Research & Reviews: Journal of Agriculture and Allied Sciences

e-ISSN:2347-226X
p-ISSN:2319-9857

Multigenic Groundnut Transgenics: An Advantage Over Traditional Single Gene Traits in Conferring Abiotic Stress Tolerance: A Review

Boya Venkatesh[1], Amaranatha Reddy V[2], Lokesh U[1], Kiranmai K[3], Anthony Johnson AM[1] Pandurangaiah M[1], Naresh Kumar A[3], Jayamma N[1], Jagadeesh Kumar N[1] and Chinta Sudhakar[1]*

[1]Department of Botany, Plant Molecular Biology Unit, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh-515003, India

[2]Department of Agronomy, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506, USA

[3]Department of Crop Physiology, UAS, GKVK, Bengaluru- 560065, India

Review Article

Received date: 03/12/2018

Accepted date: 27/12/2018

Published date: 05/01/2019

***For Correspondence**

Chinta Sudhakar, Professor, Department of Botany, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh- 515003, India

E-mail: chintasudhakar@yahoo.com

Tel: 0091 8554 255054; +919440030464

Keywords: Groundnut, Transgenics, Abiotic stress, Co-expression, Multigene transgenics

ABSTRACT

Groundnut, the third largest producing oilseed crop grown in arid and semiarid regions worldwide. The crop growth under the rainfed conditions often exposed to the abiotic stresses that severely affects crop production and yield. Introduction of stress resistance traits into crop plants may improve the tolerance capability and mitigates yield losses under stress conditions. The success of breeding approach for developing crop varieties for stress tolerance determined by the efforts of several research fields including plant physiology, molecular biology, and genetics. Also, the other major limitations for breeding programs are the species barriers and genomic incompatibilities. Hence, use of novel molecular biology tools for revealing the important mechanisms of stress tolerance and engineering the stress tolerant crops by the overexpression of stress-specific genes through transgenic approach remains a viable option. This review focuses on the groundnut transgenics developed against various abiotic stresses like drought, salt and oxidative stress by overexpression of candidate genes involved in the regulation of metabolic pathways, cellular components, and stress-responsive pathways. This review includes discussion on the significance of transgenic groundnut events and the importance of trait ability to enhance the abiotic stress tolerance. This review also emphasizes the new progress in the transgenic event developments in groundnut and the novelty of multigene transgenics in comparison with single gene transgenics for the enhanced abiotic stress tolerance.

INTRODUCTION

Abiotic stresses are the major constraints for crop plants grown in arid and semiarid regions that alters the plant's internal homeostasis and adversely affect the growth and productivity [1-3]. Amongst the abiotic stresses, drought and salinity were more prevalent in natural and agricultural systems and severely affects the crop quality thereby causes significant yield losses; hence, attained significant attraction from the researchers [4,5]. Semiarid tropics like Asia, Africa, South and North America are the leading groundnut cultivation regions with ~60% global production where drought and salinity are major stressors affecting both productivity and quality of groundnut [6-8]. Plants have adapted different tolerance mechanisms against abiotic stresses to cope with changing environmental conditions such as maintenance of cell turgor by changing cell metabolisms, osmotic adjustment [9], differential expression of genes encoding Transcription factors (TFs) [10], key enzymes in biosynthetic pathways [11,12] and proteins involved in stress perception and cell signaling [13,14].

Groundnut (*Arachis hypogaea* L.) is the third largest oilseed crop after soybean and rapeseed with a global production of 60.66 million tons under 32.20 million hectares of cultivation area worldwide with an average production of 1.88 tons/ha. India is the second largest producer of groundnut after China and produces 6.85 million tons with 5.8 million hectares of cultivation area

e-ISSN:2347-226X
p-ISSN:2319-9857

[15]. It is one of the major edible oil seed crops and contains 40-50% of oil, proteins (22-30%), minerals (P, Ca, Mg and K), carbohydrates and vitamins (E, K and B group) and also used as feed for livestock [16-19]. Groundnut is cultivated extensively in arid and semiarid regions of the world under rainfed conditions; as its production depends on annual rainfall of a particular region which varies from time to time it is more prone to abiotic stresses like drought, salinity, high temperatures and heavy metal stress [20-22].

In this scenario, there is a need to improve the groundnut productivity under abiotic stress conditions using reliable technologies for achieving maximum success rate to cope up with these adverse conditions. Conventional techniques have many limitations like species barriers, genomic incompatibilities, pollination, etc. makes them impotent for developing crops with desired agronomic traits [23,24]. Genetic engineering approaches have proven to be more versatile and comparatively fast over conventional methods and help to overcome all the barriers in developing crop plants with desired traits [25-27]. Several researchers [28-36] successfully developed transgenic groundnut against different biotic and abiotic stresses by the introgression of different genes including TFs and key enzymes of metabolic pathways through genetic engineering approach.

The abiotic stress tolerance is a multigenic trait and involves different signaling cascades and mechanisms. Even though the transgenic plants overexpressing single gene were successful, it is inadequate to achieve desired crop tolerance against abiotic stress as it is a complex phenomenon hence there is a need for co-expression of multiple genes involved in various metabolic pathways and quantitative traits [37]. From the last two decades researchers were focusing on developing transgenics with more tolerance traits by introducing more than one gene (multigene transfer, MGT) instead of single gene transfer [38-41]. Co-expression of multiple genes in model plants like Arabidopsis [42], Tobacco [43,44] have shown improved abiotic stress tolerance compared to single gene transgenics and similar results were reported in crop plants like maize [45], sugarcane [46] and groundnut [47,48].

Previous review reports on groundnut transgenics tolerant to abiotic stresses so far have focused on drought and salinity [6]. Hence, there is a requisite to document and summarize the information on other abiotic stress tolerant events and recent achievements in groundnut transgenics. This review summarizes the current progress in technology and traits used to develop the groundnut transgenics for the improvement of abiotic stress tolerance using the examples of research focused on drought, salinity, high temperature, and oxidative stress. The review is given specific attention to the advancement in transgenic technology in developing the multigene transgenics which involves the insertion of more than one gene to produce the groundnut transgenics with enhanced abiotic stress tolerance. In total seventeen groundnut transgenics, developed by manipulating with single and multiple genes which are reported to be involved in various abiotic stress tolerance mechanisms were discussed in this review **(Table 1)**.

Table 1: Groundnut transgenics developed against different abiotic stresses using genetic engineering approach.

Gene Source	Agrobacterium strain	Plasmid	Transformed gene	Promoter	Trait	Mechanism involved	Reference
1. Groundnut transgenics manipulated with a single gene against abiotic stresses							
Macrotyloma uniflorum	EHA105	pCAMBIA2301	*MuWRKY3*	CaMV35S	Drought	Low MDA, H_2O_2, superoxides, more proline, sugars and anti-oxidative enzymes	[62]
Arabidopsis thaliana	EHA105	pSARK	*IPT*	SARK	Drought	Increases in photosynthesis, stomatal conductance and transpiration and transpiration	[50]
Arabidopsis thaliana	C58	pBI29	*DREB1A*	rd29A, CaMV35S	Drought	Transpiration efficiency, root development	[51,52]
Arabidopsis thaliana	-	pCAMBIA2300	*DREB1A*	rd29A	Drought	Higher osmolyte accumulation, proline; high RWC; Low EL; less chlorophyll reduction; root:shoot ratio, root volume;	[55]
-	LBA4404	pCAMBIA1301	*PDH45*	CaMV35S	Drought	Improvement of cellular level tolerance	[56]
E. coli	-	pCAMBIA1380	*mtlD*	CaMV35S	Drought	Increased accumulation of mannitol and ROS scavenging activity	[59]
Arabidopsis thaliana	LBA4404	pBinAR	*AtNAC2*	CaMV35S	Drought	Higher CSI, RWC, and reduced RWL;	[57]
Macrotyloma uniflorum	EHA105	pCAMBIA2301	*MuNAC4*	CaMV35S	Drought	Proliferate lateral root growth, enhancement of antioxidative enzyme regulation, osmotic adjustment and reduced membrane damage	[32]

Arthrobacter pascens	LBA4404	pHS724	*COX*	2XCaMV35S	Salinity	Glycine betaine synthesis	[63]
Salicornia brachiata	LBA4404	pCAMBIA1301	*pAPX*	CaMV35S	Salinity	Higher Chlorophyll content, RWC, shoot length, shoot and root weight; reduced electrolyte leakage;	[35]
Arabidopsis thaliana	EHA105	pGreen0029, pSoup	*HDG11*	rd29	Drought & Salinity	Higher antioxidative enzymes, chlorophyll content Proline; longer root system; increased specific leaf area; reduced stomatal density; higher photosynthetic rates; increased intrinsic WUE	[72]
Arabidopsis thaliana	LBA4404	pPZP212	*AVP1*	CaMV35S	Drought & Salinity	Biomass and higher photosynthetic rate	[70]
Arabidopsis thaliana	LBA4404	pGNFA-(pAHC17)	*NHX1*	CaMV35S	Drought & Salinity	Salt and proline accumulation	[28]
Arabidopsis thaliana	GV3101	pBISN1	*NHX1*	CaMV35S	Drought & Salinity	High chlorophyll content; large photosynthetic surface area, photosynthetic rate	[65]
Salicornia brachiate	-	pCAMBIA1301	*ASR1*	CaMV35S	Drought & Salinity	Higher Chlorophyll, RWC; Lower electrolyte leakage and MDA content; Proline, sugars and Starch accumulation; Lower H_2O_2, O_2 radicals; high SOD transcript levels	[71]
Brassica carinata	-	pBinAR	*ZAT12*	LEA	Drought & Salinity	Delayed wilting of leaves, enhanced osmotic adjustment, improved water and chlorophyll retention, less electrolyte leakage.	[73]
Pennisetum glaucum	LBA4404	pGreen0229	*eIF4A*	rd29A	Drought, Salinity & oxidative stress	Superior growth performance, Chlorophyll retention and free radical scavenging.	[74]
2. Groundnut transgenics manipulated with a multiple genes against abiotic stresses							
Arabidopsis thaliana	LBA4404	pKM12GW	*DREB2A, HB7, ABF3*	CaMV35S	Drought & Salinity	Improved cellular level tolerance by increased expression of detoxifying enzymes, increased cell membrane and chlorophyll stability, ROS scavenging and Osmotic adjustment.	[47]
Oriza sativa, Pennisetum glaucum & Pisum sativum.	EHA105	pKM12GW	*OsAlfin1, PgHSF4, Pea PDH45*	CaMV35S, Rd29A, 2X35S.	Drought & Oxidative stress	Higher root growth, cool crop canopy, higher RWC, Enhanced expression of *HSPs, RBX1, Aldo reductases, LEA5* and *PRP2* stress responsive genes. tolerance to ethrel, methyl viologen	[48]

GROUNDNUT TRANSGENICS MANIPULATED WITH SINGLE GENE AGAINST ABIOTIC STRESS

Drought stress resistance

Isopentenyl transferase (IPT), the key enzyme of cytokinin biosynthetic pathway also reported to be involved in drought stress tolerance. The groundnut transgenics overexpressing Arabidopsis *IPT* gene under maturation and stress-inducible promoter, SARK [49] resulted in higher photosynthetic efficiency which is correlated with improved biomass when compared to wild type plants under drought stress in laboratory and field conditions. These transgenics also showed higher stomatal conductance and transpiration efficiency under reduced irrigation [50]. Bhatnagar et al. [51] developed groundnut transgenics by overexpressing a stress inducible transcription factor gene *DREB1A* under two different promoters; a). constitutive CaMV35s promoter and b). *rd29A* a stress inducible promoter showed different growth patterns after four weeks of seed germination. The transgenics showed higher transpiration efficiency than wild type plants under water stress conditions with higher yield [52]. Overexpression of *DREB1A* under *rd29A* promoter also enhanced harvest index in correlation with higher root to shoot ratio [53] and shown better root system in deep soil layers in lysimeter system [54]. *AtDREB1A* improved water retention capacity, enhanced chlorophyll content osmotic adjustment by the accumulation of proline when overexpressed in a drought susceptible groundnut variety GG20 [55]. Manjulatha et al. [56] overexpressed a plant DNA helicase gene *PDH45* and reported increased intrinsic cellular level tolerance in transgenic groundnuts under water stress and also reported the stay green nature of transgenics by maintaining superior mesophyll efficiency and low Δ^{13} C which can be used as a measure of water use efficiency (WUE). Overexpression of a NAC transcription factor gene, *AtNAC2* in groundnut resulted in enhanced water retention capacity with stay green nature and reduced membrane damage than wildtype under water stress conditions [57]. *MuNAC4* overexpressing transgenic groundnut revealed increased lateral root development, osmotic adjustment and better antioxidative defense mechanisms than wild type plants and thereby conferring tolerance to drought [32].

Mannitol, a compatible solute reported to be accumulated during water deficit stress in plants [58]. Bhauso et al. [59] transformed a bacterial *mtlD* gene which encodes mannitol 1-phosphate dehydrogenase, which converts mannitol 1-phosphate to mannitol [60] into groundnut. Overexpression of *mtlD* gene exhibited 1.3-1.8 fold increase in mannitol biosynthesis in transgenics when compared to wild type that were positively correlated with the drought stress tolerance. These *mtlD* overexpressing groundnut transgenics were also reported to be maintaining better photosynthetic machinery and reduced oxidative damage in comparison with wildtype plants in dry-down experiments [61]. A novel plant specific transcription factor *WRKY3* was isolated from horsegram (*Macrotyloma uniflorum*) and was used to develop groundnut transgenics. *MuWRKY3* transgenics showed improved cellular level tolerance through the accumulation of osmolytes, ROS scavenging system and antioxidative machinery under drought stress conditions [62].

Salt stress resistance

There are only two reports of groundnut transgenics against salt stress. Vadawale et al. [63] overexpressed a functional enzyme choline oxidase (COX), that is reported to be involved in glycine betaine biosynthesis. Glycine betaine is one of the osmoprotectants synthesized in plants under abiotic stresses majorly under saline stress [64]. They reported the survival of transformed plants under 100 mM NaCl stress whereas untransformed plants showed curling and burning of leaves from the margins. They correlated these results with *COX* gene overexpression and increased synthesis of glycine betaine [63]. *AtNHX1* expressing groundnut plants maintained better growth, higher photosynthetic rate, stomatal conductance and transpiration rates in 150 mM NaCl stress [65]. Overexpression of peroxisomal ascorbate peroxidase (*pAPX*) gene from halophyte *Salicornia brachiate* in groundnut resulted in higher chlorophyll and RWC with reduced electrolyte leakage than WT under 150 mM NaCl stress [35].

Resistance to multiple abiotic stresses

Drought and Salinity are the principle abiotic stresses; approximately 830 million ha. were affected by soil salinity [66] and it affects ~7.61 million ha in India [67] and in the world 20% of the land surface affected by drought at any point of time [68]. Hence the development of groundnut transgenics with multiple abiotic stress tolerance has a significant relevance for the crop improvement.

NHX1 is a Na^+/H^+ antiporter found on a vacuolar membrane and is involved in the compartmentalization of Na^+ ions into vacuole which is detrimental to the plant cells [69]. Overexpression of this *NHX1* gene from Arabidopsis in groundnut improved drought and salt tolerance through sequestration of more Na^+ in vacuole and accumulation of proline than wild type plants under stress conditions. The transgenics also survived under high concentration of NaCl (200 mM) stress and drought stress where WT plants were failed to survive [28]. Another vacuolar membrane protein from Arabidopsis, H^+- pyrophosphatase, a proton pump encoded by *AtAVP1* was transferred to groundnut by *Agrobacterium* mediated gene transformation, transgenics maintained the morphological traits under both drought and salt stress compared to wildtype. These results were positively supported by higher chlorophyll content, photosynthetic efficiency and transpiration rate in transgenics under stress conditions [70]. Tiwari et al. [71] developed groundnut transgenics by overexpressing *SbASR-1* gene, cloned from *Salicornia brachiata* which codes for abscisic acid stress ripening 1 (ASR-1) protein a group7 LEA family protein. In comparison with wildtype plants, transgenics showed less reduction in chlorophyll content, higher water retention ability and accumulation of more compatible solutes like proline, soluble sugars, etc. under salt and drought stress conditions. Overexpression of *Prd29A: AtHDG11* gene cassette in groundnut resulted in upregulation of several abiotic stress tolerance genes [72] that were responsible for expression of antioxidative enzymes, accumulation of compatible solutes, ROS scavenging enzymes and genes involved in improving intrinsic water use efficiency under drought and salt stress conditions. These findings were positively correlated with healthier morphological traits and yield under drought and salt stress conditions [72].

Groundnut transgenics overexpressing *BcZAT12* under LEA promoter is reported to be conferring multiple abiotic stress tolerance by the accumulation of more proline and improved osmotic adjustment and water conservation ability under NaCl and PEG stress. These transgenics also showed the increased expression of antioxidant enzyme system and better phenotypic traits like less wilting of leaves which is correlated with less reduction of chlorophyll under stress conditions [73]. A eukaryotic translation initiation factor gene (*eIF4A*) from *Pennisetum glaucum* transformed into groundnut and reported to confer abiotic stress tolerance by Santhosh Rama Bhadra Rao et al. [74]. The transgenics maintained the higher chlorophyll retention, increased expression of anti-oxidative enzymes and improved water conservation by maintaining higher membrane integrity and superior phenotypic characters under simulated stress conditions.

MULTIGENE GROUNDNUT TRANSGENICS DEVELOPED AGAINST ABIOTIC STRESS

Abiotic stress tolerance is a complex phenomenon and involves the participation of many genes and their products [75,76]. Scientists developed hundreds of transgenics across several plant species by manipulation of single genes involved in biochemical pathways or regulation of downstream genes. These transgenics were successfully imparted tolerance against specific stress but not up to the desired levels of multiple stress tolerance [77]. To attain the multiple stress tolerance in crop plants, researchers concentrated on introduction of multiple genes simultaneously into plant systems. There are three multigene transfer approaches mainly used by researchers, conventional method, retransformation and co-transformation among which co-transformation is the reliable and time saving option [78]. Co-transformation involves the introduction of two or more genes, each carried by a separate vector simultaneously or all the genes carried by a single vector using multisite cloning approach. In multisite cloning strategy, two or more genes linked together on a single vector either by classical restriction digestion and ligation reaction or advanced gateway cloning technology involving recombination. There are several reports of multigene transfer in crop plants, here we review groundnut transgenics developed through multigene transfer against abiotic stresses.

Pruthvi et al. [47] engineered a groundnut cultivar TMV2 by co-expression of three Arabidopsis TF genes *AtDREB2A, AtHB7 and AtABF3* under constitutive promoter CaMV35s through a modified GATEWAY cloning technology [37] and conferred improved drought and salt tolerance in transgenic plants. Co-expression of these TFs genes facilitated the reduced membrane damage and maintained higher chlorophyll content. They also altered the expression of several downstream genes involved in acquired stress tolerance (*GRX, Aldehyde reductase, Serine threonine kinase like protein, Rbx1, Proline amino peptidase, HSP70, DIP and Lea4*) and osmolyte production revealed the improved cellular level tolerance under stress conditions. Another multigene groundnut transgenics were developed by simultaneous expression of *Alfin1, PgHSF4* and *PDH45* genes cloned from different source plants and overexpressed under three different expression elements. The transgenics showed improved stress tolerance by maintaining the higher growth and productivity than wildtype plants under water limited conditions. Transgenic plants also upheld the increased root growth, cooler canopy temperature with higher RWC under moisture stress. The transgenics exhibited improved oxidative stress tolerance induced by ethrel and methyl viologen and downstream regulation of several stress responsive genes by TFs revealed the enhanced drought tolerance by reducing oxidative damage in transformed plants [48].

CONCLUSION

The current review summarizes groundnut transgenics overexpressing genes coding for enzymes, osmoprotectants and transcription factors which have a regulatory role in modifying the pathways involved in abiotic stresses like drought, salinity and oxidative stress. Transgenic plants expressing multiple genes were proven to have higher ability to withstand abiotic stresses compared to the single gene expressing transgenics. Therefore, multigene transgenic approach is the viable option for improving the abiotic stress tolerance in semiarid crops like groundnut to overcome adverse conditions in the climate changing scenario. Stacking and expression of multiple genes responsible for several traits also provides tolerance to multiple abiotic stresses. Further, multigene strategy was advantageous as it is cost-effective, time saving approach and reduces the use of several selectable markers, also stacking of multiple genes together minimizes the chances of segregation of genes in the subsequent generations.

ACKNOWLEDGMENT

BV greatly acknowledges CSIR-SRF fellowship (No: 09/383(0051)/2016-EMR-I) from CSIR-INDIA and CS acknowledges DBT-GOI for financial support (BT/PR15503/AGR/02/913/2015. dated: 09-05-2017). AMAJS acknowledges Dr. DS Kothari Post-Doctoral Fellowship (BL/16-17/0364) from UGC, New Delhi.

REFERENCES

1. Barnabas B, et al. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 2008;31:11-38.
2. Boyer JS and Westgate ME. Grain yields with limited water. J Exp Bot. 2004;55:2385-2394.
3. Boyer JS. Plant productivity and environment. Science. 1982;218:443-448.
4. Verslues PE, et al. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 2006;45:523-539.
5. Mahajan S and Tuteja N. Cold, salinity and drought stresses: an overview. Arch Biochem Biophys. 2005;444:139-158.
6. Krishna G, et al. Progress in genetic engineering of peanut (Arachis hypogaea L.) - a review. Plant Biotechnol J. 2015;13:147-162.

e-ISSN:2347-226X
p-ISSN:2319-9857

7. Reddy TY and Anbumozhi VRV. Physiological responses of groundnut (Arachis hypogaea L.) to drought stress and its amelioration- a critical review. Plant Growth Regul. 2003;41:75-88.

8. Wright GC and Nageswara Rao RC. Groundnut water relations. In: The Groundnut Crop- A Scientific Basis for Improvement (Smartt J Edn). London: Chapman and Hall. 1994;pp:281-325.

9. Blum A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ. 2017;40:4-10.

10. Kasuga M, et al. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress inducible transcription factor. Nat Biotech. 1999;17:287-291.

11. Nambara E and Marion-Poll A. Abscisic acid biosynthesis and catabolism. Ann Rev Plant Biol. 2005;56:165-185.

12. Yao D, et al. Transcriptase analysis reveals salt-stress-regulated biological processes and key pathways in roots of cotton (Gossypium hirsutum L.). Genomics. 2011;98:47-55.

13. Shinozaki K and Dennis ES. Cell signaling and gene regulation global analyses of signal transduction and gene expression profiles. Curr Opin Plant Biol. 2003;6:405-409.

14. Zhu J. Salt and drought stress signal transduction in plants. Ann Rev Plant Biol. 2002;53:247-273.

15. http://www.fao.org/faostat/en/#data.QC

16. Allen ON and Allen EK. The Leguminosae. A source book of characteristics, uses and nodulation. The University of Wisconsin Press, Madison, WI. 1981;p:812.

17. Dwivedi SL, et al. Effect of genotypes and environments on oil content and oil quality parameters and their correlation in peanut (Arachis hypogaea L.). Peanut Sci. 1993;20:84-89.

18. Knauft D and Akins OP. Recent methods for germplasm enhancement and breeding. In: Advances in peanut science (Pattee HE and Stalker IIT Edn). Stillwater: APRES. 1995;pp:54-94.

19. Savage GP and Keenan JI. The composition and nutritive value of groundnut kernels. In: The groundnut crop: a scientific basis for improvement (Smart J Edn). Chapman and Hall, London. 1994;pp:173-213.

20. Cuc LM, et al. Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (Arachis hypogaea L.). BMC Plant Biol. 2008;8:55.

21. Mace ES, et al. SSR analysis of cultivated groundnut (Arachis hypogaea L.) germplasm resistant to rust and late leaf spot diseases. Euphytica. 2006;152:317-330.

22. Nareshkumar A, et al. Effect of Pb-stress on growth and mineral status of two groundnut (Arachis hypogaea L.) cultivars. J Plant Sci. 2014;2:304-310.

23. Sharma KK and Ortiz R. Program for the application of genetic transformation for crop improvement in the semi-arid tropics. In Vitro Cell Dev Biol Plant. 2000;36:83-92.

24. Stalker HT and Moss JP. Speciation, cytogenetics, and utilization of Arachis species. Adv Agron. 1987;41:1-40.

25. Bansal S, et al. Isolation and temporal endospermal expression of γ-kafirin gene of grain sorghum (Sorghum bicolor L. moench) var. M 35-1 for introgression analysis of transgene. J Cereal Sci. 2008;48:808-815.

26. Basu S, et al. Plant adaptation to drought stress. F1000 Res. 2016;5:1-10.

27. https://www.farmprogress.com/management/traditional-plant-breeding-vs-genetic-engineering-primer

28. Asif MA, et al. Enhanced expression of AtNHX1, in transgenic groundnut (Arachis hypogaea L.) improves salt and drought tolerence. Mol Biotechnol. 2011;49:250-256.

29. Bhatnagar M, et al. An efficient method for the production of marker-free transgenic plants of peanut (Arachis hypogaea L.). Plant Cell Rep. 2010;29:495-502.

30. Chu Y, et al. Improvement of peanut (Arachis hypogaea L.) transformation efficiency and determination of transgene copy number by relative quantitative real-time PCR. In Vitro Cell Dev Biol Plant. 2013;49:266-275.

31. Iqbal MM, et al. Over expression of rice chitinase gene in transgenic peanut (Arachis hypogaea L.) improves resistance against leaf spot. Mol Biotechnol. 2012;50:129-136.

32. Pandurangaiah M, et al. Overexpression of horsegram (Macrotyloma uniflorum Lam. Verdc.) NAC transcriptional factor (MuNAC4) in groundnut confers enhanced drought tolerance. Mol Biotechnol. 2014;56:758-769.

33. Rohini VK and Sankara Rao K. Transformation of peanut (Arachis hypogaea L.) with tobacco chitinase gene: variable response of transformants to leaf spot disease. Plant Sci. 2001;160:889-898.

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

35. Singh N, et al. Ectopic over-expression of peroxisomal ascorbate peroxidase (SbpAPX) gene confers salt stress tolerance in transgenic peanut (Arachis hypogaea). Gene. 2014;547:119-125.

36. Tiwari S, et al. Expression of δ-endotoxin Cry1EC from an inducible promoter confers insect protection in peanut (Arachis hypogaea L.) plants. Pest Manag Sci. 2011;67:137-145.

37. Vemanna RS, et al. A modified multisite Gateway cloning strategy for consolidation of genes in plants. Mol Biotechnol. 2013;53:129-138.

38. Halpin C. Gene stacking in transgenic plants–the challenge for 21st century plant biotechnology. Plant Biotechnol J. 2005;3:141-155.

39. Naqvi S, et al. When more is better: multigene engineering in plants. Trends Plant Sci. 2010;15:48-56.

40. Le Martret B, et al. Tobacco chloroplast transformants expressing genes encoding dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase, exhibit altered anti-oxidant metabolism and improved abiotic stress tolerance. Plant Biotechnol J. 2011;9:661-673.

41. Zorrilla-Lopez U, et al. Engineering metabolic pathways in plants by multigene transformation. Int J Dev Biol. 2013;57:565-576.

42. Babitha KC, et al. Co-expression of AtbHLH17 and AtWRKY28 confers resistance to abiotic stress in Arabidopsis. Transgenic Res. 2013;22:327-341.

43. Garg B, et al. Simultaneous expression of PDH45 with EPSPS gene improves salinity and herbicide tolerance in transgenic tobacco plants. Front Plant Sci. 2017;8:364.

44. Singla-Pareek SL, et al. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. Proc Natl Acad Sci USA. 2003;100:14672-14677.

45. Nguyen TX, et al. Transgene pyramiding of the HVA1 and mtlD in T3 maize (Zea mays L.) plants confers drought and salt tolerance, along with an increase in crop biomass. Int J Agron. 2013;2013: 598163.

46. Augustine SM, et al. Overexpression of EaDREB2 and pyramiding of EaDREB2 with the pea DNA helicase gene (PDH45) enhance drought and salinity tolerance in sugarcane (Saccharum spp. hybrid). Plant cell Rep. 2015;34:247-263.

47. Pruthvi V, et al. Simultaneous expression of abiotic stress responsive transcription factors, AtDREB2A, AtHB7 and AtABF3 improves salinity and drought tolerance in peanut (Arachis hypogaea L.). PLoS One. 2014;9:e111152.

48. Ramu VS, et al. Simultaneous expression of regulatory genes associated with specific drought☐adaptive traits improves drought adaptation in peanut. Plant Biotechnol J. 2016;14:1008-1020.

49. Rivero RM, et al. Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. Plant Physiol. 2009;150:1530-1540.

50. Qin H, et al. Regulated expression of an isopentenyltransferase gene (IPT) in peanut significantly improves drought tolerance and increases yield under field conditions. Plant Cell Physiol. 2011;52:1904-1914.

51. Bhatnagar-Mathur P, et al. Stress-inducible expression of AtDREB1A in transgenic peanut (Arachis hypogaea L.) increases transpiration efficiency under water-limiting conditions. Plant Cell Rep. 2007;26:2071-2082.

52. Bhatnagar-Mathur P, et al. Transgenic peanut overexpressing the DREB1A transcription factor has higher yields under drought stress. Mol Breed. 2014;33:327-340.

53. Shridhar Rao J, et al. Better root: shoot ratio conferred enhanced harvest index in transgenic groundnut overexpressing the rd29A: DREB1A gene under intermittent drought stress in an outdoor lysimetric dry-down trial. J SAT Agric Res. 2012;10:1-7.

54. Vadez V, et al. DREB1A promotes root development in deep soil layers and increases water extraction under water stress in groundnut. Plant Biol. 2013;15:45-52.

55. Sarkar T, et al. Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (Arachis hypogaea L.) conferred tolerance to soil-moisture deficit stress. Front Plant Sci. 2016;7:935.

56. Manjulatha M, et al. Overexpression of a pea DNA helicase (PDH45) in peanut (Arachis hypogaea L.) confers improvement of cellular level tolerance and productivity under drought stress. Mol Biotechnol. 2014;56:111-125.

57. Patil M, et al. Overexpression of AtNAC2 (ANAC092) in groundnut (Arachis hypogaea L.) improves abiotic stress tolerance. Plant Biotechnol Rep. 2014;8:161-169.

58. Wang Y, et al. Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J. 2005;43:413-424.

59. Bhauso TD, et al. Overexpression of bacterial mtlD gene in peanut improves drought tolerance through accumulation of mannitol. ScientificWorldJournal. 2014;2014:125967.

60. Rathinasabapathi B. Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways. Ann Botany. 2000;86:709-716.

61. Patel KG, et al. Transgenic Peanut (Arachis hypogaea L.) overexpressing mtlD gene showed improved photosynthetic, physio-biochemical, and yield-parameters under soil-moisture deficit stress in lysimeter system. Front Plant Sci. 2017;8:1881.

62. Kiranmai K, et al. A novel WRKY transcription factor, MuWRKY3 (Macrotyloma uniflorum Lam. Verdc.) enhances drought stress tolerance in transgenic groundnut (Arachis hypogaea L.) plants. Front Plant Sci. 2018;9:346.

63. Vadawale A, et al. Transformation of groundnut-Arachis hypogea L. Var. GG20 with the cox gene-an attempt to develop salinity tolerance. Int J Pharma Bio Sci. 2012;3:591-598.

64. Hayashi H, et al. Enhanced germination under high-salt conditions of seeds of transgenic Arabidopsis with a bacterial gene (codA) for choline oxidase. J Plant Res. 1998;111:357-362.

65. Banjara M, et al. Expression of an Arabidopsis sodium/proton antiporter gene (AtNHX1) in peanut to improve salt tolerance. Plant Biotechnol Rep. 2012;6:59-67.

66. Martinez-Beltran J and Manzur CL. Overview of salinity problems in the world and FAO strategies to address the problem. In: Proceedings of the International Salinity Forum, Riverside, California, USA. 2005;pp:311-313.

67. Singh R, et al. Variation in sensitivity to salinity in groundnut cultivars during seed germination and early seedling growth. J SAT Agric Res. 2007;5:1-7.

68. Burke EJ, et al. Modelling the recent evolution of global drought and projections for the twenty-first century with the Hadley Centre climate model. J Hydrometeorol. 2006;7:1113-1125.

69. Yamaguchi T and Blumwald E. Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci. 2005;10:615-620.

70. Qin H, et al. Expression of the Arabidopsis vacuolar H+-pyrophosphatase gene AVP1 in peanut to improve drought and salt tolerance. Plant Biotechnol Rep. 2013;7:345-355.

71. Tiwari V, et al. Introgression of the SbASR-1 gene cloned from a halophyte Salicornia brachiata enhances salinity and drought endurance in transgenic groundnut (Arachis hypogaea) and acts as a transcription factor. PLoS One. 2015;10:e0131567.

72. Banavath JN, et al. Stress inducible overexpression of AtHDG11 leads to improved drought and salt stress tolerance in peanut (Arachis hypogaea L.). Front Chem. 2018;6:34.

73. Kumar A. Characterization of transgenic groundnut (Arachis hypogaea L.) expressing the ZAT12 transcription factor gene under abiotic stresses (Doctoral dissertation). JAU, JUNAGADH. 2017;p:174.

74. Santosh Rama Bhadra Rao T, et al. Expression of Pennisetum glaucum eukaryotic translational initiation factor 4A (PgeIF4A) confers improved drought, salinity, and oxidative stress tolerance in groundnut. Front Plant Sci. 2017;8:453.

75. Umezawa T, et al. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr Opin Biotechnol. 2006;17:113-122.

76. Zhu JK. Abiotic stress signaling and responses in plants. Cell, 2016;167:313-324.

77. Varshney RK, et al. Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends Plant Sci. 2011;16:363-371.

78. Goel P and Singh AK. Single-versus multigene transfer approaches for crop abiotic stress tolerance. In: Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants. 2018;pp:255-275.