Surface callus formation, greening, swelling, and browning were common in cultured ovules. Embryos emerged from some of the ovules and plantlets have been obtained. Cultured embryos showed similar responses, and some cultured embryos germinated, and developed into plantlets.

Résumé

Culture in vitro d'ovules et d'embryons issus de certains croisements interspécifiques incompatibles dans le genre* Arachis *L. : La culture d'ovules et d'embryons in vitro est une méthode courante de production d'hybrides à partir de croisements incompatibles. La culture d'ovules et/ou d'embryons s'avère nécessaire dans plusieurs croisements interspécifiques dans le genre* Arachis *étant donné le développement incomplet des ovules in vitro.

La taille des ovules provenant de différents croisements et ayant subi différents traitements hormonaux varie de moins de 0,3 mm à 5 mm, rarement 7,0 mm. Les ovules de taille inférieure à 3 mm doivent être cultivés entiers, les proembryons qu'ils contiennent étant trop petits pour la dissection et la culture. Les ovules de dimension supérieure à 3,0 mm sont disséqués et leurs embryons cultivés.

Tous les ovules sont cultivés sur des milieux MS gélosés ou non et à différentes concentrations de diverses hormones.

On observe des réponses différentes selon les hormones utilisées et leurs concentrations. Formation de cals en surface, verdissement, gonflement et brunissement sont courants chez les ovules cultivés. Des embryons se sont développés dans certains de ces ovules et de jeunes plants ont pu être obtenus. On constate les mêmes réactions chez les embryons cultivés : certains embryons cultivés ont germé et se sont développés en plants.

Introduction

Of the several methods available to overcome barriers to hybridization, in vitro culture of ovules and embryos, which would otherwise abort, has been the most commonly used, and hybrids have been produced in about 50 interspecific crosses by embryo culture (Raghavan 1977, Sastri 1984) and in more than ten interspecific crosses by ovule culture (Sastri 1984; Sastri et al. 1980, 1982, 1983).

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Our earlier investigations on interspecific incompatibility in the genus *Arachis* indicated the need to culture ovules or embryos for hybrid production (Nalini and Sastri 1985). In the crosses between *Arachis hypogaea* x *Arachis* sp PI 276233, and other tetraploid accessions of the section Rhizomatosae, pegs were rarely formed. If pegs were formed, they degenerated before they entered the soil, and the seeds aborted. The ovules and the proembryos in these aerial pegs were very small. Our preliminary attempts to culture such immature ovules even from selfed pegs were not successful (Sastri et at. 1980). We have induced pegs and pods from some incompatible pollinations by hormone treatments (Nalini et al. 1983; Sastri and Moss 1982; Sastri et al. 1982, 1983) in sufficient numbers and maintained growth to a stage at which it was possible to dissect and culture ovules and embryos. The objective of the present investigation was to study the hormonal requirements in the medium for survival, sustaining growth, and promoting subsequent development of immature ovules or proembryos to produce hybrid plants from these crosses.

Methods

All pegs and pods induced from incompatible crosses (Nalini and Sastri 1985) were carefully removed from the soil about 30 to 40 days after the last hormone treatment. The soil was washed from the pods in running water, surface sterilized with Clorox for ten minutes, and washed thoroughly twice with sterile water. Washing in sterile water and subsequent operations were performed under aseptic conditions.

Ovules larger than 3.0 mm were generally dissected to extract the proembryos for examination and culture.

Ovules and embryos were cultured on Murashige and Skoog's (MS) medium (1962) with or without agar, with different concentrations of sucrose, and with hormones [kinetin (Kn), Benzylamino purine (BAP), Indole acetic acid (IAA), Naphthalene acetic acid (NAA)] at different concentrations.

When liquid media were used ovules were supported on filter paper bridges in 15 mm x 50 mm screw-topped glass vials such that the placenta was in direct contact with the filter paper. When agar media were used, the placenta or the placental areas of cultured ovules were kept in direct contact with the surface of the medium in (25 mm x 150 mm) rimless glass tubes. All the cultures were incubated at 25°C ± 3° with 10h photoperiod (fluorescent and incandescent illumination at about 4000 lux).

Results and Discussion

Ovule Cultures

Most ovules were between 1.0 and 3.0 mm, with some up to 5.0 mm, but rarely reaching 7.0 mm. Since all the pods from hormone-aided pollinations on a plant were harvested on the same day their ages ranged from 50 to 70 days after pollination. There appeared to be no correlation between sizes of ovules and age after pollination.

The response of cultured ovules depended on their size at the time of culture, and the medium employed. The responses could be broadly categorized into the following:

a. increase in size of ovules with no change in color,

b. increase in size and greening of ovules,

c. increase in size and browning of ovules,

d. growth and emergence of embryos,

e. callus production from surface of ovules,

f. greening of ovules, and

g. browning (necrosis) and shrinkage of ovules.

The percentage of cultured ovules showing desirable responses on a few combinations of media are presented in Tables 1 and 2. Among the ovules from crosses of four cultivars with *Arachis* sp PI 276233 cultured on different media, cultivars MK 374 and TMV 2 were found to give better results on both liquid and semisolid media than those of other cultivars (Table 1, Plate 4b,c,d). On semisolid media the responses were slower than on liquid media. Ovules survived longer in both agar and liquid media containing BAP-NAA than when Kn-IAA combinations were used. But Kn-IAA appeared to be better than BAP-NAA at stimulating embryo growth, development and emergence from the ovules (Tables 1 and 2).

Martin (1970) observed a beneficial effect on ovule growth with kinetin and gibberellin; kinetin at 0.5-1.0 mg/l had the best effect. In our studies, even lower concentrations of kinetin, (0.1 or 0.2 mg/l), were better than BAP.

Table 1. Percentage ovules responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium with different concentrations of kinetin and IAA.

Concentrations of additives				Ovules responding[1] (%)			
				Cultivars			
Kn (mg/l)	IAA (mg/l)	Sucrose (%)	Agar (%)	Robut 33-1	MK 374	M 13	TMV 2
0.00	0.00	5	0	24.00			
0.10	0.10	5	0	22.50	20.08	16.00	85.36
0.10	0.20	5	0	41.17	29.00		75.00
0.10	0.50	5	0	0.00	5.71		
0.11	0.00	3	0	46.15	83.33		
0.22	0.00	3	0.7	9.52	66.66	28.57	92.85

1. Ovules which responded increased in size and became green.

Ovules which had swollen were examined for embryo development. Those which increased in size and remained green were generally found to possess poorly-developed embryos. On the other hand, embryos with different degrees of growth were observed in many ovules which had increased in size and turned brown. These embryos, when extracted and cultured on MS medium with agar, NAA (2 mg/l) and BAP (0.5 mg/l) produced callus and multiple shoots.

Embryo Culture

Ovules, upto 3.0 mm long had a small globular proembryo measuring 0.1 or 0.2 mm. Those larger than 3.0 mm contained slightly larger embryos. The small proembryos were likely to be affected by injury during dissection, and/or suffer desiccation during transfer to the culture vials. Many such embryos have not responded to culture. Therefore, the dissection of embryos from ovules smaller than 3.0 mm was discontinued. Even in larger ovules, the size and stages of proembryos were found to vary greatly. The embryos very rarely differentiated, typical stages of dicotyledonous embryo development were rarely observed. They were mostly amorphous and globular although they had shown an increase in size (Fig. 1a to d). Some showed both the cotyledonary initials, but a very poorly-developed embryo axis (Fig. 2e,f), some had only one well-formed cotyledonary initial, while the other was rudimentary (Fig. 2d). However, some of the embryos were successfully cultured in vitro (Fig. 2b,c,d) (Table 3). The majority of the embryos were initially cultured on MS semisolid medium with 2 mg/l of NAA and 0.5 mg/l of BAP, a medium which was good for a range of tissue and organ cultures of *A. hypogaea* and some wild species (Sastri et al. 1982, 1983). This medium with some variations in concentrations of NAA was satisfactory for callus formation and shoot regeneration from some of the embryos cultured.

Table 2. Percentage ovules responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium with different concentrations of BAP and NAA.

Concentrations of additives				Ovules responding[1] (%)			
				Cultivars			
BAP (mg/l)	NAA (mg/l)	Sucrose (%)	Agar (%)	Robut 33-1	MK 374	M 13	TMV 2
0.50	2.00	2	0	0.00			
0.10	0.10	1	0	25.00			
0.05	2.00	3	0	33.33			
0.50	0.50	3	0.7	5.45			
0.50	0.75	3	0.7	0.00		85.71	
0.50	2.00	3	0.7	11.37	40.25	37.93	100.00
0.50	0.50	3	0		72.22		

1. Ovules which responded increased in size and became green.

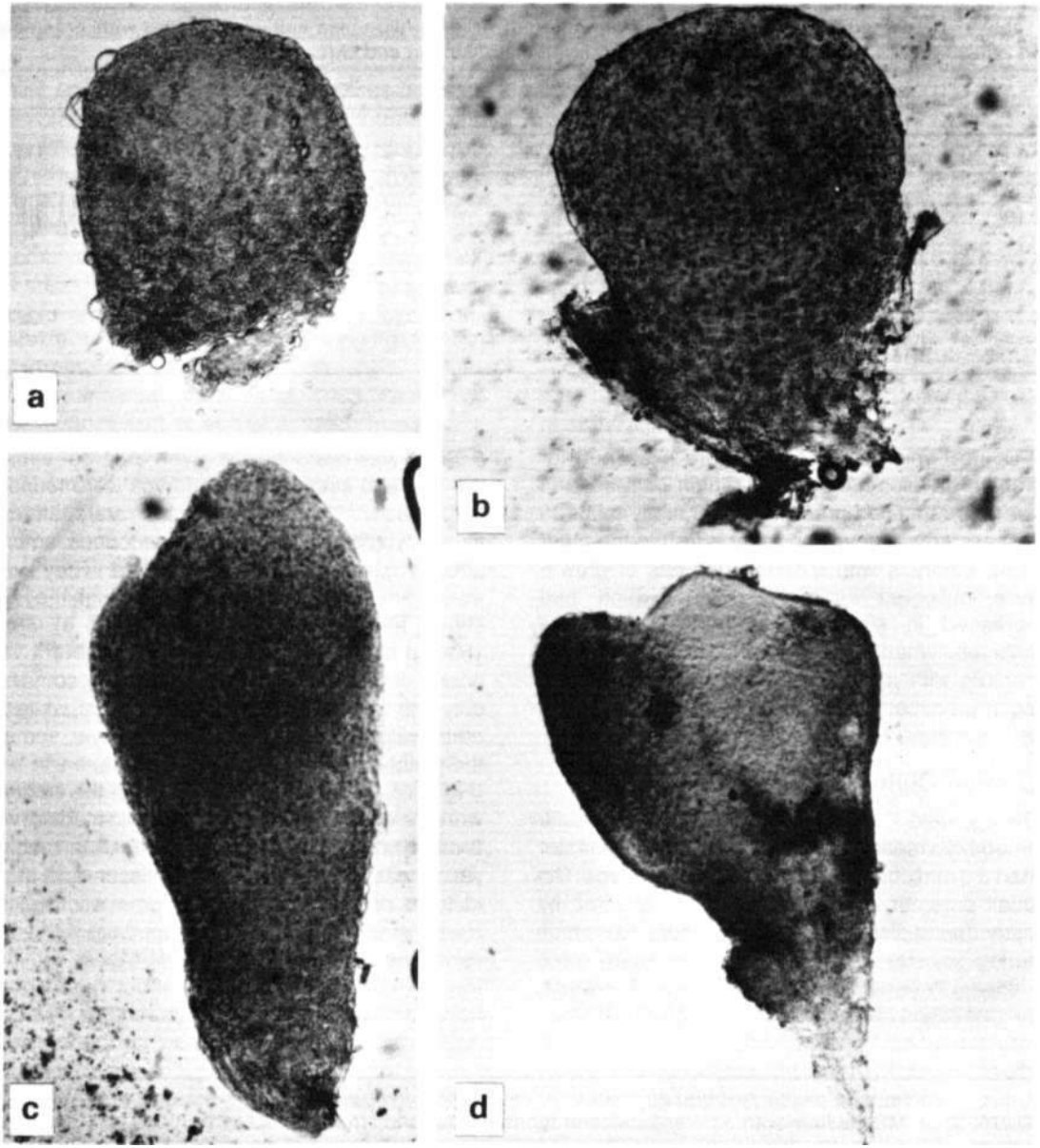

Figure 1. Poorly-differentiated embryos from *A. hypogaea* cv MK 374 x *Arachis* sp PI 276233

a. globular proembryo dissected from an ovule (1.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm + IAA 0.1 ppm) for 20 days, (x 122)

b. swollen globular proembryo dissected from an ovule (2.5 mm) cultured on liquid medium (MS + Suc. 5% + BAP 0.5 ppm) for 74 days. (x 106)

c. heart-shaped, but elongated proembryo dissected from an ovule (3.0 mm) cultured on liquid medium (MS + Sue. 5% + Kn 0.25 ppm + IAA 1.0 ppm) for 46 days, (x 96)

d. overgrown late heart-shaped proembryo from an ovule (3.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.25 ppm + IAA 1.0 ppm) for 77 days, (x 54)

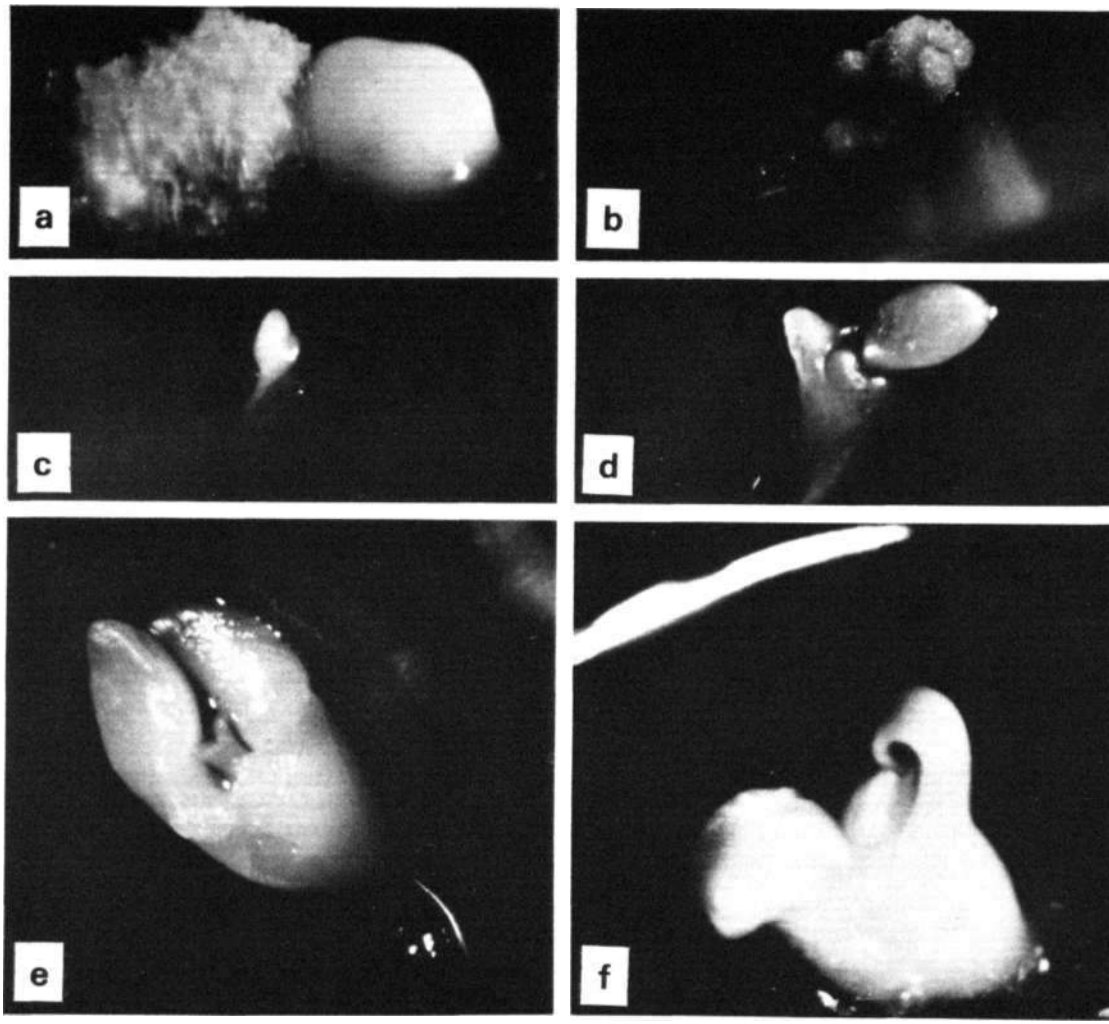

Figure 2 a. callusing ovule (0.5 mm) cultured on liquid medium (MS + Suc. 5%+Kn 0.1 ppm)for41 days from *A. hypogaea* cv TMV 2 x *Arachis* sp Coll 9649. (x 11.7)

b. differentiating callus from a globular embryo dissected from a 3.0 mm ovule cultured on semisolid medium (MS + Suc. 3% + NAA 0.75 ppm + BAP 0.5 ppm) for 79 days from the cross *A. hypogaea* cv MK 374 x *A. glabrata*. (x 11.7)

c. late heart-shaped embryo dissected from an ovule (3.5 mm) cultured on liquid medium (MS + Suc. 5%+ Kn 0.1 ppm) for 19 days from the cross *A. hypogaea* MK 374x *Arachis* sp PI 276233. (x 11.4)

d. cotyledonary embryo with one well-formed cotyledon, dissected from an ovule (3.0 mm) cultured on liquid medium (MS + Suc. 5% + Kn0.1 ppm) for 65 days from the cross *A. hypogaea* cv Chico x *Arachis* sp PI 276233. (x 13.3)

e. late cotyledonary embryo (note poorly-developed embryo axis) dissected from an ovule (2.0 mm) cultured on liquid medium (MS + Sue. 5% + Kn 0.1 ppm + IAA 0.2 ppm) for 47 days from the cross *Arachis hypogaea* cv Chico x *Arachis* sp Coll 9649. (x 12.2)

f. embryo similar to that shown in e, from the cross *A. hypogaea* cv MK 374 x *Arachis* sp PI 276233, 20 days after culture, (x 14.4)

Table 3. Percentage embryos responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium.

Concentrations of additives						Embryos responding' (%)		
						Cultivars		
Kn (mg/l)	IAA (mg/l)	BAP (mg/l)	NAA (mg/l)	Sucrose (%)	Agar(%)	Robut 33-1	MK 374	TMV 2
0.10	0.1			5	0	6.66		
0.22	0			3	0.7			25.00
0 0		0.50	2.00	2		8.33		
0	0	0.50	0.50	3	0.7	3.57	26.11	
0	0	0.50	0.75	3	0.7	0.0	50.11	
0	0	0.50	2.00	3	0.7	21.42	20.00	27.27

1. Embryos which responded increased in size and became green.

Conclusions

These studies on media and hormones have increased the survival, growth and development of hybrid ovules and embryos in culture. Changes in media necessary for continued survival, and better growth and development of ovules and embryos are being investigated, but there is now a method to produce hybrids in culture between species of Arachis which were previously incompatible. This is an important step towards transfer of desirable characters for the genetic improvement of *Arachis hypogaea*.

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Callus Induction and Morphogenesis in *Arachis hypogaea* L.

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Abstract

Seven different media with varying mineral compositions, and six different seedling explants were used in callus induction and plantlet regeneration with four groundnut genotypes. The effects of different growth regulators, (2,4-D, IAA, NAA, BAP, and kinetin) and organic additives on callus growth were studied. Shoots were regenerated with varying frequencies (11-38%) from callus cultures of epicotyl, hypocotyl, leaves and cotyledons in MS medium with BAP (1 mg/l) + NAA (0.4 mg/l) and also in kinetin (1 mg/l). Epicotyl-derived callus regenerated with high frequency compared to other explants. Rooting was induced by transferring the regenerated shoots to a medium containing NAA (1 mg/l) + Kn (0.04 mg/l). There were genotypic differences in plantlet regeneration. Flowers and flower buds were induced directly from seven different genotypes of embryonated and de-embryonated cotyledons on Blaydes' medium with cytokinins.

Résumé

Induction et morphogenèse des cals chez** Arachis hypogaea **L. : Sept milieux de composition minérale variable et 6 explants de plantules différents sont utilisés pour l'induction de cals et la régénération de jeunes plants sur 4 génotypes d'arachide. Les effets de différents régulateurs de croissance (2.4-D, IAA, NAA, BAP, kinétine) et d'additifs organiques sur la croissance des cals sont étudiés. Des pousses sont régénérées à des fréquences variables (11-38%) à partir de cultures de cals d'épicotyle, d'hypocotyle, de feuilles et de cotylédons dans un milieu MS avec BAP (1 mg/l) + NAA (0,4 mg/l) et également dans la kinétine (1 mg/l). Comparé aux autres explants les cals dérivés d'épicotyle se régénèrent à une fréquence élevée. L'enracinement est induit par transfert des pousses régénérées dans un milieu contenant du NAA (1 mg/l) + Kn (0,04 mg/l). On constate des différences génotypiques dans la régénération des jeunes plants. Des fleurs et des bourgeons floraux sont induits directement chez 7 génotypes différents à partir de cotylédons avec embryon et sans embryon sur un milieu de Blaydes avec des cytokinines.

Introduction

Crop improvement is primarily dependent on the availability of a large pool of genetic variation within the population of a given species. Since the conventional techniques employed in crop improvement may not keep pace with the demands of the expanding population, decreasing land resources, and increasing environmental stresses, the importance of plant cell and tissue culture techniques in plant improvement has been recognised. The exploitation of tissue culture techniques in the multiplication of groundnut hybrids, through direct regeneration, has already been reported (Narasimhulu and Reddy 1983). Successful application of these techniques depends on the recovery of plants that can be used in a conventional breeding program. A general knowledge of the specific media and organic requirements which promote profuse callusing, the explant types which rapidly yield callus and regenerate with high frequency, the hormonal combination effective in inducing callus, and finally the specific genotype which regenerates

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more frequently is a pre-requisite for the successful utilization of these techniques.

The present study defines the optimal nutrient and hormonal requirements for callus growth and morphogenesis in groundnut. The optimization of cultural requirements for morphogenesis may help in future studies of genetic manipulation at cellular level among regenerated plantlets to improve this important oilseed crop.

Materials and Methods

Seeds from four groundnut cultivars, ICG 4367, US 48, TMV 2, and TG 19B were surface-sterilized with 0.1% mercuric chloride for 15 min. After thorough washing in sterile distilled water, the seeds were germinated in aseptic conditions under continuous light. Leaflets, epicotyl, hypocotyl, primary and secondary roots, and cotyledons were excised from 7-day old seedlings and inoculated on media.

Callus Induction

Types of Media

Hypocotyl explants from 7-day old aseptic seedlings were cultured in seven different media; Blaydes' (1966); MS, Murashige and Skoog's (1962); B5 (Gamborg et al. 1968); SH, Shenk and Hildebrandt's (1972); Whites's (1939); Linsmaier and Skoog's (1965) and Wood's (Wood and Braun 1961), each containing 3% sucrose, and supplemented with 2-4, Dichlorophenoxy acetic acid, (2,4-D) at 2 mg/l, and kinetin (Kn) at 0.5 mg/l. All explants formed callus in Blaydes, MS and SH media, while callusing was not observed in Woods' medium (Table 1). Fresh and dry weights of 4-week old callus were maximum when grown on MS medium. The major differences in the seven media are in sodium nitrate and manganese sulphate concentrations. When sodium nitrate was substituted for ammonium nitrate in MS medium, without altering total nitrate, the frequency of callusing was reduced by about 40%.

Growth of the excised tissue in vitro is influenced by the chemical composition of the medium. The equally good growth of the callus in three different media with similar hormonal composition indicates a degree of plasticity in the culture requirements of this species. The reduction in the frequency of callusing on substituting sodium nitrate for ammonium nitrate suggests that the source and form of nitrates also influence callusing.

Table 1. Effect of different media[1] on frequency of callusing and weight of 4-week old callus and growth of hypocotyl explants.

Media[1]	Callusing frequency (%)	Callus fresh wt (g)	Callus dry wt (g)
MS	100	1.802	0.621
Blaydes'	100	1.745	0.524
B5	87	1.560	0.476
LS	92	1.452	0.428
SH	100	1.106	0.342
White's	84	0.521	0.138
Wood's	0	-	-

1. Supplemented with 2,4-D (2 mg/l) + Kn (0.5 mg/l).

Phytohormones

The effect of the three auxins 2,4-D, Indole acetic acid (IAA) and Napthalene acetic acid (NAA) on callus induction in hypocotyl explants was studied using the same concentrations of auxin (2 mg/l) along with kinetin at 0.5 mg/l in MS medium. Although all the three auxins used produced callus, the frequency of callusing as well as fresh and dry weights of 4-week old callus was high in MS media supplemented with 2,4-D (2 mg/l) + Kn (0.5 mg/l). Callus initiation occurred after 10 to 15 days in media supplemented with IAA (2 mg/l) + Kn (0.5 mg/l), compared to a shorter period of 7 to 10 days for callus initiation in media supplemented with 2,4-D (2 mg/l) + Kn (0.5 mg/l). Although exogenous supplementation of 2,4-D alone (1 and 2 mg/l) gave callus, the addition of kinetin markedly increased callus growth. The improved efficiency of 2,4-D in association with kinetin is attributed to their role in DNA synthesis and mitosis (Skoog and Miller 1957). According to Setterfield (1963) the auxin and cytokinin may act synergistically to promote either cell division or expansion depending upon other factors within the cell which react with these hormones. A study of various concentrations of 2,4-D indicated that 2,4-D at 2 mg/l is optimal for callus induction as measured by fresh and dry weights of 4-week old callus.

Organic Additives

Despite their incompletely-defined composition a number of organic complexes have greatly contributed to the success of tissue culture. The two

organic additives, coconut milk (at 5 to 20%) and casein hydrolysate (at 100 to 400 mg/l) were tested for their effect on callus growth. The addition of coconut milk to hormome-supplemented media used for growing hypocotyl-derived subcultures, yielded larger amounts of callus compared to control media without the additive. Coconut milk at 10% was found to be optimal for callus growth (Table 2). Casein hydrolysate tested in three concentrations (100, 200 and 400 mg/l) promoted callus growth. Callus growth increased with increasing levels of this protein hydrolysate. The improved callus growth in media supplemented with casein hydrolysate may have been due either to the supply of a particular amino acid, or to the provision of increased amounts of reduced nitrogen (Button et al. 1974).

Table 2. Effect of coconut milk and casein hydrolysate on weight of callus 4 weeks after culture on MS media.[1]

Additive and concentation	Callus fresh wt (g)	Callus dry wt (g)
Coconut milk		
0	2.682	0.936
5%	2.738	1.019
10%	3.014	1.031
15%	2.646	0.823
20%	2.205	0.686
Casein hydrolysate		
0	2.432	0.624
100 mg/l	2.692	0.689
200 mg/l	2.858	0.778
400 mg/l	3.217	0.801

1. Supplemented with 2,4-D (2 mg/l) and Kn (0.5 mg/l).

Source of Explant

Six different seedling explants, namely hypocotyl, epicotyl, leaves, cotyledons, primary, and secondary roots, were cultured on callus-inducing MS media. Explants were found to swell in the first 3 to 4 days and callused at the cut ends in 7 to 10 days (Fig. 1 a). In leaf explants, callus formation was more frequent in lamina portions than in the veinal region. Hypocotyl explants callused with 79 to 89% frequency, but cotyledonary explants callused with 39 to 59% frequency, depending on genotype.

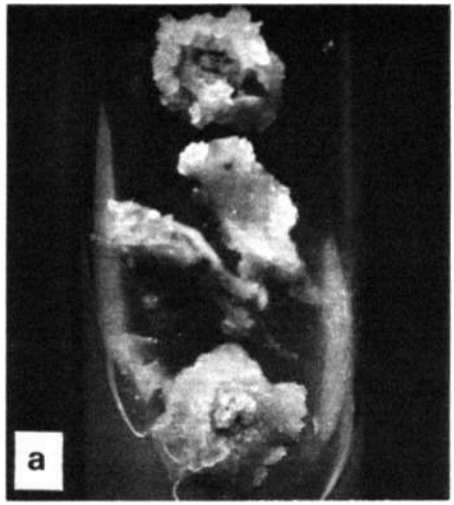
Morphogenesis

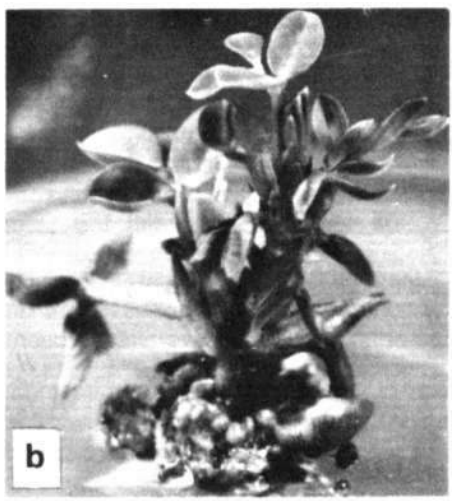
Callogenesis

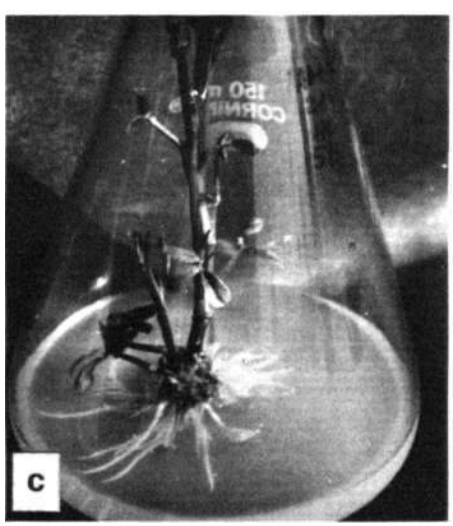
Four-week old callus was separated from the explant so as to exclude any residual fragment of the original tissue. The callus was transferred to a basal medium, and then to an organ-inducing medium containing relatively higher levels of cytokinins compared to auxins, and cultured under continuous, cool, white, fluorescent light of 2000 lux. In regeneration medium the callus grew slowly at d was compact. There was an intense greening of the callus by the seventh day. Shoot primordia appeared at the apex of the callus clump in 12 to 15 days. Of the various hormonal combinations tested, Benzylamino purine (BAP) (1 mg/l) + NAA (0.4 mg/l) was found to be favourable for shoot induction (Fig. 1 b). Regeneration has also been obtained in medium containing 1 mg/l kinetin. While the four explant calli (from epicotyl, hypocotyl, leaf, and cotyledon) regenerated, there was no morphogenesis in root callus cultures. The variability in regenerating potential shown by these different calli reflect a variable interaction of physiologically heterogenous explants with the tissue culture medium. The regeneration of plantlets from leaf callus cultures in the present study holds promising potential for protoplast studies in groundnut, where some initial studies have already been made by Oelck et al. (1982).

Rhizogenesis

When the shoots regenerated from callus cultures of four different explants reached a height of 4 to 6 cms they were transferred to a medium containing kinetin (0.05 mg/l) and varying concentrations of IAA and NAA. Occasionally one slender root was seen in cultures on basal medium without hormonal supplementation. Small and slender roots without laterals were observed with lower concentrations of auxins. In general, the number of roots per shoot increased with increasing concentrations of NAA and IAA; levels exceeding 2 mg/l resulted in short, stout roots, and callus was also formed at the base of the shoot. The combinations of IAA (2 mg/l) + Kn (0.05 mg/l) or NAA (1 mg/l) + Kn (0.05 mg/l) were found to be effective in producing healthy roots with many laterals (Fig. 1c). The regenerated shoots were transferred to vermiculite-filled pots for further studies (Fig. 1d).







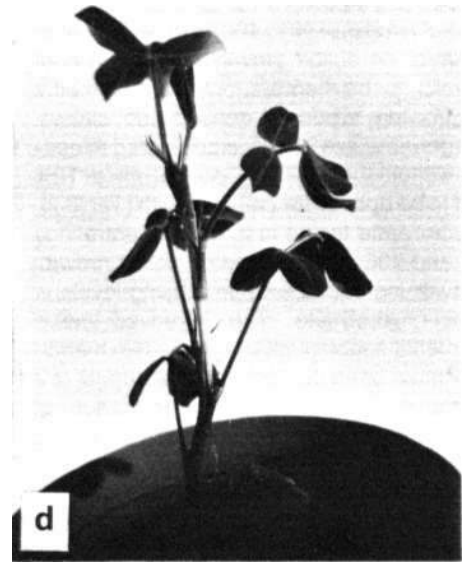


Figure 1. a. callus induction from epicotyl explant
b. shoot regeneration from epicotyl callus
c. root formation
d. plantlet which has been transferred to vermiculite-filled pot
e. flowering from de-embryonated groundnut cotyledons.

Regeneration has been successful with the two genotypes ICG 4367 and TMV 2, while the other two cultivars TG 19B and US 48 resisted all attempts at regeneration. The differences shown by the four cultivars in regenerating capacity suggest genetic control over the physiology of the plant in regard to the utilization of nutrients. Mroginski et al. (1981) reported genetic differences in the regenerating potential of immature leaflet cultures ranging from non-responding to fully-regenerating types in groundnut.

In vitro Flowering

Cotyledons, with and without embryo axes, cultured on Blaydes' medium supplemented with cytokinins, produced flower buds and flowers with varying frequencies within three weeks of culturing. Although flowering from isolated plant parts in reproductive phase has been reported (Nitsch and Nitsch 1967; Wardell and Skoog 1969), the present case is unique in that flower buds were induced directly from de-embryonated cotyledons (Fig. 1e) without intervening vegetative growth. The culture of seven different genotypes of cultivated groundnut and two other wild species, *Arachis duranensis* and *A. monticola*, indicated genotypic differences in the frequency of flowering. The present phenomenon of direct flowering offers a unique system for the study of the physiological and molecular basis of flowering, and the factors regulating the transition of mitosis to meiosis under completely defined conditions.

Acknowledgements

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In vitro Induction of Genetic Variability in Groundnut

Y. P. S. Bajaj[1]

In vitro techniques, such as embryo and anther culture, and protoplast fusion were used to generate genetic variability, and to incorporate wild germplasm (*Arachis villosa*, *A. glabrata*) into cultivated groundnut. The following approaches were adopted:

Wide Hybrldizaton through Embryo Culture: The embryos of cultivated tetraploid groundnut (*A. hypogaea*), a wild diploid species (*A. villosa*), and their hybrid embryos, which generally abort in nature, were rescued 30 to 35 days after pollination and cultured in vitro. The plants were successfully transferred to soil. The hybrids had triploid chromosome numbers (3x=30).

Protoplast Fusion and Somatic Hybridization: A comparison was made between various segments of in vitro-grown seedlings. The optimal yield of protoplasts was obtained from excised hypocotyl segments of 10 to 14-day-old seedlings treated with an enzyme mixture of cellulase and macerozyme, and incubated for 12 to 15 h. Of the two species, *A. hypogaea* was more amenable to various treatments. The mesophyll protoplasts from *A. hypogaea* were fused with callus-derived protoplasts of *A. villosa*. Treatment with 20 to 25% polyethylene glycol resulted in 11 to 13% fusion products.

Haplold Production through Anther Culture: Pollen embryogenesis was induced in anther cultures of *A. villosa* and *A. glabrata*. The pollen underwent repeated divisions resulting in the formation of pollen embryos and callus. The anther-derived callus and the regenerated plants in *A. villosa* were mixoploids, and showed wide genetic variation ranging from haploids to octoploids.

The genetic variants obtained as a result of various in vitro manipulations will be used in groundnut improvement.

Induction In vitro de la variabilite genetique chez l'arachide: Des techniques in vitro, telles la culture d'embryons, d'antheres, et la fusion de protoplastes, ont ete utilises pour l'induction d'une variabilite genetique et l'introduction du germplasme sauvage (*Arachis villosa*, *A. glabrata*) dans les arachides cultivees. Les approches suivantes ont ete adoptees:

Une hybridation large grace a la culture d'embryons: Les embryons de l'arachide tetraploide cultivee (*A. hypogaea*), d'une espece diploide sauvage (*A. villosa*) et leurs embryons hybrides, generalement avortes dans la nature, ont ete mis en culture in vitro 30-35 jours apres pollinisation. Les plants ont ete repiques avec succes dans le sol. Les hybrides ont presente des nombres chromosomiques triploides (3x=30).

Une fusion des protoplastes et une hybridation somatique: Differents fragments de plantules cultives in vitro ont ete compares. Le rendement optimal de protoplastes a ete obtenu avec des fragments d'hypocotyle excis6s de plantules agees de 10-14 jours traites avec un melange de cellulase et de macerozynrie incubes pendant 12-15 heures. Des deux especes, *A. hypogaea* est la plus susceptible d'etre traitee. Les protoplastes de mesophylle d' *A. hypogaea* ont ete fusionnes avec des protoplastes derives de cals de *A. villosa*. Le traitement a 20-25% de polyethylene glycol a permis d'obtenir 11-13% de fusion.

Production d'haploide grace a la culture d'antheres: Une embryogenese du pollen a ete induite dans des cultures d'antheres de *A. villosa* et *A. glabrata*. Le pollen a subi des divisions repetees entrainant la formation d'embryons issus directement de la microspore et de cals. Les cais derives d'antheres et les plants regeneres de *A. villosa* ont ete mixoploides, avec une importante variation genetique allant des haploides aux octoploides.

Les variants genetiques obtenus a la suite de diverses manipulations in vitro seront utilises pour ('amelioration de l'arachide.

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The Development of Somatic Hybridization Techniques for Groundnut Improvement

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Abstract

The potential of somatic hybridization as a means of overcoming incompatibility between Arachis hypogaea *L and certain wild* Arachis *species within the section* Rhizomatosae *is being assessed. Such crosses are desirable because wild species have been identified as an important source of multiple disease resistance. Since the ability to regenerate plants from protoplast-derived callus is essential for any somatic hybridization program, tissue explants of A.* hypogaea *have been screened for their regenerative potential, and as a source of viable protoplasts. Protoplasts have been isolated from precultured immature leaves, cotyledons, roots, and mature leaves of* A. hypogaea, *but only the latter have shown satisfactory levels of division. Factors affecting the yield, viability, and subsequent division of* A. hypogaea *mesophyll protoplasts have been identified, and a protocol for their isolation, purification, and culture has been developed. Plants have been regenerated from cultured immature leaves and cotyledons, but not yet from mature leaf- or protoplast-derived callus. Similar studies are in progress with six wild* Arachis *species. The implication of this work is discussed.*

Résumé

Mise au point de techniques d'hybridation somatique pour l'amélioration de l'arachide : Les possibilités d'utilisation de l'hybridation somatique pour surmonter les problèmes d'incompatibilité entre* Arachis hypogaea *L. et certaines espèces sauvages appartenant à la section* Rhizomatosae *sont évaluées. De tels croisements sont souhaitables, les espèces sauvages ayant été identifiées comme constituant une importante source de multiples résistances aux maladies. La capacité à régénérer des plants à partir de cals dérivés de protoplastes étant indispensable à tout programme d'hybridation somatique, des explants de tissus d'* Arachis hypogaea *ont été criblés pour leur pouvoir de régénération et comme source de protoplastes viables. Des protoplastes ont été isolés de précultures de jeunes feuilles immatures, de cotylédons, de racines et de feuilles adultes d'*Arachis hypogaea*; seuls ces derniers ont présenté des niveaux de division satisfaisants. Les facteurs affectant le rendement, la viabilité et la division des protoplastes de mésophylle d'* Arachis hypogaea *ont été identifiés; un protocole a été mis au point pour leur isolement, leur purification et leur culture. Des plants ont été régénérés à partir de cultures de jeunes feuilles immatures et de cotylédons mais pas encore de cals obtenus à partir de feuilles adultes ou de protoplastes. Des études similaires sont en cours sur 6 espèces d'* Arachis *sauvages. Les implications de ces travaux sont discutées.

Introduction

Arachis hypogaea, the cultivated peanut or groundnut, is a crop of major economic importance, grown in both developed and developing countries as a source of protein and oil. However, commercial groundnut cultivars are susceptible to a range of pests and pathogens (Qarren and Jackson 1973), and consequently crop yields are reduced. One of the main objectives of groundnut improvement programs is therefore the production of genetically-resistant cultivars. High levels of resistance to some diseases and pests have not been identified in *A. hypogaea* germplasm collections (Banks 1976), and so some breeders have turned their attention to the wild *Arachis* species. Wild

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relatives of some other major crops have been successfully exploited as sources of resistant germplasm (Harlan 1976; Stalker 1980), and in the case of *Arachis*, desirable traits have been identified in a number of wild species (Moss 1980; Singh et al. 1980). Some of these species have been crossed with *A. hypogaea* to produce hybrids (Moss 1980), but since the cultivated groundnut is compatible only with species within the section *Arachis*, the use of wild species in breeding programs has been limited. The wild tetraploid species within section *Rhizomatosae* have been identified as an important source of multiple disease resistance (Sastri and Moss 1982). Initial attempts to cross these species with *A. hypogaea* have been unsuccessful (Hull and Carver 1938; Gregory 1946; Johansen and Smith 1956; Moss 1980; Murty et al. 1981), and more innovative approaches to overcome the incompatibility barriers have been examined, including embryo culture, ovule culture, and ovary and gynophore tip culture (Sastri et al. 1982). However, the most promising results were reported by Sastri and Moss (1982). Hybrids were produced between *A. monticola* (section *Arachis*) and *Arachis* sp PI 276233 (section Rhizomatosae) and between *A. hypogaea* and *Arachis* sp PI 276233, when cross-pollinated flowers were treated with growth regulators.

The use of tissue culture techniques for groundnut improvement has recently received considerable attention (Sastri et al. 1982), and the potential of this technology for the production of hybrids from sexually-incompatible crosses has been suggested. Somatic hybridization by protoplast fusion is one such method which may be employed for this purpose (Cocking 1981). The initial aim of our work has been to define suitable conditions for the isolation, culture and regeneration of protoplasts from *A. hypogaea*, and species from section *Rhizomatosae*.

Isolation and Culture of Protoplasts.

Arachis hypogaea Mesophyll Protoplasts.

Isolation

Fully-expanded leaves of seedlings, grown under greenhouse conditions, were most satisfactory for protoplast isolation. Seedling age was critical, with plants between 9-and 17-days old giving optimum protoplast release (Table 1). Leaves were surface sterilized and the lower epidermis was removed by peeling. After 45 min in plasmolysing solution (9% mannito! and 0.05% $CaCl_2$ $2H_2O$ in distilled water, pH 5.8), they were transferred to an enzyme solution and floated abaxial side down. Approximately 1 g leaf material/10 ml enzyme solution was incubated for 16 h in the dark, at 26°C. Cellulase R10 (07%) and pectolyase (Y23 0.025%) in a solution oi distilled water with 9% mannitol, and 0.05% $CaCl_2$ $2H_2O$, pH 5.8, was found to be the most effective enzyme combination. Higher levels of cellulase, pectolyase, or mannitol reduced protoplast yields. Substitution of pectolyase with various levels of Macerozyme R10 was not so satisfactory, and the addition of 0.5% hemicellulase to the cellulase/pectolyase mixture had no marked effect on protoplast yield. Protoplasts were released by gentle agitation of the leaf pieces, and filtered through a 45 u m pore sieve. They were then washed twice by centrifugation (120 x g, 3 min) in 9% mannitol with 0.05% $CaCl_2$ $2H_2O$ washing solution. Intact protoplasts could not be separated from cell debris by flotation on 21% sucrose solution, even when seedlings were maintained in the dark for 24 h prior to protoplast isolation. Protoplasts were therefore purified by density gradient centrifugation using Percoll. The gradient was constructed by diluting Percoll with 9% mannitol washing solution. Percoll fractions at 40, 30, and 20% were then layered sequentially in a centrifuge tube. Protoplasts were suspended in 2 ml washing solution, and layered onto the gradient. After centrifugation (120 x g, 5 min) intact protoplasts collected at the interface between 20 and 30% Percoll, while cell debris collected between 30 and 40%, above 20% and below 40% (see Fig. 1c). Intact protoplasts were carefully

Table 1. The effect of seedling age on yield of *A. hypogaea* mesophyll protoplasts.

Seedling age (days)	Protoplast yield/g leaf tissue[1]	Viable protoplasts (%)[2]
9	1.4	65
17	0.7	72
23	0.45	73
31	0.35	71
38	0.34	75
44	0.29	69

1. x 10^6.
2. Figures are mean values of three replicated experiments.

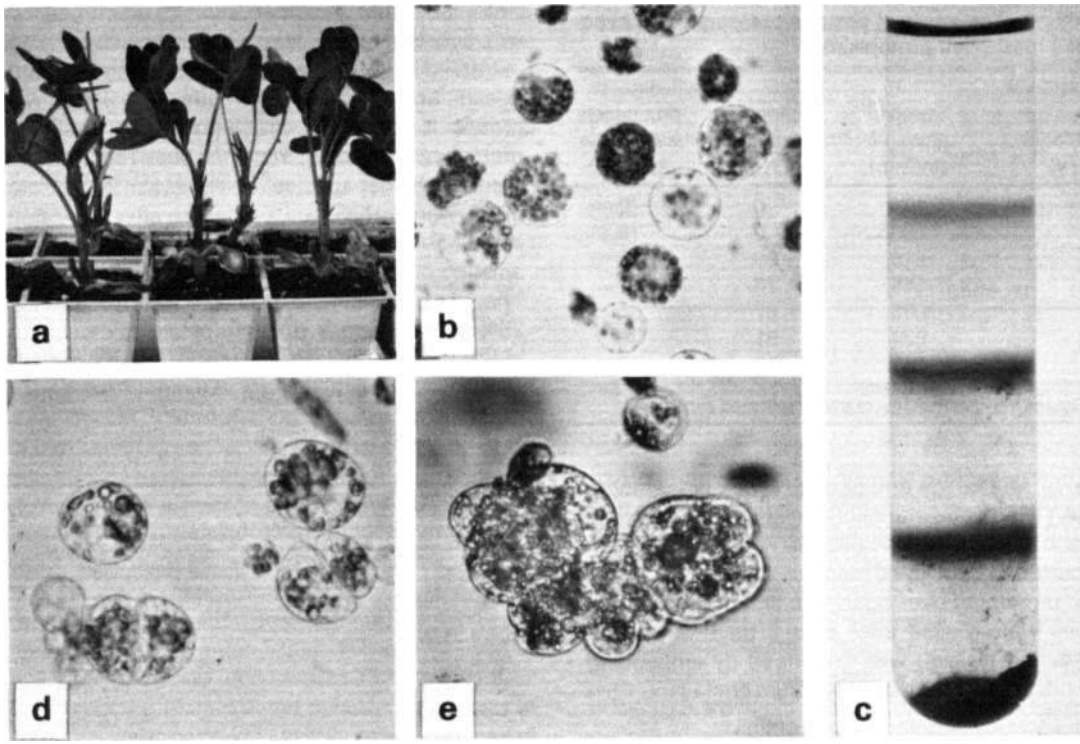

Figure 1. ***A. hypogaea*** **mesophyll protoplasts: a. seedlings used for protoplast isolation, b. freshly isolated protoplasts, x 600, c. purification of protoplasts on Percoll density gradient: from top to bottom, 9% mannitol, 20% Percoll, 30% Percoll, 40% Percoll. Intact protoplasts have layered at the interface between 20 and 30% Percoll, d. initial divisions after 5 days of culture, x 600, e. a 3-week old protoplast colony, x 600.**

removed and washed twice by centrifugation before plating. 60 to 75% of the freshly—isolated protoplasts were viable as determined by staining with fluorescein diacetate (FDA) (Widholm 1972), and observation using ultra-violet fluorescence microscopy. An osmotic pressure of 550 mOsm (determined by freezing point depression using an Osmette S osmometer) was found to maintain optimum protoplast stability and viability (Table 2). This procedure was suitable for both cultivars TMV 2 and Robut 33-1.

Culture

Protoplasts were cultured in 5 cm diameter petri dishes and maintained in the dark at 26°C. They were plated in Murashige and Skoog (1962) basal medium (MS) with 3% sucrose, 9% mannitol, 2.0 mg/l 1-naphthalene acetic acid (NAA), and 0.5 mg/l 6-benzylamino purine (BAP), KM8p medium (Kao and Michayluk 1975) and K8p medium (Kao 1977). Cell wall regeneration, as determined by staining with Tinopal fluorescent brightener and observation using ultra-violet fluorescence microscopy, was observed only in KM8p and K8p.

When the glucose osmoticum of K8p was substituted with 6% mannitol, occasional protoplast divisions were observed. Various growth regulator combinations were then screened in this basal medium in order to identify the combination capable of promoting higher levels of division (Table 3). 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l BAP was found to be the optimum, with 5.0×10^4 to 1.0×10^5 protoplasts/ml as the optimum plating density (Table 4). 1.0 mg/l NAA as the sole auxin failed to support division regardless of the cytokinin present. The basal medium of Kao (1977) was superior to that of Kao and Michayluk

Table 2. The effect of osmotic pressure on *A. hypogaea* mesophyll protoplasts.

% Mannitol (W/V)	Osmotic pressure (mOsm)	Protoplasts intact after 24 h (%)[1]	Intact protoplasts viable after 24 h (%)
3	170	0	0
5	290	0	0
7	416	39	35
9	549	70	61
11	688	67	51
13	834	61	45
15	988	56	49

1. Figures are mean values of three replicated experiments.

(1975), Murashige and Skoog (1962), or Gamborg et al. (1968), and mannitol was superior to glucose or sucrose as the osmoticum. Protoplast division occurred in both liquid medium alone, and in liquid medium plated over agar. The first protoplast divisions were visible after 5 to 7 days of culture. Sustained division was enhanced by embedding the dividing protoplasts in medium containing 1.5% Sea Plaque LMT agarose, but not when agarose was substituted with agar. Commercial agarose is extracted from agar, which consists of ionic agaropectin and agarose with attached ionic side groups. It is therefore much purer than agar. This promoting effect of agarose has been reported for a number of other species (Shillito et al. 1983). Protoplasts were transferred to agarose after 7 or 8 days in culture. Colony growth was sustained by the addition of fresh liquid medium at weekly intervals. The medium osmotic pressure was reduced by 25% at each step. Visible protoplast colonies grew to form calli when transferred to MS basal medium with 3% sucrose, 0.8% agar, 2.0 mg/l NAA, and 0.5 mg/l BAP.

Table 3. Culture of *A. hypogaea* mesophyll protoplasts[1]: growth regulator combinations supporting protoplast division in K8p basal medium with 2% sucrose, 1% glucose and 6% mannitol. Medium osmotic pressure 535 mOsm.

Growth regulators (mg/l)				Viable protoplasts[2] dividing after 7 days culture (%)[3]
2,4-D	NAA	Zeatin	BAP	
1.0		0.02		3.2(±1.8)[4]
1.0		0.2		3.0 (± 2.4)
1.0		2.0		4.4 (± 2.5)
1.0	1.0	0.02		3.0 (±1.8)
1.0	1.0	0.2		5.1 (±1.6)
1.0	1.0	2.0		4.8 (±1.6)
0.1			0.1	4.7 (±1.1)
0.1			1.0	5.1 (±1.9)
1.0			0.01	15.4 (±2.9)
1.0			0.05	29.9 (±2.1)
1.0			0.1	31.2 (±4.0)
1.0			0.5	33.9 (± 3.8)

1. Protoplast density = 5.0 x 10^4/ml.
2. Figures are the mean values of three experiments, each with two replicates *per* growth regulator combination.
3. Estimates taken from sample counts of 500 protoplasts.
4. SE given in parentheses.

Table 4. The effect of plating density on division of *A. hypogaea* mesophyll protoplasts.

Density, protoplasts/ml	Viable protoplasts dividing after 7 days (%)[1]
1.0 x 10^5	39.0 (±2.5)[2]
5.0 x 10^4	32.2 (±1.8)
2.5 x 10^4	23.3 (±1.7)
1.25 x 10^4	4.5 (±1.7)
6.25 x 10^3	0.0

1. Figures are the mean values of three replicated experiments.
2. SE given in parentheses.

Protoplasts from Roots, Cotyledons, and Immature Leaves of *A. hypogaea*

Root and cotyledon protoplasts were isolated according to the methods of Xu et al. (1981) and Lu et al. (1982). Green cotyledons and 1 cm long root tips were obtained from young seedlings grown under sterile conditions. Cotyledon protoplasts were not cultured since they were few in number and many broke up on isolation. Root protoplast yields were low, but they underwent occasional, unsustained divisions when plated in the medium developed for mesophyll protoplast culture, at a density of 5.0 x 10^4/ml.

Immature leaves for protoplast isolation were excised from sterile embryos precultured in the light for 5 days on MS basal medium with 3% sucrose and 0.8% agar. Finely-chopped leaf pieces were plasmolysed in 9% mannitol solution with 0.05% $CaCl_2$ $2H_2O$ for 30 min, before incubation in enzyme for 16 h on a rotary shaker (10 to 20 rpm) at 25 °C. Cellulase R10 2% with 0.05% pectolyase Y23 gave optimum protoplast release. Forty lea-

flets yielded 2 or 3 x 10^5 protoplasts. These regenerated cell walls in the mesophyll protoplast culture medium, but did not divide. Optimum protoplast stability and viability were maintained at 350-380 mOsm but a reduction in osmotic pressure of the medium to this level did not initiate protoplast division. Further attempts to induce division are in progress.

Cells and Protoplasts from Wild *Arachis* Species

Wild *Arachis* species were established from cuttings supplied by Dr. B. Pickersgill, University of Reading, UK. The rooted plants were subsequently grown under greenhouse conditions. A total of six accessions are currently under investigation (Table 5). Viable cells have been mechanically isolated from young leaves of these plants by grinding leaf strips gently in distilled water using a Potter homogeniser. The homogenate was sieved to remove debris, and the cells washed twice by centrifugation (120 x g, for 3 min). Isolated cells were cultured in various liquid media (Table 6) in petri dishes and incubated in the dark at 26°C. Cells of ail six accessions divided in culture when plated at a density of 5.0 x 10^5/ml (Table 7). Cells showing high levels of division were transferred to fresh medium after 14 days, and after 28 days the visible cell colonies were placed in 100 ml Erlenmeyer flasks containing 25 ml MS basal medium with 2.0 mg/l NAA, 0.5 mg/l BAP, and 3% sucrose. The flasks were placed in the light on a shaker at 80 rpm and 25°C.

Protoplast release from the following sources was assessed: a. the youngest fully-expanded leaves of greenhouse plants, with the lower epidermis removed, b. mechanically-isolated mesophyll cells, c. 3-week old cultures of dividing mesophyll cells, and d. suspension cultures established from dividing mesophyll cells, subcultured at weekly intervals. Protoplasts were isolated from the latter four days after subculture. Peeled leaves of all accessions yielded only cells, or cells and occasional protoplasts when incubated for 16 h in 0.7 to 2.0% cellulase R10 and 0.25 to 0.1% pectolyase Y23 in 9% mannitol solution with 0.05% CaCl22H20. *Arachis* sp PI 262794 yielded the highest proportion of protoplasts, whereas *Arachis* sp PI 338265 and *Arachis* sp PI 262839 only yielded cells. Mechanically-isolated cells also yielded few protoplasts. Similar difficulties concerning the digestion of mesophyll tissue have been reported in other seed legumes, including the winged bean (Evans et

Table 5. Wild Arachis species, section Rhizomatosae.

PI Number[1]	Collector Number
262844	GKP 9649
262839	GKP 9642
262807	GKP 9797
262794	GKP 9815
338263	HLKHe 560
338265	HLKHe 571

1. PI designates a plant introduction documented in the Plant Inventory records of the U.S. Department of Agriculture.

Table 7. Culture media supporting mesophyll cell division.

Wild species Coll no.	Optimum media for cell division
560	K8A, K8B
571	MSP1.UM, K8
9642	K8A, K8B
9649	K8B
9797	MSP1.K8A
9815	K8A

Table 6. Liquid culture media for cells of wild species.

Basal medium	Code	Growth regulators (mg/l)
Murashige and Skoog (1962)	MSP1	2.0 NAA, 0.5 BAP
Uchimiya and Murashige (1974)	UM	2.0 2,4-D, 0.25 kinetin
Kao and Michayluk (1975)	KM8	1.0 NAA, 0.1 2,4-D, 0.2 zeatin
Kao (1977)	K8	1.0 NAA, 0.1 2,4-D, 0.2 zeatin
Kao (1977)	K8A	1.0 2,4-D, 0.5 BAP
Kao (1977)	K8B	0.5 2,4-D, 0.5 BAP
Kao (1977)	K8C	0.5 2,4-D, 0.25 BAP
Kao (1977)	K8D	1.0 NAA, 0.5 BAP

al. 1981) and soybean *Glycine max* (Zieg and Outka 1980). Three-week old cell cultures of *Arachis* sp PI 262794 yielded a large number of viable protoplasts when incubated overnight on a slow shaker in 2% Rhozyme, 4% Meicelase P, and 0.03% Macerozyme R10 in solution with CPW salts (Frearson et al. 1973) and 13% mannitol. However, Arachis sp PI 262807 was the only other accession from which protoplasts could be isolated using this method. Cultured cells of *Arachis* sp PI 338265 grew to form a fast-growing suspension which was the most promising source of protoplasts from these wild species. Approximately 2.5 x 10^5 to 1.0 x 10^6 protoplasts/g of cells were released after 16 h incubation on a slow shaker in the enzyme mixture described above. Experiments on the culture of protoplasts from these *Arachis* species are still in progress.

Regeneration Studies

In agreement with the findings of Mroginski et al. (1981) and Sastri et al. (1983), immature leaves from seeds and very young leaves from mature plants, regenerated shoot buds when cultured on MS basal medium supplemented with NAA and BAP. Some shoot buds developed further to form shoots which were excised and rooted in medium lacking growth regulators. Rooted plantlets have been successfully transferred to the greenhouse. Mature leaves of *A. hypogaea* and the wild species formed only callus on media with all combinations of NAA and BAP tested (0.05 to 1.0 mg/l NAA, and 0.5 to 5.0 mg/l BAP). Young leaves from the wild species are currently being tested for their response on these media. Multiple shoot buds and occasional flower buds, or callus and shoot buds have been regenerated from cotyledons cultured on media with 2.0 or 4.0 mg zeatin, or 2.0 mg/l IAA and 1.0 mg/l BAP, respectively.

Callus derived from protoplasts, cultured mesophyll cells, mature and immature leaf explants, and cotyledons of *A. hypogaea* has failed to regenerate shoots on a wide range of media. Although tests have not been so extensive as in the case of *A. hypogaea*, organogenesis has not yet been induced from leaf-derived callus or cultured mesophyll cell colonies from the wild species.

Discussion

Conditions for the isolation and culture of protoplasts from a wide variety of seed legumes have recently been defined. However, reports of plant regeneration from protoplasts of these species are limited, and only in two cases has morphogenesis been induced. *Vigna mungo* mesophyll protoplasts (Sinha et al. 1981) and *Dolichos biflorus* anther-derived protoplasts (Sinha and Sen 1981) gave rise to embryoids, but these failed to develop further into plants.

Oelck et al. (1982) reported callus formation from mesophyll protoplasts of *A. hypogaea*, isolated from shoot cultures. Our work defines more precisely conditions for the isolation and culture of protoplasts from seedlings grown under greenhouse conditions. Although plants have not yet been regenerated from protoplasts, a recent report by Narasimhulu and Reddy (1983) describes the regeneration of plantlets from callus of four cultivars of *A. hypogaea*, including TMV 2. Callus derived from epicotyl, hypocotyl, leaves, and cotyledons regenerated shoots under defined conditions. Their technique is currently being assessed in our laboratory, for the ability to initiate shoot morphogenesis from protoplast-derived colonies. Successful regeneration from protoplasts would make the recovery of plants possible in a somatic hybridization program. This would offer groundnut breeders an exciting new technique, giving them access to a wider gene pool.

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Use of Incompatible Species and Tissue Culture- Discussion

Appa Rao:
Valuable germplasm is sometimes lost as Dr. Valls has pointed out. Is there any tissue culture technique by which we can raise a plant from a nonviable seed?

Moss:
Some seeds which are not viable when sown in soil do still have a living embryo but do not have green, expanded cotyledons. We have observed this in seed which has been stored under unfavourable conditions. In these cases the embryo can be cultured, and may germinate, or give rise to callus. But if the tissues are dead, they cannot be cultured.

The techniques in handling, soaking and germinating aged seed are also important in the rate of survival [see Goldworthy, A. Fielding, J.L., and Dover, M.B.T. 1982. Flash inbibition: a method for the re-invigoration of aged wheat seeds. Seed Science and Technology 10(1): 65].

However, ageing involves many processes, including loss of function in cell organelles and membranes, and damage to DNA and RNA. Although some repair can take place, genetic change may occur, and seed should be rejuvenated before this happens.

Stalker:
Arachis hypogaea will hybridize with all known diploid species of section *Arachis*. It is possible that the germplasm used in Bajaj's experiment, is probably a species other than *A. villosa*, specifically one outside section *Arachis*?

Valls:
Although I agree with Dr. Stalker that the wild species used in the crosses probably was not *A. villosa*, which as a rule hybridizes very well with *A. hypogaea*, I think it is important to make the point that genetic drift has probably occurred in most of the available germplasm of *Arachis* species and this may lead to differences in the crossing behavior of the lines used.

Stalker:
Certainly genetic drift has occurred in many populations, which resulted in departures from the original. Although I have observed sterile plants of several species, the plants in question are apparently not sterile since hybrid embryos were obtained. The material in question is most likely not *A. viliosa*.

Moss:
Embryos from *A. hypogaea* x *A. villosa* usually mature, and would be large and easily cultured.

N.C.Subrahmanyam:
Nalini has indicated that the callus route to obtaining plantlets is not advisable. I agree with this. But you have shown the successful use of 0.1 % kinetin and 0.1% auxin. Are there any studies on the chromosome numbers of the plantlets resulting from such cultures on media supplemented with auxins?

Nalini:
We have not studied chromosome numbers in callus yet, but hope to do so in future.

Satish Kumar:
Is the variation in seed and pod size reflected in ovule and embryo size, and is this the reason for cultivar variation in ability to regenerate?

Sastri:
With respect to ovule size after incompatible pollination, there are differences between cultivars, and between different hormone treatments. We generally get better responses in MK 374, not only in crosses but also in cultures.

Sharma:
Have you checked what happens to the pollen tube growth or structure after hormone treatment in incompatible crosses?

Sastri:
Our earlier studies showed that pollen tube growth was not the major limiting factor in these crosses, but we would still like to check the effect of hormones.

N.C.Subrahmanyam:
Did you succeed in getting any "culturable" ovules/ embryos from the crosses in which you have shown retardation of the pollen tube growth?

Sastri:
There is pollen tube inhibition in all the crosses we have studied so far.

Valls:
You have a large collection of section *Rhizomatosae* accessions. Is there any special reason why you used PI 276233 for your experiments?

Sastri:
There is no specific reason. *Arachis* species PI 276233 flowers longer during the year. We have also used other *Rhizomatosae* accessions.

Amin:
Calcium plays an important role in shell formation. Have you investigated the role of this element in pod and shell growth in your crosses?

Sastri:
No, we have not yet done this, and it may not be necessary because we are getting satisfactory peg and pod growth following hormone treatments.

Sharma:
What is the rate of success in generating plants from ovule and embryo cultures?

Sastri:
The rate of success seems to depend on the cultivar used for crosses and also on the pretreatments. The best observed so far has been in MK 374. Success in terms of cultured ovules from this cultivar in crosses is about 25-30%; with embryos it is much less.

Sharma:
What is the situation with regard to transferring the cultured plants to normal pot, or field environments?

Sastri:
Plantlets from selfed tissue cultures of *Arachis hypogaea* and a few other species have been transferred to soil.

N.C.Subrahmanyam:
I got the impression from the presentations that the material cultured is from interspecific crosses via peg/pod formation. I wonder whether unfertilized flowers in such crosses can form pegs or pods? Has there been any systematic study on the possibility of getting pegs/pods/seeds from emasculated flowers which are not pollinated?

Sastri:
We had some controls in which hormone treatments were given without pollination. No pegs were obtained.

Murty:
We did not observe emasculated flowers. But we certainly found several unfertilized embryos in pegs developing after natural pollination. Such pegs were observed at low frequency in cultivated groundnut varieties, at fairly high frequency in wild seed-fertile species, and at very high frequency in seed-sterile species and in incompatible pollinations.

Rees:
The induction of flower production from de-embryonated cotyledons should provide admirable opportunities for experimentation on the meiotic process in flowering plants. Hitherto the introduction of labels such as 5-bromo deoxyunidine to produce harlequin chromosomes at meiosis has been impossible in plant material. This should now be feasible, and make possible a much more sophisticated approach to investigating the mechanism of chiasma formation and of crossing over. Labelling with tritiated thymidine should also be an easy matter.

Nolt:
What is the potential of introducing genetic variability into the cultivated groundnut through regeneration of plants from callus and protoplasts?

G.M.Reddy:
Tissue cultures were being used to induct variability in plants like tomato and pigeonpea. However we have not observed much variability in callus-regenerated plants except for leaf shape and size. The use of 2,4-D, a known mutagen that also causes endoduplication of genetic material, in callus induction, as in the present case may help to generate more variability. However, plantlet regeneration from altered cells, compared to normal diploids is poor, and this may account for the low frequency of variants noticed in the present case.

Moss:
Tissue culture of *Arachis* has come of age. Five years ago little was known, now a lot is known about media, explant and conditions for growth. Most of this has work been done using semisolid media, but now the use of liquid media is being explored, though this is more difficult. Single-cell selection in culture and the induction of somaclonal variation are now distinct possibilities.

Atreya:
Since you are using cotyledon tissue which is close to the embryo axis, do you think the regeneration is de novo?

G.M.Reddy:
The regeneration of plantlets from callus cultures was certainly a de novo process. However, it was not so for the production of flower buds. The presence of flower bud initials in groundnut seeds was reported by Fortainer in 1957. The expression of flowering in the present case may be an instance of precocious manifestation by flower bud primordia.

Moss:
Have you grown out seed from these in vitro flowers in any species, to realize any variation present?

G.M.Reddy:
We have not yet grown seed from pods produced by the gynophores of the in vitro-raised flowers and as such cannot draw any inferences on the possibility of any variability being realized.

Moss:
When we place cotyledons on media, we get shoot formation - why do you think you get only flowers?

G.M.Reddy:
The production of flower buds may possibly be a result of an interaction between the mineral composition of the media with hormones. We only got shoots from cotyledons cultured on MS medium. However, alterations in the mineral composition of even MS media, has resulted in flowering indicating the importance of inorganic salts in the induction of flowering.

P.Subrahmanyam:
Did you examine the effect of temperature on callus development? We observed profuse callus development at the cut end of the petiole during our detached leaf culture, used to test reactions to various foliar diseases at ICRISAT. We observed a strong effect of temperature on callus formation; would you like to comment on this?

G.M.Reddy:
Although we have not verified the role of temperature on callus induction in groundnut, there are many reports of its association with callusing in plants like tobacco.

M.V.Reddi:
The data you presented show there are differences in regeneration between embryonated and de-embryonated cotyledons. This may be due to differences in the concentration of hormone, in as much as the embryonic plant itself will produce its own hormone, whereas the de-embryonated cotyledon will not be able to do so, and its activity will be regulated entirely by the in vitro hormone concentration. The cotyledon in close proximity to the embryo is functional, due to diffusion of in vivo hormone secreted by the embryo. The true picture can be obtained by regulating the in vitro concentration of hormone.

G.M.Reddy:
It is possible that the differences in the hormonal requirements of embryonated and de-embryonated cotyledons for flower bud induction may reflect differences in the endogenous hormonal content. Compared to de-embryonated cotyledons, that flower in the presence of low exogenous cytokinin, the embryonated cotyledons' higher exogenous cytokinin requirements may be because of the greater endogeneous auxins produced by the embryo.

N.C.Subrahmanyam:
Our results on cotyledon segment cultures suggest a regeneration gradient from the nodal segment to the tip. Thus, there are already preformed buds resulting in such shoot and flower bud formation.

Technical Review and Discussions

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Technical Review and Discussions

Chairmen : H. Rees and J.P. Moss

Technical Session I — Wild Genetic Resources

Chairman's report : L.J.G. van der Maesen

Two papers were presented in this session. The first by J.F.M. Valls from CENARGEN, Brazil, dealt with the collection, processing, and maintainance of wild germplasm. Besides describing the logistics of collecting, he mentioned some very relevant points such as, maintenance of voucher specimens, characterization of accessions, and the maintenance of detailed information on collections by computer. At present this information is confined to about 350 accessions. The other paper presented by A.K. Sadasivan dealt with the current status of *Arachis* wild germplasm at ICRISAT. He outlined the storage facilities available for seed material, and the maintenance of live rhizomatous accessions. Besides collection, and maintenance he stressed the importance of screening accessions against various pest and diseases, in collaboration with cytogeneticists, pathologists and entomologists.

However, it was quite apparent from this session that a great deal of work still remains to be done on collection, development of descriptors, and evaluation of the individual accessions to assess their potentials in groundnut improvement.

Discussion:

Rees:
It is obvious, from the many references to it during the workshop, that the basic taxonomy in the genus *Arachis* needs immediate attention.

Valls:
There are probably about 70 to 75 species in the genus *Arachis*, of which only 23 are named. These names are not published according to the rules of nomenclature in the International Botanical Code. Most publications have used numbers, and sometimes there is confusion between PI numbers and Collector numbers and this can cause mistakes. Gregory and Krapovickas are preparing a monograph, it's publication is urgently needed.

Moss:
The use of PI numbers and collection information, should continue, in addition to species names, as a mean of identifying material since all accessions of one species will not be identical.

Technical Session II — Potentials of Wild Genetic Resources

Chairman's report : H.T. Stalker

The greatest potential of wild *Arachis* species is probably for disease and insect resistance. Identification of sources of resistance has moved from 'casual' observations, except in a few instances, to replicated field trials for a series of groundnut pests. Among the diseases evaluated during recent years are rust (*Puccinia arachidis*), the leaf spots (*Cercospora arachidicola*, and *Cercosporidium personatum* (*Phaeoisariopsis personata* (Berk, et Curt.) v. Arx.), and several viruses. Resistances to several insect pests, such as aphids, thrips, *Heliothis* spp, mites and nematodes have also been observed. As new collections are made, they must also be evaluated for agronomically-desirable traits. The genetics and mechanism of resistances within and between species must be determined to fully exploit the available germplasm. Interspecific hybrid derivatives have been developed, and this work is gaining impetus. Some of these populations have been evaluated and the release of germplasm lines within the next few years is highly likely.

Discussion

Sastri:
How much multiple resistance is available in section *Arachis*?

Stalker:
There are species in section *Arachis* that have multiple resistance to different diseases and insect pests, but not at the same level as those in section *Rhizomatosae*.

Sastri:
Should the utilization of section *Rhizomatosae* species be at a low key when resistance is available in compatible species of section *Arachis*?

Stalker:
It is a question of priority. If the resources are available both could be taken up simultaneously.

P. Subrahmanyam:
For most of the fungal and viral diseases section *Arachis* species such as *A. chacoense* and *A. cardenasii* have multiple resistance. Therefore they should be given priority, without overlooking species from other sections.

Sharma:
How far we can go in our hybridization programs, depends upon the limitations and the objectives. Is the limit for *Arachis* the genus, the tribe or the family?

Moss:
Although section *Arachis* should have priority we hope the whole genus will be accessible for groundnut improvement in the near future. Wild genetic resources should be assessed in the genus *Arachis* as a whole and not by specific section.

Singh:
It is a question of priority and not of negligence towards any of the wild germplasm. As reported by P. Subrahmanyam and P.W. Amin, there are several species in section *Arachis* such as *A. chacoense* and *A. cardenasii* that have multiple resistances. Therefore, naturally the initial concentration will be on species which can be utilized by conventional cytogenticists.

Stalker:
Once the bottleneck in crossing species from different sections is removed, then concentration should be on induction of genetic transfer, if this is not possible by normal recombination.

Rees:
I agree with A.K. Singh, in the near future most of the good genetic improvement in crop species has to come from conventional cytogeneticists and breeders. In the UK, many of the premature conclusions on somatic hybridization are not so promising as they first appeared.

Moss:
We have two extremes; one where species are fully compatible, and genomes recombine to give a wide range of segregants, and the other where somatic hybridization is the only possibility. But in between, there is a lot of scope for producing hybrids by a range of techniques, and inducing recombination by genetic or physical means.

Technical Session III — Taxonomy and Means of Utilization

Chairman's report: N.C. Subrahmanyam

We heard about cytotaxonomy based on studies of the somatic complement by H.T. Stalker, and of the meiotic complement by U.R. Murthy. As emphasized by Professor Rees in his introductory remarks, the 'bread and butter' type of techniques have been used to obtain information presented on the classification of chromosome complements in *Arachis*. However, *Arachis* chromosomes are small, and difficult to study, and it is necessary to get maximum information on this important genus to facilitate gene transfer. Towards this end, we have already made a modest beginning in the molecular approach to genome analysis. When we look at data on the 2c-DNA values in different *Arachis* species, it appears that certain species (at a given

ploidy level) differ by at least 20%. Therefore *Arachis* presents excellent material in which to look for satellite DNA in different species, and to use species-specific probes to identify and analyze the chromosome complements of different *Arachis* species. This approach has already yielded fruitful results in cereals and should be followed in *Arachis* for a full understanding of the genus.

With reference to genome homologies, H.T. Stalker presented results on hybrids between *A.hypogaea* and *A.batizocoi* which differ from those of A.K. Singh. I feel that the strains that are used may be different. This aspect also mentioned earlier should be probed in a search for a diploidizing mechanism. The aneuploid approach should be continued hand-in-hand with studies on genetic markers and linkage groups, to help us understand the genomes. Emphasis should be placed on the genetics of important traits, such as disease and pest resistance.

Discussion

Moss/Singh:
The difference between our results and those of H.T. Stalker are not great, and could be due to environmental factors. Large differences occured in triploid fertility at Reading and ICRISAT when the same genotype was grown at two places. There may be a similar cause for the difference between NCSU and ICRISAT results.

Murty:
Identification of pachytene chromosomes was initially standardized in cultivated groundnut and subsequently extended to the wild species to help understand the genomic constitution of the genus.

Technical Session IV — Use of Compatible Species

Chairman's report : J.H. Williams

A total of four papers were presented in this session. The paper by J.P. Moss dealt with the utilization of wild species in crop improvement. He covered the whole range of different techniques that can be utilized in accordance with genetic distance between the parents. He also described the different manipulations affecting hybrid fertility, and maximizing meiotic recombination. He discussed the role of haploidy and aneuploidy in interspecific breeding. The paper by A.K. Singh on genetic introgression from compatible wild species into groundnut discussed in detail the relative importance of different ploidy manipulations that have been utilized at ICRISAT for transferring desired characters from the wild species into cultivated groundnut. It was concluded that the genomic relationship between the diploid wild species of section *Arachis* and cultivated groundnut are strong enough to transfer the desired characters from these wild species through meiotic recombination. However, the choice of a specific technique depends on the objective, the resources available, and the genetic nature of the trait. The paper from S.P. Tiwari dealt with maintainance and utilization of wild species, hybrids, and hybrid derivatives obtained from different sources. D.G. Krishnappa and Maria Joseph presented the cytological analysis of triploids in section *Arachis*, indicating the presence of a common genome between these wild species and *A. hypogaea*.

Technical Session V — Use of Incompatible Species and Tissue Culture

Chairman's report: D.Sharma

D.C. Sastri presented a paper dealing with the different problems of incompatibility in wide hybridization, and ways to overcome such problems. This was followed by a paper by Nalini Mallikarjuna on rescue of fertilized embryos, and ovules using tissue culture techniques. No data were presented on cytological behaviour, or on the possibilities of variation resulting from mutation, or culture conditions.

G.M. Reddy reported the induction of flowering in cotyledon culture within three weeks/This is an important discovery for the study of turnover of mitosis to meiosis using tracer elements as pointed out by H. Rees. The differences in crossability of *A. hypogaea* with *A. villosa* between Y.P.S. Bajaj's paper and other reports, with the possibility of a mistaken identity, should be conveyed to him. It is unfortunate that species description for *Arachis* are not readily available. The paper on somatic hybridization read for Emma E. Rugman and E.C. Cocking is an important beginning, but a lot of work is needed before protoplast fusion becomes a regular tool in the hands of plant breeders.

Discussion

N.C. Subrahmanyam:
It will be worthwhile to look for the chromosome elimination system in interspecific hybridization for haploid production.

Singh:
Indications of such a system were observed in an intersectional cross between *A. hypogaea* (Robut 33-1) and *A.villosulicarpa* (Ax), where a haploid plant was obtained from a small seed. However, the plant could not be sustained. This combination is being repeated to confirm these preliminary observations.

N.C. Subrahmanyam:
It is good to be informed that an autotetraploid of *A. villosulicarpa* was used since the elimination system in barley depends upon genomic ratios.

Tiwari:
More information is required on; chromosome complement; the frequency of unreduced gametes in diploid and tetraploid taxa; and the basis of restitution nuclei in triploids.

Stalker:
No unreduced gametes were observed in tetraploid and diploid crosses as they always result in triploids.

Moss:
Unreduced gametes were not observed at diploid, tetraploid or hexaploid levels, but they do occur in triploids.

Singh:
Formation of restitution nuclei in triploids is a result of spindle break down after MI, at AI or AII, and results in production of hexaploid progenies from triploids. The formation of restitution nuclei in triploids appears to be an environmental effect.

Priorities and Future Plans

Chairmen: H. Rees and J.P. Moss

Based on the discussion held during different scientific sessions and the review session, participants expressed the following opinions on priorities and future plans:

Stalker:
The following should be the priorities: the publication of a taxonomic monograph on the genus to prevent the prevailing confusion; species collection to increase the germplasm available for groundnut improvement; characterization of species, cytological and morphological, for resistance to diseases, insects, and other agronomic traits; renewed efforts to understand biosystematic relationships within the genus; renewed efforts to utilize the desirable germplasm in the genus, especially the wild species and develop tetraploid breeding populations. Creative approaches to utilize germplasm rather than simply repeating work of others, for example, we should develop methods to maximize recombination.

Appa Rao:
For a meaningful exchange of information between workers it is necessary to have uniform descriptors for wild species. A draft copy should be circulated among workers before final publication to make such descriptors effective and acceptable.

Valls.
Descriptors developed for cultivated groundnut by V.R. Rao are being checked this season on 50 wild species accessions from different sections in collaboration with V.R.Rao in Brazil.

Rees:
Attention should be paid to the more modern techniques such as DNA estimation and chromosome banding to increase our resolution in the identification of different genomes with cytological and chemical markers.

Amin:
Results differ with different accessions of a species, therefore clubbing accessions into one species may be highly dangerous.

Moss:
The knowledge as to which accessions from a species is important, but we will keep them separate to see the variability within species. Authors should always report results by accession numbers. More intensive collections within a species, and studies to increase our knowledge on variability should be priorities.

Valls:
In Georgia they had some material, which is no longer available. Therefore a list of germplasm available at different institutions is required. People with germplasm should supply a list of germplasm to prepare a complete catalog. CENARGEN are prepared to be responsible for compiling such a catalog.

Moss:
I fully agree with this, and will supply a list of what we have at ICRISAT. We should maintain any variability that exists within accessions, but this may be difficult in species which produce few seeds. If a species produces plentiful seed at one center, but not at others, that center should accept prime responsibility for distributing seed.

Stalker:
The creation of variable populations from wild germplasm should be our main objective.

Moss:
Genetics of resistance in wild species should be studied, as the production of stable, disease-resistant lines is a major objective for plant breeders.

Singh:
Techniques must be improved so that the published results can be more authentic. This could be achieved by better interdisciplinary interaction among groundnut scientists.

Stalker:
Priority and relative importance depends upon the availability of resources, but it will be better to give higher priority to the easier methods.

Moss:
This means concentrating on species in section *Arachis*, but we should not exclude other sections of the genus, and the genus should be studied as a whole. Would anybody like to make comments on meiotic recombinantion, the use of induced translocations and aneuploidy to assist gene transfer between sections?

N.C. Subrahmanyam:
These are valuable, but one should simultaneously consider genetic aspects, such as linkage groups.

Moss:
Protoplast fusion has been of little use to practical breeders as compared to conventional breeding. However, it is coming nearer to application and efforts should be made to make it more practical.

Sastri:
Success in protoplast fusion came only recently, in the last decade. More information will show which direction future work should take. I would therefore keep an eye open for the use of this technique in groundnut improvement. Molecular characterization should be given priority in cultivated groundnut and the wild species.

Rees:
If protoplast fusion is successful in groundnut we can use it. However, until now the potential use of tissue culture has been in multiplying rare genotypes and this should be given priority.

Nolt:
Production and multiplication of virus-free plants can be another application of tissue culture.

Sastri:
Studies on functional, physiological aspects of flowering, fruit morphogenesis, hormonal balance, anther culture, pollen culture, chromosome elimination, and haploid production should be given due priority.

Stalker:
We should try to investigate different problems to achieve the common objective such as the utilization of wild species in the genetic improvement of *A.hypogaea* and everybody should try to avoid duplication of effort.

N.C. Subrahmanyam:
For some approaches we need collaboration. In molecular probes, different approaches will give fruitful results in a short time if undertaken in collaboration.

Tiwari:
The undesirable segregates from interspecific derivatives may have some other desirable characters. Hence, this material should not be discarded.

Recommendations

1. Collections to increase germplasm available for groundnut improvement The exchange of information on availability of germplasm at different centers.

2. Taxonomic studies and the publication of a monograph, and/or species descriptions. The preparation of descriptor lists for wild species.

3. Full characterization of the species using all available methods including DNA estimation, chromosome banding, and DNA probes.

4. The development of tissue culture facilities and expertise, so that tissue culture can be used to multiply rare genotypes, and produce virus-free stocks, and haploids.

5. The screening of all available taxa for desirable characters, with emphasis on pest and disease resistances. Stimulation of interdisciplinary cooperation to ensure effective screening.

6. Studies on the genetical and biochemical mechanisms of resistance and other desirable characters.

7. Development of tetraploid breeding populations, from wild species having desirable traits and compatibility with *A. hypogaea*.

8. Screening of as many segregants as possible for a wide range of physiological and biochemical traits.






Workshop participants visiting ICRISAT fields, pot cultures and greenhouses to see work in progress on wild species of *Arachis*.

Workshop Organization

Organizing Committee

J.P. Moss	C. Sashikala
A.K. Singh	Nalini Mallikarjuna
D.C. Sastri	B.G. Rao

Opening Session

Chairman D.L. Oswalt
Rapporteurs D.C. Sastri and Nalini Mallikarjuna

Technical Session I - Wild Genetic Resources

Chairman L.J.G. van der Maesen
Rapporteurs S. Appa Rao and L.J. Reddy

Technical Session II - Potentials of Wild Genetic Resources

Chairman H.T. Stalker
Rapporteurs V.K. Mehan and Rasheedunisa

Technical Session III - Taxonomy and Means of Utilization

Chairman N.C. Subrahmanyam
Rapporteurs P.W. Amin and P. Subrahmanyam

Technical Session IV - Use of Compatible Species

Chairman J.H. Williams
Rapporteurs R.C. Nageshwar Rao and A.M. Ghanekar

Technical Session V - Use of Incompatible Species and Tissue Culture

Chairman D. Sharma
Rapporteurs B.L. Nolt

Technical Review and Discussions

Chairman H. Rees and J.P. Moss
Rapporteurs A.K. Singh and B.G. Rao

Priorities and Future Plans

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