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THE USE OF IN VITRO TECHNIQUES IN GROUNDNUT IMPROVEMENT

D.C. Sastri, J.P. Moss and M. Nalini

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
Patancheru
P.O. 502 324
A.P.,
INDIA

INTRODUCTION

Genetic improvement of crops by utilization of characters from wild species, which often have desirable traits not present in the cultivated forms, has been the subject of serious attention. The interspecific transfer of genes has not always been simple and straightforward because of reproductive barriers and genetic incompatibility. This is true in the genus Arachis where a number of species have been identified as sources of resistance to fungal and viral pathogens as well as to insect pests (1). A few species have been crossed with A. hypogaea and their derivatives produced (1 - 4), but there are a number of species which are not crossable with groundnut (5). This limits the scope for crop improvement, and the situation necessitates the adoption of existing methods, or development of new ones for the production of hybrids following these desirable wide crosses.

The genus is predominantly diploid (2n = 20) with its species grouped into seven sections (5). The cultivated species is a tetraploid in the section ARACHIS and cannot easily be crossed with other tetraploid species except A. monticola, the only other tetraploid in section ARACHIS. The other tetraploid species belong to the section RHIZOMATOSAE, and the attractive feature of these rhizomatous species is that they possess multiple resistance (6). Current knowledge of the genus is insufficient to decide on any specific method for breaking incompatibility. In earlier attempts to cross four wild species including A. glabrata, the one named tetraploid species

of the section RHIZOMATOSAE, with three cultivars of A. hypogaea, 25 pods were obtained which were 1/2 to 3/4 of the normal size with aborted seeds about 1/16" long (7), although no details of number of pollinations were given. Gregory (8) also reported pod production in these crosses, but no embryos or seeds were formed. Our results show the frequency of peg formation in such crosses was up to a maximum of 21.3%, none of which bore pods with well formed seeds (3). Johansen and Smith (9) observed 20 ovules of A. hypogaea, 2-7 days after pollinations with A. glabrata, and found that there were no pollen tubes in the ovaries, and fertilization had failed in all. Recently Murty et al. (10) have recorded similar observations and reported that the time taken for fertilization varied from 6 - 48 hours, and the maximum frequency of fertilization observed was 56%.

It should be emphasized here that Arachis is one of the few geocarpic taxa. After fertilization, a stalk (the gynophore) develops between the ovary and the node on which the sessile flowers are borne. This stalk elongates downwards until it has pushed the ovary into the soil. Seed development is almost absent during the aerial growth of teh gynophore and is resumed only after the peg is buried in the soil.

Therefore, it was decided that priority should be given to methods to produce plants from the fertilized ovules that were formed after wide crosses, but did not develop in vivo.

EMBRYO CULTURE

Culture of embryos in incompatible crosses in Arachis was suggested by Gregory in 1946 but was not taken up. Dissecting and culturing proembryos from about 1.5 mm long ovules is technically laborious and not always successful. Gynophore development and pod initiation and development from these incompatible crosses have been induced by hormone treatments (11, 12). Embryos in such cases grow to late globular or heart stages. Embryos at later stages were also observed in very rare instances. The younger embryos, when cultured, occasionally formed callus on MS with IAA or NAA (1 or 2 ppm) with Kinetin or BAP (0.5 or 1 ppm), but then became brown and necrotic. The embryos later than heart stages formed healthy callus and shoots. The rare well-developed embryos grew normally when cultured on White's as well as on MS medium. Culture conditions favoring the growth of embryos at globular stages are being investigated.

IN OVULO EMBRYO CULTURE

In ovulo culture of embryos has been useful in a number of plants where the dissection and culture of proembryos is difficult (13). Martin (14) cultured ovules from aerial gynophores of A. hypo-

gaea and obtained plants, using MS with Heller's microelements, vitamins, amino acids and casein hydrolysate as in Dulieu's medium, and 0.5 - 1.0 mg per litre of kinetin.

Sastri et al. (15) used a number of media but were unable to culture A. hypogaea ovules, even on Martin's (14) medium, and attempts at ovule cultures were discontinued. However, in vitro pollination of ovules had been achieved (15). Culture of whole ovaries, or gynophore tips, was, therefore, attempted.

OVARY AND GYNOPHORE TIP CULTURE

Ziv and Zamski (16) were the first to successfully culture the terminal segments of aerial gynophores and to obtain pods. Using their medium with various concentrations of kinetin and NAA and dark incubation, we failed to induce pod formation in peg tip cultures of A. monticola and A. hpyogaea. However, MS supplemented with 2 mg of NAA and 0.5 mg of BAP per litre has induced pod formation in dark incubated peg tip cultures of A. hypogaea and A. pusilla (a diploid wild species incompatible with all other species of Arachis). Some pegs from incompatible pollinations were cultured on this medium but without any success. Attempts with ovary cultures have resulted in callus formation and details have been reported earlier (15). Another observation worthy of mention is that on this medium pegs form callus which differentiates into plants. Rangaswamy et al. (17) reported root formation from callus in pericarp cultures.

THE FUTURE

The importance of groundnut as a crop in developing countries, and as a source of edible oil and protein for developing and developed countries, necessitates an objective realization of the problems reducing productivity and demands diversification of approaches to crop improvement, particularly when conventional methods of breeding have been unable to alleviate certain problems. The recent interest in, and application of, tissue culture methods in the improvement of some crops, notably potato, rapeseed and sugarcane, has stimulated groundnut scientists to turn their attentions to a wider range of approaches. Some of the initial problems associated with utilization of wild species have been overcome. Despite the ploidy differences between some wild species and the cultivated groundnut, Spielman et al. (18) produced fertile interspecific hybrids in large numbers. More recent reports of plant regeneration in tissue and organ cultures (19, 20) have now created hopes of regeneration from single cells and application of these techniques to some of the refractory problems in groundnut breeding.

The importance of haploids in plant breeding is now too well

recognized to elaborate. The success of Mroginski and Fernandez (21, 22) in inducing plantlets in anther cultures from the cultivated and a few diploid species of Arachis has stimulated our efforts to produce haploids at ICRISAT. Plantlets have been obtained from anther cultures of A. pusilla and A. sp. P.I. No. 276233.

• Production of plants form meristem cultures (19, 23) and their survival after freezing for 28 days (24) should be utilized for generation of disease-free populations, long term storage and international transfer of germplasm. Whilst our present knowledge is being utilized to realize these objectives, efforts to explore other possibilities will be initiated. Foremost amongst these must be spontaneous and induced variability in cultures and selection of cell lines with desirable traits. In a very recent report Bajaj et al. (25) have obtained genetically variable plants from the anther derived calli of A. hypogaea and a diploid wild species, A. villosa

The recent developments in methodologies should, therefore, soon lead to realization of hybrids in large numbers from incompatible crosses as was achieved in compatible species (2, 18). In the meantime optimization of conditions for culturing a range of tissues should stimulate efforts to produce agriculturally desirable plants by other methods.

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