Biotechnological Options for Sustainable Groundnut Production : Prospects and Future Strategies

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Modern biotechnological tools for crop improvement in general, and groundnut in particular, have the most immediate potential.

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Despite the significant improvement in production of agricultural commodities worldwide over the last few decades, the challenge of producing sufficient food remains daunting because of the pressure on the production system by biotic and abiotic factors. The 20th century has seen a widespread usage of chemical pesticides, inventions of the human wit for sustaining human needs. Though, use of chemical pesticides has been spectacularly successful, their immediate and long-term flaws draw immediate attention of the scientific communities to devise alternative strategies to combat the menace of epidemics by eco-friendly means.

Groundnut has to continue to be an important commercial crop for the semi-arid and arid zones if, "Production with Sustainability" has to be the motto of Oilseeds-agriculture in India. It contributes about 60-65% of the total edible oil production of the country. To meet the demand of edible oil and to lessen the import bill in the near future, the production of groundnut has to be increased by 2.2% annually. Increasing the productivity can bridge the gap between the present level of production and the future demand. The erratic trend in productivity due to predominantly rain-fed and low input cultivation of the crop, relatively high cost of cultivation, the associated financial risk and the non-availability of quality seed in sufficient quantities, and a tough competition from cheaper oils and easy import of them portend a tough future for groundnut farming in India. Productivity of rain-fed groundnut is also low because of a large number of biotic and abiotic stresses. Increase in the productivity will have two components viz. realisation of existing yield potential by management of stresses and by increasing inherent productivity level. On the other hand groundnut has an advantage over other oilseeds in that it is a highly valued food with excellent nutrient qualities (Table 1). Realisation of existing yield potential by management of stresses in a sustainable manner while keeping the cost down and profitability high is the challenge where biotechnology can play a major role to complement the conventional system. Biotechnology can also be of significance in improving nutritional quality, particularly, alteration of the composition of fatty acids and amino acids and enhancement of the content of carbohydrates. Considering the present constraints of the production and the demand of sustainable use of fertiliser and pesticides, integration of eco-friendly components like application of bio-fertilisers, bio-control agents, and organic matter (through recycling or addition) in groundnut farming could provide stability in production while providing profitability and sustainability to the system. This by definition will include more input use efficiency system-the plant itself or its environment. The use of biotechnological approaches for ameliorating the stresses and introduction and expression of novel gene(s) could provide both an alternative and a supplement to achieve sustainability in groundnut production. Moreover, the recycling and utilisation of the by-products of groundnut for the farm and for the industry and augment the qualities of groundnut as a food and feed can, therefore, diversify the utility of the produce and hence system efficiency.

TABLE 1 : **Nutritional Characteristics of Groundnut**

Characteristics	Content/100g		
	Raw	Roasted	Defatted Flour
Calories (g)	564.0	582.0	371.0
Proteins (g)	26.0	26.0	45.0
Fat (g)	47.5	48.7	5.8
Carbohydrates (g)	18.6	20.6	30.0
Calcium (mg)	69.0	72.0	127.0
Phosphorus (mg)	401.0	401.0	800.0
Iron (mg)	2.1	2.2	3.5
Thiamine (B_1) (mg)	1.14	0.32	0.75
Riboflavin (B_2) (mg)	0.13	0.13	0.35
Niacin (mg)	17.2	17.2	2.5

Source : Burn and Huffmann (1975)

We attempt here to examine the status of biotechnology vis-à-vis the increase in productivity of groundnut by the realisation of its other potentialities.

Two major biotechnological approaches needs examination:

a. Genetically modifying the crop
b. Modifying the environment

GENETICALLY MODIFYING THE CROP

Biotic Stresses

Diseases:

Early leaf spot, late leaf spot, rust, alternaria leaf spot, alternaria blight, collar rot, stem rot, dry root rot caused by fungi, peanut bud and stem necrosis, peanut mottle, peanut clump, peanut stripe caused by viruses, pod and root lesion, and pod and root lesions are some of the important diseases of groundnut, causing considerable yield losses (10-15%). Host resistance is one of the most essential components of Integrated Pest Management strategy, as it is cost effective and environment-friendly. Despite constant efforts, the rate of success in conventional disease resistance breeding so far has been low. Though, some good sources of resistance to foliar diseases are available, a very high degree of resistance has not been able to transfer to a high yielding background mainly because of the inherent complexity of the genetics of resistance.

An encouraging degree of success has been obtained in incorporating resistance to virus pathogen for which resistance is not available even in the wild species. Attempts have been made to transfer coat-protein mediated resistance against tomato spotted wilt virus (TSWV), peanut clump virus (PCV), and peanut stripe virus (PStV), through *Agrobacterium,* particle bombardment, and electroporation.

Though some progress has been made in developing genotypes resistant to viral diseases through breeding means, the progress in imparting resistance to fungal and bacterial pathogens is still in its infancy. Efforts are on to incorporate the genes for chitinase and b-1,3-glucanase in the cultivated groundnut.

The two major alternative strategies being tried today are the incorporation of genes for toxic proteins of bacterial origin, and the other incorporating genes for insecticidal proteins of plant origin.

Insect Pests:

Genes of Bacterial Origin

The bacterial toxins with insecticidal properties are the highly specific Bt toxins active against Lepidoptera, Diptera and Coleoptera but have no toxic effects on other organisms. These are crystalline proteins (Cry) produced by strains of Bacillus thuringiensis. Transgenic crop plants capable of producing the toxin are quite effective in resisting the attack of pests even though only minute quantities of the toxins are produced.

In groundnut, however, only one report is available on the production of transgenics with *cry* gene(s) (Singsit *et al.,* 1997) with an aim at controlling aflatoxin contamination by incorporating resistance against pod borers. Though a good number of cry genes are now available, they have not been screened for the groundnut insect-pests. Recently at NRCG, work has been initiated to screen Bt toxins against leaf miner, a major pest of groundnut in South India, and other foliage feeding insects. New strains of Bt isolates varying in insecticidal properties, are being added every year to the large number of Bt isolates already available.

Thus, there is great scope of identifying the suitable cry genes and incorporating them into the cultivated groundnut to combat the incidence of the economically important insects in an eco-friendly manner.

Genes of Plant Origin

The anti-feedant effects of the plant proteases have been exploited in this approach. The insecticidal action of protease inhibitors has been widely tested and proved against a wide range of economically important field and storage pests including *Lepidoptera, Coleoptera* and *Orthoptera*. Moreover, toxicity towards mammals is not significant (Gatehouse *et al.*, 1991). This approach also remains un-exploited so far in groundnut improvement, and it may open up new vistas in crop breeding using genes of plant origin, with products that are more acceptable and less of ethical problems

Recently other plant products like GNA (the mannose specific lectin snow drops, *Galanthus nivialis*), and even products of animal origin like cholesterol oxidase (Corbin et al, 1996) have been reported to be having insecticidal properties and these genes, being tried for incorporation in other plants, can also be considered for the improvement of groundnut.

Resistance Development

One of the major concerns limiting the adoption and large scale cultivation of transgenic crops is the development of resistance among the insect population against the Bt. Resistance development to Bt pesticides have already been reported in laboratory (Mallet and Porter, 1992). Various strategies were suggested to overcome/manage the resistance development of Bt transgenics (Whalon and McGaughey, 1993). Transgenic plants with multiple genes (pyramiding) and other management strategies of IPM might slow resistance development. The other strategies involve the provision of refuges, ultra high doses of toxin, temporal and spatial expression of the *Bt* genes in transgenic plants, etc.

In case of the protease inhibitors, the ability of the insects to evolve a resistance mechanism based on mutation would be minimal, as the target site of action of the toxin is the catalytic site of an enzyme. The available information on the transformation studies in groundnut is summarised in Table 2.

Bio Safety of the Transformed Plants

The major concerns in this regard are dispersal of the engineered gene from the transformed plants to the environment, ecological consequences resulting from the non target effects, genesis of 'super weeds' and 'super viruses', toxigenic and allergic reactions on human beings and animals, unexpected phenotypes, and the erosion of the biological diversity. Groundnut being a strictly self-pollinated crop, the likelihood of the gene getting in to other groundnut cultivars grown nearby would be minimum. However, if required, this can be taken care of by using trap crops and maintaining isolation distances. Studies are yet to be conducted to find out if any changes are occurring in the non-targeted species. Use of genes for herbicide resistance in transgenics can create problems resulting from the weeds developing herbicide resistance. While choosing a gene construct for transformation, this aspect is to be kept in mind. The threats of formation of super virus are still inconclusive and may have to be thought of in the light of the information, which may be generated in the future. Though groundnut is considered to be a food crop, which has allergenic properties, it is not a problem in India. However,

TABLE 2. **An Update of Genetic Transformation Studies in Groundnut**

Gene(s) used	Delivery System	Target Tissue	Plant Recovery	Reference
TSWV nucleo capsid protein	Biolistics	Shoot meristem of embryonic axis	Transgenic plants, upto R2 generation	Brar et al., 1994
PStV genome	Agrobacterium	Embryonic axis	Putative transformants	Cassidy and Ponsamuel 1996
PStV coat protein	Electroporation	Protoplast callus	Protoplast derived colonies	Li et al., 1996
PCV coat protein	Agrobacterium	Cotyledon and leaf explants	T2 generation with viable seeds	ICRISAT 1996
Cry I Ac	Biolistics	Somatic embryos	Transgenic plants	Singsit et al., 1997
TSWV nucleo capsid protein	Biolistics	Somatic embryos	T2 generation plants	Yang et al., 1998
IPCV coat protein	Agrobacterium	Cotyledon	T1 plants	Sharma and Anjaiah, 2000

TSWV: tomato spotted wilt virus; PStV: peanut stripe virus; IPCV: Indian peanut clump virus specific guidelines for food safety of transgenic crops have already been formulated by DBT, which may have to be adhered to.

Abiotic Stresses

Salinity causes osmotic and ion specific effects leading to reduction in growth and yield. A gene *sal T* appears to have a protective role in plants and the inductive response of this gene to NaCl seems to be organ specific. Proline, one of the most commonly investigated osmoprotectants, induced *sal T* even in the absence of NaCl and had a synergistic effect in its presence. It is visualized that stress induced proline production naturally contributes to the induction of the *sal T* gene. These genes can be profitably exploited in order to evolve groundnut strains that can be more salt tolerant than originally was.

Quality Improvement

A number of quality traits which are now being considered for genetic enhancement include modification of O/L ratio, composition of amino acids and fatty acids, contents of raffinose and stachyose, etc. Log chain fatty acids like arachidic (20:0), behenic (22:0) and ligoceric (24:0) are present in groundnut oil are reported to contribute to atherosclerosis. Prevention of further elongation of steric acid would free groundnut oil from these hazardous fatty acids. Groundnut is poor in S containing amino acids. Hence, enhancement or engineering of the groundnut with the gene(s) encoding the methionine can improve the quality of groundnut. Modification of O/L ratio is essential for prologation of shelf life which can be achieved by expression of additional copies of gene for stearoyl-ACP desaturase. This may, in turn, enhance the content of oleic acid and hence O/L ratio. Aflatoxin load in the final produce is also a major concern considering its carcinogenic effects. Since conventional breeding could not make much headway in solving these problems, biotechnological approaches may be employed for possible enhancement after identification and isolation of the desired gene(s). Biotechnological approaches may be more direct, further less costly than the conventional means. The modifications which may be useful for enhancing quality in groundnut is presented below (from Misra, J. B., 1997):

Modification of the Environment

Modification of the growing environment of the crop includes the temporal or partial shift of the dynamics of the soil microbiota during the crop season and soil health by replenishing the depleted soil carbon and nutrients. Direct use of the beneficial microorganisms, which are normally a part of the natural soil microflora, to promote plant growth and to control plant pests continues to be an area of great attention in sustainable agriculture. The ability of the specific root colonizing bacteria, or rhizobacteria, to increase growth and yield of crop plants currently is

TABLE 3. **Gene(s) to be Engineered for Enhancing the Quality of Groundnut**

Objective	Modifications	Gene/activity to be engineered	Transgenic species with a similar approach
Prolongation of shelf-life	Increase in oleic acid	Stearoyl desaturase	Transgenic tobacco with yeast (Polashock 1992) and rat (Garyburn 1992)
Reduction in the risk for atherosclerosis	Reduction in long chain saturated fatty acids	Antisense of Stearoyl-CoA: B-ketoeicosanoyl CoA synthetase	Transgenic Brassica by antisense expression of stearoyl-ACP desaturase gene (Knutzon *et al.*, 1992)
Reduction in flatus properties	Reduction in raffinose and stachyose	Galactinol:sucrose-6-galactosyl transferase	Not yet attempted
Reduction in aflatoxin load	Increase in stilbenes	Stilbene synthase	Transgenic tobacco (Hain *et al.*, 1990)
Improvement in nutritive value of protein	Increase in polypeptides rich in S-containing amino acids	Gene encoding Brazil nut methionine-rich protein	Transgenic tobacco (Altenbach *et al.*, 1989)

attracting considerable attention. In the era of rhizosphere manipulation, modification of both the host and its microenvironment are essential to attain maximum benefit by harnessing nutrients from a limited pool and by providing healthy environment for growth. Though a myriad of microorganisms are present in a given ecosystem in a balanced manner, the introduction of a group of beneficial microorganisms by artificial means can shift the dynamics spatially and temporally towards beneficial organisms to exhibit desired effect with high sustainability at a low cost. The immediate need of the hour is to enhance the efficiency of the meagre amount of the externally applied inputs and make the best use of the nutrients present in the soil system to achieve sustainability in a production system. Use of plant growth promoting rhizobacteria especially fluorescent pseudomonads increased the groundnut growth and yield substantially (Pal *et al.* 2000 b)

Considering the above facts, application of the biofertilizers, biocontrol agents and recycling of the organic matter could stabilize the sustainable production of groundnut in an eco-friendly manner. Utilization of the groundnut by-products for producing industrially important products by microbial means will enhance the profitability of the produce further and will enhance the efficiency of the production system.

Biofertilizers / Biopesticides / Biocontrol Agents:

The status of the microorganisms used in groundnut is given below:

Genetic Enhancement

Use of biotechnological and genetic engineering tools can enhance the efficiency of the organisms further. Erratic biological nitrogen fixation in groundnut is a major problem because of being nodulated by the inefficient native rhizobia. Attempts are now being made to isolate more efficient and competitive strains which can eliminate the native inefficient strains which are otherwise nodulating in a large number. The competitiveness traits like production of antibiotics (e. g. trifolitoxin) in *R. leguminosarum* has been isolated, cloned and expressed in non-producing strains for enhancing the nodulation competitiveness. Similar approach can also be adopted in groundnut once competitiveness factor is identified. The alternative strategies which are emerging now-a-days include introduction of an addition copy of *nif* genes.

TABLE 4. **Status of Biofertilizers / Biocontrol Agents in Groundnut**

Organisms/ Antagonists	Nature	Utility	Aagainst Pathogens / Pests
Bradyrhizobium *Bacillus polymyxa*	Nodulating bacteria	N_2-fixation	-
Pseudomonas striata	Phosphate solubilizing bacteria	Inorganic phosphate solubilization	-
Pseudomonas fluorescens	Plant growth promoting rhizobacteria	N_2-fixation, Inorganic phosphate solubilization, iron availability, biocontrol against pathogens, IAA production	*Aspergillus niger* *Aspergillus flavus* *Sclerotium rolfsii*
Vesicular-Arbuscular Mycorrhizae	Root colonising fungi	P and micronutrient uptake, drought tolerance	-
Bacillus subtilis	Antifungal rhizobacteria	Biocontrol	*Aspergillus niger*
Trichoderma harzianum	Antifungal	Biocontrol	*Aspergillus niger* *Sclerotium rolfsii*
Trichoderma hamatum	Antifungal	Biocontrol	*Aspergillus niger* *Sclerotium rolfsii*
Penicillium islandicum	Antifungal	Biocontrol	*Cercospora* leaf spot
Verticillium lecanii	Antifungal	Biocontrol	*Cercospora* leaf spot
Bacillus thuringiensis var. kurstaki	Entomo-pathogen	Biocontrol	*Heliothis* spp. *Spodoptera* spp. *Helicoverpa* spp.

As symbiosis is governed both by the host and micro-symbiont, plant factor has also to be considered to develop superior cultivars with high nodulation. As non-nodulating lines are available and the specific genes are known, signal molecules (flavonoids/ isoflavonoids) that trigger the *nod* D functions can be identified and be transferred to cultivated varieties for better nodulation and nitrogen fixation by the inoculants strains.

The production of organic acids by microorganisms is reported to be responsible for phosphate solubilization. Development of regulatory mutants by chemical mutagens can overproduce the organic acid responsible for phosphate solubilization constitutively and thus better phosphate solubilization.

The genes responsible for siderophore production (in *E. coli* and *Pseudomonas fluorescens*) and IAA production in *Azospirillium brasilense* are known and have been isolated and cloned. Similarly, chitinase genes from *Serratia mercecens* have been isolated and cloned in *E. coli.*

Therefore, there is a great potential of expressing these genes heterologously from constitutively expressed promoters in rhizosphere competent PGPR strains to enhance the production of IAA and siderophore for plant growth promotion and nutrient uptake, and to enhance the biocontrol abilities against *A. niger, A. flavus* and *S. rolfsii.*

The enhancement of the production of IAA and siderophore can also be made by altered expression by inserting promoter (ptac) upstream of biosynthesis genes of IAA and siderophore or by gene dosage by increasing the copy number in the wild type of the plant growth promoting rhizobacteria.

Biotechnological tools are now being used for studying the survival, dispersal and tracking down the introduced organisms in the environment. Molecular markers like *lacZ, gus A, ina Z, lux AB, xyl E* and TFD monooxygenase are used for majority of the organisms (Pal *et al.* 2000a). For evaluating the rhizosphere competence of the plant growth promoting rhizobacteria and competitiveness of rhizobia, *lac Z* is now being used at NRCG under controlled condition.

The beneficial effects of PGPR involve an array of mechanisms and multiple genes. Therefore, the transfer of PGP genes into plants will be very difficult. Understanding and unravelling the bases of plant growth promotion by PGPR will encourage any speculation in this direction.

Reported development of resistance in the pest population can limit the large-scale application of *Bacillus thuringiensis.* To overcome this problem, expressing the Cry proteins in *Pseudomonas fluorescens* has evolved an alternative strategy. This organism is also found on the leaf surface abundantly. Mycogen has commercialized two such *Pseudomonas fluorescens* formulations containing Cry proteins active against catterpillers. In groundnut also, attempts can be made for expressing both Cry and chitinase proteins in leaf colonizing *Pseudomonas fluorescens* for showing both insecticidal and fungicidal action against both the pests and leaf diseases.

As genetic manipulation and transfer of biocontrol genes in fungi are very difficult, bacterial antagonists can be engineered with multiple biocontrol traits production of antifungal antibiotics, chitinase etc. for better biocontrol abilities. Plant growth promoting rhizobacteria are the possible candidates.

Ecological, Ethical and Environmental Issues Regarding Organisms and GMO:

There is misperception regarding the Bt and its proteins. However, worldwide testing of Bt on its persistence and all kinds of toxicity against human, animals and birds failed to provide any evidence of its harmful effects. Bt is degraded very rapidly when exposed to UV light. Its half-life under normal sunlit conditions is 3.8 hours. Because this material readily biodegrades in the environment, it poses little or no disposal problem. Bt is rapidly inactivated in soils that have a pH below 5.1. Because of their rapid biological breakdown and low toxicity, they pose no threat to groundwater.

However, genetic engineering techniques involve the use of high dose of antibiotic resistance maker for selection of the transformants/clones. If the desired genes are not incorporated into chromosome, the antibiotic resistance genes used for cloning the desired genes can be transferred to pathogenic organisms through natural transformation systems and can cause health hazards.

If the alien gene transfer is transposon based, it is generally incorporated into the chromosome of the recipient strains. Though the genes after incorporation into chromosome of the recipient strains become stable, horizontal and verticle transfer of the genes into other organisms via natural transformation process can not be ruled out. This is why one should be very cautious while using such genes.

Maintaining the Balance of Organic Carbon

Soil organic carbon content is the determinant of the maintenance of nutrient dynamics in the soil and its health. Continuous cultivation and depletion of organic carbon are threatening the equilibrium. It is highly essential to maintain the balance of the organic carbon in soil to stabilize the soil health for ensuring the sustainable crop production. The ways by which it can be maintained is replenishing the organic carbon by recycling the crop residues and application of organic matter. Two types of crop residues are available, legume and non-legumes. Leguminous residues are easily decomposable because of low C:N ratio. But the C:N ratio of the non-legume residues are very high and thus takes lot of time to decompose. Thus, the efficiency of the decomposition of the non-leguminous crop residues can be enhanced by using organisms having constitutive expression of cellulase enzymes and supplementing the residue with N source. This will help, to some extent, in maintaining the organic C (depleted during the preceeding crop) in the soil for the succeeding crop.

Utilization of Groundnut By-Products:

The crop residues after harvest can be recycled in agriculture not only to conserve energy but also to minimize pollution. Large quantities of agricultural wastes, at present, are either left completely un-utilized or used in an uneconomic manner. Thus, there is a need for promoting more economic as well as purposeful utilization of agricultural residues.

Groundnuts, as they are produced, have an outer thick woody shell which constitutes 20-40% of the pod weight. Even at the lowest level of 20%, the annual availability of (with the production of around 75 lakh tonnes of groundnut per annum) shells would be around 15 lakh tonnes in India. At present, most of the shell is utilized as fuel either directly or in combination with dung. Thus, there exists a great scope for utilizing shells for more useful purposes. The major constituent of groundnut shell is cellulose (65%). Soluble carbohydrates account for about 21% of the shell. Thus, the constituents of shell can be commercially exploited for the production of alcohol, organic acids and cellulases by employing cellulolytic and fermentating organisms like *Phanerochete chrysosporium, Aspergillus niger* and *Saccharomyces cerevisae.*

As bulk of the groundnut produced in the country is crushed for oil expulsion, the oil-cake that is left behind is another major byproduct of groundnut. Expeller cakes generally contain oil around 6%. The residual oil is extracted through solvent extraction, leaving cakes rich in protein (60%) and starch (12.5%). The available starch in the de-oiled groundnut cake can be used as a substrate for the production of a-amylases by using *Bacillus licheniformis.* On the other-hand, the proteins present in the cake can be used as substrates for the proteolytic action of microbes like *Bacillus licheniformis* for the production of proteases.

Attempts are now being made at the NRCG, for utilizing the groundnut shell/cakes for producing of organic acid, cellulases, amylases and proteases. All these products have a number of applications in industry and pharmaceuticals. Therefore, this will enhance the profitability of the produce and diversity and sustainability of the production system as a whole.

FUTURE STRATEGIES

Modern biotechnological tools for crop improvement in general, and groundnut in particular, have the most immediate potential. However, the major biotechnological emphasis has to be given to the following areas for sustainable production of groundnut in an eco-friendly manner.

- Development of transgenics showing resistance to economically important pests, diseases and abiotic stresses
- Development of transgenics for enhancing the nutritional quality of groundnut
- Development/construction of strains for enhanced biocontrol ability against pathogens like *A. niger, A. flavus* and *S. rolfsii*
- Development/genetic enhnacement of biofertilizer of beneficial microbes for enhancing the nutrient use efficiency in groundnut under low input systems
- Genetic enhancement of groundnut or *Bradyrhizobium* or both for the enhancement of biological nitrogen fixation
- Enhancement of the decomposition potential of the crop residues by constitutive expression of the cellulase genes in potent cellulolytic organism as cellulase is under catabolic repression
- Exploring the possibilities of identifying potent microbes for utilizing groundnut shell/cake for producing cellulase, proteases, a-amylases, organic acids and alcohols etc. Once the organisms are identified, the efficiency of those organisms can be enhanced further by genetic engineering or mutations.

While applying the molecular and biotechnological methods for crop enhancement, equal importance should also be given to risk assessment from all the possible threats to be posed by the transgenics to avoid environmental hazards and to monitor the hazards of the genetically modified microbes.

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