Arunachalam, V.
Some Areas where Tissue Culture can Help to Widen the Breeding Base in Groundnut (Arachis Hypogaea L.).
Proceedings Indian National Science Academy, 54 B, 54 (1988): 261-263.

Proc. Indian natn. Sci. Acad. **B54** No. 4 pp. 261–263 (1988)

Some Areas where Tissue Culture can help to widen the Breeding Base in Groundnut (*Arachis hypogaea* L.)

V ARUNACHALAM
Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

(Accepted on 7 June 1988)

Modern concepts on improving productivity in groundnut lay emphasis on breeding genetically broadbased populations particularly to provide some risk cover against pests and diseases. Breeding methods involve making single and multiple crosses and evaluating F_2 populations of adequate sizes. Selection in early segregating generation is optimally based on an index involving key physiological, biological nitrogen fixation and yield components. Destructive sampling at earlier stages required for measuring some of those characters makes it difficult to relate them with the yield of plants on which they were measured. Field estimation of nitrogenase activity, an important character indicative of nitrogen fixation, is found to be efficient if it is done on a group of few plants of each genotype; this requirement poses its problems in F_2 generation. This paper highlights the utility of tissue culture in increasing population sizes for tackling some of these problems.

Key Words: Peanut, *Arachis hypogaea* L., **Tissue culture, Nitrogen fixation**

Introduction

Work in the past decade for improving and sustaining high productivity in groundnut (*Arachis hypogaea* L.) has laid emphasis on developing "heterogeneously homozygous" (Hammons 1976) genotypic blends for enhanced buffering against a number of biotic and abiotic stresses. The variety 'Florunner' is such a blend and is very popular in USA. In breeding for coadaptive components of such blends, and also in developing productive pure lines, it is essential to arrive at decisions based on large F_1 sample sizes and adequately large populations of F_2. Often this requirement is hard to meet in groundnut. In addition, a number of other relevant situations also exist where rapid multiplication through tissue culture methods has a good scope. This paper brings to focus such situations.

Germplasm: Genotypes with rare characteristics like aerial podding (EC 21137-1) are available in the germplasm bank but have low survival capacity under normal field conditions. These can be multiplied by tissue culture techniques and maintained by cryopreservation, if needed.

Hybridisation: Despite the fact that techniques of hybridisation in groundnut have been well set (Norden 1980), the rate of success is not consistently high. In the well-organised hybridisation programme at ICRISAT, during 1977–78, 3500 pollinations were made in 2-months period between rust resistant PI 259747 and PI 298115 and a number of high-yielding but rust susceptible parents. It yielded 2074 pegs and 1188 pods, giving 3.4% success (ICRISAT Annual Report 1977–78). With increased field infrastructure, providing improved insect control, a perfo-irrigation system to maintain high humidity at the time of pollination and many-fold increase in the number of pollinations, success rate averaged 50–67% in rainy and post-rainy seasons of 1979 (Nigam et al. 1980).

Such infrastructural facilities are still beyond the reach of national programmes in operation today. The rate of success in hybridisation is consequently low. In groundnut, despite precautions a certain per cent of selfing cannot be ruled out. Studies on hybridisation do not usually report the percentage of true hybrids after

testing the F_2 segregation. In two typical instances, it was noted that percentage of true F_1 hybrids based on F_2 segregation was low and ranged from 1 to 10.

Due to such problems in hybridisation, it often happens that F_1 plants in each cross are either too few or vary wildly. In the former case, the material is advanced to F_2 without F_1 analysis and in the latter, the data are converted in terms of cross means. This results in inappropriate and inefficient estimates of various genetic components and consequent errors of inference and judgement. Through tissue culture, it is possible to generate adequate number of F_1 plants per cross so that an effective statistical analysis can be made. The problems associated with unequal sample sizes can then be eliminated. Since the number of required tissue culture raised plants will be of the order of 50 to 100, somaclonal variation may not be of great consequence in vitiating judgement.

The utility of tissue culture is particularly relevant when crosses made with certain parents (e.g. cross Robut 33–1 × NC Ac 17090 and 87/4/7 (2) × PI 298115) produce very few seeds. In general, hybrids produce ill-filled and shrivelled seeds. In field conditions there could be germination and growth problems and in the presence of pests and diseases, not all of them survive to maturity. Tissue culture generation of adequate number of F_1 plants can help to overcome such difficulties.

Further, natural outcrossing occurs in groundnut (Gibbons & Tattersfield 1969, Hammons 1963) and some of the natural hybrids derived from Robut 33–1 were productive (Nigam et al. 1983). One of these, ICGS-11, was released for general cultivation in India. Such natural hybrids which occur in low frequency can escape detection in low population sizes. If through regeneration, the population sizes are enlarged, the frequency of such hybrids, and, in turn, the possibility of detecting such hybrids increase.

Moreover, hybrids with exceptional attributes can be utilised as parents of multiple crosses. But hybridisation to produce multiple crosses using F_1 as a parent has to be undertaken at the same time as the single cross F_1 hybrid is evaluated. If the number of F_1 plants is too low, such an exercise may not be feasible. Tissue culture generation of adequate F_1 plants can be of great help in such situation.

The need for tissue culture in making successful inter-specific crosses is well-documented in several crop plants and also in groundnut (Sastri et al. 1981). Cotyledons have been cultured from immature seeds formed in an incompatible cross, *Arachis monticola* × A. sp. PI 276233. Embryo, ovule and ovary cultures have also been successfully employed to produce hybrid plants from incompatible crosses. Anthers and microspores have been cultured to produce haploids; pure homozygous diploids could then be obtained by colchicine treatment. This will save considerable time that would otherwise be spent in pure diploid production by conventional methods.

Selection in Segregating Generations

Epistatic variance is usually found associated with characters directly related to yield in groundnut. Two situations are of particular interest. Sometimes an exceptionally heterotic hybrid is obtained with twice or thrice the yields of better parent (Hammons 1973). Such hybrids segregate out rapidly in the next few generations nullifying the F_1 advantage.

Further, the size of F_2 population usually grown is too small to trap transgressive segregants occurring in low frequency, especially in the presence of pest, disease and environmental hazards. Increased F_2 population sizes (through tissue culture) would increase the possibilities of detecting and retaining such trangressive segregants. Somaclonal variation can be an advantage so long as substantial variability is maintained and becomes available for selection.

Population improvement: As pointed out earlier, productivity in groundnut can be maintained at a stable level under normal growing conditions, if genetically broad-based genotypic blends are used. Multiple crosses, convergent crosses using genotypes with a high degree of disease tolerance, recurrent selection based on general combining ability, reciprocal recurrent selection and intra-gene pool intermating (Dutta et al. 1986) are some vital steps in such programmes. In all these cases population size will be a crucial factor. Tissue culture can be of certain utility in the maintenance of adequate population sizes in such breeding steps.

Nitrogen Fixation

One character of direct relevance to nitrogen-fixing ability is nitrogenase activity measured easily by acetylene reduction technique. But about 5 plants are needed for efficient field estimates. Thus, measurement of nitrogenase activity in F_2 generation is a problem, especially when each plant can be of a different genotype. By tissue culture, from each plant, a few plants can be regenerated and nitrogenase activity estimated. This process opens up new avenues for selecting high nitrogen-fixing segregants in F_2 and later generations.

Selection for productivity is profitably made not only on direct yield components but on key physiological parameters like biomass, leaf area and harvest index and characters indicative of nitrogen fixation—like nitrogenase activity and nodule mass. Since these characters can be measured only by destructive sampling, it is not possible to measure all these characters and yield on the same plant. Tissue culture generation of copies of plant would make it possible to measure all these characters on the same genotype (if

not the same plant) to enable selection of efficient types.

It has been reported that non-nodulating progenies occur in the F_2 generation of certain crosses (Nambiar & Dart 1980). These plants produce fewer pods compared to normal plants under intensive fertilization and care. Tissue culture multiplication of such plants may be worthwhile for making crosses with genotypes like NC Ac 2821 which has high nitrogenase activity and good general combining ability for (traits associated with N_2-fixation) nitrogenase activity, total nitrogen, leaf area and top weight (ICRISAT Annual Report 1982). Evaluation of such crosses in F_1 and further generations will not only help in establishment of the genetics of non-nodulation but also in seeking improvement in nitrogen fixation, photosynthesis, partitioning of carbohydrate to nodules and ultimately yield (Ruschel & Vose 1980).

Disease Resistance

Finally, there are areas where exploratory work using tissue culture is worth attempting. In groundnut, genetic basis of resistance to rust and leafspots is yet to be understood since physiological races are still to be identified. Field-resistant genotypes are, however, available that show stability in resistance. As in sugarcane where micropropagation of shoot apices of the sugarcane variety CO-740 gave mosaic virus-free plants (Hendre et al. 1975) with 20% higher yields, it is worthwhile to attempt similar work on suitable plants isolated in segregating generations of crosses between high yielding but susceptible cultivars and rust resistant genotypes. Work on segregating generations is suggested since the rust-resistant genotypes are usually Valencia land races ill-adapted to growing environment in India and as such their yields are not high.

Race-specific resistance was reported to have been expressed in tissue cultures in the cases of potato and tomato to *Phytopthora infestans,* soybean to *P. megasperma* and tobacco to *Pseudomonas* spp. and tobacco mosaic virus (Miller & Maxwell 1983). These reports encourage such investigations in groundnut too for leafspots, rust and bud necrosis caused by Tomato Spotted Wilt Virus.

Lastly, somaclonal variation generated through tissue culture is a viable route to identify superior and new variants that could directly become potential cultivars. Screening of calli for disease and stress resistance, early flowering and other desired traits and the association between *in vitro* selection and field performance are some open but potential areas of research.

References

Dutta M, Arunachalam V, Bandyopadhyay A and Prabhu K V 1986 Early generation intermating for yield improvement in groundnut (*Arachis hypogaea* L.); *Theor. Appl. Genet.* **71** 662-666

Gibbons R W and Tattersfield J R 1969 Out-crossing trials with groundnuts (*Arachis hypogaea* L.); *Rhod. J. Agric. Res.* 7 71–75

Hammons R O 1963 Artificial cross-pollination of the peanut with bee-collected pollen; *Crop Sci.* **3** 562–563

—— 1973 Genetics of *Arachis hypogaea* in Peanuts—*Culture and Uses* (APREA) 135

—— 1976 Peanuts: Genetic vulnerability and breeding strategy; *Crop Sci.* **16** 527–530

Hendre R R, Mascarenhas A F, Nadgir A L, Pathak M and Jagannathan V 1975 Growth of virus-free sugarcane plants from apical meristem; *Indian Phytopath.* **28** 175–178

Miller S A and Maxwell D P 1983 Evaluation of disease resistance; in *Handbook of Plant Cell Culture* Vol. 1 *Techniques for Propagation and Breeding* ed. D A Evans, W R Sharp, P V Aminirato and Y Yamada (Mac Millan) 853

Nambiar P T C and Dart P J 1980 Studies on nitrogen fixation by groundnut at ICRISAT; in *Proc. Int. Workshop on Groundnut,* ICRISAT, ed. R W Gibbons (India: ICRISAT) pp 110

Nigam S N, Dwivedi S L and Gibbons R W 1980 Groundnut breeding at ICRISAT; in *Proc. Int. Workshop on Groundnuts,* ICRISAT ed. R W Gibbons; (India: ICRISAT) pp 62

—— Ramanatha Rao V and R W Gibbons 1983 Utilization of natural hybrids in the improvement of groundnuts *(Arachis hypogaea* L); *Expl. Agric.* **19** 355-359

Ruschel A P and Vose P B 1980 Nitrogen fixation as a source of energy in tropical agriculture in *FACO/SIDA Workshop on Organic Recycling in Agriculture;* FAO

Sastri D C, Nalini M S and Moss J P 1981 Tissue culture and prospects for improvement of *Arachis hypogaea* L; in *Proc. COSTED Symp. on Tissue Culture of Economically Important Plants* ed. A N Rao p 42