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Plant Biotechnologies for Developing Countries

**Proceedings of an
International Symposium
organized by CTA and FAO,
26-30 June 1989, Luxembourg**

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*Proceedings of an international symposium organized jointly by
the Technical Centre for Agricultural and Rural Co-operation (CTA)
and the Food and Agriculture Organization of the United Nations (FAO)
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Technical Centre for Agricultural and Rural Co-operation (CTA)
and Food and Agriculture Organization of the United Nations (FAO)

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- to improve the production and distribution of all food and agricultural products;
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*The Application of Biotechnologies to Groundnut, *Arachis hypogaea* L., with Special Reference to Developing Countries*

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Groundnut is the twelfth most important crop in the world, occupying some 19 million hectares in the warm temperate and tropical zones bounded by the 40° line of latitude. It is usually grown under rainfed conditions, but there are some areas in which it is grown under irrigation. World production in 1987 was 20 million tonnes in shell, most of which (12.8 million tonnes) was produced in Asia; of this amount, China produced 6.1 million tonnes while India produced 4.5 million tonnes. Africa produced 4.7 million tonnes. Worldwide, average yields by country range from 0.43 tonnes/ha to 4.60 tonnes/ha (FAO, 1987). Rainfed crops are often grown under low-input conditions, and even in those developing countries where fertilizers and pesticides are available on the market, many small farmers do not have sufficient resources to make use of them. There is thus a strong incentive to develop varieties with pest and disease resistance, tolerance to poor soils, and the ability to yield under low-moisture regimes.

The genus *Arachis* is a member of the sub-tribe Stylosanthinae of the tribe Aeschynomeneae in the Leguminosae. Related genera are *Stylosanthes*, *Chapmannia*, *Arthrocarpum* and *Pachecoa*, but there are no reports of successful intergeneric hybridization. Fruit development in *Arachis* differs from these genera, as the fruit is formed underground. The genus *Arachis* has been divided into seven sections, and there are some strong crossability barriers between certain sections (Gregory et al., 1973).

The closely related diploid species of *Arachis* have been crossed with cultivated groundnut, but cytogenetic manipulations were needed to overcome problems because of the ploidy differences. A wide range of derivatives have been produced (Moss, 1985). Hybrids with other species of *Arachis* have only rarely been produced using hormone treatments and embryo rescue (Moss et al., 1988). The primary gene pool consists of the cultivated accessions of *A. hypogaea*, while the secondary gene pool consists of the closely related wild species. There is therefore a strong tendency to use the recent technological advances in gene identification and transfer which hold considerable promise for the genetic improvement of groundnut.

BIOTIC CONSTRAINTS

Fungal diseases

Rust

Rust, caused by *Puccinia arachidis* Speng., occurs in almost all groundnut-growing areas and causes serious yield losses. It can be controlled by fungicides, but repeated applications are needed for effective control. Resistance has been identified in the cultivated species; 14 lines have been released as resistant germplasm, and resistant cultivars have been produced. Resistance has also been identified in the wild species, and the gene involved is different from that in cultivated groundnut (Singh et al., 1984). Tetraploid germplasm lines incorporating resistance from the wild species have been produced at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (Moss et al., 1988). They are being used in breeding programmes in many groundnut-growing countries and should lead to more stable resistance. However, rust is one of the most important constraints to production, and there are indications that physiological races occur (Kalekar, et al., 1985). Efforts to identify more sources of resistance, and to transfer them to *A. hypogaea*, must therefore continue.

Leaf spot diseases

Early leaf spot, caused by *Cercospora arachidicola* Hori, and late leaf spot, caused by *Phaeoisariopsis personata* (Berk. & Curt.) v. Arx, occur in most groundnut-growing countries, but their relative importance varies with location and, to some extent, with season (McDonald et al., 1985). Both pathogens cause lesions on the leaves, petioles, stems and pegs. Severe attacks lead to extensive defoliation and thus to yield losses. Some resistant cultivated germplasm has been identified, but a greater level of resistance has been found in the wild *Arachis* species. *A. cardenasii*, a diploid wild species, has been used extensively in crosses with *A. hypogaea* to transfer genes conferring resistance to late leaf spot into tetraploid cultivars. Many interspecific lines have been produced at ICRISAT incorporating this resistance (Moss et al., 1988).

Resistance to early leaf spot has been identified in diploid species of section *Erectoides*. These species do not, however, cross with *A. hypogaea*, although pollination results in normal pegs and pods but with restricted development of the ovule, which results in abortion of embryos. Efforts are being made to use exogenous hormone application to support the *in vitro* growth of ovules and embryos, and to use *in vitro* embryo rescue techniques to produce intersectional hybrids. Another approach is to use diploid wild species of section *Arachis* that are compatible with *A. hypogaea* as bridge species. These have been crossed with species from section *Erectoides*, using *in vitro* techniques to transfer the resistance to *A. hypogaea*. Application of restriction fragment length polymorphism (RFLP) technology is expected to lead to the isolation of the gene for resistance and the development of probes for gene detection and of vectors for asexual transfer, all of which would benefit breeding efforts.

Aspergillus flavus and aflatoxins

Aflatoxins, which include some of the most powerful carcinogens known, are produced following infection of groundnut seed by the aflatoxigenic fungi of the *Aspergillus flavus*

group. Aflatoxin contamination is a serious problem in most groundnut-producing countries (ICRISAT, 1988), especially in developing countries where improving harvesting techniques and post-harvest technology is difficult because of inadequate extension services, storage facilities and marketing infrastructure. Most infection occurs in the field during growth of the pod, and disease build-up is severe under late-season drought stress. Infection can also occur during post-harvest field drying and in storage. Genetic resistance to seed infection by the toxigenic fungi is important in preventing aflatoxin contamination. Screening to date has identified some field resistance and dry-seed resistance in cultivated groundnut. Genotypes have been identified which produce low levels of aflatoxin when infected by *A. flavus* (Mehan et al., 1987). This important constraint to consumption and the serious health risk warrant a concerted effort to combine available resistances, to search for new sources and to develop new approaches, based on the prevention of aflatoxin production in the seed.

Viral diseases

In the case of viruses infecting groundnut, symptoms of 'green rosette' and 'clump' in West Africa resemble each other, as do 'mottle' and 'stripe' (Reddy, 1988). It is essential to use serological techniques such as enzyme-linked immuno-sorbent assay (ELISA) and other physico-chemical techniques to distinguish viruses. To undertake ELISA tests, polyclonal antisera are suitable in most cases. Nevertheless, in the case of peanut clump virus (PCV), which exists as several serologically distinct isolates, monoclonal antibodies with broader specificity or cDNA probes are required to detect the clump virus. It has been demonstrated that a probe prepared for one PCV isolate can detect several PCV isolates (Reddy et al., 1985; Naidu et al., unpubl.).

Peanut clump is an economically important disease in India and West Africa. No sources of resistance have so far been identified in *A. hypogaea* or in those wild *Arachis* species tested to date. Transgenic *A. hypogaea* expressing the coat protein gene, or incorporation of a portion of a viral gene which can synthesize defective interfering particles, would contribute significantly to finding a solution to this problem.

Groundnut rosette virus (GRV) disease is by far the most important virus disease of groundnut in sub-Saharan Africa. Resistant varieties have been bred using landraces collected in Côte d'Ivoire and Burkina Faso. The causal agents of GRV disease were not known until recently. Two viruses and a viroid are involved in the etiology. A luteovirus assists in aphid transmission. GRV, the particle structure of which is not known, assists in the multiplication of a satellite RNA which is responsible for symptom production (Murant et al., 1988). When several genotypes were screened under high disease pressure, most rosette-resistant cultivars showed resistance only to GRV and the satellite, but not to the luteovirus. Nevertheless, two wild *Arachis* species, one in section *Arachis* (cross compatible with *A. hypogaea*) and the other in section *Erectoides*, showed resistance to the luteovirus. This type of resistance is desirable because it can eliminate the luteovirus, which is chiefly responsible for aphid transmission. A quicker approach to obtain resistance to the luteovirus would be to incorporate luteo coat protein into the genome of selected groundnut cultivars.

Until the early 1970s, peanut stripe virus (PStV) had been reported from very few countries. With increased germplasm transfer without stringent quarantine procedures, the virus has since spread to several countries and is now regarded as the most important virus

disease in South and South-East Asia. It is essential to locate a source of resistance to PStV. Although over 8,000 *A. hypogaea* genotypes were screened in Indonesia, none were found to be resistant. However, several derivatives from crosses involving wild *Arachis* species showed resistance in preliminary tests, and resistance has been identified in wild species (Culver et al., 1987). As PStV is a potyvirus, it may be possible to induce resistance by transferring viral coat protein gene. It is essential to explore this possibility.

Peanut mottle virus (PMV) occurs in all groundnut-growing countries, and is aphid- and seed-transmitted. Genotypes of *A. hypogaea* have been identified in which there is no seed transmission, and tolerant lines have been selected in which yield is not seriously reduced by the presence of PMV. Conventional breeding efforts are under way to combine these traits, but biotechnological approaches can be used to identify and introduce a third level of resistance, based on the introduction of viral coat protein genes to further limit the incidence of PMV.

In contrast, tomato spotted wilt virus (TSWV), which has a wide host range and causes bud necrosis disease in groundnuts, is being tackled successfully by conventional breeding and management approaches.

Insect pests

Spodoptera litura is an important foliage feeder and is widespread from the Middle East to Japan (Wightman and Amin, 1988). It is difficult to control, and the use of pesticides has often increased the levels of resistance of *Spodoptera* to insecticides (Chou et al., 1984; Ramakrishnan et al., 1984). The groundnut leaf miner, *Aproaerema modicella*, is also important in South-East Asia. One way of controlling these pests is to use parasexual means to introduce the toxin-producing gene from *Bacillus thuringiensis* (*B.t.*).

Termites (*Microtermes* and *Odontotermes* species) occur in dry areas of Africa, in India and in the drier parts of Asia, and there is no economically feasible control method available. White grubs occur in all areas of the semi-arid tropics (Feakin, 1973) and are serious pests in northern India (Wightman and Amin, 1988). Pests such as millipedes are also important as pod borers in West Africa (Johnson et al., 1981). The extent of the losses caused by these pests justifies a search for suitable toxins and transformation of groundnut to produce these in the roots.

Aphids, primarily *Aphis craccivora* transmit GRV, PStV and PMV, and thrips are the vectors of TSWV. Resistance to these vectors has been identified in cultivated as well as wild *Arachis* species, and this vector resistance should be combined with virus resistance.

ABIOTIC CONSTRAINTS

Drought is a major constraint to rainfed production of groundnuts. End-of-season drought can be avoided by using short-duration varieties. There is a need to improve water extraction ability and water use efficiency of the genotypes. Genotypic differences exist for both these traits (Nageswara Rao et al., unpubl.) and identification, isolation and enhancement of the activity of the genes involved could increase yields in drought-prone areas. There is also a need for varieties which will survive and quickly recover from mid-season drought. This characteristic is typical of some of the wild species.

Research is needed on producing groundnut varieties which are tolerant to acid soils. Some areas of southern Africa (for example, Zambia) are particularly prone to the problem of 'pops' (empty pods), associated with unavailability of calcium at pod filling. These problems may be amenable to techniques of *in vitro* selection, as may the problem of lime-induced chlorosis under high pH conditions in a large area of Vertisols.

USING BIOTECHNOLOGIES IN ADDITION TO CONVENTIONAL BREEDING TECHNIQUES

Cultivated groundnut is a self-pollinated, inbreeding allotetraploid ($2n = 4x = 40$), with genomes AABB where A and B genomes are homoeologous, as a result of which many characters show tetrasomic or multi-allelic inheritance. The production of haploids by microspore culture would be a useful tool in genetic analysis of economically important traits, and may reduce the time needed to produce new cultivars. Worldwide production of groundnut, a valuable source of oil and protein, justifies the production of RFLP maps and the further use of this technology to produce probes for identification of genes. These probes, in addition to their acknowledged benefits in breeding programmes, would also be useful in germplasm enhancement.

CONCLUSION

Breeders have used a range of conventional techniques to improve the groundnut crop, and varieties with increased yield and/or resistance to some pests and diseases are available. However, there are several constraints, such as early leaf spot, for which there is no known source of resistance, or only low-level resistance in the cultivated germplasm. Biotechnological techniques provide the opportunity to solve some of these problems, to improve existing resistance and to explore alternatives to conventional breeding.

For all major constraints, whether amenable to conventional breeding or not, biotechnology can play an important role in germplasm enhancement, whereby the breeder is provided with germplasm with multiple resistance and can concentrate on using conventional techniques in breeding for adaptability to specific climatic-edaphic zones.

The wild species of *Arachis* are a valuable source of resistance to most major diseases and pests (Moss et al., 1988). Some of these species have been crossed with *A. hypogaea* and valuable derivatives have been produced, but others have defied attempts to transfer genes into cultivated groundnut. Transfer of genes from these species into *A. hypogaea* by asexual means would be advantageous, because a gene is introduced into a related nuclear and cytoplasmic background with less disruption to the genetic background of the cultivated species than is the case with sexual hybridization.

Viruses are major constraints, and the isolation and introduction of coat protein genes should be a major priority.

The *B.t.* may be of value for *Spodoptera* and *Approaerema* but there should also be a concentrated effort to find other genes for resistance to non-lepidopteran insects, with emphasis on soil-dwelling insects.

Much basic work on interspecific hybridization and on regeneration from *in vitro* cultures has already been undertaken, and provides scope for biotechnology to assist the breeder in groundnut improvement.

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