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REVIEW

Genetic engineering approaches to understanding drought tolerance in plants

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Abstract

Abiotic stresses such as drought, salinity, frost, etc., affect plant yield manyfold. These stresses can decrease the plant yield of important major crops up to 50%. The abiotic stress-related genes or other transcription factors (TFs) have multiple functions, as it increases proline content, leads closing of stomata to decrease the transpiration rate, enhances the production of some important stress-related protective enzymes, etc. and hence increases abiotic stress tolerance. Many TFs and other stress-related genes have been identified and characterized and transformed to many important cultivated plants against drought and others abiotic stresses. The transformed plants show better morpho-biochemical and physiological performances than non-transgenic plants. Many genetically engineered plants have been developed against drought stress including wheat, rice, tomato, soybean, cotton and many more. The efficiently engineered clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system is now becoming a preferred choice of researchers to edit plant genomes for introgression natural resistance against a range of abiotic stresses. It leads genome editing by precise manure with minimal or no effect on growth and development of plants. Very limited reports are available to develop drought-tolerant plants using CRISPR/Cas9 system. Here we discuss transgenic plant technology and new [CRISPR Cas9 and Virus-Induced Gene Silencing (VIGS)] techniques to confer drought tolerance in important plant species.

Keywords Abiotic stress · CRISPR/Cas9 · Drought stress · Transcription factor · Transgenic plants · VIGS

Introduction

Global food security is one of the key issues due to change of world climate condition and by the increase of population. Drought, salinity, heat, etc., stresses significantly affect plant yield and productivity (Nouri et al. 2015; Singh et al. 2018). Drought and salinity affect about one-third of our cultivated land and it cause loss of approximately 1,500,000 ha crop land/year (Peng et al. 2011). In recent years, drought stress significantly decreased the plant yield manyfold by disturbing its morpho-biochemical processes (Azevedo et al. 2011). Even lower heat and drought stress affect plant yield. These stresses reduced crop yield by up to 50%. The plants in reproductive stages are more sensitive to these stresses and thus affect yield of many important plant species (Lamaou et al. 2018).

Plants are more susceptible to these extreme environmental stresses as compared to any other living organisms. Plants respond poorly to high environmental stresses, as it affects both biochemical and physiological processes. So, the development of new engineered plants is important to fight against these stresses (Ramonell and Somerville et al. 2002). Decreasing the water amount up to 40% declines the yield of maize and wheat by as much as 40% and 21% (Daryanto et al. 2016). The important cultivated cowpea yield is affected up to 68% by drought stress (Farooq et al. 2017). About 40% of soybean yield loss occurs due to drought stress (Specht et al. 1999). It also reduces cell division, spreading of leaf surface, retards stem growth and root propagation (Anjum et al. 2015). Long-term

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exposure of drought stress may cause death of plants (Gill and Tuteja 2010). In sunflower, it affects the chlorophyll a, b and a + b content (Manivannan et al. 2007). Drought stress enhances oxidative damage, stomatal closure, affects other cellular structures and decreases the exchange rate of gases (Hasanuzzaman et al. 2017). It also increases the ROS and leads to breakage of cellular physiological homeostasis (Fernández-Ocana et al. 2011).

The high-level drought and frost stresses in soil affect plants morpho-biochemical processes. Plants' response to these environmental stresses has been extensively studied through various genetic engineering methods (Gosal et al. 2009). The stress genes regulate important drought stress-related pathways and hence increase drought tolerance in plants. Several drought-resistant genes have been characterized in many plant species whose function is still unknown. Therefore, reverse genetics approaches are useful for proper identification of these abiotic stress-related genes (Azevedo et al. 2011). The conventional breeding techniques are not enough to provide long-term resistance against abiotic stresses. Therefore, identification and cloning of important stress-related gene families is important to provide long-term resistance against abiotic stresses (Shinwari et al. 1998; Narusaka et al. 2003; Jan et al. 2016a, 2017).

Thus in this review, we focus on the negative impact of drought stress on plants and discuss various genetic engineering approaches to confer resistance against drought stress.

Regulons involved in abiotic stress tolerance in plants, transcription factors (TFs) and specific drought-related genes

Abiotic stresses disturb normal morpho-biochemical and physiological processes, hence affect yield. The response to these stresses varies according to genotype (Wang et al. 2018). The plant genes are activated by TFs in combination with other transcription binding sites (Chaves and Oliveira, 2004; Kimotho et al. 2019). These TFs attached the *cis*-acting elements of upstream regions of all gene promoters (Ciarmiello et al. 2011). In additions, TFs activate or suppress the activity of DNA polymerase enzyme and play a key role in gene expression (Riechmann et al. 2000). TFs activate many stress-related genes and enhance drought tolerance response. The key basic amino acids are involved to confer resistance to plants against abiotic stress (Annunziato 2008). TFs are useful by providing protection to plants in both biotic and abiotic stress conditions (Umezawa et al. 2006). In model *Arabidopsis* plants, about 34 families have been identified containing approximately 1533 TFs which have been classified (Riechmann et al. 2000). Recently, Wen et al (2019) reported the tolerant response of an important tree species *Betula platyphylla* (birch). They identified 2917 drought stress-related genes through RNA-Seq method. Among these genes, some drought-related TFs families, i.e., ethylene responsive factor and myeloblastosis oncogene were found to be maximum. In addition, they found that *BpERF2* and *BpMYB102* TFs enhance plant response against drought. These two TFs further activate several other stress-related genes and provide drought tolerance. Sakuma et al (2002) described various types of *DREBs* transcription factors in model *Arabidopsis* plant. They envisaged that *DREB1A* and *DREB2A* bind to a specific six-nucleotide sequence (A/GCCGAC) of DRE and increase drought and cold tolerance in *Arabidopsis* plant. However, the specificity of these transcription factors varies with change in second and third nucleotides in the sequence (A/GCCGAC) of DRE. They further classified these proteins into different classes including AP-2 subfamily, RAV subfamily, *DREB* subfamily, ERF subfamily, and others. Agarwal et al. (2010) reported that the *DREB* genes belong to AP2/ERF (apetala2 and ethylene responsive factors) class of TFs and present in different plant species. The *DREB1* and *DREB2* activate other stress-related genes which leads enhanced tolerance in plants against these environmental extreme conditions. Shinozaki et al. (2003) have clarified that incorporation of a single transcription factor has enhanced tolerance in plants against salt, drought and cold stresses though activation of several stress-tolerant gene against these stresses (Fig. 1). They noted that *DREB* gene with DRE-containing promoter enhances tolerance in plants against abiotic stresses. The detailed process of activation of biotic and abiotic stress-related genes via different TFs is given in Fig. 2.

These TFs are classified into different families, i.e., AP2 transcription factors (Liu et al. 1998), B ZIP transcription factors (Uno et al. 2000), MYB transcription factors (Fujita et al., 2007) and zinc finger transcription factors (Sugano et al. 2003). Grill and Himmelbach (1998) stated that the two pathways adopted by the plant for the stress response, i.e., abscisic acid-dependent and abscisic acid-independent gene expression are activated by a number of transcription factors such as ABFs proteins (ABRE-binding factor), ABA responsive element binding protein (AREB proteins), MYB/MYC proteins, DREB factors (dehydration responsive element binding factors) and NAC proteins (NAM, ATAF1-2, and CUC domains). The *CBF/DREB1* transcription factors act in abscisic acid-independent pathway for the induction of stress-responsive gene by cold stress signal. The bZIP and ABRE transcription family activate stress-responsive gene in abscisic acid-dependent stress signal pathway. Most of the abscisic acid-inducible genes have a nine base pair conserved *cis*-acting sequence (PyACGTGG C) namely ABA responsive element in their promoter.

Several other *DREB*-related genes have been characterized and transformed to important cultivated plant

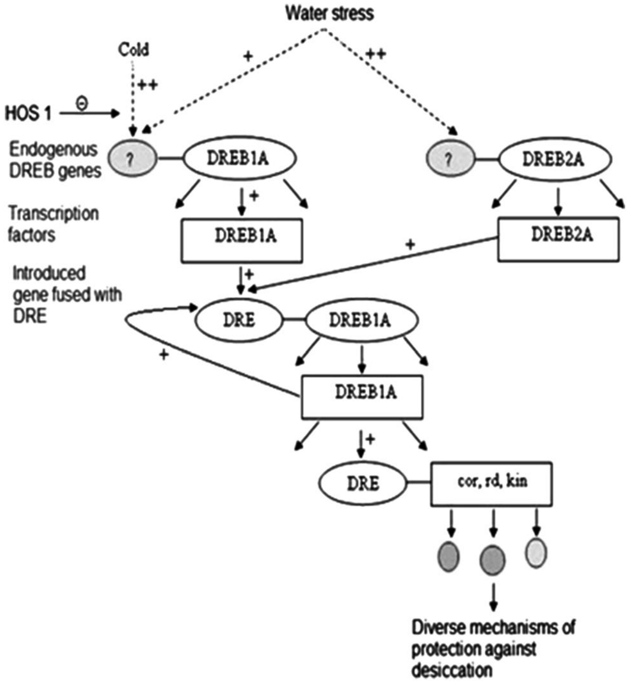

Fig. 1 Effect of cold, salt loading and dehydration on expression of stress-related genes. The activation of stress-related genes enhanced plant tolerance against drought (Shinozaki et al. 2003)

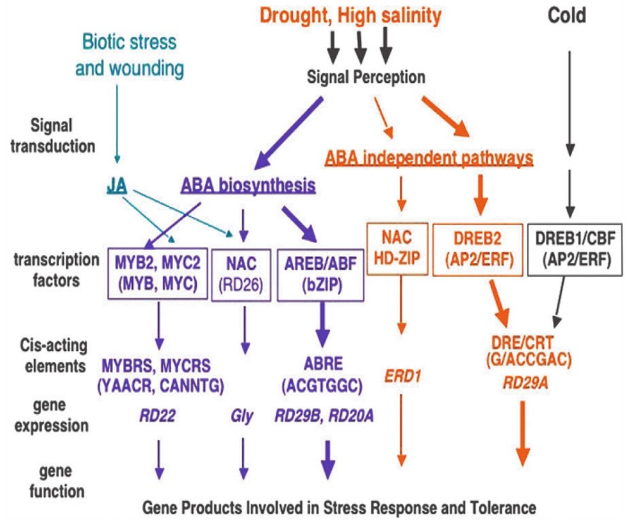

Fig. 2 Activation of transcription factors to improve biotic and abiotic stress tolerance in plants (Fujita et al. 2007)

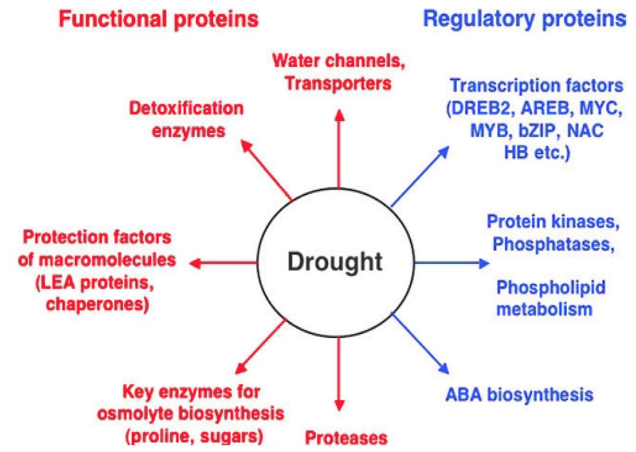

Fig. 3 The functions of drought stress-inducible genes in stress tolerance and response. Two types of proteins are produced in response to drought stress. The first group includes function proteins that probably function in stress tolerance and the second group includes regulatory proteins that function in signal transduction and gene expression of other abiotic stress-related genes (Shinozaki and Yamaguchi-Shinozaki 2007)

species against abiotic stresses. Like, Maruyama et al (2004) reported 38 different classes of *DREB* genes. They also showed that the transgenic plants express these genes that have multiple functions against drought and salt. Dubouzet et al (2003) isolated and constructed five different CDNAs of *DREB1A* genes: *OsDREB1A, OsDREB1B, OsDREB1C, OsDREB1D,* and *OsDREB2A* from rice and their functions were also characterized. Among these genes, *OsDREB1A* and *OsDREB1B* were highly activated in drought and cold conditions while *OsDREB2A* showed a high level of expression in drought and high salt condition. The resulting *OsDREB1A* from rice was transformed to *Arabidopsis* plant and the resulting transgenic plant showed multiple abiotic stress tolerance. From their work, they concluded that *OsDREB1A* gene show functional similarity with *AtDREB1A* gene and it is very useful to transform *OsDREB1A* gene in monocots to produce tolerance against these extreme environmental stresses. The *DREBs* transcription factors are further classified into three *DREB1A* and five *DREB2A* proteins, these two proteins have a main role in the activation of multiple genes against abiotic stresses. Chen et al (2008) isolated *GmDREB2* gene from Soybean (*Glycin mix*) and was transformed to *Arabidopsis* plant. The resulted transformed *Arabidopsis* plant showed better response and higher survival rate under drought, salt and cold stress conditions than control plants. Schramm et al (2008) reported that *DREB2A* transcription factor has a key role in the activation of several heat stress-related proteins especially HsfA3 which provide protection to tested *Arabidopsis* plant under extreme thermal conditions. Wang et al (2008) reported that *DREB* gene enhances abiotic stress tolerance in plants. Yamaguchi and Shinozaki (2007) also recorded similar results in *Arabidopsis* plants by expressing *DREB1A* gene. The expression analysis revealed that transformed plants showed enhanced tolerance to abiotic stress but also retarded normal plant growth. The *DREB1A* cDNA has an important role in many agriculturally important crops by gene transfer method. They described that the 35S CaMV promoter also leads to several morphological changes in transgenic *Arabidopsis* plants. Li et al (2005) isolated three different types of *DREB* genes from Soybean (*Glycin mix*) namely *GmDREBa*, *GmDREBb*, and *GmDREBc* under drought, and salt stress conditions. The expression analysis showed that *GmDREBa*, *GmDREBb* genes are expressed in leaves under salt and drought stress condition, while *GmDREBc* is only expressed in roots under extreme drought and salt condition. From their results, they concluded that these three types of *DREB* genes promote plant response against drought and salt. Kasuga et al. (1999) studied transformation of *DREB1A* gene to tobacco and found enhanced tolerance against both drought and cold. Details about the function of *DREB1A* and *DREB2B* are given in Fig. 3.

The maize WRKY TF (*ZmWRKY4*) provides drought, salinity and temperature stress resistance in transgenic *Arabidopsis*. The gene up-regulates other abiotic stress-tolerant genes in transformed plants. In addition, it also increases important stress-related enzymes, i.e., peroxide dismutase and catalase and increases drought tolerance (Wang et al. 2018). Similarly, wheat TF (*TaWRKY33)* enhances resistance in transformed *Arabidopsis* plants against both drought and salt (He et al. 2016). The *DREB2* and antioxidant enzyme gene (*CAT1*) in bread wheat enhance drought resistance. The relative water content (RWC), total chlorophyll content and catalase activity was found to be maximum in wheat cultivar (Kavir) under drought stress condition. So, these two genes are important for drought resistance (Eftekhari et al. 2017). The transgenic wheat lines expressing mutated TF (*HaHB4*) of sunflower enhance water use efficiency under abiotic stress (González et al. 2019). The high expression of *ERF1-V* in wheat provided tolerance against powdery mildew disease and salt and drought stresses (Xing et al. 2017).

The rice Rab family proteins, i.e., *Rab7* (*OsRab7*) enhance drought and heat stress response in transformed rice genotypes. The overall morpho-biochemical and physiological responses were higher in transformed plants than wild rice genotypes under both stresses. In addition, malondialdehyde (MDA), hydrogen peroxide and electrolyte leakage was lower in transgenic plants. High yield was noted for transgenic rice than non-transgenic plants (Masood et al. 2005; El-Esawi and Alayafi 2019).The TFs (*CUC2, ATAF1-2,* and *NAM*) play a key role in plant developmental stages and abiotic stress conditions. At vegetative stage, the high level of *OsNAC14* expression increases tolerance in drought condition in engineered rice plants. RNA-sequencing data showed that *OsNAC14* increased the expression of other stress-related genes, plant defense, DNA repair system and biosynthesis of strigolactone (Shim et al. 2018). Also, *ZmWRKY58* increases drought tolerance in transformed rice (Cai et al. 2014). The higher expression of *SNAC1*gene increased drought tolerance in rice. The engineered plants showed higher seed setting (22–34%) at reproductive stage than non-transgenic plants. In addition, no adverse effect was found in plant morphology and yield. The DNA chip data also showed that overexpression of SNAC1 gene activates other TFs (*NAM, ATAF,* and *CUC* (*NAC*) hence, improved drought and salt tolerance (Hu et al. 2006). The transgenic indica rice genotype expressing *AtDREBIA* gene shows drought tolerance at both vegetative and reproductive stages in T1, T2, and in following generations. The transgenic rice shows better physiological responses than wild plants. The tolerance, spikelet fertility and grain yield response were found to be maximum in homozygous lines than non-transgenic plants (Ravikumar et al. 2014). In another report, the overexpression of *OsERF71* enhances drought tolerance in transgenic rice (Lee et al. 2016).

The drought tolerance is found maximum in cotton TM-1 genotype due to high expression of several coding TFs and other regulatory and enzymes controlling genes (*ERF*, *ERFB*, *DREB*, etc.). The TM-1 genotype showed maximum biochemical and physiological performance and healthy chloroplast structure under drought condition (Nakashima et al. 2000; Mosfeq-Ul Hasan et al. 2018). *WRKYs* in soybean promote drought tolerance. The activation of *GmWRKY12* in tissues is minimal under normal conditions than drought condition. In hairy root culture, *GmWRKY12* is responsible for higher proline and MDA content production, thus provides tolerance against drought and salt stresses in transgenic plants seedlings (Shi et al. 2018). Transgenic soybean expressing *AtABF3* shows both drought and salt tolerance even at the end of 20 days of stress. Various physiological changes were noted during these stress conditions. The chlorophyll amount was found to be higher in transformed plants than control plants. Maximum stomata were found closed at stress condition. In addition, the engineered plants showed normal and stable cell membrane structure and lower transpiration rate at both stress conditions. The transgenic plants showed more total seed weight than NT plants. However, the drought and salt stress responses varied with types of genotype (Kim et al. 2018).

The transgenic tomato genotypes overexpressing the *AnnSp2* gene show tolerance to both drought and salinity stresses. In severe stress condition, the *AnnSp2* gene enhances ABA content, hence increased rate of closing stomata and decreased water loss. In addition, the transgenic plants showed better biochemical and physiological responses and lower ROS content (Ijaz et al. 2017). The *ATAF1* gene from NAC family may increase drought resistance in tomato (Awais et al. 2018).

The *SsDREB* TFs of *Suaeda salsa* enhance drought and salt tolerance in tobacco plants. The chlorophyll, proline and soluble sugar content and photosynthetic rate were found to be maximum in transgenic plants as compared to control plants (Zhang et al. 2015). The high expression of *Pennisetum glaucum Rab7* in tobacco plant increases both salt and osmotic stress resistance (Agarwal et al. 2008). Cong et al (2008) reported that transgenic Tobacco plant expressed the *Brassica juncea DREB1B* gene. The resulted transgenic plants showed a high level of proline content as compared to non-transgenic plant which leads to enhanced abiotic stress tolerance in transformed plants.

Many TFs belonging to family AP2/ERF were overexpressed in plants and provide resistance to different types of stresses (Mizoi et al. 2012; Phukan et al. 2017). The high expression of *SpERF1* in engineered *A. thaliana* improves drought resistance (Yang et al. 2016). The *AhDREB1*gene in *Arabidopsis* increase ABA level and activate many drought-tolerant genes *(RD29A*, *P5CS2, NCED1,* and *P5CS1).* The resulted transgenic plant showed higher drought tolerance as compared to non-transgenic plants. In addition, at osmotic stress condition, the histone acetylation significantly affects the regulation of *AhDREB1* gene, hence increases tolerance against drought (Zhang et al. 2018).The overexpression of *AtRabG3e* in *Arabidopsis* increases both salt and osmotic stresses tolerance (Mazel et al. 2004).The engineered *Arabidopsis* plants having a low amount of lignin and xylan acetylation shows maximum drought tolerance response as compared to control plants. The lower lignin content is due to expression of QsuB, which enhance abscisic acid (ABA) response for germination of seed and stomata closing. In addition, the low xylan-modified plants showed high amount of galactose and sugar (Yan et al. 2018). *PYR1/PYL/RCARs* play a key role in ABA signal transduction. The *ZmPYLs* is important in various abiotic stress conditions. Transgenic *Arabidopsis*-expressing *ZmPYL* increased sensitivity to ABA. The transformed plants having *ZmPYL12, ZmPYL9,* and *Zmpyl8* show more resistance to drought. The accumulation of a high amount of protective protein (proline) further justifies the drought tolerance capability of *ZmPYL* genes in plants (He et al. 2018). *MtMYBS1* increases both drought and salt tolerance in engineered *Arabidopsis* plants (Dong et al. 2017). The high expression of *GaMYB62L* in transformed *Arabidopsis* plants increases drought tolerance (Butt et al. 2017). Triple mutation of *snrk2.2/3/6* removes the ABA response and increases drought susceptibility (Fujii and Zhu 2009). *SnRK2.6* enhances the expression of regulating the ubiquitin E3 ligase activity of RZFP34/CHYR1 and promotes drought tolerance (Ding et al. 2015). *HAI PP2Cs* enhance the content of proline (an important osmo-regulator) and promote drought tolerance (Bhaskara et al. 2012). The high-level expression of epidermal patterning gene (*OsEPF1*) from rice increased drought tolerance by decreasing stomatal density in transgenic plants. The low stomata density allows rice plant to store about 60% of water between weeks 4 and 5 post-germination. Elevated CO_2 level enables transgenic plants to survive in high drought and temperature (40 °C) conditions for a longer time period. In addition, low stomatal density enhances plant yield and helps plant to adapt better under warm climate condition (Caine et al., 2019). The overexpression of Cycling Dof Factor 3 enhances drought, salt and frost tolerance in *Arabidopsis* (Corrales et al. 2017). Details of different TFs or stress-related genes transformed into important plant species are given in Table 1.

Advanced genomic methods used to confer drought resistance in important plant species

Transgenic technology is very expensive, time consuming and difficult. In addition, this technology is not successful in many important cultivated crops (Slade et al. 2005). Various techniques like acetylation (Kim et al. 2012), methylation (Fu et al. 2013, 2017) and ubiquitination (Chen et al. 2018) are important to produce drought-tolerant genotypes. Some other efficient and quick technologies have been developed recently for crop improvement and for specific gene analysis like VIGS and CRISPR/Cas9 (Senthil-Kumar and Mysore 2014; Khatodia et al. 2016) (Tables 2, 3).

VIGS is an RNA-based antiviral defense technique commonly used to confer resistance against viruses. However, the virus vectors having genes can target the corresponding mRNAs. This method has been exploited in plants for analysis of gene function and has been adapted for high-throughput functional genomics. Various RNA viruses were engineered as viral vectors to check the function of abiotic stress-related genes (Lu et al. 2003). The knock-down of drought-inducible variant (*H1-S)* in tomato increased drought tolerance and stomata closing (Scippa et al. 2004). The overexpression of *TaH2A.7* variant in *Arabidopsis* increased drought response and decreased water loss efficiency (Xu et al. 2016). The knock-down *TaH2B-7D* gene via VIGS in common wheat increases the relative electrolyte leakage rate and malondialdehyde amount. In addition, it decreases proline and percent relative water content, hence provides drought tolerance. The knock-down plants shows dwarf phenotype and symptoms of wilting as compared to non-knock-down genotypes. It means that *TaH2B-7D* gene provides tolerance under drought condition in common wheat (wang et al. 2019). The upregulation of *AtHUB2* gene enhances cotton response against drought (Chen et al. 2018). The knock-down of three important genes (*SpMAPK1, SpMAPK2,* and *SpMAPK3)* in *Solanum pimpinellifolium* reduced drought tolerance. The silencing of *GhWRKY27a* gene enhances drought tolerance in cotton (Yan et al. 2015a, b). The VIGS of two important genes (*GhNAC79* and *JUB1*) decrease drought response in cotton and tomato (Tasaki et al.

Table 1 Detailed functions of different stress-related genes/TFs against drought and other abiotic stresses in important plant species

Gene/TF	Crop	Stress response	Reference
OsRab7	Rice	Drought and heat	El-Esawi and Alayafi (2019)
OsNAC14	Rice	Abiotic stresses including drought	Shim et al. (2018)
OsERF71	Rice	Drought	Lee et al. (2016)
ZmWRKY58	Rice	Drought	Cai et al. (2014)
AtDREBIA	Rice	Drought	Ravikumar et al. (2014)
SNAC1	Rice	Drought and salt	Hu et al. (2006)
ERF, *ERFB*, *DREB*, others	Cotton	Drought	Mosfeq-Ul Hasan et al. (2018)
GmWRKY12	Soybean	Drought and salt	Shi et al. (2018)
AtABF3	Soybean	Drought and salt	Kim et al. (2018)
ATAF1	Tomato	Drought	Awais et al. (2018)
AnnSp2	Tomato	Drought and salt	Ijaz et al. (2017)
AtNHX1 and *TVP1*	Tomato	Drought and salt	Khoudi et al. (2009)
HaHB4	Wheat	Drought	González et al.(2019)
*DREB2*and *CAT1*	Wheat	Drought	Eftekhari et al. (2017)
ERF1-V	Wheat	Drought, salt and powdery mildew	Xing et al. (2017)
AtHDG11	Wheat	Drought	Li et al. (2016)
ZmPEPC	Wheat	Drought	Qin et al. (2016)
DREB1A	Wheat	Drought, salt and frost	Pellegrineschi et al. (2004)
DREB1A	Wheat	Drought and salt	Shen et al. (2003)
OsMYB55	Maize	Drought and heat	Casaretto et al. (2016)
ZmARGOS1	Maize	Drought	Shi et al. (2015)
AtLOS5	Maize	Drought	Lu et al. (2013)
ZmPIS	Maize	Drought	Liu et al. (2013)
NPK1	Maize	Drought	Shou et al. (2004)
SoP5CS	Sugarcane	Drought	Li et al. (2018)
BcZAT12	Sugarcane	Drought and salt	Saravanan et al. (2018)
AtHDG11	Groundnut	Drought and salt	Banavath et al. (2018)
MuWRKY3	Groundnut	Drought	Kiranmai et al. (2018)
AtDREB1A	Groundnut	Drought and salt	Sarkar et al. (2014)
SsDREB	Tobacco	Drought and salt	Zhang et al. (2015)
AtNPR1	Tobacco	Oxidative	Srinivasan et al. (2009)
DREB1B	Tobacco	Drought and salt	Cong et al. (2008)
DREB1A	Tobacco	Drought and cold	Kasuga et al. (2004)
Rab7	Tobacco	Salt and osmotic stress	Agarwal et al. (2008)
ZmWRKY4	*Arabidopsis*	Drought, salt, and temperature	Wang et al. (2018)
ZmPYL12, ZmPYL9, and *Zmpyl8*	*Arabidopsis*	Drought	He et al. (2018)
AhDREB1	*Arabidopsis*	Drought	Zhang et al. (2018)
MtMYBS1	*Arabidopsis*	Drought and salt	Dong et al. (2017)
GaMYB62L	*Arabidopsis*	Drought	Butt et al. (2017)
Cycling Dof Factor 3	*Arabidopsis*	Drought, salt, and frost	Corrales et al. (2017)
TaWRKY33	*Arabidopsis*	Drought and salt	He et al. (2016)
SpERF1	*Arabidopsis*	Drought	Yang et al. (2016)
DREB	*Arabidopsis*	Drought, salt, and cold	Wang et al. (2008)
GmDREB2	*Arabidopsis*	Drought, salt, and cold	Chen et al. (2008)
DREB1A	*Arabidopsis*	Drought, salt, and cold	Wang et al. (2007); Dubouzet et al. (2003)
DREB	*Arabidopsis*	Drought and salt	Li et al. (2005)
AtRabG3e	*Arabidopsis*	Salt and osmotic stress	Mazel et al.(2004)

Table 2 List of some important abiotic stress-related gene silenced in model and other comically important plant species using VIGS

Target gene	Crop	Stress response	Reference
TaH2B-7D	Wheat	Drought	Wang et al. (2019)
G18431620 (GH 3.5)	Cotton	Drought	Kirungu et al. (2019)
AtHUB2	Cotton	Drought	Chen et al. (2018)
GhNAC79 and *JUB1*	Cotton and tomato	Drought	Thirumalaikumar et al. (2018); Tasaki et al. (2016)
ApDRI15	*Alternanthera philoxeroidsi*	Drought	Bai et al. (2017)
TaH2A.7	*Arabidopsis*	Drought	Xu et al. (2016)
GhWRKY27a	*Nicotiana benthamiana*	Drought and salt	Yan et al. (2015a, b)
CaMLO2	*Chili pepper*	Drought	Lim and Lee (2014)
TaEra1	*Wheat*	Drought	Manmathan et al. (2013)
TdAtg8	Wild emmer wheat	Drought	Kuzuoglu-Ozturk et al. (2012)
HvHVA1	Barley	Drought	Liang et al. (2012)
SlSR1L and *SlGRX1*	Tomato	Drought	Guo et al. (2010)
H1-S	Tomato	Drought	Scippa et al. (2004)

Table 3 Recent examples of some important drought stress-related genes studied via CRISPR/Cas9 technology (modified from Singh et al. 2018)

Gene name	Crop	Reference
SlNPR1	Tomato	Li et al. (2019)
AREB1	*Arabidopsis*	Paixao et al. (2019)
GT79B2, UGT79B3	*Arabidopsis*	Li et al. (2017)
PtoMYB216	*Arabidopsis*, Poplar	Xu et al. (2017)
ARGOS8	Maize	Shi et al. (2017)
slmapk3	Tomato	Wang et al. (2017)
OsSAPK2	Rice	Lou et al. (2017)
mir169a	*Arabidopsis*	Zhao et al. (2016)

2016; Thirumalaikumar et al. 2018). The silencing of two important genes (*SlSR1L* and *SlGRX1*) led to lower drought tolerance in tomato (Guo et al. 2010). The list of abiotic stress-related genes silenced through VIGS technique is given in Table 2.

The CRISPR/Cas9 system was discovered early in prokaryotic organisms that act as source of defense against the foreign phages by cleaving its genome through RNA-guided DNA nuclease by specialized mechanism (Sorek et al. 2013). Recently, this system has been used as a major source of genome editing for many living organisms including plants (Belhaj et al.2015; Khurshid et al. 2017; Shinwari et al. 2017; Xing et al. 2014). CRISPR/Cas9 technology has been extensively used as a novel technique for conferring drought resistance in plants (Singh et al. 2018).The *SlNPR1 cis*-acting element provides drought tolerance in tomato. The CRISPR/Cas9-based mutation of mutant tomato *slnpr1* mutants showed lower resistance to drought with a higher amount of electrolyte leakage and stomatal opening, hydrogen peroxide and MDA content than wild type. These findings showed that *SlNPR1* is involved in drought tolerance response in tomato (Li et al. 2019). The maize *ARGOS8* variants produced by CRISPR/Cas9-based method showed tolerance to drought and gave maximum yield (Shi et al. 2017). The detailed importance of CRISPR/Cas9 system against drought stress in important plant species is given in Table 3.

Conclusions

Drought condition has been increased globally over the last few decades and it is one of the major constraints that limit our crop productivity and sustainable agriculture. The drought condition decreased food and feed dramatically especially in the last 2 decades. Therefore, it is vital to develop novel drought resistance varieties for further crop improvement. The transgenic technology can solve this problem by the production of new drought-resistant genotypes with no or minimal effect on plant morpho-biochemical and physiological performances. A large number of environmental stress-related genes have been isolated and transformed to various cultivated plants and the resulted engineered plants showed enhanced drought tolerance. Detailed steps of genetic transformation are given in Fig. 4a. New in planta (tissue culture free)-based transformation protocols should be developed for genotypes having problems with tissue culture protocol and long germination to maturity time periods (Fig. 4b). Many other novel techniques have been developed that are more efficient, precise and quick than genetically modified (GM) technology. VIGS is a quick and robust method to check the function of genes, involved in abiotic stress tolerance in plants. However, this technique has been successfully used for only few model plants and there is a need to extend this technique to check the function of others abiotic stress-related genes in the model

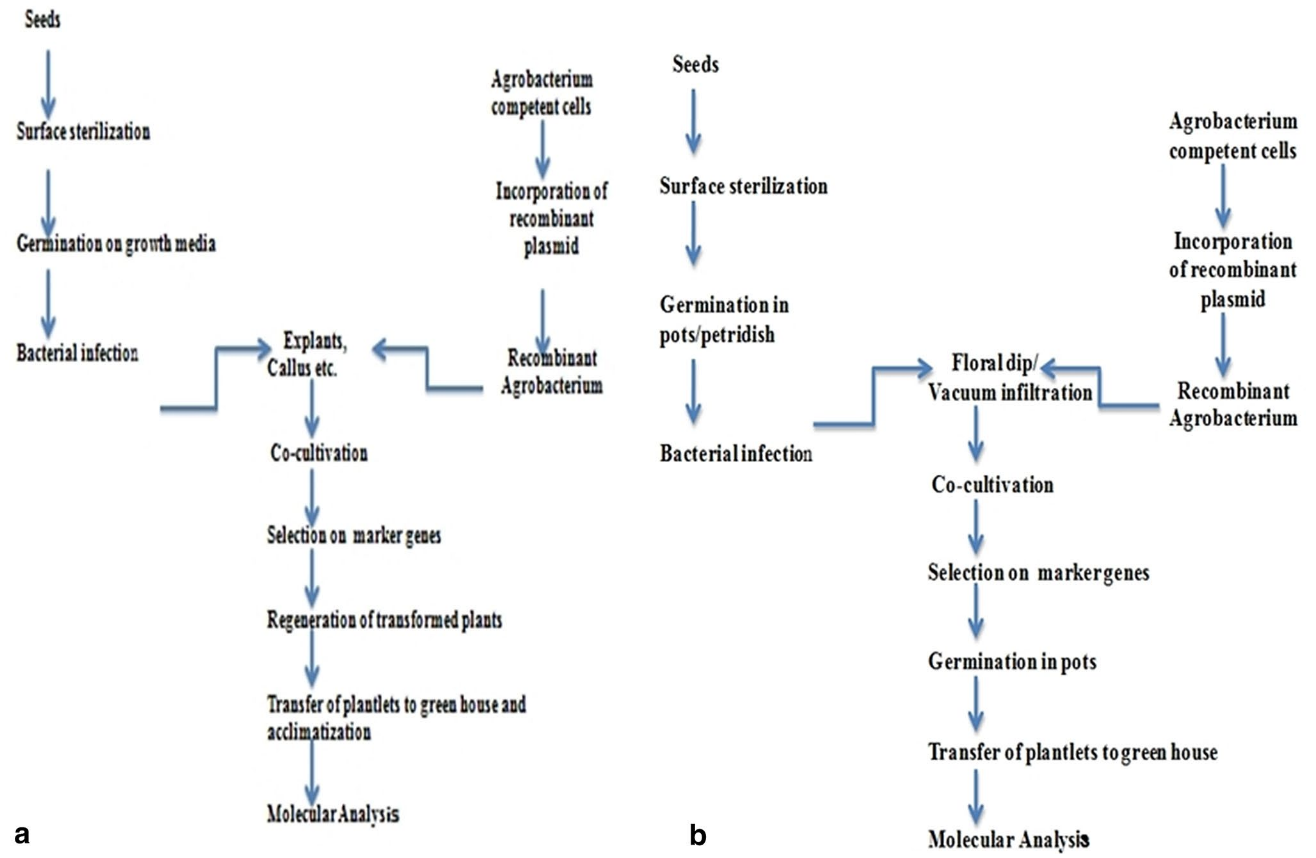

Fig. 4 **a** Various steps involved in the development of stable transgenic plants via Agrobacterium-based transformation into different plant species. This protocol is useful for plants having tissue culture protocol optimized. The protocol is expensive and time consuming and needs a long time period. **b** In planta transformation is a direct transformation method to develop transgenic plants in a short time. The protocol does not need any tissue culture method and is less expensive. The production of chimera plants is a major drawback of this method (Jan et al. 2016b)

and other economically important crop species. Therefore, developing new viral vectors for model and other organisms need to be well optimized. Recently, novel CRISPR/Cas9 plant genome editing protocols have been developed by different researchers. It is one of the novel methods of plant genome editing and provides resistance against drought stress. This system also helps us to know about the gene knock outs/knock in, epigenetic mechanism and gene regulation. However, several modifications are needed to develop some new drought-resistant engineered plants using CRISPR technology. The CRISPR/Cas9 method may produce some new transgenic plants against drought stress with minimal or no biosafety issues in near future. Only few transgenic plants have been developed against drought stress using CRISPR/Cas9 system. Therefore, new drought-resistant plants should be developed using this technology. In addition, new stress models and multiple stress markers data should be developed. The new drought avoidance, escape and tolerance strategies should be used for providing long-term drought tolerance in plants.

Author contributions Z-KS and S-AJ outlined and conceived the idea. Z-KS, S-AJ, K-N and K-YS helped in manuscript writing, editing and proof reading.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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