Chapter 16

Agrobacterium-Mediated Genetic Transformation of Peanut

P. Bhatnagar-Mathur
V. Anjaiah
P. B. Kirti
Kiran K. Sharma

INTRODUCTION

Legumes are important sources of dietary proteins and fats in developing countries of the semiarid tropics where peanut (*Arachis hypogaea* L.) is one of the important food legume crops. It is a rich source of protein (23 percent) and edible oil (43 percent; Norden, 1980) and is considered one of the principal economic crops of the world. The genus *Arachis* belongs to the subfamily Papilionaceae of the family Fabaceae. It is native to South America and comprises diploid ($2n = 20$), tetraploid ($2n = 40$) and octaploid ($2n = 80$) species.

Peanut is a seed-propagating, self-pollinating crop originating from Brazil. The geographical classification of peanut is delineated into six regions: the Americas, Africa, Asia, Near East, Europe, and Oceania (Gregory et al., 1980). The total area under peanut cultivation is over 24.8 million hectares and the world's production is over 32.8 million tons per year, with an average yield of 1.32 tons per hectare (Rao and Nigam, 2001). India is the major producer of peanut, with a total production of 8.9 million tons per year. Peanuts are utilized in several ways; the edible oil is important for human consumption and the meal is used for livestock feed. It is also used directly for food in industrial countries including the United States, Canada, and the European Union.

Since the mid 1970s edible peanuts have increased in both domestic consumption and export trade. In contrast, production in Africa has declined by 17 percent over the last two decades. The major reasons for such low production are various biotic and abiotic stresses. Resource-poor farmers who obtain low yields of 500-800 kg·ha^{-1} due to various biotic and abiotic constraints grow about 93.8 percent of the world's production of peanut. *Aspergillus flavus,* which produces aflatoxins, for which no adapted resistant genotype is available, adversely affects peanut quantity and quality. Foliar diseases such as early and late leaf spot caused by *C. arachidicola* and *Phaeoisariopsis personata* (= *C. personata*) respectively are the most damaging diseases (Subramanyam et al., 1985). Among the insect pests, Spodoptera, legume pod borer, aphids, and thrips cause the greatest losses to the peanut crop (Wightman and Ranga Rao, 1993).

Conventional plant breeding techniques and methodologies have not been proven successful in imparting resistance against various biotic and abiotic stresses due to species barriers in the natural system. Peanut improvement has been limited due to the lack of integration of resistance to many diseases and pests from wild species of peanut (Stalker and Moss, 1987) because of problems with sterility barriers and genomic incompatibilities associated with traditional breeding. Genetic engineering approaches have been shown to be comparatively fast, leading to better isolation and cloning of genes controlling desired traits and their introduction into crop plants for combating biotic and abiotic stresses. Several gene transfer approaches have been employed to improve stress tolerance in different crop plants (Holmberg and Bulow, 1998). The application of recombinant DNA technologies for crop improvement in the semiarid tropics has shown great potential (Sharma and Ortiz, 2000). The advent of biotechnological tools including marker-assisted selection and gene transfer across the species barrier has opened up novel opportunities for enhancing seed quality, disease and pest resistance, viral resistance, abiotic stress tolerance, and nutritional improvement that are not accessible normally by conventional breeding, that is, they are limited by sexual incompatibility (Sharma and Ortiz, 2000). However, for successful genetic transformation, a reliable and effective regeneration system adaptable to transformation methods is needed. The transformation protocols for peanut are now well established and development of transgenic peanut expressing desirable foreign genes is going to be a reality soon (Sharma and Anjaiah, 2000). This chapter briefly reviews progress with tissue culture and genetic transformation of peanut and its possible applications for improvement of this important legume crop.

TISSUE CULTURE METHODS

Advances in plant tissue culture techniques have been exploited for in vitro regeneration of peanut plants. In vitro regeneration in peanut occurs through organogenesis or embryogenesis. Regeneration by organogenesis occurs by the development of shoots directly on the surface of cultured explants (McKently et al., 1991; Hazra et al., 1989) or by an intervening callus phase, that is, the development of shoots from the callus tissue (Bajaj et al., 1981; Bajaj and Gosal, 1983, 1988). In earlier reports, the organogenic systems that regenerated shoots from immature leaflets, seed explants, de-embryonated cotyledons, hypocotyls, epicotyls, and anther-derived callus (Li et al., 1994; McKently et al., 1990; Mroginski and Fernandez, 1980; Mroginski et al., 1981; Narasimhulu and Reddy 1983; Pittman et al., 1983; Willcox et al., 1991) had a low frequency and plants were not realized in good frequencies. There are numerous reports of tissue culture and regeneration of peanut from diverse explants with various combinations of phytohormones in culture media (Table 16.1). However, not much success with genetic transformation of *Arachis* species was achieved until recently (Sharma and Anjaiah, 2000) due to the lack of efficient protocols to obtain whole plants through in vitro regeneration of adventitious shoot buds from the transformed tissues. This has prompted some workers to adopt non-tissue-culture-based approaches that do not depend on the regeneration of adventitious shoot buds for generating transgenic plants of peanut (Rohini and Rao, 2000). Direct regeneration systems have advantages due to the rapidity of morphogenesis and no requirement for frequent subculture; besides, de novo production of shoot primordia is extremely rapid and initially synchronous with the period of cellular differentiation. Such a regeneration system favors easy accessibility for *Agrobacterium*-mediated genetic transformation. Sharma and Anjaiah (2000) successfully obtained high-frequency direct shoot regeneration from mature cotyledon explants in various peanut genotypes. Shoot organogenesis and plants were also successfully obtained using immature leaflets (McKently et al., 1991; Sharma et al., unpublished data). Regeneration via somatic embryogenesis has also been reported (Cucco and Jaume, 2000; Gill and Saxena, 1992; Zhuang et al., 1999), which has been used in transformation studies in peanut (Ozias-Akins and Branch, 1990; Sellars et al., 1990; Chengalrayan et al., 1994, 1997). However, conversion of somatic embryos into plants remains inefficient and limits the application of somatic embryogenesis in many systems, including genetic transformation (Wetzstein and Baker, 1993).

TABLE 16.1. Responses of various explants and hormones on in vitro shoot regeneration in peanut.

Explant	Genotype cultivar	Medium	Growth regulators	Morphogenic response	Reference
Apical meristem		MS + B5 vitamins	NAA (10 µM) + BA (0.1 µM)	Single shoots with many roots	Kartha et al., 1981
			NAA (10 µM) + BA (1 µM)	Shoots without any further development	Kartha et al., 1981
Mesocotyl		MS	IAA (11 µM) + kinetin (2.3 µM)	Shoots with roots	Bajaj, 1982
Epicotyl		MS	Casein hydrolysate	Multiple shoots, roots	Bajaj, 1982
	New Mexico Valencia	MS	BA (10 mg·l^{-1}) + NAA (1 mg·l^{-1})	Organogenesis	Cheng et al., 1992
Hypocotyl	ICG 4367, US 48, TMV 2, TG 19B	MS	IAA (2 mg·l^{-1}) + kinetin (2 mg·l^{-1})	Shoots	Narasimhulu and Reddy, 1983
De-embryonated cotyledons	ICG 4367, US 48, TMV 2, TG 19B	MS	2,4-D (2 mg·l^{-1}) + kinetin (2 mg·l^{-1})	Multiple shoots	Narasimhulu and Reddy, 1983
	TG- 17	Moist cotton wool	BA (1 mg·l^{-1})	Multiple shoots	Bhatia et al., 1985
Cotyledonary nodes		MS	NAA (1 mg·l^{-1}) + BA (3 mg·l^{-1})	Multiple shoots	Banerjee et al., 1988

Explant	Genotype	Medium	Growth regulators	Response	Reference
Mature cotyledons	JL-24, J-11, ICGS-11, ICGS 44, Robut-33-1	MS + B5 organics	BA (20 µM) + 2,4-D (10 µM)	Adventitious shoot buds	Sharma and Anjaiah, 2000
Immature leaflets		MS + Gamborg vitamins	NAA (1 mg·l⁻¹) + BA (1 mg·l⁻¹)	50% shoots	Pittman et al., 1983
	JL-24	MS	NAA (4 mg·l⁻¹) + BA (5 mg·l⁻¹)		Chengalrayan et al., 1994
	NC-7	MS	NAA (2 mg·l⁻¹) + BA (4 mg·l⁻¹)	Shoots	Utomo et al., 1996
Embryo axis		MS	None	Shoots regenerated into plantlets	Atreya et al., 1984
Immature embryos	New Mexico Valencia	-	TDZ (10 mg·l⁻¹)	-	Kanyand et al., 1994
	Several varieties	B5	Picloram (0.5-1 mg·l⁻¹)	Shoots with roots	Ozias-Akins et al., 1993
Embryos	MK 374, M 13, TMV 2, Robut-33-1	MS or White's medium	None	Whole plants	Sastri et al., 1980
	Span cross, Dixie Spanish	MS	IAA (0.5 mg·l⁻¹) + GA$_3$ (0.05 mg·l⁻¹) + Zeatin (0.5 mg·l⁻¹)	Shoots	Ozias-Akins and Branch, 1990
Ovules		MS	Kinetin + GA$_3$	Shoots and roots	Martin, 1970
Ovaries	MK 374, M 13, TMV 2, Robut-33-1	MS	BA (0.5 mg·l⁻¹) + NAA (2 mg·l⁻¹)		Sastri et al., 1980

TABLE 16.1. *(Continued)*

Explant	Genotype cultivar	Medium	Growth regulators	Morphogenic response	Reference
Epicotyl	ICG 4367, US 48, TMV 2, TG 19B	MS	None	9-28% shoots	Narasimhulu and Reddy, 1983
De-embryonated cotyledons	MK 374, M 13, TMV 2, Robut-33-1	MS	Zeatin (4 mg·l^{-1}) or kinetin (4 mg·l^{-1})	Multiple shoots	Sastri et al., 1980
Leaflets		MS	NAA (1 mg·l^{-1}) + BA (1 mg·l^{-1})	Organogenic callus	Mroginski et al., 1981
	TMV 2	MS	BA (2 mg·l^{-1}) + NAA (0.5 mg·l^{-1})	Shoot primordia	Venkatachalam et al., 1999
Plumule	Okrun	MS	BA (30 μM) + NAA (5 μM) + brassin (1 μM)	Multiple shoots	Ponsamuel et al., 1998

GENETIC TRANSFORMATION

Genetic transformation for incorporation of novel genes into the peanut gene pool has opened up new opportunities for crop improvement in this important legume. The transformation and regeneration protocols for peanut are now well established. Transformation efficiencies frequently are directly related to the tissue culture response and therefore highly regenerative cultures are often transformation competent. The developments in genetic transformation in peanut have emboldened researchers to pursue the development of transgenic peanut plants capable of producing high-quality peanuts resistant to various diseases, insect pests, and abiotic stresses (Sharma and Anjaiah, 2000; Rohini and Rao, 2001). Peanut tissue is susceptible to infection by wild-type strains of *A. tumefaciens* (Lacorte et al., 1991). Several methods for DNA transfer are used for the genetic transformation of peanut (Table 16.2). Novel genes can be introduced into actively growing peanut cells biologically through *Agrobacterium*-mediated gene transfer or through direct and physical DNA delivery methods such as electroporation or microprojectile bombardment. However, *Agrobacterium*-mediated gene transfer is the most widely applied system in peanut. Increasingly, there is a trend toward the use of *A. tumefaciens* for DNA delivery in crop improvement programs compared with microprojectile bombardment. This is driven by the development of highly virulent strains and binary vectors that are useful for genetic transformation and their ease of use and researcher familiarity. There is also the consensus that because *A. tumefaciens* generally delivers only the T-DNA, transgene loci resulting from *A. tumefaciens* infection are less complex than those produced via direct DNA delivery methods. A unique advantage of *Agrobacterium* T-DNA transfer is the accurate processing of the T-DNA between the right and left borders and its precise transfer and integration into the plant genome. Historically, both microprojectile bombardment and *A. tumefaciens* have been used for DNA delivery into either organogenic or embryogenic cultures of peanut (Table 16.2).

Key Elements of Efficient Transformation

Despite significant advances over the past decade, development of efficient transformation methods can take many years of painstaking research. Peanut transformation, like all other transformation systems, relies on common key elements. The major components for the development of transgenic plants are:

1. the development of reliable tissue culture regeneration systems
2. preparation of gene constructs and transformation with suitable vectors
3. efficient techniques of transformation for the introduction of genes into the crop plants
4. recovery and multiplication of transgenic plants
5. molecular and genetic characterization of transgenic plants for stable and efficient gene expression
6. transfer of genes to elite cultivars by conventional breeding methods if required
7. evaluation of transgenic plants for their effectiveness in alleviating the biotic and abiotic stresses in the field condition
8. biosafety assessments including health, food, and environmental safety
9. deployment of genetically modified plants.

Transformation of plants involves the stable introduction of DNA sequences, usually into the nuclear genome of cells capable of giving rise to a whole transformed plant. Transformation without regeneration and regeneration without transformation are of limited value. The very basis of regeneration in tissue cultures is the recognition that somatic plant cells are totipotent (i.e., capable of giving rise to a whole plant) and can be stimulated to regenerate into whole plants in vitro, via organogenesis or somatic embryogenesis, provided they are given the correct hormonal and nutritional conditions (Skoog and Miller, 1957). Adventitious shoots or somatic embryos are thought to arise from single cells and thus provide identifiable totipotent cells that are both competent and accessible for gene transfer and will give rise directly to nonchimeric transformed plants. Transformation techniques reliant on plant regeneration from in vitro cultured tissues have been described for many species (Lindsey and Jones, 1989; Dale et al., 1993; Birch, 1997). There are numerous reports of tissue culture and transformation of peanut from various explants (Kartha et al., 1981; Sastri and Moss, 1982; Kanyand et al., 1994). Regeneration via somatic embryogenesis has also been reported as one of the promising methods for transformation studies in peanut (Ozias-Akins et al., 1993; Sellars et al., 1990; Baker and Wetzstein, 1995; Chengalrayan et al., 1994, 1997).

A suitable system for selection of transgenic tissues and plants is one of the most important aspects of any transformation system. The utility of any particular gene construct as a transformation marker varies depending on the plant species and explant involved.

TABLE 16.2. Genetic transformation in peanut.

Explant	Mode of gene transfer	Strain/plasmid	Gene of interest	Transformation frequency	Reference
Cotyledon	At			3.30%	Rohini and Rao, 2000
Leaf	At	pBI121	gus, nptII	0.2-0.3%	Cheng et al., 1997
Embryonic axis	At	EHA 101/ pMON9793	uidA, nptII	9%	McKently et al., 1995
	Pb			0.9-1%	Brar and Cohen, 1994
	Pb	pAC2MR/ pACH2MR	MerApe9, hph/MerApe9, mercuric ion reductase		Yang et al., 2003
Embryonic axis, cotyledon, leaf, petiole explants	At	pTiBo542/ pTiT37	uidA, nptII		Lacorte et al., 1991
Mature cotyledons	At	PBI121/ pROKII:IPCVcp	IPCV (coat protein)	55%	Sharma and Anjaiah, 2000
	At		H protein gene		Khandelwal et al., 2003
Cotyledons	At	LBA4404/pBI121	uidA, nptII	47%	Venkatachalam et al., 2000
	Pb	pCAMBIA-1301	uidA, hph	1.6%	Yang et al., 2001
	Pb	pMOG617/pxVGH	uidA, hph	168 hygomyin resistant lines recovered	Wang et al., 1998

235

TABLE 16.2 (Continued)

Embryogenic callus	Pb			1%	Ozias-Akins et al., 1993
	Pb	pDO432/pHygr/ pGIN	*Luc, hph hph*	54 independent transgenic lines	Livingstone and Birch, 1995
Immature cotyledon	Pb		*cry 1Ac*		Singsit et al., 1997
Somatic embryos	Pb	pCB13-N+ pCB13-N++	*hph* gene nucleocapsid protein gene of TSWV	52 hygromycin resis-tant cell lines	Yang et al., 1998
Embryonic leaflets	El				Padua et al., 2000
Leaf, epicotyl	At	EHA 101	*uidA*	12-36% (leaves) 15-42% (epicotyl)	Egnin et al., 1998
Leaf discs	At	pBI121	*gus, nptII*	6.7% putative shoots; 20 confirmed sterile transgenic plants	Eapen and George, 1994
Epicotyl	Pb	pKYLX80-N11 pTRA140	*uidA, hph*		Magbanua et al., 2000

Note: At: *Agrobacterium tumefaciens*; Pb: particle bombardment; El: electroporation.

Selection System

Most vectors used for the genetic transformation of plants carry marker genes that allow the recognition of transformed cells by either selection or screening. The most popular selectable marker genes used in plant transformation vectors include constructs providing resistance to antibiotics such as kanamycin and hygromycin, and genes that allow growth in the presence of herbicides such as phosphinothricin, glyphosate, bialaphos, and several other chemicals (Wilmink and Dons, 1993). For successful selection, the target plant cells must be susceptible to relatively low concentrations of the antibiotic or herbicide in a nonleaky manner. Choice of antibiotic and selective concentration varies across different explants and genotypes in peanut. Clemente et al. (1992) have shown kanamycin to be an effective selection agent to select stably transformed callus tissue obtained from immature leaflets of peanut. To date, kanamycin resistance is the most widely used selectable marker. Judicious choice of selection levels may be an important criterion for the recovery of transformed cells, because too high a level would be deleterious even to the transformed cells at initial stages of screening. Preculture of inoculated explants for 2 weeks in the absence of selection was important for enhanced efficiency of transformation, although absence of selection at initial stages may also result in very low recovery of transformants (Moloney et al., 1989).

Screenable marker reporter genes have also been developed from bacterial genes coding for easily assayed enzymes, such as chloramphenicol acetyl transferase (*CAT;* Herrera-Estrella et al., 1983), β-glucuronidase (*GUS;* Jefferson, 1987), luciferase (*LUX;* Olsson et al., 1988), green fluorescent protein (*GFP;* Reichel et al., 1996), nopaline synthase, and octopine synthase (Herrera-Estrella et al., 1988). However, in peanut transformation β-glucuronidase (Jefferson, 1987) is the most widely used screenable marker. The optimization of selection and identification systems is crucial for improving transformation efficiency. The development of a selection system based on hygromycin B greatly increased transgenic soybean production and reduced both the number of nontransformed escapes and time in culture (Olhoft et al., 2003).

Agrobacterium-*Mediated Genetic Transformation*

The naturally evolved unique system of *Agrobacterium* transfers the foreign DNA sequences precisely into plant cells using Ti plasmids. An *Agrobacterium*-based DNA transfer system offers many unique advantages in plant transformation: (1) higher frequency of stable transformation with

many single-copy insertions, (2) a precise transfer and integration of DNA sequences with defined ends, (3) a linked transfer of genes of interest along with a transformation marker, (4) a reasonably low incidence of transgene silencing, and (5) the ability to transfer long stretches of T-DNA.

Preliminary evidence in peanut transformation suggests gene transfer into the calli on coculturing seedling-derived hypocotyl explants with *Agrobacterium* (Dong et al., 1990; Lacorte et al., 1991; Mansur et al., 1993). *Agrobacterium*-mediated transformation using leaf explants of peanut resulted in a transformation frequency of 2 percent (Eapen and George, 1994). The immature embryonic axis has also been employed as an explant for *Agrobacterium*-mediated transformation in peanut. McKently (1995) developed a procedure whereby embryonic axes from mature seeds of peanut cocultivated with *A. tumefaciens* were stably transformed.. Cheng et al. (1997) obtained fertile transgenic plants with 0.3 percent frequency using leaf segments. However, a high transformation frequency was obtained with cotyledonary node explants precultivated on medium for 3 days followed by 4 days of cocultivation with *A. tumefaciens* strain LBA4404 carrying marker *gus* and *npt*II genes (Venkatachalam et al., 1998). Shoot regeneration occurred within 4 weeks. Besides, Yang and co-workers (1998) introduced the nucleocapsid gene of tomato spotted wilt virus along with the *uid*A and *npt*II marker genes in a sense orientation, into peanut variety New Mexico Valencia, using *Agrobacterium*-mediated transformation.

Precultured peanut cotyledons cocultivated for 2 days with *Agrobacterium* strain LBA 4404, harboring pBI121 containing *uid*A and *npt*II genes, followed by transfer on an embryo induction medium containing NAA, BA, kanamycin, and cefotaxime resulted in transformed embryos, which efficiently gave rise to shoots (47 percent) on MS medium containing BA and kanamycin (Venkatachalam et al., 2000). A non-tissue-culture-based transformation method involving direct cocultivation of cotyledon-attached embryo axis with *Agrobacterium* treated with wounded tobacco leaf extract resulted in a stable 3 percent transformation frequency (Rohini and Rao, 2000). An efficient system with high transformation frequency, above 55 percent, based on cotyledon explants forming adventitious shoot buds (>90 percent) has been developed by Sharma and Anjaiah (2000). A number of independently transformed peanut plants with coat protein gene of IPCV were produced by this method. Besides, *Agrobacterium*-mediated transgenic peanut plants expressing the hemagglutinin (H) protein of rinderpest virus have also been developed as an expression system for the delivery of recombinant subunit vaccine through fodder as a means of mass immunization of domestic ruminants as well as wildlife (Khandelwal et al., 2003). More recently, Swathi Anuradha et al. (2006) produced promoter

tagged transgenic plants of peanut using the cotyledonary nodes as explants and a promoterless fusion gene *npt*II:*gus*.

DIRECT GENE TRANSFER

Direct DNA transfer methods can circumvent the genotype dependence of *Agrobacterium* infection. Direct gene transfer has been accomplished by several methods such as microprojectile bombardment, electroporation of protoplasts and intact tissues, microinjection of protoplasts or meristems, and polyethylene glycol–mediated transformation of protoplasts. Of these methods, microprojectile bombardment is the most widely deployed method for genotype-independent genetic transformation. Microprojectile bombardment or particle gun bombardment has a number of characteristics that make it an attractive alternative for DNA delivery in peanut and has been demonstrated as a practical means of introducing a number of agronomically important genes.

Particle bombardment, developed by Sanford and his co-workers (Sanford, 1990; Sanford et al., 1987; Klein et al., 1988), has been successfully used for direct introduction of genes into a number of plant species including peanut. Choice of the explant for bombardment can be made on the basis of criteria such as regeneration potential, favorable metabolic conditions for the expression of a particular genetic construction, or cellular organization that facilitates unambiguous selection of the transformants (Schnall and Weissinger, 1995). Transient expression (Li et al., 1995) and stable transformation have been observed in callus lines from immature peanut leaflet tissue bombarded with microcarrier particles carrying plasmid DNA (Clemente et al., 1992). Of 875 leaflets of the cultivar UPL PN 4 bombarded, 202 kanamycin-resistant calli were recovered but only one untransformed shoot was produced. Similar observations were reported by Schnall and Weissinger (1995) where regenerated plants from slow-growing brown callus as well as green clusters formed by bombarding leaflets did not show any stable transformation. However, bombardment of 1-2-year-old embryogenic callus derived from immature embryos followed by stepwise selection for resistance to hygromycin B in solid and liquid media produced transgenic shoots at a frequency of 1 percent (Ozias-Akins et al., 1993), while the shoot meristems of mature embryonic axis produced transgenic plants at a relatively low transformation frequency of 0.9-1.0 percent (Brar and Cohen, 1994). Transgenic peanut plants expressing the *cry1Ac* gene for resistance to the cornstalk borer (*Elasmopalpus lignosellus*) have been reported (Singsit et al., 1997) by using the somatic embryos from immature cotyledons of peanut bombarded with vectors containing the codon-modi-

fied *cry1Ac* gene along with the *hpt* gene for antibiotic resistance with an efficiency of 0.85 to 2.3 transgenic lines per bombardment. ELISA of Cry1Ac protein from the putatively transformed plants showed the expression of Cry1Ac protein up to 0.18 percent of the total soluble protein. Insect bioassays conducted at a temperature of 27°C, light/dark cycle of 16:8 hours and 70 percent relative humidity also indicated various levels of resistance to *E. lignosellus*. The transient gene expression as assayed by GUS assay has been found to be affected by both particle size and amount of DNA used for coating and to be positively correlated with gene copy number (Lacorte et al., 1997). Livingstone and Birch (1995) efficiently transformed both Spanish and Virginia types of peanut by particle bombardment into embryogenic callus derived from mature seeds, followed by single-step selection for hygromycin B resistance resulting in 3 to 6 independent transformants per bombardment of 10 cm^2 embryogenic calluses with copy number ranging from 1 to 20 with a mean of 4 copies. Recent reports show further increased transformation efficiencies, ranging from 2.6 ± 3.5 to 19.8 ± 18.5 hygromycin B-resistant lines per bombardment (5 cm^2) with fertility rates of 32 percent (Wang et al., 1998).

Among the different genes that have been introduced by particle gun bombardment is the 2S albumin gene from Brazil nut (Lacorte et al., 1997). A high-frequency transformation and regeneration of somatic embryos via microprojectile bombardment has been achieved with constructs containing the *hpt* gene and the nucleocapsid protein (*N*) gene of the lettuce isolate of tomato spotted wilt tospovirus (Yang et al., 1998). The primary transformant containing a single copy of the transgene expressing the N protein, indicating a gene-silencing mechanism operating in the primary transgenic lines with multiple gene integration, has been observed. More recently, peanut lines exhibiting high levels of resistance to peanut stripe virus (PStV) were obtained following cobombardment of embryogenic callus derived from mature seeds of the commercial cultivars Gajah and NC7 with the hygromycin resistance gene and one of two forms of the PStV coat protein (*CP*) gene, an untranslatable full-length sequence (*CP2*) or a translatable gene encoding a *CP* with an N-terminal truncation (*CP4;* Higgins et al., 2004). More recently, Bhatnagar-Mathur et al. (2007) developed transgenic peanut plants by expressing the *AtDREB1A* gene under the stress inducible *rd29A* gene promoter and demonstrated that one of the transgenic events showed 40 percent higher transpiration efficiency than the control plants under water limiting conditions.

Preculture and osmotic treatments have important effects on transformation. Rinsing leaf and epicotyl explants of var. New Mexico in half-strength MS medium prior to infection has been reported as more conducive to

Agrobacterium transformation than the runner-type cultivars. The transient transformation efficiency significantly increased from 12 percent to 36 percent for leaf explants and 15 percent to 42 percent for epicotyls (Egnin et al., 1998). The preculture process influences the competence for transformation of bombarded epidermic cells and subepidermic cells on the adaxial surface of peanut cotyledons. Cotyledons precultured for 3 days on half-strength MS medium followed by 3 hours treatment in osmosis medium before particle bombardment with a plasmid containing a chimeric *hph* gene conferring resistance to hygromycin and a chimeric intron-*gus* gene resulted in a high transformation frequency (Yang et al., 2001). The biolistic-based systems for gene delivery into embryogenic calluses and embryo axes are labor intensive and require the bombardment of a large number of explants to obtain a few transformed cell lines (1 percent) which produce transgenic plants at low frequencies that are often chimeric or result from a few transformation events.

The advantages of particle bombardment system are: (1) DNA may be transferred without using specialized vectors; (2) the introduction of multiple DNA fragments or plasmids can be accomplished by cobombardment, thus eliminating the necessity of constructing a single large plasmid containing multiple transforming sequences; and (3) organelle transformation is achieved only by particle bombardment. Though the biolistic gene delivery system has been successfully used to create transgenics, certain drawbacks of the technique have been observed, for example, high copy number and rearrangements of transgenes, thus causing gene silencing or genomic rearrangements.

Different methods based on biological or direct DNA transfer have been developed for the production of transgenic peanut over the last few years. Padua et al. (2000) employed an electroporation method for direct gene transfer into intact embryonic leaflets of peanut in a modified electroporation buffer (EPRm) supplemented with 75 μM NaCl. A positive effect on the number of shoots and regeneration efficiency was observed using electric strengths of 500-625 v/cm. Research is being carried out globally with single or multiple gene introductions to produce pest-resistant, healthier, and high-quality peanuts.

GENETICALLY ENGINEERED PLANTS FOR PEANUT IMPROVEMENT

Genes for transformation can be broadly divided into those that will be used to overcome agronomic limitations (high yield potential, resistance to biotic and abiotic stresses) and ones that could be used to enhance value-

added traits (Schnall and Weissinger, 1995). Although major emphasis is currently being placed on improving the primary constraints, the manipulation of value-added traits, such as flavor and nutrition will be of much concern for peanut improvement using transgenic technology. Transgenic technology could conceivably be used in peanut for the introduction of disease and pest resistance as well as value-added traits such as improved vitamin, protein, and oil quality, enhancing the crop product value, quality, and safety. The genus *Arachis,* which itself is a repository for most of the valuable pest- and disease-resistance genes, could be used to transform cultivated peanut varieties. Current efforts include incorporating immunity or very high resistance to several viral and fungal diseases through transformation of peanut cultivars that have very high demand for which no adapted resistant peanut genotypes are available. Improved crop protection through the transfer and expression of disease resistance genes will decrease or eliminate the usage of pesticides, which are costly to the grower and may be harmful to the environment.

Fungal and Bacterial Disease Resistance

Peanuts are susceptible to aflatoxin contamination; peanuts contaminated with aflatoxins cannot be used for human consumption. In addition to their direct impact on the yield and quality of agricultural crops, they are often indirectly related to the introduction of plant pathogens, which produce aflatoxins, a group of potent carcinogens. Development and integration of plant resistance, biological control, and genetic approaches for application in localized and area-wide pest management programs is a must for the eradication of this fungus. Peanut produces stilbene phytoalexins in response to fungal infection. Stilbenes inhibit fungal growth and spore germination of *Aspergillus* species and aflatoxin contamination does not occur as long as kernels have the capacity to produce stilbenes. Stilbene synthase has been identified as the key enzyme for the biosynthesis of stilbene. The gene encoding of this enzyme has already been characterized and even successfully expressed in tobacco. Organ-specific expression of multiple copies of a gene for stilbene synthesis is likely to enhance production of stilbenes in peanut kernels and hence make them less prone to colonization by *Aspergillus flavus* and coincident aflatoxin contamination. Hydrolytic enzymes such as chitinases and glucanases, which degrade the fungal cell wall, also pose as attractive candidates for development of disease-resistant peanut plants (Eapen, 2003). A novel approach of introducing microbial toxins (phytotoxins) such as tabtotoxin acetyl transferase and glucose oxidase into the plant has emerged as an efficient way to develop resistance in a wide

range of host species (Eapen, 2003). This approach can be conveniently used to impart resistance against bacterial wilt of peanut caused by *Burkholderia solanacearum,* formerly known as *Pseudomonas solanacearum.*

Virus Resistance

Viruses pose a great threat to peanut production throughout the world. Viruses such as the peanut clump virus, peanut bud necrosis virus (IPCV), groundnut rosette assistor virus (GRAV), peanut mottle virus (PMV), peanut stripe virus (PStV), tobacco streak virus (TSV), and tomato spotted wilt virus (TSVV) cause considerable damage to the crop. Genetic transformation has been used to develop peanut varieties with total resistance and not just tolerance to these viral diseases. The insertion of genetic material from the virus confers resistance to infection by preventing virus replication and spread. The development of genetically transformed peanut cultivars with resistance to viruses and other biotic constraints should have tremendous impact on crop productivity, especially in the resource-poor agricultural systems of the semiarid tropics. Franklin et al. (1993) reported transformed callus expressing the PStV coat protein gene through *Agrobacterium*-mediated genetic transformation. The introduction of coat protein gene of IPCV by using *Agrobacterium*-mediated transformation has led to the production of virus-resistant peanut plants (Sharma and Anjaiah, 2000). Besides, peanut transgenics for resistance to GRAV, TSV, and PBNV are being produced and evaluated in ICRISAT (K.K. Sharma, unpublished results).

Biofortification

Peanut is deficient in the essential amino acid methionine, besides lysine, threonine, and isoleucine, which lowers its dietary and nutritional value. The nutritional quality of peanut can be improved by either raising the level of sulphur-containing amino acids of storage proteins or by changing the proportion of methionine-rich proteins already present in the peanut seed. High methionine levels cannot be produced by conventional breeding methods because of their failure to detect genotypes containing desirable levels of methionine. Hence, genetic transformation is an alternative approach for developing methionine-rich-peanuts. Attempts have been made to produce transgenic peanut plants with improved protein quality by transferring genes like the Brazil nut 2S albumin gene (Lacorte et al., 1997). The regenerated transgenic plants are being tested for the incorporation of the methionine-rich protein genes. The success in peanut transformation technology enabled researchers to address more complex and important aspects

of biofortification in peanut for enhanced levels of beta-carotene (pro-vitamin A) by using bacterial *crtB* and maize *psy1* genes (ICRISAT, unpublished results).

Improvement in Quality of Oil

The long chain saturated fatty acids (LSFAs) amyl arachidic (20:0), behenic (22:0), and lignoceric (24:0), present predominantly in the sn-3 position, have been reported to contribute to arteriosclerosis. If further elongation of stearic acid can be prevented, peanut oil would be free from these hazardous fatty acids. The elongation of the chain behind C18 is catalyzed by membrane-bound enzyme steoryl-CoA-: α-ketoeicosanoyl-CoA synthetase. Engineering a gene coding for antisense RNA in peanut may help reduce activity of this enzyme and hence of LSFA. For enhancing shelf-life of peanut products, a higher oleic/linoleic (O/L) ratio is considered desirable. Increasing the protein of oleic acid in peanut oil can attain this. The introduction of the first double bond in the plant fatty acids occurs by the action of enzyme stearoyl-ACP desaturase. Expression of additional copies of the gene for this enzyme may enhance the content of oleic acid and hence the O/L ratio.

CONCLUSION

Plant regeneration from somatic cells is essential for successful in vitro genetic manipulation techniques, since transformation efficiencies are directly related to tissue culture response. Genetic transformation offers a complementary means to conventional crop breeding, especially for characteristics that are rare or may not be available in the genetic resources of peanut. We consider genetic transformation to be a tool that may allow the breaking of gene transfer barriers for high productivity and nutritional quality of the crop. With the rapid progress in genetic mapping and the isolation of new genes from various organisms, there will be new opportunities to modify plants using a range of genetic strategies. It is important that internationally accepted biosafety standards and local regulatory capacities be strengthened within developing countries. Development and deployment of transgenic plants in an effective manner will be an important prerequisite for sustainable use of biotechnology for crop improvement. The gains in crop productivity through scientific advancement will help to achieve sustainable food security, poverty reduction, and environmental protection. Research on transgenic crops provides new tools to improve agriculture in

areas of the world where low rainfall and biotic stress are the major constraints on crop productivity.

REFERENCES

Atreya, C.D., J.P. Rao, and N.C. Subrahmanyam (1984). In vitro regeneration of peanut (*Arachis hypogaea* L.) plantlets from embryo axes and cotyledon segments. *Plant Science Letters* 34:379-383.

Bajaj, Y.P.S. (1982). Regeneration of plants from pollen embryos of *Arachis*, *Brassica* and *Triticum* species cryo-preserved for one year. *Current Science* 52:484-486.

Bajaj, Y.P.S. and S.S. Gosal (1983). Somatic hybridization and embryo culture studies on *Arachis hypogaea* and *Arachis villosa*. *American Journal of Botany* 70:83-84.

Bajaj, Y.P.S. and S.S. Gosal (1988). Isolation and fusion of protoplasts of *Arachis hypogaea* and Arachis *villosa. International Arachis Newsletter* 3:13-14.

Bajaj, Y.P.S., P. Kumar, K.S. Labana, and M.M. Singh (1981). Regeneration of plants from seedling explants and callus cultures of *Arachis hypogaea* L. *Indian Journal of Experimental Biology* 19:1026-1029.

Baker, C.M. and H.Y. Wetzstein (1995). Repetitive somatic embryogenesis in peanut cotyledon cultures by continual exposure to 2,4-D. *Plant Cell Tissue and Organ Culture* 40:249-54.

Banerjee, S., S. Bandyopadhyay, and P.D. Ghosh (1988). Cotyledonary node culture and multiple shoot formation in peanut: Evidences for somatic embryogenesis. *Current Science* 57:252-257.

Bhatia, C.R., G.S.S. Murty, and V.H. Mathews (1985). Regeneration from de-embryonated peanut (*Arachis hypogaea*) plants from elite cultivars using ACCELL technology. *Plant Journal* 5:745-753.

Bhatnagar-Mathur, P., M.J. Devi, D.S. Reddy, M. Lavanya, V. Vandez, K. Yamaguchi-Shinozaki, and K.K. Sharma (2007). Stress inducible expression of *AtDREB1A* in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water limiting conditions. *Plant Cell Reports* (EPub-ahead of publication).

Birch, R.G. (1997). Plant transformation: Problems and strategies for practical application. *Annual Review of Plant Physiology and Molecular Biology* 48:297-326.

Brar, G.S. and B.A. Cohen (1994). Recovery of transgenic peanut plants from elite cultivars utilizing ACCELL technology. *Plant Journal* 5:745-753.

Cheng, M., D.C.H. His, and G.C. Phillips (1992). In vitro regeneration of Valencia-type peanut (*Arachis hypogaea* L.) from cultured petiolules, epicotyl sections and other seedling explants*. Peanut Science* 19:82-87.

Cheng, M., R.L. Jarret, Z. Li, and J.W. Demski (1997). Expression and inheritance of foreign genes in transgenic peanut plants generated by *Agrobacterium* mediated transformation. *Plant Cell Reports* 16:541-544.

Chengalrayan, K., V.B. Mhaske, and S. Hazra (1997). High-frequency conversion of abnormal peanut somatic embryos. *Plant Cell Reports* 16:783-786.

Chengalrayan, K., S.S. Sathaye, and S. Hazra, S. (1994). Somatic embryogenesis from mature embryo-derived leaflets of peanut (*Arachis hypogaea* L.). *Plant Cell Reports* 13:578-581.

Clemente, T.E., D. Robertson, T.G. Isleib, M.K. Beute, and A.K. Weissinger (1992). Evaluation of peanut (*Arachis hypogaea* L.) leaflets from mature zygotic embryos as recipient tissue for biolistic gene transfer. *Transgenic Research* 1:275-284.

Cucco, M.F. and A.D.R. Jayme (2000). Protocol for regeneration in vitro of *Arachis hypogaea* L. *Electronic Journal of Biotechnology* 3:154-160.

Dale, P.J., J.A. Irwin, and J.A. Scheffler (1993). The experimental and commercial release of transgenic crop plants. *Plant Breeding* 111:1-22.

Dong, J.D., Y.P. Bi, L.S. Xia, S.M. Sun, Z.H. Song, B.T. Guo, X.C. Jiang, and Q.Q. Shao (1990). Teratoma induction and nopaline synthase gene transfer in peanut. *Acta Genetica Sinica* 17:13-16.

Eapen, S. (2003). Regeneration and genetic transformation in peanut: current status and future prospects. In *Applied genetics of leguminosae biotechnology,* P.K. Jaiswal and R.P. Singh (eds.). Dordrecht: Kluwer Academic Publishers, pp. 165-186.

Eapen, S. and L. George (1994). *Agrobacterium tumefacien*s mediated gene transfer in peanut (*Arachis hypogaea* L.). *Plant Cell Reports* 13:582-586.

Egnin, M., A. Mora, and C.S. Prakash (1998). Factors enhancing *Agrobacterium tumefaciens*-mediated gene transfer in peanut (*Arachis hypogaea* L.). *In Vitro Cellular and Developmental Biology–Plant* 34:310-318.

Franklin, C.I., K.M. Shorrosh, A.N. Trieu, B.G. Cassidy, and R.S. Nelson (1993). Stable transformation of peanut callus via *Agrobacterium*-mediated DNA transfer. *Transgenic Research* 2:321-324.

Gill, R. and P.K. Saxena (1992). Direct somatic embryogenesis and regeneration of plants from seedling explants of peanut (*Arachis hypogaea* L.): Promotive role of TDZ. *Canadian Journal of Botany* 70:1186-192.

Gregory, N.C., A. Krapovickas, and P.M. Gregory (1980). Structural variation, evolution and classification in *Arachis*. In *Advances in Legume Science*, R.J. Summerfield and A.H. Bunting (eds.). Kew, UK: Royal Botanical Garden, pp. 469-481.

Hazra, S., S.S. Sathaye, and A.P. Mascarenhas (1989). Direct somatic embryogenesis in peanut (*Arachis hypogaea* L.). *Nature Bio/Technology* 7:949-951.

Herrera-Estrella, L., A. Depicker, M. Van Montagu, and J. Schell (1983). Expression of chimeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* 303:209-213.

Herrera-Estrella, L., T.H. Teeri, and J. Simpson (1988). Use of reporter genes to study gene expression in plant cells. In *Plant molecular biology manual: B1,* S.B. Gelvin, R.A. Schilperoort, and D.P.S. Verma (eds.). Dordrecht: Kluwer Academic Publishers, pp. 1-22.

Higgins, C.M., R.M. Hall, N. Mitter, A. Cruickshank, and R.G. Dietzgen (2004). Peanut stripe potyvirus resistance in peanut (*Arachis hypogaea* L.) plants carrying viral coat protein gene sequences. *Transgenic Research* 138:1-9.

Holmberg, N. and L. Bulow (1998). Improving stress tolerance in plants by gene transfer. *Trends in Plant Science* 3:61-66.

Jefferson, R.A. (1987). Assaying chimeric genes in plants: The *Gus* gene fusion system. *Plant Molecular Biology Reporter* 5:387-405.

Kanyand, M., A.P. Desai, and C.S. Prakash (1994). Thidiazuron promotes high frequency regeneration of peanut (*Arachis hypogaea*) plants in vitro. *Plant Cell Reports* 14:1-5.

Kartha, K.K., K. Pahl, N.L. Leung, and L.A. Mroginski (1981). Plant regeneration from meristems of grain legumes, soybean, cowpea, peanut, chickpea and bean. *Canadian Journal of Botany* 59:1671-1679.

Khandelwal, A., K.J.M. Vally, N. Geeta, P. Venkatachalam, M.S. Shaila, and G. Lakshmi Sita (2003). Engineering hemagglutinin (H) protein of rinder pest virus into peanut (*Arachis hypogaea* L.) as a possible source of vaccine. *Plant Science* 165:77-84.

Klein, T.M., M.E. Fromm, and T. Gradziel (1988). Factors influencing gene delivery into *Zea mays* cells by high velocity microprojectiles. *Biotechnology* 6:923-926.

Lacorte, C., F.J.L. Aragu, E.R. Almeida, E. Mansur, and E.L. Rech (1997). Transient expression of GUS and 2S albumin gene from Brazil nut in peanut (*Arachis hypogaea* L.) seed explants using particle bombardment. *Plant Cell Reports* 16:619-623.

Lacorte, C., E. Mansur, B. Timmerman, and A.R. Cordeiro (1991). Gene transfer into peanut (*Arachis hypogaea* L.) by *Agrobacterium tumefaciens. Plant Cell Reports* 10:354-357.

Li, Z., R.L. Jarret, M. Cheng, and J.W. Demski (1995). Improved electroporation buffer enhances transient gene expression in *Arachis hypogaea* protoplasts. *Genome* 38:58-863.

Li, Z., R.L. Jarret, R.N. Pittman, and J.W. Demski (1994). Shoot organogenesis from cultured seed explants of peanut (*Arachis hypogaea* L.) using thidiazuron. *In Vitro Cell and Developmental Biology–Plant* 30:187-191.

Lindsey, K. and M.G.K. Jones (1989). Plant biotechnology in agriculture. Milton, UK: Open University Press.

Livingstone, D.M. and R.J. Birch (1995). Plant regeneration and microprojectile-mediated gene transfer in embryonic leaflets of peanut (*Arachis hypogaea* L.). *Australian Journal of Plant Physiology* 22:585-591.

Magbanua, Z.V., H.D. Wilde, J.K. Roberts, K. Chowdhury, J. Abad, J.W. Moyer, H.Y. Wetzstein, and W.A. Parrott (2000). Field resistance to tomato spotted wilt virus in transgenic peanut (*Arachis hypogaea* L.) expressing an antisense nucleocapsid gene sequence. *Molecular Breeding* 6:227-236.

Mansur, E., C. Lacorte, and V.G. De Freitas (1993). Regulation of transformation efficiency of peanut (*Arachis hypogaea* L.) explants by *Agrobacterium tumefaciens. Plant Science* 99:89-91.

Martin, J.P. (1970). A contribution to the study of certain hereditary characters of agronomic importance in the groundnut. *Oleagineux* 22:673-676.

McKently, A.H. (1995). Effect of genotype on somatic embryogenesis from axes of mature peanut embryos. *Plant Cell Tissue and Organ Culture* 42:251-254.

McKently, A.H., G.A. Moore, H. Doostdar, and R.P. Niedz (1995). *Agrobacterium*-mediated transformation of peanut (*Arachis hypogaea* L.) embryo axes and the development of transgenic plants. *Plant Cell Reports* 14:699-703.

McKently, A.H., G.A. Moore, and F.P. Gardner (1990). In vitro plant regeneration of peanut from seed explants. *Crop Science* 30:192-196.

McKently, A.H., G.A. Moore, and F.P. Gardner (1991). Regeneration of peanut and perennial peanut from cultured leaf tissue. *Crop Science* 31:833-837.

Moloney, M.M., J.M. Walker, and K.K. Sharma (1989). An efficient method for *Agrobacterium*-mediated transformation in *Brassica napus* cotyledon explants. *Plant Cell Reports* 8:238-242.

Mroginski, L.A. and A. Fernandez (1980). Obtencion de plantulas por cultivo in vitro de anteras de escpecies silvestres *Arachis* (Leguminosae). *Oleagineux* 35:89-92.

Mroginski, L.A., K.K. Kartha, and J.P. Shyluk (1981). Regeneration of peanut plantlets by in vitro culture of immature leaves. *Canadian Journal of Botany* 59:826-830.

Narasimhulu, S.B. and G.M. Reddy (1983). Plantlet regeneration from different callus cultures of *Arachis hypogaea* L. *Plant Science Letters* 31:157-163.

Norden, A.J. (1980). Use of the pedigree breeding method to develop multiline peanut varieties. *Proceedings of Peanut Breeding Symposium.* American Peanut Research and Education Society 1980. Research Report No. 80, North Carolina State University, Raleigh.

Olhoft, P.M., L.E. Flagel, C.M. Donovan, and D.A. Somers (2003). Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. *Planta* 216:723-735.

Olsson, O., C. Koncz, and A.A. Szalay (1988). The use of the *luxA* gene of the bacterial luciferase operon as a reporter gene. *Molecular and General Genetics* 215:1-9.

Ozias-Akins, P. and W.D. Branch (1990). Incompatibility during late embryogeny in some crosses of *Arachis hypogaea-A. stenosperma* and the utility of the tissue culture methods for hybrid rescue. *Proceedings of American Peanut Research and Education Society* 22:51.

Ozias-Akins, P., J.A. Schnall, W.F. Anderson, C. Singsit, T.E. Clemente, M.J. Adang, and A.L.Weissinger (1993). Regeneration of transgenic peanut plants from stably transformed embryogenic callus. *Plant Science* 93:185-194.

Padua, V.L.M., M.C. Pestana, M. Margis-Pinheiro, and D.E. De Oliveira (2000). Electroporation of intact embryonic leaflet of peanut: Gene transfer and stimulation of regeneration capacity. *In Vitro Cell and Developmental Biology–Plant* 36:374-378.

Pittman, R.N., D.G. Banks, J.S. Kirby, E.D. Mitchell, and P.E. Richardson (1983). In vitro culture of immature peanut leaves: Morphogenesis and plantlet regeneration. *Peanut Science* 10:21-25.

Ponsamuel, J., D.V. Huhman, B.G. Cassidy, and D. Post-Beittenmiller (1998). In vitro regeneration via caulogenesis and brassin-induced shoot conversion of dormant buds from plumular explants of peanut (*Arachis hypogaea* L. cv 'Okrun'). *Plant Cell Reports* 11:373-378.

Rao, R.C.N. and S.N. Nigam (2001). Genetic options for drought management in groundnut. In *Management of agricultural drought—agronomic and genetic options,* N.P. Saxena (ed.). New Delhi: Oxford & IBH Publishing, pp. 123-142.

Reichel, C., J. Mathur, and P. Eckes, K. Langenkemper, C. Koncz, J. Schell, B. Reiss, and C. Maas (1996). Enhanced green fluorescence by the expression of an *Aequorea victoria* green fluorescent protein mutant in mono- and dicotyledonous plant cells. *Proceedings of National Academy of Sciences USA* 93:5888-5893.

Rohini, V.K. and K. S. Rao (2000). Transformation of peanut (*Arachis hypogaea* L.): A non-tissue culture based approach for generating transgenic plants. *Plant Science* 150:41-49.

Rohini, V.K. and K. S. Rao (2001). Transformation of peanut (*Arachis hypogaea* L.) with tobacco chitinase gene: Variable response of transformants to leaf spot disease. *Plant Science* 160:889-898.

Sanford, J.C. (1990). Biolistic plant transformation. *Physiologia Plantarum* 79: 206-209.

Sanford, J.C., T.M. Klein, E.D. Wolf, and N. Allen (1987). Delivery of substances to the cells and tissues using a particle gun bombardment process. *Particle Gun Technology* 5:27-37.

Sastri, D.C. and J.P. Moss (1982). Effects of growth regulators on incompatible crosses in the genomes *Arachis hypogaea* L. *Journal of Experimental Botany* 33:1293-1301.

Sastri, D.C., M. Nalini, and J.P. Moss (1980). *Arachis* ovary and ovule culture in vitro. In *Proceedings of symposium on plant tissue culture, genetic manipulation and somatic hybridization of plant cells,* P.S. Rao, M.R. Heble, and M.S. Chadah (eds.). Trombay, India: Bhabha Atomic Research Centre, pp. 366-374.

Schnall, J.A. and A.K. Weissinger (1995). Genetic transformation in *Arachis hypogaea* L. Vol 34, In *Biotechnology in agriculture and forestry,* Y.P.S. Bajaj (ed.). Heidelberg: Springer-Verlag, pp. 135-144.

Sellars, R.M., G.M. Southward, and G.C. Philips (1990). Adventitious somatic embryogenesis and cultured immature zygotic embryos of peanut and soybean. *Crop Science* 30:408-414.

Sharma, K.K. and V. Anjaiah (2000). An efficient method for the production of transgenic plants of peanut (*Arachis hypogaea* L.) through *Agrobacterium tumefaciens*-mediated genetic transformation. *Plant Science* 159:7-19.

Sharma, K.K. and R. Ortiz (2000). Program for the application of genetic transformation for crop improvement in the semi-arid tropics. *In Vitro Cell and Developmental Biology–Plant* 36:83-92.

Singsit, C., M.J. Adang, R.E. Lynch, A. Wang, G. Cardineau, and P. Ozias-Akins (1997). Expressions of a *Bacillus thuringiensis cry IAc* gene in transgenic peanut plants and its efficiency against lesser cornstalk borer. *Transgenic Research* 6:169-176.

Skoog, F. and C.O. Miller (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposium of the Society of Experimental Biology* 11:118-131.

Stalker, H.T. and J.P. Moss (1987). Speciation, genetics, and utilization of *Arachis* species. *Advances in Agronomy* 41:1-40.

Subramanyam, P., J.P. Moss, D. McDonald, P.V. Subba Rao, and V.R. Rao (1985). Resistance to leaf spot caused by *Cercosporidium personatum* in wild *Arachis* species. *Plant Disease* 69:951-954.

Swathi Anuradha, T., S.K. Jami, R.S. Datla, and P.B. Kirti (2006). Genetic transformation of peanut (*Arachis hypogaea* L.) using cotyledonary node as explant and a promoterless *gus::npt*II fusion vector. *Journal of Biosciences* 31:1-12.

Utomo, S.D., A.K. Weissinger, and T.G. Isleib (1996). High efficiency peanut regeneration using a non-imbibed immature leaflet culture method. *Peanut Science* 23:71-75.

Venkatachalam, P., N. Geeta, and N. Jayabalan (1998). Induction of somatic embryos and plantlet development in cell suspension cultures of *Arachis hypogaea* L. *Breeding Science* 48:231-236.

Venkatachalam, P., N. Geetha, A. Khandelwal, M.S. Shaila, and G. Lakshmi Sita (1999). Induction of direct somatic embryogenesis and plant regeneration from mature cotyledon explants of *Arachis hypogaea* L. *Current Science* 77:269-273.

Venkatachalam, P., N. Geeta, A. Khandelwal, M.S. Shaila, and G. Lakshmi Sita (2000). *Agrobacterium*-mediated genetic transformation and regeneration of transgenic plants from cotyledon explants of groundnut (*Arachis hypogaea* L.) via somatic embryogenesis. *Current Science* 78:1130-1136.

Wang, A., H. Fan, C. Singsit, and P. Ozias-Akins (1998). Transformation of peanut with a soybean VspB promoter-*uid*A chimeric gene. 1. Optimization of a transformation system and analysis of GUS expression in primary transgenic tissues and plants. *Physiologia Plantarum* 102:38-48.

Wetzstein, H.Y. and C.M. Baker (1993). The relationship between somatic embryo morphology and conversion in peanut (*Arachis hypogaea* L.). *Plant Science* 92:81-89.

Wightman, J.A. and G.V. Ranga Rao (1993). Groundnut insects. In *The groundnut crop,* J. Smart (ed.). London: Chapman Hall, pp. 394-469.

Willcox, M.C., S.M. Reed, J.A. Burns, and J.C. Wynne (1991). Effect of microspore stage and media on anther culture of peanut (*Arachis hypogaea* L.). *Plant Cell Tissue and Organ Culture* 24:25-28.

Wilmink, A. and J.J.M. Dons (1993). Selective agents and marker genes for use in transformation of monocotyledonous plants. *Plant Molecular Biology Reporter* 11:165-185.

Yang, H.Y, J. Nairn, and P.Ozias-Akins (2003). Transformation of peanut using a modified bacterial mercuric ion reductase gene driven by an actin promoter from *Arabidopsis thaliana. Journal of Plant Physiology* 160:945-952.

Yang, H.Y., C. Singsit, A. Wang, D. Gonsalves, and P. Ozias-Akins (1998). Transgenic plants containing nucleocapsid protein gene of tomato spotted wilt *tospovirus* show divergent levels of gene expression. *Plant Cell Reports* 17:693-699.

Yang, D.X., Z.M. Wei, and H.L. An (2001). Transgenic peanut plants obtained by particle bombardment via somatic embryogenesis regeneration system. *Cell Research* 11:156-160.

Zhang, W.J., S.B. Zhang, S.H. Liu, and L.L. Cai (1999). Somatic embryogenesis and plant regeneration from axes of peanut embryos. *Journal of Tropical and Subtropical Botany* 7:153-158.