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Review on; Application of Biotechnology on Peanut (Arachis hypogaea L.) Improvement

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Abstract

The extent of crop improvement dependent on the number of genes that control the trait of interest. Quantitative traits are very difficult to transfer under conventional plant breeding techniques, because they are polygenic traits. Segregation occurs at a large number of loci affecting a trait in such kinds of polygenic trait inheritance. The phenotypic expression of polygenic traits is highly affected by the variation in environ- mental factors to which plants in the population are subjected. However, conventional plant improvement has been contributing a lot for the existing peanut breeding achievements. This method takes about five to twelve years to develop new variety, thus it could not be able to address the increasing world population food demand as well as future crop improvement programs. Peanut has cross incompatibilities and ploidy barriers between diploid wild and tetraploid cultivated along with poor agronomic performance of interspecific material. In such scenario, different cell and tissue culture techniques, genomics and genetic engineering methods needs to be implemented in an integrated manner with conventional improvement approach. Therefore, genes of interest can be efficiently incorporated into new crop varieties. This paper reviewed the developments and applications of plant tissue culture and molecular biology and genetic modification in peanut improvement.

Keywords; Genetic transformation; Molecular markers; Peanut; Tissue culture

INTRODUCTION

Peanut is allotetraploid (2n = 4x = 40) with little polymorphism at the molecular level (*Janila et al., 2016)*, with a large genome (3.2Mb). It has originated through a single hybridization and polyploidization event. It is valued for its good quality cooking oil, energy and protein rich food, and nutrient-rich fodder (Acquaah, 2012), which grown in over 100 countries with 33.16 million ha with a total production of 63.34 million tons during 2018 (FAOSTAT, 2019). The production of peanut is being threatened by both biotic

and abiotic factors. These factors still have not been mitigated solely through conventional plant breeding approaches. Transfer of desired traits using conventional breeding is a time consuming and difficult task due to the cross incompatibilities and ploidy barriers between diploid wild and tetraploid cultivated along with poor agronomic performance of interspecific material (Holbrook *et al.*, 2011), which is based on attempting crosses between desirable parents followed by selection of promising recombinants in subsequent segregating generations. Repeated cycle of selection is required for fixation of the genotype to produce true to type breeding lines. This procedure is time consuming and tasks anywhere 6 to 8 years (Chopra and Sharma, 1991). Repeated cycle of selection of plants resulted in a highly narrow genetic base of the cultivated species (Young *et al*., 1996). Only few qualitative traits such as resistance to Sclerotinia blight, root-knot nematode and Tomato Spotted Wilt Virus (TSWV) are improved through conventional breeding, which is benefitting US peanut producers >$200 million annually (George, 2012; Stalker and Wilson, 2016). Nowadays, tissue culture and molecular genetic technology has been employed on peanut (*Arachis hypogaea*L.) cultivar development, but lack of investment, low levels of molecular polymorphism among cultivated varieties are the major challenges. Recent advances in biotechnology/molecular genetic technology have allowed researchers to more precisely measure genetic polymorphism and enabled the development of low-density genetic maps for *A. hypogaea* and the identification of molecular marker or QTL's for several economically significant traits (Sharma *et al*., 2002; Corley *et al*., 2011). Genetic maps for diploids and tetraploids have been developed using Simple Sequence Repeat (SSR) and Diversity Array Technology (DArT) markers (Pandey *et al*. 2012; Varshney *et al*., 2013). Generally, Marker Assisted Selection (MAS) has been achieved in peanut breeding for most important traits like high yield, high oil content, high oleic acid, resistance to leafspot, rust, bacterial wilt, Tomato Spotted Wilt Virus (TSWV), Peanut Rosette Virus (GRV), aflatoxin contamination and drought stress (Mallikarjuna and Varshney, 2014). Peanut genetic transformation has been accomplished by several different methods. In general, there are two methods, Direct DNA transfer methods such as, biolistic and electroporation, and indirect DNA transfer method through

Agrobacterium-mediated have been used for genetic transformation. It enables to crate peanut varieties having biotic and abiotic resistance, vaccine producing ability etc. (Janila*et al., 2016)*. Genetically modified peanut accommodates higher kernel yield, biomass and better resistance to biotic and tolerant to abiotic stresses as compared to wield relatives. Transgenics has the potential for improving the plants with desired traits. Stress tolerant peanuts could provide an opportunity to the restoration of loss due to severe drought or salinity conditions (kishor*et al*., 2018). This paper reviewed the application of biotechnological techniques on peanut improvement.

BIOTECHNOLOGICAL IMPROVEMENT OF PEANUT

Nowadays, biotechnology has been implemented on peanut improvement, but lack of investment, and low levels of molecular polymorphism among cultivated varieties are the most challenges (Sharma *et al*., 2002). It helps to shorten the time taken by conventional breeding, which is 10 to 15 years. With the help of biotechnology, it is possible to transfer genes of interest from distantly related organisms and nearly any organism, including plants, animals, bacteria, or viruses, and introduce those genes into another organism. An organism that has been transformed using genetic engineering techniques is referred to as a *transgenic* organism, or a genetically engineered organism (sun *et al*., 2013). Peanut genomic breeding requires identification of genes/QTLs linked to traits of interest. The first step in genomic breeding is development of mapping populations (Pandey *et al*., 2012).

A. MOLECULAR TECHNIQUES

i. Diversity analysis and QTL mapping

Genetic diversity analysis is the basis for molecular breeding. Peanut genomic breeding requires identification of genes/QTLs linked to traits of interest, first it requires mapping populations development. Different study showed that, there was very low levels polymorphism among cultivated peanut accessions using random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) markers, DNA amplification fingerprinting (DAF), and isozymes (Grieshammer and Wynne, 1990; Kochert*et al*., 1991; Bhagwat *et al*., 1997; He and Prakash, 1997; Subramanian *et al*., 2000; Stalker, 2001). Similarly, Moretzsohn*et al*. (2004) confirmed that there was low level of polymorphism among Brazilian cultivars using microsatellite markers. However,

Microsatellites have become one of the most widely used molecular markers for genetic studies in recent years (Edwards *et al*., 1996).

QTL mapping can increase biological knowledge of the inheritance and genetic architecture of quantitative traits. It was initiated with the development of DNA (or molecular) markers (Bernardo, 2008). According to li *et al*. (2019) study on QTL mapping on cultivated peanut using SLAF-seq (specifc length amplifed fragment sequencing) techniques having 2,808 markers on the 20 LGs with a total length of 1,308.20 cM and an average inter-marker distance of 0.47 cM, they identified a total of 39 QTLs associated with growth habit-related traits using RIL population. Similarly, QTLs for oleic acid (C18:1), linoleic acid (C18:2) and the ratio of oleic acid to linoleic acid (O/L) were identified and positioned on linkage groups (Hu *etal.*, 2018). On the other hand, Liu *et al*. (2020) identified Seven QTLs for oil content were identified on five linkage groups.

ii. Marker assisted breeding

Quantitative trait improvement is difficult to transfer and takes longer time through conventional breeding. However, marker-assisted selection (MAS) techniques have been overcome such problems and many genes can be pyramided either for the same trait or for different traits along with faster recurrent parent genome recovery through intense background selection. MAS can be used to transfer many recessive genes in less time than is possible through conventional breeding (Pandey *et al*., 2012). Marker assisted breeding (MAB) developed peanut variety was registered in 2003.

Fatty acid composition is the most determinant factor in peanut nutritional quality of oil and storage period. High oleic acid trait improvement using different marker technologies has been developed (Chen *et al*., 2010). Similarly, Root-knot (M. arenaria) resistance also improved through molecular markers linked with root-knot nematode resistance (Simpson, 2001).

B. TISSUE CULTURE

Genetic improvement of peanut is dependent on the establishment of a high efficiency in vitro regeneration system. Many studies have reported the establishment of peanut regeneration systems using different explants and medium compositions (Shan *et al*., 2009), like longitudinally halved cotyledons with removed plumule and radical (Hassan *et al*., 2013); and leaf sections, cotyledonary nodes, longitudinal cotyledon halves,

embryo axes, embryo leaflets, and hypocotyls have been tested for *A. tumefaciens* transformation. *A. tumefaciens* gene transfer uses apical or axillary meristematic cells in these tissues allow for multiple shoot regeneration. However, conditions for adventitious shoot formation through organogenesis vary widely, and cocultivation protocols with or without virulence inducing agents (Sharma and Anjaiah, 2000). Grain legumes in general and Vigna species in particular are highly recalcitrant to in vitro regeneration (Jaiwal and Singh, 2003). According to Matand*et al*. (2013) study, peanut cotyledon tissue might be more efficiently manipulated *in vitro* for increasing multiple shoot formation, similar to standard explants such as leaf, stem, or embryo axis. They suggest that both cotyledon and root might not be the tissues of choice for callus formation.

i. In vitro regeneration

Grain legumes in general and *Vigna* species in particular are highly recalcitrant to in vitro regeneration (Jaiwal and Singh, 2003). Extensive studies on peanut in vitro culture and plant regeneration have been implemented to addressed different issues involved in plant tissue culture. Regeneration rate depends on selection of proper explants, basal medium composition, types and concentrations of plant growth regulators, and type of explants as well as the culture conditions. Different types of explants are investigated in peanut regeneration studies, from them mature seeds, hypocotyls or epicotyls from mature seeds have been selected for several studies (Shan *etal*., 2009). The embryonic leaflet is more juvenile than other explants, so it is easy to differentiate and dedifferentiate. According to Li *et al*. (2008) experiment, they used 2,4-D for somatic embryo induction from leaflets on different varieties, they showed different frequencies of embryogenesis. The shoot induction rate reached up to 81.5% in suitable medium. however, there was significant differences between two genotypes for regeneration. Cotyledons have been used as peanut regeneration explants in the 1990s.

ii. Callus formation

Experimental result on peanut experiment indicates, callus formation varied based upon explant type, preparation, and treatment. Good friable callus was only observed in diced cotyledon explant, but both cotyledon and root might not be the tissues of choice for quality callus formation. Transformation efficiency was evaluated based on callus age and whisker quantity on. Transformation efficiency

(6.88%) was highest when 200 mg of whiskers were used with 5 µg plasmid for 2 g of 20-day-old callus (Holbrook *et al*., 2011).

iii. Shoot organogenesis in root and cotyledonary explants

peanut root tissue has been successfully used to induce direct adventitious plant formation *in vitro*. The greatest response observed (65 shoots/root explant) occurred in explants treated with 20 mg/l BA. Range of regeneration was 2 to 65 shoots per explant. (Matand*et al.,* 2013).

Research finding showed that the dry-mature cotyledon is potentially a reliable tissue for *in vitro* micro-propagation, when proper preparation and conditioning are applied. However, shoot organogenetic responses differ with explant and treatment. Similar observations have previously been reported in peanut and other plant species. Except di-side-cut cotyledon, all explants tested formed shoots *in vitro*. Mono-side-cut cotyledon was overall the most responsive explant for direct shoot formation, considering that 92% of the treatments applied to this explant, excluding the control, caused shoot formation (Vadawale*et al*., 2011).

C. GENETIC TRANSFORMATION OF PEANUT

i. Peanut transformation systems

Genetic transformation of peanut has been accomplished by several different methods. In general, there are two methods, Direct DNA transfer methods such as, biolistics and electroporation and indirect DNA transfer method through Agrobacterium-mediated have been used for genetic transformation in peanut (Cattivelli*et al*., 2008). The same techniques that have been used in other crops can be used in peanut (Zhang *et al*., 2004)

Two most commonly used means of delivering genes to plant cells are via *Agrobacterium tumejaciens*or direct gene transfer using microprojectile bombardment. The selection of delivery means is determined by several factors including the laboratory facilities and technical skills available, the species and/or cultivar to be transformed (Baker *et al*., 1995).

a. microprojectile bombardment

Microprojectile bombardment involves the coating of gold or tungsten particles with DNA and accelerating them at high velocity into target plant tissue, then the DNA particles will penetrate into the cell and integrate to plant genome. Cells that survive the impact and are able to regenerate can give rise to whole

transgenic plants (Baker *et al*., 1995). Micro bombardment technique is the first successful transformation of peanut with accompanying plant regeneration. Micro-bombardment has since been completed in peanut with a number of genes conferring disease resistance (Yang *et al*., 1998). However, its efficiency levels remain low and the process takes several months from when the initial transformation event is induced until plant maturity (Egnin*et al*., 1998) cited in (Cattivelli*et a*l., 2008). To date, biolistic methodologies are more reliable in peanut than other transformation methodologies and single constructs can be inserted into the peanut genome (Holbrook *et al*., 2011).

b. Agrobacterium tumefaciens

Genetic transformation of peanut needs highly efficient and faster technique, thus *Agrobacterium*-mediated transformation appears to offer the possibility to achieve this goal, and it is the most common for introduction of foreign genes in to selected plants. *Agrobacterium tumefaciens* is a soil-borne bacterium, which causes tumours to infected plants through the integration of part of the plasmid, the tumour inducing (Ti) plasmid (Gardner *et al*., 1991; Holbrook *et al*., 2011).

Explants like leaf sections, cotyledonary nodes, longitudinal cotyledon halves, embryo axes, embryo leaflets, and hypocotyls were used for *A. tumefaciens* mediated peanut transformation. From these tissues apical or axillary meristematic cells are used for multiple shoot regeneration for gene transfer. However, conditions for adventitious shoot formation through organogenesis vary widely, and cocultivation protocols with or without virulence inducing agents (Sharma and Anjaiah, 2000).

D. ACHIEVEMENTS IN PEANUT GENETIC TRANSFORMATION

Genetic transformation is one of the modern technologies which enhance the introduction of desired genes of the desired trait into plants for manipulating several beneficial traits associated with crop improvement. This technology creates a path to transfer important genes into peanut genome for enhancing resistance against fungal, viral pathogens, other pests, drought, and salinity as well as silencing undesirable genes and improvement in nutrient acquisition (Mallikarjuna*etal*.,2016).

a. Oil content improvement

Peanut kernel is known for its high oil content, which contains 36% to 54% oil, 16% to 36% protein and 10 to 20% carbohydrates (Gregory *et al*., 1980).Oil content Improvement has been a major

target of peanut breeding programs. Transgenics using key regulator of Fatty Acid biosynthesis, the *AtLEC1* gene (Guiying*et al*., 2018) and *AhLPAT2 gene* (Chen *et al*., 2015), can provide peanuts with higher oil content and heavier seeds than the untransformed control. Previous studies indicated that, the overexpression of some seed development-related genes, such as *LEC1*, *LEC1-like*, and *WRI1*, via either constitutive expression or expression at a higher level could increase the oil content in dicots and monocots, but also led to a series of disorders of agronomic traits (Mu et al., 2008; Shen et al., 2010; Tan et al., 2011). There was no phenotype difference observed in transgenic *Arabidopsis* overexpressing LEC1-like genes of *B. napus*. However, transgenic seedlings showed markedly reduced growth when germinated and grown in the presence of estradiol (Mu et al., 2008). Additionally, the overexpression of *ZmLEC1* gene under two promoters, a strong *OLEOSIN* (*OLE*) promoter and a weaker *EARLY EMBRYO PROTEIN* (*EAP1*) promoter, similarly increased the seed oil accumulation and embryo size. However, it causes reductions in seed germination and leaf growth (Shen et al., 2010). According to Chen et al., (2015), they elaborated that seed-specific overexpression of *AhLPAT2* in Arabidopsis results in a higher percentage of oil in the seeds that leading to higher oil yield per plant.

b. Fatty acid improvement

Fatty acid composition is the most determinant factor in peanut nutritional quality of oil and storage. It has mainly eight fatty acids such as, oleic, linoleic, palmitic, stearic, arachidic, eicosenoic, behenic and lignoseric. Among them, oleic acid, a monounsaturated fatty acid and linoleic acid, a polyunsaturated fatty acid account for 75 to 80% of the total fatty acids in peanut oil, the remaining 20% is contributed as other fatty acids, among them; palmitic acid (10%) has the largest proportion (Kavera, 2008). High oleic acid resulting in high oleic/linoleic acid (O/L) ratio which is responsible for longer stability or shelf life. Cultivars with high O/L ratio, low oil/fat and high protein are suitable for confectionary purpose (Kavera, 2008). Because of high oleic content peanut oil can be excellent cooking medium and stored at room temperature for 18 months without significant deterioration in quality (Misra*et al.*, 2000). Chen *et al*., (2015) confirmed that, over expression of *AhLPAT2* gene increased the total fatty acid (FA) content and the proportion of unsaturated FAs also increased.

Marker assisted back crossing introgression lines of elite peanut genotypes study showed that, there was the possibility to increase in oleic acid up to 97%, and reduce linoleic acid content up to 92% as compared to recurrent parent (Bera*et al*., 2019). Huang *et al*. (2019) elaborated that, in superior genotypes reduced linolenic content was obtained up to 6%. According to Pasupuleti et al., (2016), study also showed oleic acid increased by 0.5–1.1 folds, with concomitant reduction of linoleic acid by 0.4–1.0 folds and palmitic acid by 0.1–0.6 folds among ILs compared to recurrent parents.

c. Drought-Tolerant Peanut development

Peanut cultivation is challenged by several abiotic stresses. Abiotic stresses bring about morphological, physiological, biochemical, and molecular changes in the plant systems. Environmental stresses such as drought and salinity are major factors that limit peanut production in the world (Stansell and Pallas, 1985).

Genetically engineered crops that enhance stress tolerance could be a promising approach to address the challenges faced in the peanut crop. Drought stress can be modified using genes like DREB1A, DREB2A, ABF, MuWRKY3, AtHDG11, IPT, NHX1, SbNHXLP, AVP1, and SbVPPase, (Kishor *et al.* 2018), *DREB, PDH45, NAC, mtlD, NHX* *(*Mallikarjuna et al., 2016), it is necessary to search for new genes and transcription factors.

According to Kishor *et al*. (2018), study on over expression of transcription factor AtDREB1A gene based, they identified transgenics have similar transpiration rate was identical to that of wild-type plants. Transpiration efficiency (TE) was higher in transgenic events with a lower stomatal conductance. On the other hand, transgenic peanut with the AVP1 gene, which resulted in increased salt and drought stress tolerance and also higher yields under reduced irrigation conditions (Park *et al.* 2005; Qin *et al*. 2013). AVP1-transgenic peanut exhibited 37% higher yield on an average in comparison with the wild-type plants, which showed the potential for enhanced yield production when grown under water-limited (Qin *et al*. 2013).

Abiotic stress related genes isolated from Sorghum bicolor conferred drought and salt tolerance in transgenic peanut plants. Transgenic peanut plants (variety JL-24) overexpressing S. bicolor plasma membrane-bound sodium proton antiporter-like protein (SbNHXLP) exhibited higher biomass, yield,

chlorophyll, proline, K^+ but lower Na^+ content under salt stress conditions (Venkatesh, 2016). According to Amareshwari, (2017), transgenic peanut plants (JL-24 variety) osmolytes and proline accumulation. over-ex-pressing vacuolar H^+ -pyrophosphatase (SbVPPase) isolated from S. bicolor recorded elevated levels of Na^+ and K^+ ions in roots, stems, and leaves and displayed altered root phenotypes under both drought and salt stress conditions.

Sharma's group in India reported that transformed peanut using Arabidopsis transcription factor gene called *AtDREB1A* into a drought sensitive peanut line can increase drought tolerance. The transcription factor *AtDREB1A* acts onstress responsive genes, thereby activates expression of DRE (dehydration responsive elements) containing genes under stressful conditions. One transgenic line demonstrated a 40% increase in transpiration efficiency (TE) in a greenhouse drought tolerance test (Holbrook *et al*., 2011: Kambiranda*et al*., 2011; Datta *et al*., 2012).

d. Biomass improvement

Drought stress causes early senescence in plants, which is advantageous for plants to survive under severe drought conditions in nature as they can quickly finish their life cycle (Taiz and Zeiger, 2002). Under drought conditions delayed senescence could reduce the yield penalty. Genetically engineering peanut using cytokinin biosynthetic gene (*IPT)* for drought tolerance, which encodes iso-pentenyl transferase, an enzyme that plays a critical role in a rate-limiting step of cytokinin biosynthesis (sun *et al*., 2013). Transgenic lines demonstrated improved biomass retention in a greenhouse drought tolerance test and an average of 58% yield increase in a two-year field test (Holbrook *et al*., 2011).

Transgenics with *IPT* gene showed that, there was no difference between wild-type and transgenic peanut plants under normal growth conditions for agronomic traits, but the experiment under reduced irrigation conditions showed that, there was higher yield than wild-type peanut. *AtNHX1* over expression could also improve biomass (sun *et al*., 2013).

e. Salt-Tolerant Peanut development

Peanut growth is very sensitive to salt because it is a glycophytic plant. Therefore, salt tolerant variety improvement is vital. In 1999, overexpression of *AtNHX1* that encodes the vacuolar membrane-bound sodium/proton (Na+/H+) antiporter in Arabidopsis could improve salt tolerance

in transgenic plants. The increased Na+/H+ antiporter activity could lead to increased Na+ sequestration into vacuole, which reduces Na+ toxicity in cytoplasm and at the same time reducing water potential in the vacuole, leading to increased salt tolerance in *AtNHX1*-overexpressing plants (Zhang and Blumwald, 2001). This approach was successfully used to increase salt tolerance in other plant species such as tomato, rapeseed, cotton, and soybean. According to sun *et al*. (2013) experiment in both field and green house, they introduced *AtNHX1* into peanut and observe peanut could tolerate up to 150 mM NaCl in soil. Additionally, amounts of biomass could increase, as a result photosynthetic rates and stomatal conductance will be higher during salt treatment.

f. Creation of Both Drought and Salt Tolerant Peanut

Recently, researchers demonstrated that by overexpressing an Arabidopsis vacuolar pyrophosphatase gene *AVP1*, they could increase drought and salt tolerance simultaneously in transgenic peanut plants. Gaxiola's group demonstrated that overexpression of *AVP1* in transgenic plants could increase both drought- and salt-tolerance. The increased drought tolerance in the *AVP1*- over expressing plants was due to robust root development, which is caused by increased auxin polar transport in transgenic plants. (Kambiranda*et al*., 2011; Datta *et al.*, 2012). Whether or not *PSARK*:*IPT*-transgenic peanut and *AVP1*-expressing peanut plants would increase peanut yield under field conditions in large-scale trials is not known (Sun *et al*., 2013).

g. Disease resistance peanut development

i. Fungal resistance

Fungal diseases are majorly affecting peanut production and quality through aflatoxin production, which is carcinogen produced by Aspergillus species. To overcome this problem several genes were introduced into peanut through genetic engineering. Peanut kernel produces stilbean phytoalexins in response to fungal infections and it has been shown to inhibit fungal growth and spore formation. Stilbene synthase has been isolated from peanut and expressed in tobacco resulted in production of resveratrol (Hain *et al.*,1990).

Overexpression of a tobacco glucanase gene in peanut has increased its resistance towards Cer-cosporaarachidicola and Aspergillus flavus in three peanut cultivars, JL 24, ICGV 89104 and ICGV 86031 (Sundaresha*et al.*, 2010). Peanut plants expressing b-1–3,glucanase gene

showed the enhanced fungal disease resistance evaluation of some transgenic lines was advanced to field studies such as resistance to *Sclerotinia minor*. Synthetic *cry*1 EC and cry1AcF gene transformed peanut was shown to confer resistance to the larvae of *Spodoptera litura*(Keshavareddy et al., 2013; Hassan *et al*., 2016).

ii. Virus resistance

The most common yield determinant viruses of peanut are Peanut Stripe Virus (Pstv), Indian Ground Nut Rosette Virus (GRV), Peanut Clump Virus (IPCV), Peanut Bud Necrosis Virus (PBNV), Tobacco Streak Virus (TSV) and Peanut Mottle Virus (PMV). No sources of resistance to this virus were found in more than 10 000 accessions of the world *Arachishypogaea*germplasm collection in collaboration with ICRISAT (sun *et al*., 2013).

Tobacco Streak Virus (TSV) resistance could be improved by introducing Tomato spotted wilt virus nucleocapsid protein (N gene). Most diseases caused by the virusescould be minimized by coat protein genes. Coat protein of IPCV was also introduced into peanut through Agrobacterium mediated transformation and obtained lines resistance to Indian peanut clump virus (Sharma and Anjaiah, 2000). The transgenic peanut plants expressing the TSV-Coat Protein (TSV-CP) gene were developed and these plants showed resistance against PSND virus under field conditions up to the T_3 generation. These transgenic lines showed minimal symptoms, which indicated their tolerance against TSV infection (Mehta *et a*l. 2013).

iii. Insect resistance development

Insect pests on peanut remain a great challenge to manage. Crystal (Cry) genes derived from *Bacillus* thuringenesis are being widely used to develop insect resistant plants. It was first transformed into peanut for cornstalk borer resistance (Singsit*et al*. 1997). Peanut with chimeric Btcry1AcF and synthetic Cry1EC genes showed resistance against Spodoptera litura (Tiwari *et al*. 2008; Keshavareddy*et al*. 2013). A synthetic cry8Ea1 gene, which is effective against Holotrichia parallela larvae, was expressed in peanut roots and transgenics exhib-ited insecticidal activity (Geng*et al*. 2012). Vain *et al*. (1998), confirmed that *OC*-1 gene expression in transgenic plants confer resistance to coleopterans and nematode *Globodera pallida* ssp (Vaine*t al*., 1998).

iv. Vaccine production

peanut transformation has a potential application in vaccine development and

peanut allergen silencing. Recent development in the transformation technology and controlled an efficient expression of foreign genes in plants have resulted in the development of transgenic plants for producing edible vaccines for chronic infections, inhibiting allergies, and for producing therapeutic antibodies. Urease subunit B (UreB) under the control of oleosin promoter has been overexpressed in peanut through Agrobacterium mediated transformation. Edible oral vaccine has been produced for controlling the human bacterial pathogen Helicobacter pylori through transgenics (Yang *et al*., 2011).

v. Allergen silencing

Peanut could cause allergies and it is a serious challenge in food processing industries. Peanut caused Immunoglobulin E (IgE) mediated allergic reactions in 0.6 % of total population and children are more sensitized (Sicherer*et al*. 2003). There are eleven peanut proteins that have been identified of which Ara h 2 and Ara h 6 were shown to be potent peanut allergens, they are silenced by the introduction of RNAi construct targeting homologous coding sequence. Ara h 2 was shown to have some trypsin inhibitor function, but silencing Ara h 2 did not promote *Aspergillus flavus* fungal growth. However, to date no released peanut cultivars are transgenic for Allergen silencing (Holbrook *et al*., 2011).

vi. Bio-fortification studies in peanut

It is a new technique to enriching nutritional values in staple food crops to combat malnutrition through breeding and transgenic approaches. Bio-fortification studies has a particular importance to the undernourished millions of people in the developing countries. Peanut is a poor source of essential sulfur containing amino acids like methionine, iron, zinc and vitamin A, which are limiting its nutritional value. A gene coding for 2S albumin seed protein that is enriched with methionine from Brazil nut was characterized (Gander *et al*., 1991). Expression of 2S albumin gene for enriching methionine content can improved peanut, which was detected by ELISA (Lacorte*et al.* 1997). Bioavailability of nutrients like iron, zinc, vitamin A in daily consuming foods could be a solution to health problems like anemia or cataract especially in developing countries (Shen *et al*. 2014).

II. CONCLUSION

Peanut is a very rich source of edible oil, proteins and essential biochemical products which have a major economic

importance. Its production of peanut is being threatened by both biotic and abiotic factors. These factors still have not been mitigated solely through conventional plant breeding. Recent advances in biotechnology/molecular genetic technology have created an opportunity for researchers to more precisely measure genetic polymorphism and enabled the development of low-density genetic maps for peanut. QTL's were identified for several economically important traits. Nowadays, Genetic modification strategies are being employed to improve biotic and abiotic stresses. Peanut plants have been regenerated from explants such as mature and immature embryonic axes, cotyledons and leaves by either organogenesis or embryogenesis.

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