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REVIEW

Genetic engineering for peanut improvement: current status and prospects

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Abstract Peanut is an important source of edible oil, dietary minerals, and vitamins, and proteins. Environmental stresses like biotic and abiotic factors are main constraints for peanut production and productivity. Generation of resistant varieties against these stresses can lead to quantum leap in the crop productivity. Potential biotechnology approaches through engineering and expression of novel genes can improve stress tolerance/resistance mechanisms that ultimately lead to crop adaptation and yield enhancement. There are several genetic engineering techniques are adopted, among them *Agrobacterium* mediated and biolistic methods are widely employed for transforming peanut varieties. Expression of abiotic stress related genes or transcription factors like *DREB*, *PDH45*, *NAC*, *mtlD*, *NHX* etc. in different peanut varieties resulted in enhanced tolerance to drought, salinity, temperature extremes and osmotic adjustments. In addition, expression of transgene could regulate the biochemical pathways which lead to scavenge free radicals, lipid peroxidation, increase photosynthetic efficiency, water use efficiency and transpiration rate. To address the biotic stresses, genes like glucanase, chitinase, chloroperoxidase, coat proteins, crystal proteins, and pathogen related genes have been expressed in peanut which exhibited enhanced resistance towards different fungal, bacterial pathogens and pests. Interestingly, plant based edible vaccines and therapeutic antibodies are produced in plants through the expression of *urease B* and *oleosin* to combat chronic infections. Allergy is a prime concern in peanut that is to be inhibited through RNA interference mechanisms. We review here factors influencing the transformation, recent progress in peanut transgenic research, development and future perspectives.

Keywords *Agrobacterium* · Biolistic · De embryonic cotyledons · *β-Glucuronidase* (*uidA*) · Biotic · Abiotic stress · Edible vaccines · Allergens

Introduction

Legumes are the most important crops next to cereals which are a vital source of dietary proteins and edible oil. Among the grain legumes, peanut (*Arachis hypogaea* L.) is the fifth most important oil seed crop in the world after soybean, cotton, sunflower and rapeseed. It is also known as groundnut, earthnut, monkey-nut or goobers nut, which is native of South America (Brazil). It is widely cultivated in tropical and subtropical regions of Asia, Africa and North and South America. Peanut is cultivated on over 25.67 million hectare worldwide with a total production of 42.31 million metric tons with an average yield of 2.34 metric tons per hectare (FAOSTAT 2014). Yields of peanuts are mainly limited by abiotic stresses like extreme temperatures, drought, soil factors and biotic stresses like pod borers, aphids, mites as insect pests and leaf spots, rusts and toxin producing *Aspergillus* fungus among the diseases (Kumar and Kirti 2015a; Sundaresha et al. 2010).

Researchers across the world have been employing several approaches for crop improvement against biotic, abiotic stresses, nutrient use efficiency and nutritional value of the produce. Significant improvements of different traits are achieved through conventional and Marker

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Assisted Selection (MAS). However, these approaches have certain limitations. The progress of peanut breeding has been hampered due to the non-availability of sources of desired traits and low level of polymorphism in cultivated varieties. The genus *Arachis* contains several wild relatives of the cultivated peanut along with the allotetraploid species, *A. monitcola* and *A. hypogaea* in the section *Arachis.* The diploid species are a rich repository of genes for resistance against various biotic and abiotic stresses. However, breeders were not able to access these genes because of the genetic load carried by the introgression lines derived from the crosses of the wild relatives with the cultivated peanuts. The introgression was associated with undesirable gene blocks making it unuseful so far in peanut improvement. This problem can be overcome through the identification of suitable genes from the wild peanuts through differential gene expression studies and genetic transformation of these genes is the best option in long term peanut improvement (Kumar and Kirti 2015a, b).

Genetic transformation allows introduction of desired genes across species barrier (Sharma and Anjaiah 2000). Many successful genetic transformation protocols have been reported in peanut via *Agrobacterium tumefacience* (Sharma and Anjaiah 2000; Tiwari et al. 2008, 2011; Bhatnagar et al. 2010), *Agrobacterium rhizogenes* (Geng et al. 2012) and biolistic/particle bombardment (Singsit et al. 1997; Chu et al. 2008a). Employing *Agrobacterium* as a tool for transformation, both tissue cultured—callus mediated and *in planta* protocols have been successfully adopted. Transgenic research has picked up the momentum during last decade and resulted in development of several transgenic lines with novel traits and improved plant performance. However, no transgenic peanut has been released for commercial cultivation so far. Nevertheless, peanut is recalcitrant to regeneration and genetic transformation posing several hurdles for fresh researchers. In this review, we summarize different methods of peanut regeneration, factors influencing transformation efficiency, successful attempts of transformation with improved agronomic traits as a guide to beginners and underscore the areas of research that needs focus in near future.

Factors influencing the regeneration and transformation

Transformation and regeneration are influenced by various physical and chemical factors such as choice of genotypes, explants, co-cultivation time, virulence inducing agents, hormonal combinations and selectable markers (Manjulatha et al. 2014). An ideal transformation protocol should be qualified as the genotype independent and rapid, with minimal presence of chimeras in the regenerated transgenic plants, with high frequency of transformation. Various factors influencing transformation efficiency are listed in Tables 1, 2 and 3.

Explants

Transformation and regeneration are highly influenced by the choice of explant (Cheng et al. 1996; Bhatnagar-Mathur et al. 2008; Singh and Hazra 2009). Cotyledon, cotyledonary node, de-embryonated cotyledon (DEC), embryonic axes, leaflets, hypocotyls, epicotyls, auxiliary bud, embryogenic calli, immature leaves, zygotic embryos etc. are being used as explants for generating transgenic plants through somatic embryogenesis and organogenesis. Among these explants, DEC or cotyledon explants are the most amenable for regeneration. Even the recovery of transformed multiple shoots was ($\sim$10) higher in DEC when compared to immature embryos (Mehta et al. 2013). Highly efficient in vitro regeneration protocols (>90 %) were developed using DEC explants of peanut (Tiwari et al. 2008). Tiwari et al. (2008) optimized a genotype independent protocol using DEC explants which performed superior in shoot bud formation and high regeneration. The orientation of DEC explants and auxin polarity on shoot induction medium played the key roles in efficient regeneration (Tiwari et al. 2008). The probability of multiple shoot induction from cut surface of DEC (half cotyledon) explants was significantly high in comparison with full cotyledons. However, the Immature zygotic embryos were pre-treated with an osmotic solution (0.4 M Mannitol) for 4 h and 80 % of hygromycin resistant leaflets were showed positive for GUS activity and PCR (Deng et al. 2001). In another study, genetic transformation protocol was optimized using hypocotyls, cotyledons and cotyledonary node explants. However, the highest frequency of transformation was observed with cotyledonary node (58 %) upon these explants incubated for 4 weeks of culture on 75 mg l^{-1} kanamycin (Venkatachalam et al. 1998).

Duration of co-cultivation and *vir* gene inducers

During co-cultivation, *Agrobacterium* infects the plant cells, simultaneously gene construct mobilizes into the genome through *vir* gene induction (McCullen and Binns 2006). It is a very crucial and common step in conventional tissue culture as well as *in-planta* transformation approaches. Co-cultivation period was optimized by incubating the explants with the *Agrobacterium* cells at different time intervals. For instance, shoot regeneration was significantly higher in cultures co-cultivated for 24 h, whereas the regeneration efficiency decreased gradually in cultures that were co-cultivated for 48 h or more, due to increased

Table 1 Tissue culture based *Agrobacterium* mediated transformations

Variety	Strain of *Agrobacterium*	Gene/source	Explants	Marker	% of regeneration/transformation	Factors influencing regeneration and transformation efficiency	Reference
JL-24 TMV-2 TAG-24 Dh-3-30	EHA101, LBA4404	*Uid-A/E.coli*	De-embryonated cotyledons (DEC)	*hpt*	81 %	Orientation, period of co-cultivation (5 days), anti oxidant (L-Cysteine)	Tiwari and Tuli (2012)
JL-24	C58	*Chitinase*/rice, *phytoene synthase*/maize	DEC	Marker free	75 %	Nature of the genes and promoters used	Bhatnagar et al. (2010)
JL-24	GV2260	*Uid-A/E.coli*	Embryogenic axis	*npt II*	31 %	Hormonal combinations of BAP, 2,4-D	Anuradha et al. (2006)
JL-24 Spanish (J-11 ICGS-11) Virginia (Robut-33-1 ICGS-76 ICGS-44)	C58	*IPCV Cp/Indian peanut clump furovirus,* *Uid-A/E.coli*	DEC	*npt II*	55 %	Cotyledons halves with induced shoot buds	Sharma and Anjaiah (2000)
Valencia Florunner Georgia Runner Sunrunner South runner	*EHA101*	*Uid-A/E.coli*	Leaflets and epicotyls	*npt II and hpt*	12 % to 36 % for leaf lets, 15–42 % for epicotyls	Polarity of explants and co-cultivation media with high auxin and low cytokinin promoted higher transformation	Egnin et al. (1998)
VRI-2 TMV-7	LBA4404	*Uid-A/E.coli*	Hypocotyl, epicotyl, axillary bud, immature leaves, cotyledonery nodes	*npt II*	58 %	Pre-culture for 3 days	Venkatachalam et al. (1998)
New Mexico (Valencia)	EHA105	*β-Glucuronidase (uidA)/E.coli*	Leaflets	*npt II*	0.2–0.3 at T_1 generation	Hormonal combinations of BAP, 2,4-D	Cheng et al. (1996)
New Mexico (Valencia)	None	None	Zygotic embryos, hypocotyl and cotyledon, root segment, internode segment, leaf	Marker free	None	Thidiazuron	Kanyand et al. (1994)
Tatu (Valencia group)	A281(pTDO2) A281(pTDE4)	*Uid-A/E.coli*	Leaf (apical half) Leaf (basal half) EC DEC	*npt II and bar*	56 % 35 % 3 % 5 %	Co-cultivation period	Mansur et al. (1993)

Table 2 *Agrobaterium* mediated *in-planta* transformations

Variety	Strain of *Agrobacterium*	Gene/source	Explants	Marker	% of transformation	Factors influencing regeneration and transformation efficiency	References
Luhua11 Huayu16 Huayu28 Baisha1016 Yuanhua8 Xinhua1 Xinhua5	*A. rhizogenes* K599	*Cry8Ea1/Bacillus thuringiensis, GFP/ Aequorea victoria and Uid-A/E.coli*	Embryonic axes	*hpt*	61 %	Co-cultivation period, acetosyringone concentration	Geng et al. (2012)
TMV-2	LBA4404	*Uid-A/E.coli*	Embryonic axes	*npt II*	3.3 % *gus* positive	Wounded tobacco leaf extract and time of infection	Rohini and Rao (2000)

Table 3 Biolistic or particle bombardment transformations

Variety	Tool for transformation	Gene/source	Explants	Marker	% of transformation	Factors influencing regeneration and transformation efficiency	References
Georgia Green	Spermidine, protamine	*Ara h1/peanut, Uid-A/E.coli*	Zygotic embryos	*Hpt*	64	Embryogenic tissue and desiccation during bombardment	Chu et al. (2013)
Luhua 9 YueYou 116	Gold particle, helium	*Uid-A/E.coli*	Zygotic embryos	*Hpt*	80	Pre-culture and osmosis	Deng et al. (2001)
Florunner Florigiant	Gold beads (ACELL gene delivery system)	*Uid-A/E.coli, tswv-np*/tomato spotted wilt virus	Shoot meristems of mature embryonic axes	*Bar*	44	Different concentrations of DNA with gold particles	Brar et al. (1994)
Toalson Florunner	Gold particle and helium	*Uid-A/E.coli*	Embryogenic callus	*Hpt*	1	Osmotic shock	Ozias-Akins et al. (1993)

necrosis in the explants. In spite of this, 72 h co-cultivated explants showed highest (31 %) transformation frequency and also evidenced by transient GUS staining (Anuradha et al. 2006). Co-cultivation of *Agrobacterium* with leaf and cotyledon explants for 48 h in solid medium exhibited higher transformation efficiency rather than liquid medium (Mansur et al. 1993).

Transfer of T-DNA is regulated by *vir* (Virulence) gene expression, which is induced by phenolic compounds. Supplementation of phenolic compounds or signaling molecules in co-cultivation medium has been used to enhance *vir* gene expression, which increases the transformation efficiency in some cases. Acetosyringone, a *vir* gene inducing chemical has been successfully tested to aid in the transfer of genes. Various concentrations of acetosyringone were tested ranging from 30 to 200 μM for different plant species (Chen et al. 2006; Costa et al. 2006). Geng et al. (2012) suggested the optimum concentration of acetosyringone as 50 μM for *A. rhizogenes* mediated peanut transformation. In some cases, supplementation of acetosyringone did not show any effect on increasing transient *gus* gene expression (Mansur et al. 1993), whereas addition of wounded tobacco leaf extract induced *gus* gene expression in transgenic plants (Cheng et al. 1996; Rohini and Rao 2000).

Phyto hormones and anti-oxidants

Endogenous hormones of plants can be modulated by the external supplementation of growth regulators, which can improve regeneration efficiency of in vitro cultures (Tripathi et al. 2013). Exogenous supplementation of

auxin/cytokinin ratio in shoot induction medium enables regeneration of shoots and roots. Thidiazuron induced multiple shoots with high frequency and repetitive production of multiple shoots from different kinds of explants (Kanyand et al. 1994). Supplementation of antioxidants in the medium can protect cells from oxidative stress by scavenging free radicals (Dutt et al. 2011). Supplementation of 100 mg l^{-1} L-Cysteine during co-cultivation minimized the oxidative stress generated from cut surface of DEC explants by scavenging free radicals. Moreover, it enhanced the transient transformation efficiency 4.5 fold (Tiwari and Tuli 2012). The antioxidants such as glutathione, DL-α-tocopherol and selenite were enabled to increase the regeneration and transformation efficiency, which confirmed by GUS assay. Significantly, these transgenic peanut plants exhibited enhanced levels of superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities (Qiusheng et al. 2005). Apart from hormones, optimized concentration of antibiotics such as augmentin, and timentin in combination with cefotoxime reduced the *Agrobacterium* over growth and subsequently helped in inducing multiple shoots with high frequency (Tiwari and Tuli 2012).

Selectable markers

Binary vectors used in genetic transformation carry antibiotic or herbicide marker genes, which allow the selection of putative transformed cells. Optimal concentration of selection pressure and choice of appropriate antibiotics are important components for recovery of transformed cells. Supplementation of antibiotics at higher levels could be deleterious for the transformed cells even at the initial stage. However, exclusion of antibiotics at initial stage resulted in very low frequency of transformation (Moloney et al. 1989). Efficiency of genetic transformation based on proper selection of marker genes allows the recovery of transformed cells. Usually kanamycin, hygromycin, phosphinothricin, bialaphos, glyphosate and spectinomycin are the preferred selectable agents used in plant transformation. Callus tissue derived from immature leaflets of peanut was stably transformed in kanamycin selection medium and *npt II* demonstrated as an effective selective agent (Clemente et al. 1992). Hygromycin based system has been optimized for recovery of large number of fertile transformants in three months duration (Olhoft et al. 2003; Tiwari and Tuli 2012). McKently et al. (1995) reported that *npt* II marker system was not successful in selecting the transformed tissues, it failed to eliminate untransformed plants resulting in the occurrence of false positives and chimeras (McKently et al. 1995; Cheng et al. 1996; Sharma and Anjaiah 2000; Dodo et al. 2008). Genetically transformed shoots were recovered on 175 mg l^{-1} kanamycin medium and the presence of *npt II* gene and a single copy number in putative transformed lines were confirmed by PCR and Southern blot analysis (Anuradha et al. 2006).

Methods of peanut transformation

Genetic transformation technology for transfer of novel genes into the peanut genome made a new platform for agronomic trait improvement or functional validation of genes. A decade research has led to development of protocols for transformation and regeneration in peanut. In general, *Agrobacterium* mediated and particle bombardment methods are widely used for genetic transformation of peanut. Apart from these, direct generation of plantlets showed great advantages of being genotype independent, which can be achieved through *in planta* or germ line transformation methods.

Tissue culture based *Agrobacterium* mediated transformation

Agrobacterium transformation is widely accepted technology in peanut transformation in comparison to other techniques. *A. tumefaciens* mediated transformation was initially based on tissue culture method and takes less time (usually 4–5 months) to obtain primary putative transgenic plants. Various strains of *Agrobacterium* such as LBA4404, EHA105, EHA101, C58 and A281 were used for the transformation of peanut. *Agrobacterium* mediated transformation protocols have been standardized using different explants including leaf sections, zygotic embryos, cotyledonary nodes, embryo axes, leaflets, DEC and hypocotyls (Li et al. 1997; Tiwari and Tuli 2012; Anuradha et al. 2008). Transformation of peanut was standardized by using zygotic embryo axes of matured seed via *Agrobacterium* EHA101 carrying binary vector with *α-glucuronidase* (*uidA*) and *npt II* genes which resulted in 9 % of seedlings confirmed with *gus* positive shoots and T-DNA was shown to be integrated in the progeny of T_1 and T_2 generations (McKently et al. 1995).

Cotyledonary nodal explants of VR1-2 and TMV-7 were infected with *Agrobacterium* (LBA4404) strain carrying *uid A* and *npt II,* which resulted in 58 % of regeneration frequency (Venkatachalam et al. 1998). First fertile transgenic plants were obtained in New Mexico Valencia variety in which leaflet explants infected with EHA105 strain showed 10 % regenerated on selection medium as *gus* positives and also these plants showed stable integration of transgenes (*uid A* and *npt II*) at T_1 generation with 0.2-0.3 % of transformation frequency (Cheng et al. 1996).

Cotyledonary node explants of JL-24 variety were co cultivated with GV2260 strain carrying *p35SGUSINT* and

npt II construct and resultant plants showed 3.54 % of transformation frequency, which was confirmed through PCR and Southern blotting (Anuradha et al. 2006). Bhatnagar-Mathur et al. (2007) reported *Agrobacterium* (C58) mediated transformation using the DEC of JL-24, where 75 % of plants appeared to be PCR positives for the *DREB1A* gene. Another transformation using EHA101 carrying *35S Intron-uidA* construct with DEC of JL-21 recorded 81 % of transformation efficiency (Tiwari and Tuli 2012).

Another species *A. rhizogenes*, which is the causative agent of hairy root disease, was used for transformation of peanut to study the root nodule formation and resveratrol synthesis (Akasaka et al. 1998; Sinharoy et al. 2009). *A. rhizogenes* mediated transformation of peanut was standardized as a genotype independent protocol with four optimized parameters including embryonic axes as explants, *Agrobacterium* culture density at (at OD_{600}: 0.6), acetosyringone 50 μM l^{-1} and the co-cultivation time of 2 days, which achieved up to 61 % of transformation efficiency (Geng et al. 2012). The embryogenic axes along with cotyledons of peanut were injected with the suspension of *A. rhizogenes* (K599) harboring *Cry8Ea1* gene and expressed it in the roots, which conferred resistance against the beetle *Holotrichia parallela* (Geng et al. 2012).

Agrobacterium mediated transformation offers unique advantages such as higher efficiency of stable transformation with single copy integrations, low occurrence of gene silencing, transfer of gene of interest linked to transformation marker and ability to transfer longer fragments of T-DNA. Despite the advantages of this method, recurring problems like less frequency of gene integration and low regeneration of transformants might lead to development of false positive/transgene escape and sterility in regenerated plants (Sharma and Anjaiah 2000; Tiwari et al. 2008, 2011).

In planta transformation

In planta transformation is a non-tissue culture based method for generating transgenic plants. In general, embryo axes of peanut are excised, and further wounded by pricking with a fine needle to infect with *Agrobacterium* and grow the plants under field conditions. Embryo axes of mature seeds were pricked and infected with *Agrobacterium* and then incubated in the presence of tobacco leaf extract and these explants were reported to be 3.3 % of *gus* positive by histochemical assay and PCR (Rohini and Rao 2000). Significantly, *in-planta* approach has been attempted widely for validating the novel biotic and abiotic stress genes in peanut (Keshavareddy et al. 2013; Pandurangaiah et al. 2014; Manjulatha et al. 2014). Recently, Kumar and Kirti (2015a) reported the transformation of *AdSGT1* (Suppressor of G2 allele of Skp1) gene into JL-24 variety through *in-planta* method and generated late leaf spot pathogen resistant transgenic peanut plants. In another investigation, 2 days old peanut seedlings were immersed and co-cultivated with *Agrobacterium* in liquid and solid medium for 3 days respectively and kanamycin resistant plants were primarily confirmed by PCR analysis of *gus* and *npt II* genes and twelve lines exhibited *MuNAC4* gene integration and drought tolerance even in T_5 generation (Pandurangaiah et al. 2014). Apical meristem of K-134 cultivar was pricked and infected with EHA105 harboring chimeric *cry1AcF* and *npt II* genes and integration of these genes analyzed by PCR and Southern blotting at T_2 generation (Keshavareddy et al. 2013).

A reliable and efficient *in-planta* transformation approach is more advantageous than the conventional tissue culture based transformation, since it is genotype independent and applicable to different crop species. It does not require long duration for regeneration of shoots and roots. Hence, tissue culture induced somaclonal variations could be avoided. Thus *in planta* transformation can be an effective and alternative to tissue culture based direct transformation approach. Of late, *in-planta* method of transformation is being more widely applied to different crop species with success. However, *in-planta* transformed lines require high throughput screening methodology to identify the positive transformants. The results obtained through different methods of transformation were described in Table 2.

Marker free transgenics

Plant transformation vectors containing marker genes conferring resistance to antibiotics or herbicides are used for selecting the primary transformants. These maker genes have no role in the plant once the transgenic plants are identified and stabilized for the expression of the target gene(s). Biosafety concerns suggest that the presence of marker gene might lead to the development of antibiotic and herbicide resistance in pathogenic microorganisms and weeds, respectively through horizontal gene transfer. Hence, there are concerted efforts to develop marker free transformation methods (Puchta 2003; Darbani et al. 2007). Marker free transgenic plants could be the best solution for the issues of biosafety concerns especially with genetically engineered food crops. Selectable marker genes, which are being used in transgenic selection, confer the resistance to appropriate selective agents like antibiotics and herbicides, e.g. BAR, PAT, EPSP, CSR1, NPT II and HPT. Various approaches have been developed for the elimination of selectable markers such as multi-auto-transformation system (MAT), co-transformation, site specific recombination system, transposon based marker method, intrachromosomal recombination

system and transplastomics (Miki and McHugh 2004; Darbani et al. 2007). Only limited efforts were so far made on the development of marker free transgenics in peanut. Transformation of peanut by using marker-free binary vectors harboring either the *phytoene synthase* gene from maize (*Zmpsy*1) or *chitinase* gene from rice (*Rchit*) showed high transformation frequency of about 75 % (Bhatnagar et al. 2010). Marker free transgenics has been developed in several other crops, tobacco (Li et al. 2009), wheat (Doshi et al. 2007), where transformation frequency varied from 2.2 to 25 %. Transformation frequency obtained through marker free approach was comparable to the results generated using selectable marker system (Sharma and Anjaiah 2000; Sharma and Bhatnagar-Mathur 2006). This approach would reduce the risk of introduction of unwanted genetic changes and increase regeneration efficiency. In spite of this, recovery of transformation events would be very low as compared with selection of the explants screening under selection pressure.

Biolistics (or) micro projectile particle bombardment

Direct DNA transfer technology has been achieved by different methods including microprojectile bombardment, electroporation of protoplasts, microinjection of meristems and polyethylene glycol mediated protoplast transformation (Li et al. 1995). Among these, microprojectile bombardment method is most widely deployed method of transformation. In biolistic method, the gene of interest is transferred into plant cells by delivering micro projectiles coated with the target plasmid DNA at high velocity (Klein et al. 1987). The biolistic method is the most effective approach since it is least influenced by genotype, explant recalcitrance and is independent of *Agrobacterium*. The transgenic peanut plants were generated successfully through the bombardment of embryogenic calli with *35S-gus* gene, which showed 12 % of transformation frequency (Ozias-Akins et al. 1993). Schnall and Weissinger (1993) developed a methodology for producing fertile peanut plants using zygotic embryos as explants under high concentration of agar medium. The human *Bcl-xL* gene was transformed into Georgea Green variety, where ten fertile transgenic lines with multiple integrations were obtained (Chu et al. 2008a). The repetitive somatic embryos of VC1 and AT120 cultivars were bombarded with gold particles coated with DNA construct encoding tomato spot wilt virus nucleocapsid protein, and 77 and 31 % of frequency of transformation obtained respectively (Magbanua et al. 2000). Somatic embryos from immature cotyledons of peanut bombarded with codon optimized *cry1Ac* along with *hpt* antibiotic marker and transgenic peanut conferred resistance against cornstalk borer with an efficiency of 0.85 to 2.3 per bombardment (Singsit et al. 1997).

Direct DNA transfer using electric discharge particle acceleration (ACCELL) technology was proven to be an efficient, genotype independent method of transformation. Two transgenic peanut transgenic varieties generated, Florunner and Florigiant through ACELL gene delivery approach exhibited chimeric transgenic shoots at frequency of 8.8 and 6.4 % respectively (Brar et al. 1994). McCabe and Christou (1993) also demonstrated genotypic independent direct DNA transformation technology through ACCELL. A codon modified bacterial *mercuric ion reductase* gene (*MerA reductase*) was transferred to peanut through the bombardment of embryonic cultures to study the phytoremediation in mercury contaminated soils and these transgenic lines showed resistance towards $HgCl_2$ (Yang et al. 2003). A modified biolistic method with protamine rather than spermidine in combination with 70 ng/shot DNA and 50 µg/shot gold particles significantly enhanced 4.6 fold transformation efficiency in Georgia Green variety (Chu et al. 2013).

Even though particle bombardment has many advantages, it has certain limitations. The transformation frequency is a limiting factor and gene integration is random and the process of gene delivery itself might cause damage to the DNA during transformation. This methodology often results in integration of multiple copies of the transgene into the target genome; hence, the expression of the gene is unregulated or silenced (Singsit et al. 1997; Livingstone et al. 2005). Apart from these observations, the methodology is highly cost intensive and the technology is not freely available to the workers interested in genetic transformation. The biolistic transformed methodologies are listed in Table 3.

Agronomic trait development through genetic manipulation in peanut

Genetic transformation can facilitate the introduction of potential candidate genes into plants for manipulating several beneficial traits associated with crop improvement. Transformation technology developed 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.

Abiotic stress tolerance

In plants, response to abiotic stress involves activation and coordination of stress responsive genes and their networks, which contribute to increased tolerance against drought,

salt, cold and several other stresses by modifying proteins or enzymes involved in biosynthesis of osmolytes (proline, glycine betaine), sugars (mannitol, trehalose, galactinol) and polyamines (Shinozaki and Yamaguchi-Shinozaki 1997; Vinocur and Altman 2005). Peanut is cultivated mostly in rain fed regions, where higher temperature prevail and prolonged drought conditions recur which contribute to the limited productivity in peanut crop. Development of drought tolerant varieties is needed to mitigate the environmental stresses (Bhatnagar-Mathur et al. 2007; Chu et al. 2008a).

For instance, over expression of *AtNHX1* gene in peanut (a vacuolar Na^+/H^+ antiportar) improved tolerance against high salinity and water deprived conditions by compartmentalization of Na^+ ions in the vacuoles (Asif et al. 2011). Expression of human *Bcl-xL* gene introduced into the peanut genome, resulted transgenics showed significant tolerance towards oxidative and salt stresses (Chu et al. 2008a). Similarly, *PDH45*, pea DNA helicase homologous to *eiF4A* showed abiotic stress tolerance and improved peanut productivity at T_3 generation under field conditions (Manjulatha et al. 2014). Transgenic peanut plants showed higher survival, chlorophyll capacity and recovered under PEG simulated dehydration stress conditions.

Drought tolerant peanut generated through the transformation of *rd29A:AtDREB1A* (Responsive to Dehydration promoter: Dehydration Responsive Element Binding protein) which showed 40 % increase in transpiration efficiency under limited water conditions in comparison to the control plants. The seeds expressing *rd29A:AtDREB1A* plants showed normal germination and positive growth effects, whereas *35S:AtDREB1A* expressing seeds showed delayed germination with plants exhibiting severe growth retardation (Bhatnagar-Mathur et al. 2007, 2014). In another study, transgenic peanut developed with *AtNAC2* and *MuNAC4* (NAM, ATAF and CUC) transcription factors conferred tolerance against drought, moisture stress, salinity with improved crop yield under limited water conditions (Pandurangaiah et al. 2014; Patil et al. 2014). Expression of *NAC3* from Chickpea caused enhanced accumulation of proline and photosynthetic pigments along with lower levels of malondialdehyde concentration in transgenic poplars (Movahedi et al. 2015).

The peroxisomal *ascorbate peroxidase* genes of *Salicornia brachiate* displayed salt and drought stress tolerance in tobacco and it was reconfirmed in peanut (Singh et al. 2014). Moreover, these transgenic plants rendered with chlorophyll, relative water content (RWC) under normal and also under stress conditions. Transgenic peanut plants expressing *SbASR* (Abscisicacid Stress Ripening) gene constitutively showed enhanced drought and salinity tolerance. In addition, *SbASR* gene was functioned as LEA protein and also as a transcription factor (Tiwari et al. 2015).

The osmolytes and osmoprotectants play key role in protecting the plant cells through scavenging free radicals. Mannitol, an osmoprotectant plays an important role in scavenging hydroxyl radicals generated during abiotic stresses. Peanut transgenic expressing *mtlD* (mannitol-1-phosphate dehydrogenase) displayed drought stress tolerance with enhanced RWC compare to non transgenic control under limited water conditions (Bhauso et al. 2014). Overexpression of *mtlD* gene in other plants including eggplant, sorghum, and maize, resulted in enhancing plant growth parameters apart from drought and salinity stress tolerance (Prabhavathi et al. 2002; Maheswari et al. 2010; Nguyen et al. 2013). Simultaneous expression of genes responsible for regulating different OsAlfin (alfalfa zinc finger),PDH45 and PgHSF4(Heat shock factor) which stimulated drought, moisture stress tolerance and yield improvement in peanut by way of up regulating several stress responsive genes (Ramu et al. 2015). This was the first report demonstrated by simultaneous expression of multiple genes in peanut for enhancing the abiotic stress tolerance. Research on development of abiotic stress tolerant peanut varieties were represented in Table 4.

Biomass improvement

Iso pentenyl transferase (IPT) is an enzyme in the cytokinin biosynthetic pathway and its expression in peanut increased tolerance to drought stress (Qin et al. 2011). Transgenic lines expressing *IPT* under the control of drought inducible SARK promoter (Senescence Associated Receptor protein Kinase) showed improved biomass retention in drought tolerance test under green house conditions and 58 % of yield increase under field conditions (Qin et al. 2011).

Manipulation of biotic stress tolerance in peanut

Peanut is prone to biotic factors like viral, fungal, bacterial infections and to several of other pests. Among them, the major yield devastating agents are tomato spot wilt virus, *Cercospora* leaf spot, and white mold, *Aspergillus flavus* (Brar et al. 1994), which are widely distributed in environment and cause severe disorders in peanut. As mentioned earlier, identification of suitable genes that confer stress tolerance from wild relatives and using them in making transgenic plants expressing them would be the ideal option in peanut genetic advancement.

Fungal resistance

Peanut production and quality are predominantly affected by fungal diseases through aflatoxin production. Several

Table 4 Manipulation of abiotic stress tolerant varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene/source	Selectable marker	Trait evaluation	References
GG-20	LBA4404	Shoot apices	*CaMV35S/Cauliflower mosaic virus,* *SbpAPX/Salicornia brachata*	*hpt*	Transgenic peanut plants exhibited adequate tolerance against salt and oxidative stress in T_1 generation	Singh et al. (2014)
Narayani	EHA105	Seedlings (*in-planta*)	*CaMV35S/Cauliflower mosaic virus,* *MuNAC4*/Horsegram	*npt II*	Improved water/drought stress tolerance, osmotic adjustments reduced membrane damage and balancing the redox status	Pandurangaiah et al. (2014)
K-134	LBA4404	Embryonic axes (*in-planta*)	*CaMV35S/Cauliflower mosaic virus,* *AtNAC2 Arabidopsis*	*npt II*	Enhanced tolerance to drought and salinity with yield under limited water conditions	Patil et al. (2014)
JL-24	LBA4404	Cotyledon	*Rd29A/Arabidopsis, DREB1A/Arabidopsis*	*npt II*	Transgenic plants showed 40 % higher transpiration efficiency (TE) than control and exhibited drought stress tolerance during T_2–T_6 generations	Bhatnagar-Mathur et al. (2007, 2009, 2014)
GG-20	LBA4404	DEC and immature explants	*CaMV 35 S/Cauliflower mosaic virus, mtlD/ E.coli*	*npt II*	Increased the amount of mannitol in transgenic plants and exhibited tolerance against water deficit stress	Bhauso et al. (2014)
K-134	LBA4404	Embryonic axes (*in-planta*)	*CaMV 35 S/Cauliflower mosaic virus, PDH45/ Pea*	*hpt*	Increased WUE and also yield under stress conditions at T_3 generation	Manjulatha et al. (2014)
Golden BARI-2000	LBA4404	DEC	*CaMV 35 S/Cauliflower mosaic virus, AtNHXI/ Arabidopsis*	*npt II*	Transgenic plants resistant to higher salt and water deprivation conditions due to accumulation of salt and protein	Asif et al. (2011)
New Mexico Valencia A	EHA104	Cotyledon	*SARK/bean Isopentenyltransferase/ Arabidopsis*	*npt II*	Showed higher photosynthetic rate, stomata conductance, and higher transpiration rate under limited water conditions than control plants	Qin et al. (2011)
Georgia Green	Microprojectile bombardment	Embryogenic calli	*CaMV 35 S/Cauliflower mosaic virus, Bcl-xL/* human	*hpt*	Enhanced tolerance against biotic and abiotic stresses	Chu et al. (2008a)

genes were introduced into peanut through transgenic approaches for providing resistance against fungal diseases. Aflatoxin, has been identified to be a potent carcinogen produced by *Aspergillus* species. 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). Chitinases and glucanases are hydrolytic enzymes which degrade the fungal cell wall and spore formation and these enzymes are attractive candidate genes for development of fungal resistant plants. Overexpression of a tobacco *glucanase* gene in peanut has increased its resistance towards *Cercospora arachidicola* and *Aspergillus flavus* (Sundaresha et al. 2010) in three peanut cultivars, JL 24, ICGV 89104 and ICGV 86031. *Oxalate oxidase* gene showed increased resistance to *Sclerotinia minor* disease in peanut (Livingstone

et al. 2005). Leaf spot disease is one of the major concerns in peanut, because of its potential devastating impact on crop yield. Tobacco chitinase gene showed resistance in peanut against leaf spot or tikka disease caused by fungal pathogen *C. arachidicola* (Rohini and Rao 2001). Over-expression of rice *chitinase* gene in peanut showed fungal resistance against *C. arachidicola* and good correlation was observed between *chitinase* activity and fungal resistance at the laboratory level (Iqbal et al. 2012). Defensin gene such as *RsAFP-2* (*Raphanus sativum* antifungal protein-2) was transferred into peanut and the resulting transgenic peanut plants showed enhanced resistance against the pathogens, *Pheaoisariopsis personata* and *C. arachidicola*, which jointly cause serious late leaf spot disease (Anuradha et al. 2008). Plant based pathogenesis related (PR) proteins are toxic to the fungal pathogens which infect the plant cells. The combination of PR genes, *SniOLP* (*Solanum nigrum* osmotin like protein) and *RsAFP2* showed enhanced resistance to late leaf spot disease in transgenic peanut plants at laboratory and as well as at green house level (Vasavirama and Kirti 2012). Peanut plants expressing *β-1–3, glucanase* gene showed the enhanced fungal disease resistance (Qiao et al. 2014). In a recent study, several genes that get upregulated in the wild peanut *A. diogoi* upon challenge with the late leaf spot pathogen have been characterized (Kumar and Kirti 2015b). Constitutive expression of *AdSgt1*{Suppressor of G2 allele of SKP1 (suppressor of Kinetochore Protein)} in transgenic peanut plants obtained through *in planta* transformation showed enhanced resistance to the late leaf spot pathogen. Similarly, transgenic tobacco plants expressing *AdSgt1* also displayed enhanced resistance to multiple pathogens (Kumar and Kirti 2015a). Fungal resistant peanut varieties showed in Table 5.

Virus resistance

In the year 2000, a viral disease PSND in association with TSV crop devastated over $64 million in Anantapur district alone, a major peanut producing region of Andhra Pradesh in India (Mehta et al. 2013) Tomato spotted wilt virus nucleocapsid protein (N gene) when introduced into peanut conferred resistance to TSV (Yang et al. 1998). Coat protein mediated resistance has been engineered in many corps earlier with an impressive success so far. Yield of peanut was restricted by various viruses including Indian Peanut Clump Virus (IPCV), Ground Nut Rosette Virus (GRV), Peanut Stripe Virus (Pstv), Peanut Bud Necrosis Virus (PBNV), Peanut Mottle Virus (PMV), Tobacco Streak Virus (TSV) (Reddy et al. 2002). Coat protein genes have proven to be effective in minimizing the diseases caused by the viruses (Gonsalves and Slightom 1993). 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 al. 2013) (Table 6).

Pest resistance/insect resistance

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. *CryIA* gene was first transformed into peanut with improved efficiency against cornstalk borer (Singsit et al. 1997). Expression of chimeric *Btcry1AcF* (fused domains of *cry1Ac* and *cry1F*) and synthetic *Cry1EC* genes showed resistance against *Spodoptera litura* in peanut (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 exhibited insecticidal activity (Geng et al. 2012). Pest tolerant varieties of peanut described in Table 7.

Vaccine development

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 inhibiting allergies, chronic infections, and for producing therapeutic antibodies. *Urease* subunit B (*UreB*) under the control of *oleosin* promoter has been overexpressed in peanut through *Agrobacterium* mediated transformation. The transgenic seed for use as an edible oral vaccine has been produced for controlling the human bacterial pathogen *Helicobacter pylori* (Yang et al. 2011). *UreB* gene was overexpressed in other plants also like tobacco, rice and carrot. However, these plants produced very low amounts of protein (Gu et al. 2005, 2006; Zhang et al. 2010). The transformation with *VP2* (Bluetongue Virus Protein) gene coding for capsid of bluetongue virus (a sheep pathogen) into peanut was used for vaccine development. In other crop plants, the recombinant human α1 proteinase inhibitor was expressed in chickpea which could be useful for possible therapeutic applications (Mishra et al. 2013). However, the transgenic plants have not been tested for the effectiveness of vaccination (Athmaram et al. 2006) (Table 8).

Allergen silencing

Peanut causes one of the common life threatening food allergies and it is a serious challenge in food industries.

Table 5 Fungal resistant varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene/source	Selectable marker	Trait evaluation	References
Huaya 20 Huaya 22	EHA105	Embryonic leaflets	*CaMV 35 S/Cauliflower mosaic virus, β 1–3 glucanase/Arabidopsis*	None	Transgenic plants showed resistance against *C. personata*	Qiao et al. (2014)
JL-24	EHA105	Somatic embyro	*CaMV 35 S/Cauliflower mosaic virus, AdSGT1/ A. diogoi*	*npt II*	Peanut plant with enhanced resistance against the late leaf spot disease	Kumar and Kirti (2015a)
JL 24 ICGV 89104 ICGV 86031	C58	Shoot buds	*CaMV 35 S/Cauliflower mosaic virus, Rchit*(class 1 chitinase)/Rice	*hpt*	Transgenics showed enhanced resistance against three fungal pathogens causes foilers diseases including late leaf spot and rust	Prasad et al. (2013)
JL-24	GV2260	Cotyledon	*CaMV 35 S/PR-5/SniOLP/ Solanum nigram, CaMV 35 S/PR-12/Rs-AFP2/ Raphanus sativum*	*hpt*	Enhanced late leaf spot disease resistance and reduced number and size of lesions on leaves	Vasavirama and Kirti 2012
Golden BARI-2000	LB4404	Cotyledonary node	*CaMV 35 S* and *A. thaliana oleosin/chitinase-3/*Rice	*hpt*	Inoculation with *C. arachidicola*	Iqbal et al. 2012
TMV-2	LB4404	Two-day old seedlings	*CaMV 35 S/Cauliflower mosaic virus, uidA/ E.coli, β 1–3 glucanase/ Arabidopsis*	*npt II*	Resistance towards *C. arachidicola* and *A. flavus* and reduced number of spots and delay on set of disease	Sundaresha et al. (2010)
JL-24	EHA105	Embyro axes	*CaMV 35 S/Cauliflower mosaic virus, RsAFP1 and RsAFP2/*Mustard	*npt II*	Transgenics showed enhanced resistance against leaf spot disease	Anuradha et al. 2008
Okrun	Biolistic	Somatic embyro	*CaMV 35 S/Cauliflower mosaic virus, chitinase/* Rice and *CaMV 35 S/ glucanase/*Alfalfa	*hpt*	Fungal resistance observed under field conditions	Chenault et al. (2006)

Peanut caused Immunoglobulin E (*IgE*) mediated allergic reactions in 0.6 % of total population (Sicherer et al. 2003) and children are more sensitized. There are eleven peanut proteins that have been identified of which *Ara h 2* and *Ara h 6* are reported as predominant allergens (Maleki et al. 2003; Chu et al. 2008b). First successful application of RNAi mediated approach was demonstrated in pea by targeting the immunodominant allergen *Ara h2*, which reduced the growth of *A. flavus* in seeds (Dodo et al. 2008). Ozias-Akins et al. (2009) conducted a case study for silencing *Ara h2* through RNAi mechanism in peanut. In another study, *Ara h2* and *Ara h 6* genes were silenced by introducing the RNAi construct targeting homologous coding sequence and resulted in reduction of *A. flavus* growth in peanut (Chu et al. 2008b) (Table 9).

Bio-fortification studies in peanut

Biofortification is an emerging research field for enriching nutritional values in staple food crops to combat malnutrition through breeding and transgenic approaches. This area of research is of particular importance to the undernourished millions of people in the developing countries. Peanut is an excellent source of dietary proteins and essential oils. However, it is a poor source of essential sulfur containing amino acids like methionine, iron, zinc and vitamin A, which are limiting its nutritional value. Biofortification of peanut enhances the micronutrient accumulation in kernel and it induces the productivity. Gander et al. (1991) characterized a gene coding for 2S albumin seed protein that is enriched with methionine from Brazil nut (*Bertholetia excels*). Transgenic peanut plants were improved with the enhanced expression of *2S albumin* gene for enriching methionine content, 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. Intercropping of gramineous species (peanut/maize and chickpea/wheat) induce biofortification of Fe and Zn metal ions as nutrients through a series of inter specific root interactions (Shen et al. 2014). Iron is a primary nutrient for plant growth and development involved in several biochemical reactions. Gene coding for *AhDMT1* (*Arachis hypogaea* Divalent

Table 6 Viral resistant varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene	Selectable marker	Trait evaluation	References
Kadiri 6 (K6) Kadiri 134 (K134)	LB4404	DEC, immature leaves	*CaMV35 S/Cauliflower mosaic virus, TSV-CP/tomato spotted virus*	*hpt*	Symptomless, no systemic accumulation of virus indicated the resistance to TSV infection	Mehta et al. (2013)
JL-24	Particle bombardment/ C58	Cotyledon explants and Embryonic leaves	*CaMV35 S/Cauliflower mosaic virus, PBNV-N/peanut bud necrosis virus, uidA/E.coli.*	*hpt*	T_2 generation plants exhibited resistance to PBNV and reduced the 20 % disease incidence under nature field conditions	Rao et al. (2013)
Gajah NC7	Microprojectile bombardment	Embryogenic callus	Dual *CaMV35 S/PStV coat protein CP2 and CP4/Potato spindle tuber viroid*	*hpt*	Peanut lines exhibited high levels of resistance to PStV	Higgins et al. (2004)
VC1 AT120	Microprojectile bombardment	Embryo axes	*CaMV35 S/Antisense NC* gene of *TSWV/tomato spotted wilt virus*	*hpt*	Peanut lines exhibited resistance to TSWV	Magbanua et al. (2000)
Florunner Georgia Runner MARC-1	Microprojectile bombardment with helium	Embryogenic callus	*CaMV35 S/TSWV N/tomato spotted wilt virus*	*hpt*	Plants showed resistance to TSWV	Yang et al. (1998)

Metal Transporter gene 1), $Fe2^+$ transporter was induced in the nodules of peanut during intercropping of peanut/maize under Fe deprivation conditions and it is also involved in N_2 fixation (Shen et al. 2014). Likewise, some more genes coding for metal iron transporters such as *AhIRT1* (Iron Regulated Transporter 1), *AhNRAMP1* (Natural Resistance Associated Macrophage Protein 1) were induced strongly in roots during peanut intercropped with maize under Fe deficit conditions (Xiong et al. 2012, 2014). *AhNRAMP1* is a Fe transporter and induced strongly in roots especially under Fe deficient conditions and its expression in tobacco enhanced the Fe deposition and showed tolerance towards Fe deprivation (Xiong et al. 2012).

Functional validation of peanut genes

Defense mechanism results in activation of different stress related genes ultimately leading to the production of reactive oxygen species (ROS), synthesis of pathogen related proteins, and accumulation of phytoalexins. Some of the peanut varieties also tolerate both biotic and abiotic stresses by modulating cellular metabolisms. Multiple gene families associated with abiotic and biotic stress have been identified by constructing EST library (Govind et al. 2009) and Suppressive Subtraction Hybridization (SSH) libraries (Ding et al. 2014). Understanding molecular mechanism in response to water deficit stress would be more rewarding as it would help identify key native genes in peanut that may be more helpful in generating transgenic plants resistant to stress conditions.

Arachis diogoi, a wild relative species of peanut is a source of novel genes related to biotic and abiotic stress tolerance. Ectopically expressing salt induced pathogenesis related protein of Arachis hypogaea (*AhSIPR10*) was alleviated the broad spectrum of abiotic stress tolerance in tobacco. Moreover, these plants showed higher photosynthetic CO_2 assimilation rates under drought, salt and metal stress conditions (Jain et al. 2012). Transgenic tobacco plants expressing a pathogen induced thaumatin like protein of *A. diogoi AdTLP* gene showed resistance against fungal pathogen *Rhizoctonia solani* and seedlings exhibited enhanced tolerance to salt and oxidative stress (Singh et al. 2013). Pathogen induced *SGT1* gene was identified from *A.diogoi* through differential gene expression studies and its overexpression induced hypersensitive like cell death in tobacco (Kumar and Kirti 2015a). *AhAREB1* (*Arachis hypogea* Abscisic acid Responsive Element Binding protein) belongs to the family of leucine Zinc finger (*bZIP*) type transcription factors in peanut. The constitutive expression of *AhAREB1* conferred water stress tolerance and scavenges the reactive oxygen species (ROS) in transgenic Arabidopsis (Li et al. 2013). Expression of peanut iron regulated transporter 1 (*AhIRT1*) in tobacco and rice plants conferred improved iron nutrition (Xiong et al. 2014). *Chitinase* gene from peanut was expressed and its regulatory elements were characterized for pathogen induced expression. The transcriptional activation of the

Table 7 Insect/pest resistant varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene/source	Selectable marker	Trait evaluation	References
K-134	EHA105	Shoot apical meristem (In-planta)	*CaMV 35 S/Cauliflower mosaic virus, Cry1AcF/ Bacillus thuringiensis*	*npt II*	High % of larval mortality in T_1 and T_2 generations and *Cry1AcF* showed effective against *S. litura*	Keshavareddy et al. (2013)
Luhua11 Huayu16 Huayu28 Baisha1016 Yuanhua8 Xinhua1 Xinhua5	*A. rhizogenes* K599	Cotyledons	*CaMV 35 S/Cauliflower mosaic virus, cry8Ea1/ Bacillus thuringiensis*	None	Transgenic roots were tested for insecticidal activity against *H. parallela*	Geng et al. (2012)
Wilson Perry NC-7	Biolistic	Somatic embryo	*CaMV 35 S/Cauliflower mosaic virus, Oxlate oxidase*	*hpt*	Field evaluation of transgenic lines for *S. minor* resistance	Livingstone et al. (2005), Partridge-Telenko et al. (2011)
JL-24	EHA105	DEC	*CaMV 35 S/Cauliflower mosaic virus, PR-1a/ Cry1EC/Bacillus thuringiensis*	*hpt*	Salicylic acid induced transgenic plants showed 100 % mortality of *S. litura* during all stages of larval development	Tiwari et al. (2011)
Toalson Florunner MARC-1	Microprojectile bombardment with helium	Cotyledons	*CaMV 35 S/Cauliflower mosaic virus, CryIA(c)/ Bacillus thuringiensis*	*hpt*	Peanut plants were protected from damage caused by lepidopteron insect larvae of lesser crosstalk borer	Singsit et al. (1997)

Table 8 Vaccine producing varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene/source	Selectable marker	Trait evaluation	References
–	EHA105	Embryo leaflets	*Oleosin/Brassica napus Ure B/H. pylori*	*npt II*	Transgenic plants potentially used as an edible vaccine against *H. pylori*	Yang et al. (2011)
JL-24	Biolistic	Somatic embryo	*CaMV 35 S/Cauliflower mosaic virus BT-VP2/ Bluetongue virus*	*npt II*	Produced blue tongue outer coat protein that comprises the neutralizing the epitopes	Athmaram et al. (2006)

regulatory elements after fungal infection was demonstrated in tobacco (Kellmann et al. 1996).

Transgene segregation, stability of progenies and assessment

Since last two decades the scientists have focused on studying gene transfer mechanism and process of transformation for trait improvement and crop protection like disease resistance, herbicide, drought, salinity tolerance. The gene sequence is responsible for expressing particular phenotypic trait. The stability of transgene expression is influenced on locus of gene integration and segregation. The gene or its phenotypes can be transmitted from one generation to next by following Mendelian law of segregation. Though the transgenic plants developed under identical conditions by transferring same DNA expression construct, the phenotypic variations can be displayed because of copy number and location of transgene integration into any chromosome(s) and also many other factors like gene deletions, inversions and duplications in the chromosome (Zhu et al. 2010).

As per many reports, the stress tolerance was demonstrated under laboratory or glass house conditions, but only in few cases these plants established with stress tolerance

Table 9 Allergen silencing varieties

Peanut genotype	Transformation method	Explant	Promoter/transgene/source	Selectable marker	Trait evaluation	Reference
Georgia Green	Biolistic	Somatic embyro	*CaMV 35 S/Cauliflower mosaic virus, Arah 2 RNAi/peanut*	*hpt*	Produced hypo allergenic peanut by silencing *Arah 2* and *Arah 6* genes	Chu et al. (2008b)
Georgia Green	EHA105	Hypocotyls	Drought inducible *SARK/ bean, Arah 2 RNAi/Peanut*	*npt II*	Alleviated peanut allergy	Dodo et al. (2008)

and enhanced productivity under field conditions. For instance, Bhatnagar-Mathur et al. (2014) reported that the DREB expressing peanut plants were segregated homozygous even at progenies of T_6–T_9 generations and displayed drought tolerance and also more yield than non transgenic plants under limited soil moisture field conditions. Similarly, regulated expression of IPT in peanut was conferred drought tolerance and more yields in both in vitro and also under reduced irrigated field conditions (Qin et al. 2011). Peanut plants expressing *Cry1AcF* gene was segregated and showed stable integration and homozygous nature at T2 and T3 generations. These plants performed with reduced damage to the leaves and increased larval mortality of *S. litura* as the generations advanced which indicated stability and efficacy of the transgene (Keshavareddy et al. 2013).

Though there are many genetic tools available for producing transgenic plants and trait evaluation, still there is a lacuna in expression, gene stability in further generations. So there is an urgency to establish the plants with improved traits at field level. The genetic attributes may mitigate major concerns and associated problems in peanut production.

Conclusion and prospects

Peanut is a very rich source of edible oil, proteins and essential biochemical products which have enormous economic importance. In general, the peanut is cultivated under rain fed conditions of tropical and sub-tropical regions in the world. Under such conditions, the crop has to counter the inevitable adverse conditions like biotic, abiotic and nutrient deficient conditions. These stresses can be overcome through suitable crop management strategies. However, there is no adequate genetic diversity in peanut. Hence, genetic transformation by introducing foreign genes into peanut is an alternative to breeding approach. Advances in methods of transformation and recombinant DNA technologies facilitated the gene transfer into several crop species.

Peanut has been demonstrated as a recalcitrant legume crop for in vitro regeneration and transformation. However, exploitation of cotyledonary nodes and embryo axes explants tackled the recalcitrance and expedited the process of genetic transformation with high efficiency. Methods like *Agrobacterium* and biolistic transformations have been developed by standardizing different variables like the combinations of different explants, co-cultivation period, selectable marker, hormonal combinations. In genetic transformation, promoters are also play key role in driving the expression of genes. Majority of the researchers demonstrated the expression of genes with constitutive promoters in peanut. Tissue specific expression is more rewarding than the constitutive expression. Research should take place in this direction for achieving better specific expression at various stages of plant development. Isolation and characterization of novel promoters in peanut is prerequisite for successful genetic engineering applications.

The genome sequence of peanut is yet to be disclosed completely. However, some research groups constructed cDNA libraries through subtractive hybridization. They provide an opportunity to study the role and functional significance of some important genes controlling agronomic traits. The genus *Arachis* is warehouse of novel drought, pest, and disease resistant genes. Peanut is a partially drought tolerant crop and it can perform osmotic adjustments at cellular level. cDNA libraries were generated in drought imposed peanut which evidenced genes expressed in immature pods (Devaiah et al. 2007; Luo et al. 2005), 25 day old plants (Govind et al. 2009), and roots (Ding et al. 2014). Taken together of these findings, several genes related to stress adaptation and signaling components have been identified in groundnut. However, the functional relevance of these genes needs to be studied in near future to reveal their role in response to stress conditions.

Interestingly, drought stress triggers the expression of hormone signaling genes like Auxin responsive proteins (ARP), cytokinin repressed (CR9), and counteracting brassinosteroid responsive (BRH1) genes, which play important roles in desiccation tolerance (Govind et al. 2009). Another

wild species related to peanut, *A. diogoi* is an important source of genes related to biotic and abiotic stresses.

Stress responsive genes and transcription factors such as *DREB*, *NAC*, *mtlD*, *NHX1* and *PDH45* exhibited the multiple stress tolerance and these plants showed the increased transpiration efficiency under limited water conditions (Bhatnagar-Mathur et al. 2014; Pandurangaiah et al. 2014; Bhauso et al. 2014; Asif et al. 2011; Manjulatha et al. 2014). However, peanut crop should be evaluated under field conditions to score the tolerance towards stresses.

Development of virus resistant peanut cultivars has tremendous impact on crop productivity, especially in the resource poor agricultural systems of the semi-arid tropic regions. RNAi silencing mechanism is suitable strategy to knockdown the peanut allergen genes to eliminate or control the peanut allergy. Peanut kernel, roots and shells are rich sources of resveratrol, a major stilbean phytoalexin which acts as nutraceutical agent and minimize the risk of cardiovascular disease, anti-aging and anti-cancerous agent. Considering the economic importance of resveratrol in herbal, food, health industry and plant defense mechanism, the effective strategies for production of resveratrol needs to be established in future. Organ specific expression of stilbene synthase can enhance the production of resveratrol in peanut kernels which can minimize the colonization of *A.flavus* and occurrence of aflatoxin contamination. Transformed hairy root lines of peanut were utilized as bioreactors for large scale production of trans resveratrol (Halder and Jha 2016). Since there are no adopted resistant genotypes is available, improvement of viral, fungal, allergen silencing or incorporation of immunity through the transformation of peanut cultivars have major demand. Improved crop protection through the expression of disease resistance genes may minimize the usage of pesticide spray, which can reduce the economic burden to the grower and improve the environment safety.

So far, major focus has been laid on developing biotic and abiotic stress tolerance in peanut. However, peanut being an important food crop needs the manipulation of value added traits including vitamin, iron, nutrient enrichment, protein, phytoalexins, and oil quality enhancement. Low productivity in semiarid regions is due to rain fed cultivation, poor soil fertility and mismanagement of micronutrients. Zinc and iron deficiency causes maximum yield losses in peanut. Suitable crop improvement and agronomic strategies need to be developed to improve the uptake and bioavailability of these micronutrients in peanut. However, collaborative research, government policies to enhance bioavailability of nutrients in regular food crops are much desired to address hunger problems in the world. Better understanding of molecular, physiological mechanisms of stress tolerance and nutrient assimilation pave the way for enhancing the crop yield and productivity that could ensure the food security and environmental protection.

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Compliance with ethical standards

Conflicts of interest The authors declare that have no conflict of interest.

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