UTILIZATION OF WILD RELATIVES IN GENETIC IMPROVEMENT OF *ARACHIS HYPOGAEA* L. I. TECHNIQUES

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SUMMARY

Many of the wild relatives of *Arachis hypogaea* are potentially useful in groundnut improvement, but there are a number of problems to be overcome in transferring genes to the cultivated groundnut. Studies on karyotypes, meiosis in hybrids, seed protein electrophoresis, polyploidisation and incompatibility contribute to the full knowledge of the genus, the genomes present and the relationships between species that will be useful to breeders and cytogeneticists in utilizing wild species characters.

INTRODUCTION

In the genus *Arachis*, only species of section *Arachis* are cross compatible with *A. hypogaea*, but the majority of them are diploids and therefore produce sterile triploid hybrids when crossed with the cultivated groundnut (Moss *et al.*, 1981). Considerable progress has been made in transferring resistance to leafspots caused by *Cercospora arachidicola* Hari. and *Cercosporidium personatum* (Berk. and Curt.) Deighton, and rust caused by *Puccinia arachidis* Speg., from three wild diploids in section *Arachis* (Abdou *et al.*, 1974; Moss, 1980) to *A. hypogaea.* The problems encountered in transferring genes from these closely related taxa are those of producing hybrids in sufficient numbers, and rendering large numbers of these hybrids fertile by colchicine treatment. However there are six other sections of the genus that are almost reproductively isolated from *A. hypogaea* (Gregory & Gregory, 1979). Many species in these six sections have characters, such as disease resistance, that would be useful if they could be transferred to *A. hypogaea* (Moss, 1980).

In this situation there is justification for building up a detailed fundamental knowledge of the cytogenetics of the genus *Arachis* to enable efficient and speedy incorporation into *A.hypogaea* of genes from the potentially useful wild species, using conventional or new cytogenetic and breeding techniques. *Arachis* has not received the same attention as many other crop plants, and much of our basic knowledge is still incomplete. The first interspecific hybrids (*A. hypogaea* × *A. villosa correntina* [Benth.]) were

produced by Krapovickas & Rigoni in 1951, but we still do not know the number of genomes within the genus, nor even within section *Arachis* (Stalker & Wynne, 1979). This paper describes some of the techniques used by cytogeneticists at ICRISAT to increase our basic knowledge of the genus and attempt to utilize all the desirable characters in the wild species.

KARYOTYPE

Husted (1933,1936) identified two marker chromosomes in some species. Smarit *et al.* (1978) separated the wild diploid species into two groups, each with one of the marker chromosomes, and postulated that *A. hypogaea* was an amphiploid from two species, one from each of the groups. The long time interval between these reports was partly due to the difficulties of studying *Arachis* chromosomes, which are small (1.8-3.0μ) and not easy to stain (Banks, 1970; Stalker *et al.*, 1979).

The modified techniques used at ICRISAT repeatedly give preparations that can be analyzed in detail. Root tips are first fixed in Carnoy's Fluid II for 24 to 48 hours, where the chloroform helps in rapid penetration and fixation, and in removal of oil or fat globules from the cytoplasm to obtain a clear background. Root tips are then transferred to Carnoy's Fluid I with a drop of 5% ferric chloride. A longer period of hydrolysis (30-35 min) in N HCl gives better staining and easier separation than conventional times. Following hydrolysis, root tips are stained in Feulgen reagent and squashed in 1% aceto-carmine, using an iron needle (Singh & Moss, 1979). The clarity of preparations is evident from Figure 1. Our studies have revealed a wider range of types of marker chromosomes (Fig. 2). *Arachis batizocoi* and *A. duranensis* have a secondary constriction with a large satellite in the second pair of chromosomes; *A. villosa, A. correntina* and *A. chacoense* have a secondary constriction with a small satellite in the third pair of chromosomes; secondary constrictions have been seen on the ninth and fifth pair of chromosomes in other species, PI 262141 and 338280.

HYBRIDIZATION

Optimum environmental conditions must be provided to obtain maximum crossability. The humidity from the time of emasculation to a few hours after pollination is important, and sprinkler irrigation is given after emasculation (S.N. Nigam, personal communication). The optimum time for emasculation and pollination depends on the season and working hours of staff are adjusted to coincide with these optimum times. This has enabled us to produce hybrids among all diploid species in section *Arachis* available to us and to group these, according to crossability, in two groups with good crossability within groups but poor crossability between species in different groups.

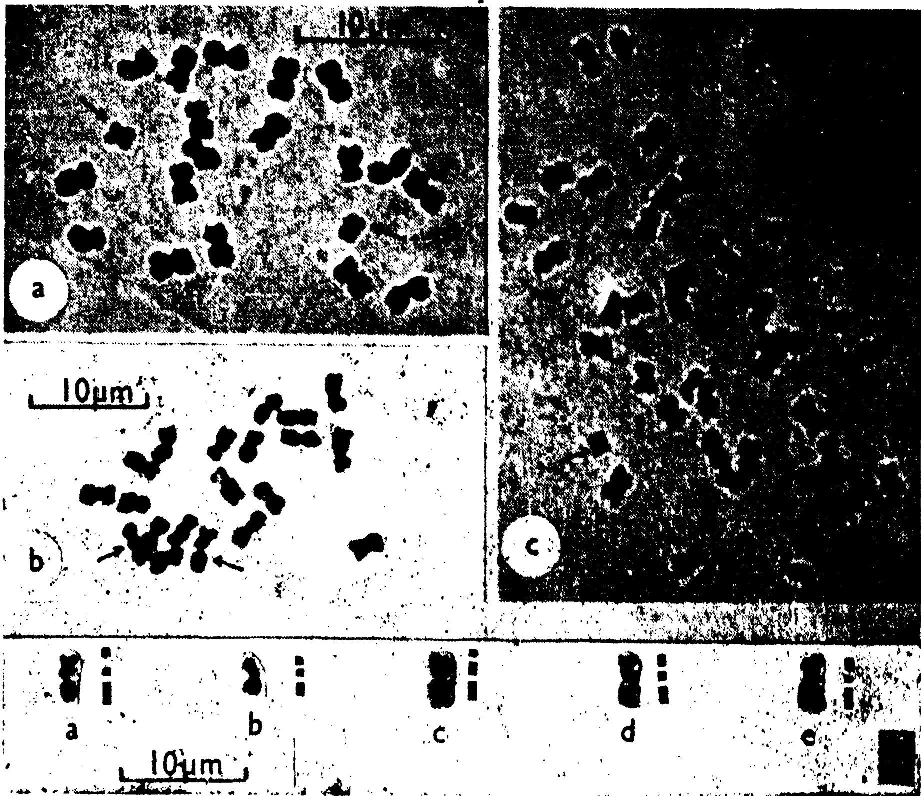

FIG. 1. Somatic cells at metaphase. (*a*) *Arachis correntina* ($2n=20$) with a small pair of chromosomes (arrows). (*b*) *A. batizocoi* ($2n=20$) showing secondary constrictions in a pair of chromosomes (arrows). (*c*) *A. hypogaea* ($2n=40$) with a small pair of chromosomes (arrows) and another with secondary constriction (arrows).

FIG. 2. Chromosomes with different types of secondary constrictions in five species of section *Arachis*. (a) *A. batizocoi* chromosome II. (b) *A. duranensis* chromosome II. (e) *A. villosa* chromosome III. (d) PI 338280 chromosome V. (e) PI 262141 chromosome IX.

ANALYSIS OF CHROMOSOMES AND POLLEN STAINABILITY IN HYBRIDS

These analyses have confirmed the presence of two groups of diploid species, with regular chromosome pairing and good pollen stainability in hybrids within groups, but univalents and poor pollen stainability in hybrids between species in different groups. There is some seedling lethality in one of the latter type of hybrids.

Analysis of chromosome pairing in hybrids between any species in either group and *A. hypogaea* indicate that the two different diploid wild species genomes can each pair with one of the genomes of *A. hypogaea* The frequent observation of one or two multivalents in hybrids with *A.*

hypogaea, and of a few bivalents in hybrids between species in different groups, indicates some homology between the two genomes, but the precise relationships are still to be evaluated.

There are many different ways of utilizing wild species in the genus *Arachis* (Banks, 1972; Moss, 1980). The knowledge gained from the above studies indicates which crossing program is most promising, by enabling predictions of the probable fertility of future hybrids and the amount of meiotic recombination expected in them.

POLYPLOIDIZATION

Incorporating diploid wild species directly gives sterile triploid hybrids because of the difference in ploidy level with tetraploid *A. hypogaea.* To regain fertility in them through the induction of polyploidy, the colchicine treatment technique developed by Spielman & Moss (1976) was used. Actively growing branches of sterile triploid hybrids were cut 20 to 30 mm above a node of young lateral, and leaves, buds and petioles were removed from the next two or three nodes. A glass tube that fitted the stem

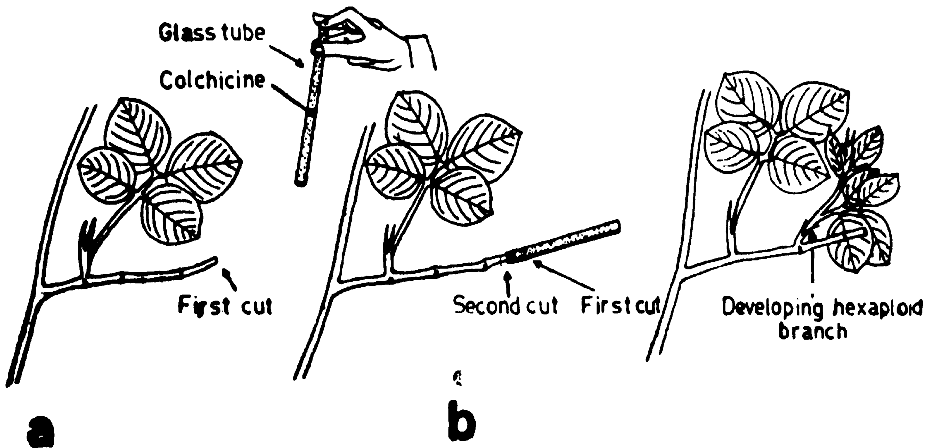

FIG. 3. Method of colchicine treatment of young laterals.

tightly was filled with colchicine solution. A second cut was then made 5 mm below the original cut, and the glass tube was immediately placed over the cut end (Fig. 3). Prevention of air bubbles was important to maintain the flow of colchicine. Tubes with colchicine were left for 24 to 48 hours.

ELECTROPHORETIC STUDIES

Karyotypic and taxonomic studies can contribute to our knowledge of the relationships between species of section *Arachis*, including *A. hypogaea*, and species in other sections with which they are not cross-compatible. Obviously, the information from analysis of hybrids is not available in the latter case.

Electrophoretic studies of seed proteins for resolving taxonomic and evolutionary problems have been regularly used and had proved useful in evolutionary analyses (Ladizinsky & Hymowitz, 1979). Preliminary studies were undertaken by Cherry (1975) on five species in section *Arachis*. We have standardized the polyacrylamide gel electrophoresis technique for use with *Arachis* seeds with high oil content, and are studying all sections of the genus.

DISEASE SCREENING

The cooperation between Reading University and ICRISAT between 1973 and 1978 (Spielman & Moss, 1976; Moss, 1980) produced large number of plants, originating from interspecific crosses, to be screened in the field (2000 in kharif 1978, and 20000 in kharif 1979).

To guarantee sufficient inoculum for proper screening, an infector row technique was used, but as more than one pathogen was present in the field, a detached leaf technique has been used; this enables a genotype to be tested against a single pathogen. Initially disease resistance was rated on a 0 to 5 scale, but now a 1 to 9 scale is being used (Subrahmanyam *et al.*, 1980).

(*a*) Infector Row Technique (Singh *et al.*, 1977; Subrahmanyam *et al.*, 1980).

Plants to be tested were grown such that each test row was adjacent to an infector row, consisting of alternate plants of local susceptible cultivars of early and late maturity (i e. one infector row to two test rows). The spread of the pathogen was facilitated by sprinkler irrigation from perforated pipes to increase humidity. When natural inoculum was low, additional inoculum was provided by spreading foliage from diseased plants along the infector rows.

(*b*) Detached Leaf Technique.

Clean healthy leaves were incubated with inoculum of leaf spot or rust as follows:

(1) Leaf with petiole was rooted in Hoagland medium (Melouk & Banks, 1978; Subrahmanyam *et al.*, 1980).

(2) Leaf was kept on sterilized moist sand spread in plastic trays and covered with polythene sheet (Nevill, 1979).

(3) Leaf kept in moist petridishes.

Observations were started one week after incubation and continued for two weeks.

INCOMPATIBILITY

Pistils pollinated with compatible or incompatible pollen were fixed in acetic alcohol (1:3) for 24 hours, cleared in 8N NaOH overnight, washed and stained in 0.01% decolorised aniline blue (0.15M Na_2HPO_4, pH9.0) and observed in UV light (Currier, 19.7). Failure of pollen germination

on the stigma or of pollen tube growth through the style was observed by this technique. Ovaries fixed at various intervals after pollination were dehydrated and embedded for microtomy to study details of embryo and endosperm development and their failure.

Germination of pollen on the incompatible stigma can be brought about by application to the stigma of sucrose and/or boron, some organic solvent (Willing & Pryor, 1976) or mentor pollen or its leachates (Knox *et al.*, 1972) or by wounding the stigma. Acceleration of the pollen tube growth through the style can be achieved by mentor pollen or its leachates (Sastri, 1978) or by application of growth substances to ovary or style (Matsubara, 1977). We were successful in overcoming intersectional incompatibility with the mentor pollen technique and also with the application of kinetin to the pistil. The seeds or embryos from this technique have been cultured to obtain seedlings in *in vitro* conditions. We are trying the technique of *in vitro* pollination and fertilization of the ovules as well as the culture of ovules from flowers pollinated *in vivo*.

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