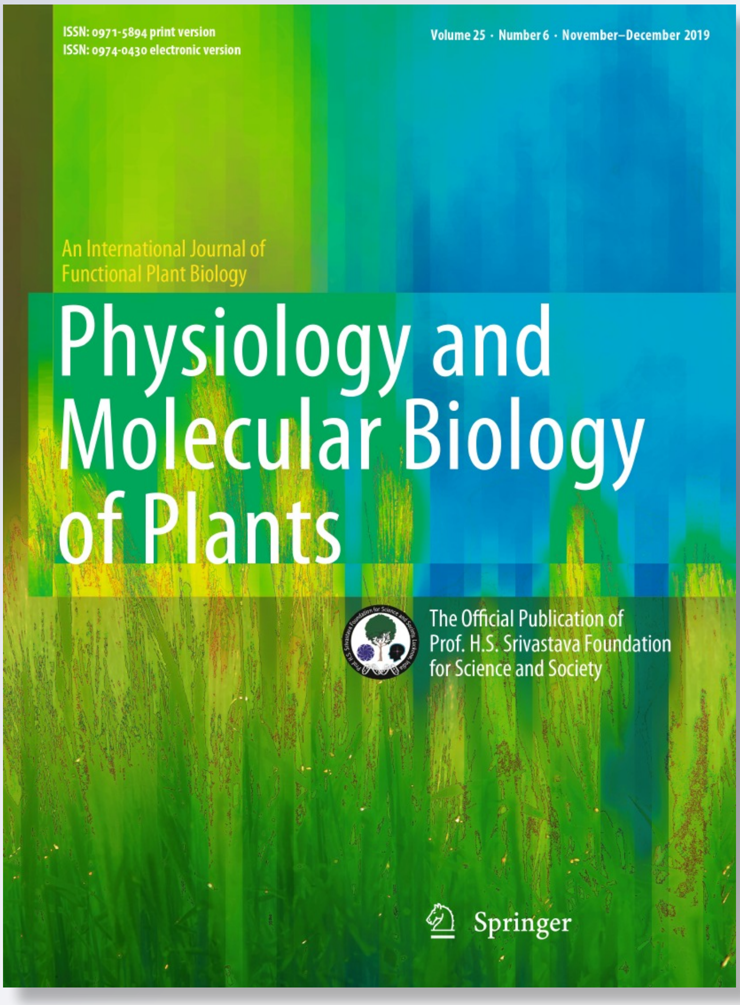
Advances in the development and use of DREB *for improved abiotic stress tolerance in transgenic crop plants*

Tanmoy Sarkar, Radhakrishnan Thankappan, Gyan P. Mishra & Bhagwat D. Nawade

Physiology and Molecular Biology of Plants
An International Journal of Functional Plant Biology

ISSN 0971-5894
Volume 25
Number 6

Physiol Mol Biol Plants (2019)
25:1323-1334
DOI 10.1007/s12298-019-00711-2



Physiol Mol Biol Plants (November–December 2019) 25(6):1323–1334
https://doi.org/10.1007/s12298-019-00711-2

REVIEW ARTICLE

Advances in the development and use of *DREB* for improved abiotic stress tolerance in transgenic crop plants

Tanmoy Sarkar[1,2] · **Radhakrishnan Thankappan**[1] · **Gyan P. Mishra**[1,3] · **Bhagwat D. Nawade**[1]

Received: 19 January 2019 / Revised: 7 May 2019 / Accepted: 29 August 2019 / Published online: 4 October 2019

Abstract Abiotic stresses negatively influence the survival, biomass production, and yield of crops. Tolerance to diverse abiotic stresses in plants is regulated by multiple genes responding differently to various stress conditions. Genetic engineering approaches have helped develop transgenic crops with improved abiotic stress tolerance including yields. The dehydration-responsive element binding protein (DREB) is a stress-responsive transcription factor that modulates the expression of downstream stress-inducible genes, which confer simultaneous tolerance to multiple stresses. This review focuses on advances in the development of *DREB* transgenic crops and their characterization under various abiotic stress conditions. It further discusses the mechanistic aspects of abiotic stress tolerance, yield gain, the fate of transgenic plants under controlled and field conditions and future research directions toward commercialization of *DREB* transgenic crops.

Keywords Climate resilience · Dehydration-responsive element binding protein · Functional markers · Omics · Transgenic crop · Yield traits

Introduction

Abiotic stresses like drought, salinity, and temperature fluctuations, negatively affect the growth and development of plants, and are responsible for the reduced productivity of major crops and significant economic losses, especially in the changing climatic scenario (Bhatnagar-Mathur et al. 2008; Patel et al. 2017). Globally, drought and salinity affect around 20% and 22% of the arable land, respectively; resulting in more than 50% reduced crop productivity in the affected areas (Agarwal et al. 2017; Bhatnagar-Mathur et al. 2008; Sarkar et al. 2016). Abiotic stresses can trigger various morpho-physiological alterations, such as leaf-wilting, leaf-abscission, reduction in relative water content (RWC), a decrease in chlorophyll content and membrane stability, increased electrolyte leakage (EL), and generation of reactive oxygen species (ROS) (Patel et al. 2016). Therefore, stress tolerant varieties of major crops need to be developed to enhance their productivity under multiple abiotic stresses (Bosamia et al. 2015; Sarkar et al. 2014).

Both classical and marker-assisted breeding methods have been employed to improve the abiotic stress tolerance of crops but have resulted in limited success (Sarkar et al. 2014). Stress tolerant quantitative trait loci (QTL) have been used to develop a stress-tolerant, high yielding variety (Bhatnagar-Mathur et al. 2008). The complex response mechanism of plants to various abiotic stresses involves the expression of multiple stress-inducible genes, which result in morphological, cellular, biochemical and molecular changes. Stress-inducible genes can be grouped into structural and regulatory proteins encoding genes (Fig. 1). Structural genes code for proteins that have structural, enzymatic, or other functions. Regulatory genes code for proteins that control the expression of other genes.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12298-019-00711-2) contains supplementary material, which is available to authorized users.

✉ Radhakrishnan Thankappan
radhakrishnan.nrcg@gmail.com

[1] ICAR-Directorate of Groundnut Research,
Post Box 1, Junagadh, Gujarat 362001, India

[2] Central Sericultural Research & Training Institute (CSRTI),
Mysuru, Karnataka 570 008, India

[3] Division of Genetics, ICAR-Indian Agricultural Research
Institute, New Delhi, Delhi 110012, India

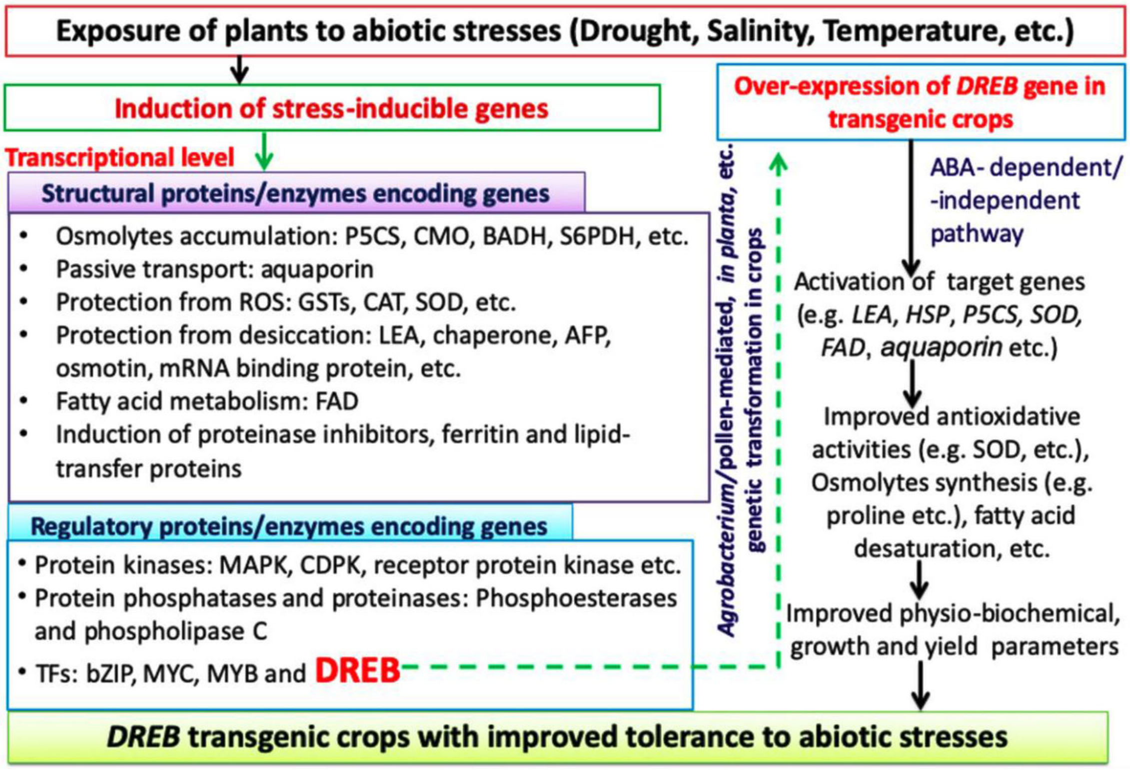


Fig. 1 An outline of the induction of various inducible genes involving abiotic stress response pathways and mechanisms of *DREB*-regulated abiotic stress tolerance in transgenic plants. Superoxide dismutase (SOD), glutathione-S-transferases (GSTs), pyrroline-5-carboxylate synthase (P5CS), choline monooxygenase (CMO), betaine aldehyde dehydrogenase (BADH), sorbitol-6-phosphate dehydrogenase (S6PDH), catalase (CAT), mitogen-activated protein kinase (MAPK), calcium-dependent protein kinase (CDPK), glutathione reductase (GR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), cold-regulated (COR), dehydrin (DHN), late embryogenesis abundant (LEA), antifreeze protein (AFP), fatty acid desaturase (FAD), basic leucine zipper domain (bZIP), myelocytomatosis related proteins (MYC), myelocytomatosis related proteins (MYB) and heat shock protein (HSP)

Since abiotic-stress tolerance is polygenic in nature, and stress responses in plants have overlapping signaling mechanisms, transferring any single-action gene will likely not be sufficient to induce the desired level of tolerance (i.e. the level at which transgenic plant can show optimal growth and yield). Regulons, that are regulated as a unit by a single gene coding a transcription factor (TF), can be harnessed to simultaneously induce the expression of multiple stress-inducible genes. The dehydration-responsive element (DRE) binding protein (DREB) or CRT (C-Repeat) binding factor (CBF) is a *trans*-acting TF, which activates multiple downstream stress-inducible genes via the *cis*-acting DRE/CRT motifs on their respective promoter regions (Yamaguchi-Shinozaki and Shinozaki 1994). DREBs belong to the ERF (ethylene-responsive element binding factor) family of TFs and consist of two main subclasses—DREB1/CBF and DREB2—that respectively mediate cold and salinity/dehydration tolerance (Liu et al. 1998; Agarwal et al. 2006).

Expression of *DREB1* or its target genes are regulated by up-stream TFs like ICE1 (Chinnusamy et al. 2003), HOS1 (Lee et al. 2001), DEAR1 (Tsutsui et al. 2009), and others (Akhtar et al. 2012) (Fig. 2). The *DREB* gene is the master regulator of an array of downstream stress-inducible genes, and therefore a promising candidate for its utilization in engineering stress-tolerant transgenic crops. Transcriptome, proteome, epigenome, and metabolome studies have shown that the DREB can regulate various stress-responsive pathways in transgenic crops (Table S1). Some epigenomic analyses have shown the involvement of various microRNAs in the fine-tuning of *DREB*-regulated expression of the downstream gene(s) (Hackenberg et al. 2012; Jiang et al. 2017). Furthermore, a recent meta-analysis has shown that the promoter sequence, type, and duration of stress and genus of donor and recipient plants can significantly influence the *DREB*-regulated pathways under drought stress (DS) (Dong et al. 2017).

So far, the reports and reviews on the use of the *DREB* regulon have mainly focused on the functional validation of novel *DREB* genes and identifying the upstream and downstream regulatory mechanisms of *DREB* regulon in various transgenic plants under various abiotic stresses.

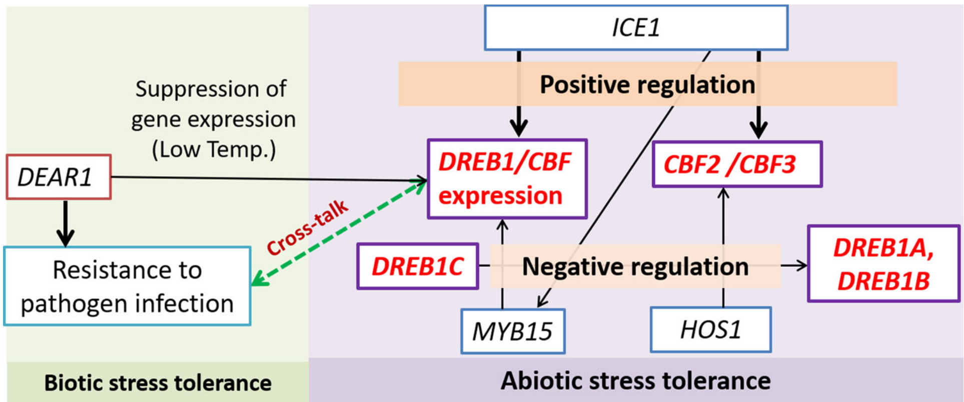


Fig. 2 Regulation of *DREB1* gene(s) by various signaling pathways under abiotic/biotic stress. ICE1 positively regulates expression of *DREB1/CBF*, HOS1 negatively regulates expression of *CBF2* and *CBF3* genes by interfering with the functioning of ICE1 protein, MYB15 negatively regulates expression of *DREB1/CBF*, ICE1 negatively regulates expression of *MYB15* and positively regulates expression of *DREB1/CBF*, DREB1C negatively regulates the expression of *DREB1A* and *DREB1B* genes, DEAR1 lead to suppression of *DREB1/CBF* expression under low-temperature stress and mediates the cross-talk between biotic and abiotic stress response pathways and confers resistance to pathogens in plants. *HOS:* high expression of somatically responsive; *ICE1:* inducer of CBF expression 1 protein; *EAR:* ethylene response factor-associated amphiphilic repression

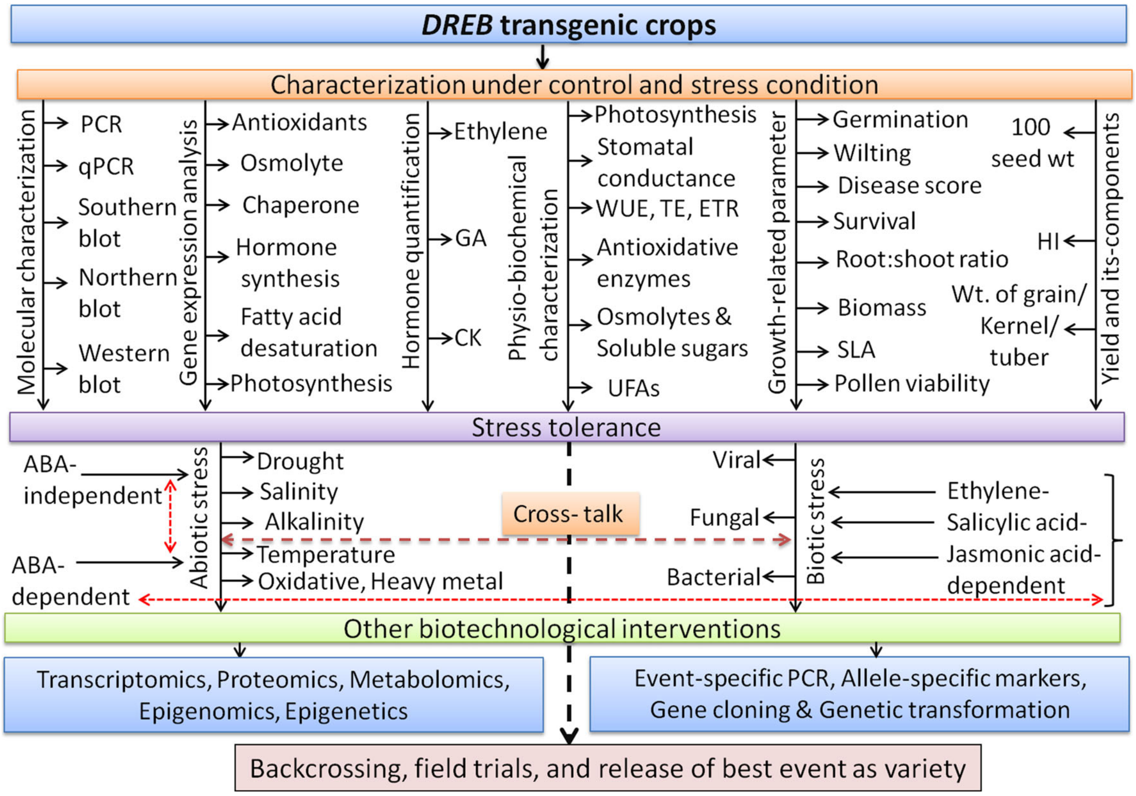


Fig. 3 Schematic representation of integrated approaches for the development and characterization of *DREB* transgenic crops for stress tolerance. GA: Gibberellic acid; HI: Harvest index; CK: Cytokinin; PCR: Polymerase chain reaction; WUE: Water use efficiency; TE: Transpiration rate; ETR: Electron transport rate; SLA: Specific leaf area; UFAs: Unsaturated fatty acids, qPCR: Quantitative PCR

This review was aimed to summarize the following aspects of *DREB* transgenic crops: (i) Detailed characterization of *DREB* transgenic plants; (ii) The fate of *DREB* transgenic crops under both confinement facility and field like conditions; (iii) Potential use of *DREB* transgenic plants in ongoing breeding programs (Fig. 3, Table S2).

The *DREB* regulon and the ABA-associated gene cascades

Abscisic acid (ABA) has its role in not only regulating the stomatal functions but also in coordinating various stress-responsive pathways. The *DREB1/CBF* and *DREB2* proteins are known to regulate the ABA-independent-pathway, while the DRE/CRT *cis*-element also functions in an ABA-dependent-pathway, indicating a cross-talk between the two (Agarwal et al. 2006; Agarwal and Jha 2010; Agarwal et al. 2017). Expression analysis of downstream stress-inducible genes in *DREB* transgenic plants and promoter analysis of these target genes elucidated the molecular mechanisms involved in cross-talk between ABA-dependent and ABA-independent abiotic stress responsive pathways (Narusaka et al. 2003; Oh et al. 2005). However, exogenous ABA treatment could not induce the expression of a heterologous *HvCBF4* gene in transgenic rice, indicating the latter's possible role in the ABA-independent pathway (Oh et al. 2007). *ARAG1*, an ABA-responsive *DREB* gene in transgenic rice, mediates DS tolerance via the ABA-dependent signaling pathway (Zhao et al. 2010). *DREB1* transgenic rice showed stomatal closure under DS via the ABA-dependent pathway (Datta et al. 2012), and exogenous ABA up-regulated *StDREB1* in transgenic potato (Bouaziz et al. 2012), thus indicated causative role of ABA-dependent *DREB* regulon to abiotic stress responses.

Three *AtABF3*-target genes—*Hsp70*, *PP2Ca,* and receptor kinase—are induced by *AtCBF3* in transgenic rice indicating a partial overlap between the ABA-dependent and independent pathways (Oh et al. 2005). However, many abiotic stress-inducible genes (e.g., *rd29B*) are specifically regulated by ABA, while others like *COR15a* are not, indicating parallel involvement of the ABA-dependent and independent pathways (Yang et al. 2011a; Zhang et al. 2013). The promoter of *rd29A* contains a DRE/CRT element that also functions as a coupling element (CE) of ABRE (abscisic acid response element), which mediates the ABA responsiveness of stress-inducible genes. Both these elements are interdependent in the stress-induced expression of *rd29A*, which can be activated by both ABA-dependent and independent pathways (Narusaka et al. 2003). Nevertheless, limited information is available regarding the underlying mechanisms of *DREB*-induced abiotic stress tolerance.

DS tolerance in *DREB* transgenic plants

The most common approach used to evaluate DS tolerance is potting the plants in dry soil (low water potential), which mimics in-field drought conditions. Pellegrineschi et al. (2004) compared the performance of the *DREB1A* lines and wild type (WT) variety of wheat by withholding water for 15 days and re-watering the plants until maturity. Similar strategies were also used for the *AtDREB1A* tomato and rice cultivars (Rai et al. 2013; Ravikumar et al. 2014). The DS tolerance of the *AtDREB1A* peanut lines was evaluated under progressively declining soil moisture regimes or varying vapor pressure deficits in pots/lysimeters/field, which can help quantify the severity of DS (Bhatnagar-Mathur et al. 2007, 2014; Sarkar et al. 2016).

Heterologous expression of *DREB1A* in transgenic rice helped in alleviating the deleterious effects of DS even at the reproductive stage as indicated by better morpho-physiological traits than WT varieties. For example, *AtDREB1A* rice (T_3) showed greater tolerance in the booting and anthesis stages compared to the WT variety and displayed stomatal closure under DS indicating DREB-dependent regulation of stomatal behavior (Datta et al. 2012). Similarly, high chlorophyll SPAD value and more greenish leaves were observed in *AtDREB1A* rice, indicating better yield and 'stay green' traits across various transgenic lines as compared to the WT variety under DS (Ravikumar et al. 2014).

Pollen-viability and bud-sprouting are important agronomic traits that should be evaluated under stress conditions. Although the agronomic traits of *TsCBF1* and WT maize were not significantly different under control conditions, most transgenic lines grew taller (10–15%), showed more tassel branches, shorter anthesis-silking interval (ASI), and better pollen viability than the WT during DS (Zhang et al. 2010). Furthermore, Sakuma et al. (2006) showed constitutive expression of *DREB2A* gene, if there is a deletion in the negative regulatory domain consisting of 29 amino acids. Also, the *AtDREB2A CA* sugarcane showed not only improved DS tolerance but also enhanced initial bud sprouting and higher sucrose content without biomass penalty (Reis et al. 2014).

Expression of *DREB* in transgenic plants modulated the up-regulation of water transport protein, thus help in maintaining cellular water status under various abiotic stress conditions. *AtDREB1B* expression in *Salvia miltiorrhiza* up-regulated the *aquaporin* gene, which modulates water permeability in plant cells (Wei et al. 2016). *DREB1A* transgenic crops maintained a conservative pattern of water use under DS as indicated by enhancement in the transpiration efficiency (TE) over its WT genotypes. Transgenic peanut (Bhatnagar-Mathur et al. 2007) and chickpea (Anbazhagan et al. 2015b) cultivars overexpressing *DREB1A* showed a substantial increase in TE under DS. Devi et al. (2011) reported a significant correlation between TE and its surrogate traits such as specific leaf area (SLA) and SPAD chlorophyll meter readings (SCMR) but none between TE and Δ^{13}C (carbon isotope discrimination), indicating that the latter cannot be used to assess TE under DS.

Root architecture is a critical parameter for evaluating DS tolerance since it is associated with water mining capacity. Ban et al. (2011) observed higher *LbDREB* gene expression in the tobacco roots compared to the leaves under DS. Furthermore, the DS-induced expression of *AtDREB1A* gene in transgenic peanut was also found associated with improved root/shoot ratio, which enhanced their harvest index (HI) compared to the WT genotype due to greater water uptake from the deeper soil layers (Jagana et al. 2012; Vadez et al. 2007). Under drought-like conditions, *AtDREB1A* peanut (Sarkar et al. 2016) and chickpea (Anbazhagan et al. 2015b) showed enhanced root growth in deeper soil layers, which might have resulted from improved DS tolerance and increased water extraction capacity. The *DREB* transgene, however, does not always improve all parameters related to stress tolerance and yield. The *AtDREB1A* peanut lines did show variations in RWC and total biomass under DS, but no significant difference was observed for pod-yield (Sarkar et al. 2014).

Similarly, the *AtDREB1A* soybean line did not outperform the WT variety in terms of yield, but the number of seeds per pod and the total number of pods were higher in the former under DS (de Paiva Rolla et al. 2014). The presence of transgene in the genome may not always show its expression in transgenic plants (Shavrukov et al. 2016). Various transgenic lines expressing the same *DREB* gene in same genetic background may show variations in gene expression pattern, physio-biochemical responses, yield and yield-components under abiotic stress conditions. This is likely due to the positional effects of transgene integration in the genome, copy numbers, positive pleiotropic effects of the tissue culture process, variations in promoter activity, the regulatory function of heterologous *DREB* gene itself and genotype × environment interactions (Anbazhagan et al. 2015a; Datta et al. 2012; Sarkar 2014).

Antioxidant mechanisms are an important line of defense against DS. Rai et al. (2013) reported a significant increase in the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), and monodehydroascorbate reductase (MDHAR) in the *AtDREB1A* tomato under DS, indicating better tolerance compared to WT variety. In addition, the levels of antioxidants such as ascorbic acid, glutathione, and their reduced forms were also higher in the transgenic tomato and increased with ROS levels (Rai et al. 2013). Although *DREB* upregulates the antioxidant enzyme genes, it does not play any causative role in improving the TE in *AtDREB1A* peanut under DS (Bhatnagar-Mathur et al. 2009), indicating that the antioxidant mechanism does not regulate the TE.

In addition to physio-biochemical assays, visual observations are used to characterize abiotic stress tolerance. The *AtDREB1A* transgenic rice and peanut showed less severe, as well as delayed wilting/curling of leaves during drought. After stress withdrawal, both *AtDREB1A* transgenic rice (Ravikumar et al. 2014) and peanut (Sarkar et al. 2014) showed speedy recovery compared to their respective WT varieties, possibly due to the up-regulation of genes involved in energy and carbohydrate metabolism (Paul et al. 2015).

Although, *DREB* transgenic crops are generally tolerant to DS with improved physio-biochemical and growth-related parameters, yet some transgenic events do show dwarfism and pleiotropic effects on leaf ultrastructure and phenology as effective water conservation mechanisms (Li et al. 2012; Tang et al. 2017). Such altered phenotypic traits could be due to the DREB-induced down-regulation of some phytohormones biosynthesis pathways. The *DREB* gene regulates the response to multiple abiotic stresses, which influences the crop physiology during its entire life cycle. Since drought and salinity are the most prevalent abiotic factors affecting the major crops, considerable efforts have aimed at developing transgenic crops tolerant to DS, followed by salinity stress (SS) (Fig. S1).

Phenotyping of stress tolerant *DREB* transgenic lines

The abiotic stress tolerance of transgenic plants is evaluated in terms of physiological parameters such as total water use, water use efficiency (WUE), transpiration, TE, RWC, membrane stability (Table S2), osmotic adjustment, surrogate traits of photosynthesis like SLA and SCMR, specific leaf nitrogen (SLN), photochemical efficiency, photosynthetic rate, and stomatal conductance (Anbazhagan et al. 2015a; Bhatnagar-Mathur et al. 2007; Devi et al. 2011; Reis et al. 2014; Sarkar et al. 2014). Under abiotic stress, the heterologous expression of *DREB* in various transgenic crops showed better stomatal regulation, water status, osmotic homeostasis, protection of photosynthetic apparatus and photosynthetic efficiency (Table S2) over its WT genotype (Datta et al. 2012; Oh et al. 2007; Sarkar et al. 2016). Delayed wilting or curling of leaves in the transgenic crop is a morphological sign of DS tolerance, as it signifies slower water usage due to lower water potential in the tissues (Sarkar et al. 2016). Hence, this phenotypic index could be used to assess the optimal level of stress tolerance in transgenic crops.

The leaf chlorophyll content, photochemical efficiency (F_v/F_m), chlorophyll fluorescence and electron transport rate (ETR) are related to photosynthetic rate and net photosynthesis in transgenic crops (Jin et al. 2010; Ravikumar et al. 2014; Yang et al. 2010). The decrease in chlorophyll SPAD values or chlorophyll content under abiotic stress is a likely result of its photo-oxidation and degradation

(Farooq et al. 2009; Jin et al. 2010; Yang et al. 2010), and therefore can be considered a symptom of oxidative stress. Thus, the improved photosynthetic rate, ETR and turnover rates of dark reactions in *DREB/CBF* transgenic plants might be due to less photo-oxidative damage and degradation of photosynthetic machinery than WT cultivars under drought and cold stresses (Ravikumar et al. 2014; Yang et al. 2010). Taken together, quantitative estimation of various physio-biochemical and molecular (gene expression analysis) indicators can help elucidate the mechanisms underlying stress-tolerance in *DREB* transgenic crops (Bhatnagar-Mathur et al. 2007, 2014; de Paiva Rolla et al. 2014; Sarkar et al. 2016).

Salinity stress (SS) tolerance in *DREB* transgenic crops

SS refers to the stress caused by high levels of sodium chloride (NaCl), the major component of most saline soils. It can be simulated experimentally by irrigating the plants with saline water (in pots) or by transferring the seedlings to salt-containing media or nutrient solution having electrical conductivity (EC) > 4. The effects of SS should be evaluated both at the seedling and mature plant stages (Bhatnagar-Mathur et al. 2008). Datta et al. (2012) and Sarkar et al. (2014) observed an improved SS tolerance, physio-biochemical parameters and yield of *DREB* transgenic rice and peanut cultivars when exposed to NaCl (12–18 EC or 100–200 mM)-supplemented nutrient solution for 28 days and 12 days, respectively.

Transgenic *StDREB1* potato maintained large green leaves (stay-green trait) compared to the WT variety, which showed necrotic lesions and subsequently died after 20 days of SS (Bouaziz et al. 2013). The *GmDREB1* transgenic wheat showed a higher level of osmolytes such as proline and glycine betaine for retaining macromolecule homeostasis, better seedling traits along with longer roots, higher fresh-weight, and more tillers than the WT variety, with increasing duration of SS (Jiang et al. 2014). The heterologous expression of the *DREB* gene might regulate the expression and function of ion transporters in a stress-specific manner for maintaining ion homeostasis at the cellular level. *LbDREB* transgenic tobacco showed significantly more K^+/Na^+ ratio than its WT counterparts under $CuSO_4$ induced-oxidative stress, suggesting over-expression of *DREB* gene can significantly reduce Na^+ content in transgenic tobacco under stress conditions (Ban et al. 2011). However, over-expression of *PgDREB2A* in transgenic tobacco could not activate ion exchange antiporters under SS (Agarwal et al. 2010).

Salinity-susceptibility index (Oraby and Ahmad 2012) and salt-injury index (Jiang et al. 2014) are critical parameters for evaluating SS. Constitutive expression of heterologous *DREB* genes, however, may result in salinity sensitivity as opposed to tolerance (Table S2), indicating the requirement of a stress-inducible promoter (Tang et al. 2017).

DREB imparted thermo-tolerance in transgenic crops

A number of studies have demonstrated thermo-tolerance in various *DREB* transgenic crops. *DREB2A* is a heat shock (H) stress-inducible gene containing an H element upstream of its promoter, which transcriptionally activates the downstream *HsfA3*, a heat shock TF which in turn activates the downstream heat shock proteins (HSPs) and confers thermo-tolerance (Sakuma et al. 2006; Schramm et al. 2008). The *DREB* transgenic tobacco showed short-term (6 h) tolerance to a sudden drop in temperature (4 °C) (Agarwal et al. 2010), as well as long-term tolerance (33 days) to gradually declining temperatures (12 °C to − 5 °C) under open field conditions (Wei et al. 2006). Similarly, the *TaDREB2* and *TaDREB3* transgenic barley showed less damage from frost stress and improved survival rate of 45–50% compared to the WT variety (Morran et al. 2011).

Other examples include the *AtDREB1A* tomato that showed improved stomatal conductance, transpiration rate, RWC, and osmotic adjustments compared to the WT variety when exposed to low temperatures (4, 6, and 8 °C) for one week (Shah et al. 2015). Also, *MtDREB1C Medicago truncatula* and China Rose cultivars showed better survival compared to their WT counterparts when exposed to − 6 °C for 10 h and − 6 °C for 60 h, respectively (Chen et al. 2010). The heterologous expression of *CfCBF3* in tobacco also conferred tolerance to low-temperature stress by enhancing the level of total unsaturated fatty acids in the leaves, which helped protect its photosynthetic apparatus (Yang et al. 2011b).

In some cases, synthetic/modified *DREB* gene, rather than its native form have been used for achieving the desired level of ectopic gene expression and stress tolerance in transgenic crops (Reis et al. 2014; Wang et al. 2014). The *RdreB1BI* transgene was synthesized by overlap extension PCR on the *DREB1B* gene of rice using primers with optimized codons and decreased GC content, and induced low-temperature tolerance in transgenic strawberry (Wang et al. 2014). DREB also regulates the expression of heat shock proteins (HSPs), as well as photosynthetic/metabolic enzymes that confer high-temperature stress tolerance (Table S2, Fig. S1). Hong et al. (2009) reported high-temperature tolerance in *AtDREB1A* chrysanthemum when exposed to 45°C for 36 h. However, the thermo-tolerance and overall improved physiological traits of various *DREB* transgenic crops have not been associated with an improved yield so far.

Multiple stress tolerance in *DREB* transgenic crops

Since crops are simultaneously exposed to multiple stresses under field conditions, it is of prime importance to develop multi-stress tolerant varieties of important crops to increase productivity. Various *DREB* transgenic lines do show tolerance to drought, salinity, alkalinity, and high/low temperature. For example, heterologous expression of *AtDREB1A* in peanut (Sarkar et al. 2014) and maize (Al-Abed et al. 2007) improved the RWC and membrane stability (low ion leakage) compared to the WT, thus indicating better cellular water status under drought, salt, and low-temperature stresses. Similarly, *AtDREB1A* peanut (Sarkar et al. 2014), *AaDREB1* rice (Zong et al. 2016) and *GhDREB* wheat (Gao et al. 2009) also showed higher chlorophyll retention capacity, indicating a higher rate of photosynthesis, under drought, salt, and low-temperature conditions compared to their WT counterparts. The better performance of *DREB* transgenic plants could be attributed to the DREB-activated expression of downstream genes preventing the chlorophyll degradation and thereby maintaining the normal photosynthesis and energy metabolism under multiple stress conditions.

As discussed earlier (Fig. 1), osmolyte biosynthesis and antioxidant enzyme activity are vital responses underling physio-biochemical and growth-related improvements in transgenic plants under various abiotic stresses. For example, *AtDREB1B* potato (Movahedi et al. 2012), *AtDREB1A Lolium perenne* (Li et al. 2011), *AaDREB1* rice (Zong et al. 2016) and *IbCBF3* sweet-potato (Jin et al. 2017) showed higher proline levels, soluble sugar accumulation and antioxidant enzymatic activities due to the activation of macromolecule homeostasis and detoxification mechanisms under drought, salt and low-temperature stresses (Table S2). Also, *AtDREB1A* peanut showed better RWC under DS and SS than the WT variety; following stress withdrawal, the transgenic plants also recovered faster and performed better than the WT genotype (Sarkar et al. 2014). Furthermore, *AtDREB1A* peanut showed improved osmotic adjustment in terms of higher osmolality or lower osmotic potential under DS and SS (Sarkar et al. 2014), which again indicated the possibility of cross-talks among various stress-response mechanisms.

The constitutive co-expression of three stress-responsive TFs genes viz. *AtDREB2A*, *AtHB7,* and *AtABF3* in peanut was reported conferring tolerance to various abiotic stresses (Pruthvi et al. 2014). However, since each transgene expression may increase the metabolic load on the plant, thus the incorporation of a single TF gene imparting the desired level of stress tolerance and yield under DS and SS might be a more feasible option (Sarkar 2014). In fact, studies on transgenic crops have demonstrated that individual physiological responses are inter-linked and eventually lead to stress tolerance, improved growth, and yield. For example, *AtDREB1A* peanut exposed to DS and SS showed elevated levels of proline, which resulted in better osmotic adjustment. Both phenomena increase water retention capacity and lower the level of ion leakage due to improved membrane integrity and better protection of photosynthetic mechanisms (Sarkar et al. 2014).

In addition, *OsDREB1B* tobacco shows improved resistance to tobacco streak virus and tolerance to drought, salt, low-temperature, as well as methyl viologen-induced oxidative stress (Gutha and Reddy 2008). Similarly, various transgenic crops expressing heterologous *DREB* gene also showed tolerance to oxidative stress in combination with biotic and abiotic stresses (Table S2) (Bouaziz et al. 2013; Charfeddine et al. 2015; Gutha and Reddy 2008; Pruthvi et al. 2014; Rai et al. 2013). Thus, the *DREB* transgenic crops show multi-stress tolerance likely due to the cross-talk between various biotic and abiotic stress-responsive mechanisms, and also due to the interaction between ABA-dependent and independent signal transduction pathways (Fig. S1).

Evaluation of yield-components and yield in *DREB* transgenic crops

The ultimate goal of developing stress-tolerant transgenic crops is to improve crop yield under harsh environmental conditions. However, most studies so far have only reported the short-term expression of heterologous *DREB* gene and survival of the transgenic plants under stress. In addition, the experimental acute stress used to study these transgenic plants does not reflect the in-field conditions. Therefore, the physiological, biochemical and growth-related data of transgenic plants may not always be reliable (Bhatnagar-Mathur et al. 2008). Thus, it is essential to determine the threshold level of any abiotic stress which a transgenic can tolerate and show optimal growth and yield as compared to its WT counterparts (Sarkar 2014).

Some reports have shown improved physio-biochemical characteristics and growth of *DREB* transgenic plants that resulted in improved yield, likely due to stress escape, avoidance, tolerance, and recovery mechanisms (Table S2). Yield parameters like daily transpiration (T), TE and HI have been recorded to determine the effect of DS on transgenic plants (Anbazhagan et al. 2015a, b; Bhatnagar-Mathur et al. 2010; Sarkar et al. 2014). Nevertheless, very few studies have evaluated transgenic crop yield as a function of stress tolerance under containment facility or open field conditions (Anbazhagan et al. 2015a; Bhatnagar-Mathur et al. 2014; de Paiva Rolla et al. 2014; Ravikumar et al. 2014; Sarkar et al. 2014) (Table S2). For example, *AtDREB1A* peanut (Bhatnagar-Mathur et al. 2014), rice (Ravikumar et al. 2014), and chickpea (Anbazhagan et al. 2015a) showed yield gain under DS and SS compared to the WT genotypes, without any yield penalty. Anbazhagan et al. (2015a) observed early flowering and seed setting in *AtDREB1A* transgenic chickpea line as compared to WT counterparts under DS, which was indicative of a drought escape mechanism.

Rice being an important cereal crop of the world, most studies on transgenic plants have focused on the development of high-yielding stress-tolerant rice cultivars (Fig. S2). *DREB* rice showed improved agronomic traits, such as an increase in the number of reproductive tillers and spikelets at the pre-flowering and vegetative stages, respectively, compared to the WT variety under DS and SS (Datta et al. 2012). As stated earlier, the DREB transgenic line could show multiple abiotic stress tolerance due to overlapping stress responsive mechanisms, with the exception of a few instances. For example, two *TaDREB3* transgenic wheat lines (F_3BC_3) showed higher seed yield (18.9% and 21.5%) and better survival rate than WT varieties under DS, but no significant difference was observed in survival rate under low-temperature stress (Shavrukov et al. 2016).

On the contrary, *AtDREB1A* peanut showed improved HI, an important yield parameter under DS and SS (Bhatnagar-Mathur et al. 2014; Sarkar et al. 2014). In addition, improved RWC in *AtDREB1A* peanut was found positively correlated with improved HI ($r = 0.64$ and 0.91) compared to WT peanut under DS and SS (Sarkar et al. 2014). Therefore, improved physiological traits under abiotic stresses may improve the yield of crop plants.

Conclusions

Single gene action based transgenic approaches have not always shown much encouraging results due to the polygenic nature of abiotic stress-tolerance mechanisms. *DREB* gene was introduced into plants to develop stress-tolerant crops. This review discussed in-depth molecular, physio-biochemical mechanisms responsible for abiotic stress tolerance in transgenic crops and the fate of transgenic crops under confinement/contained facilities and field conditions (Table S2). This review also highlighted the use of *DREB* transgenic plants in back-cross breeding programs and future strategies towards commercialization of *DREB* transgenic crops (Fig. 3).

Although many *DREB* transgenic crops with varying degrees of abiotic and biotic stress tolerance have been developed worldwide, none of them are currently under large-scale cultivation (Table S2; Fig. S1, S2). In addition, limited reports are available showing improved yield under field conditions (Table S2). The recovery and yield of the transgenic plants after stress withdrawal are also critical parameters for evaluation of stress tolerant lines (Sarkar et al. 2014). Therefore, integrated approaches are needed to assess the tolerance and yield potential of the homozygous and stable *DREB* transgenic lines following the withdrawal of individual or multiple stresses under field-like conditions, in order to identify the best *DREB* transgenic lines for further characterization and large-scale cultivation (Fig. 3).

In addition, novel genetic engineering strategies like the use of stress-inducible, tissue-specific promoters to minimize excessive metabolic load on the transgenic crops, chloroplast transformation, and targeted and marker-free gene transfer can be introduced into the *DREB* regulon technology to nullify the environmental, ethical and health concerns associated with transgenic crops.

Future prospects

Advanced 'omics' approaches should be used to dissect the *DREB* regulon controlled downstream signaling pathways involved in stress responses in transgenic crops. The identification of novel genes that are up-regulated during stress and their respective promoters can help engineer new stress-tolerant crops with improved photosynthetic machinery, water conservation, WUE and root traits and cellular level tolerance, without any growth and yield penalty (Fig. 3). The downstream genes of *DREB* regulon that is up-regulated under abiotic stress could be mapped to the linkage groups of different transgenic crops. Furthermore, the identification of quantitative trait loci (QTL) based on bi-parental mapping populations or genome-wide association studies (GWAS) will help develop allele-specific markers (ASMs) of these candidate genes (Lata et al. 2011; Rao et al. 2015; Zhuang et al. 2015) and enable marker-assisted transgenic crop development for abiotic stress tolerance (Fig. 3).

The identification of novel promoters of stress-inducible genes could be utilized for driving expression of heterologous genes in transgenic plants. Further, the *TaDREB3* gene was transferred into four elite bread wheat cultivars by repeated backcrossing and the resultant lines (F_2BC_3) showed 12–18% higher survival rates and yield than the WT varieties under DS. The backcross breeding programs also generated marker-free *TaDREB3* transgenic wheat lines (F_1BC_2) due to recombination events that occurred in the genomic regions surrounding the transgene (Shavrukov et al. 2016). Such backcross-based gene introgression can also be extended to the other *DREB* based transgenic crops.

So far, very few abiotic stress-tolerant *DREB* transgenic plants have been tested to understand their resistance to biotic stresses (Table S2, Fig. S1). Hence, *DREB* transgenic crops need to be evaluated for their possible resistance to biotic stress and to unravel the in-depth molecular mechanisms responsible for cross-talks between biotic and abiotic stress responses. Further, each transgenic crop needs critical evaluation upon exposure to the appropriate level of stress, under confinement/contained facility or field trials (multi-year, multi-location), at the appropriate developmental stage, for evaluating its potential utility at commercial scale in near future.

Alternatively, the transgenic crops can also be evaluated for their tolerance, growth, and yield under sub-optimal and/or optimal stress exposure for their entire life span (Wang et al. 2016). However, upscaling from transgenic development to field trials requires continuous and consorted efforts, scientific networking, adequate fund flow, and availability of land, technical expertise and biosafety clearance (Mishra et al. 2017; Sarkar et al. 2017). Event-specific qualitative and quantitative PCR-based detection assays need to be developed for meeting the demands for field trials and curb the use of unauthorized transgenic crops (Liu et al. 2014). The currently available stress-tolerant *DREB*-based transgenic crops ought to be made accessible for small and resource-poor farmers. However, political will and simplification of biosafety regulations are also simultaneously required to facilitate the commercialization of transgenic crops.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Agarwal PK, Jha B (2010) Transcription factors in plants and ABA-dependent and independent abiotic stress signaling. Biol Plant 54(2):201–212. https://doi.org/10.1007/s10535-010-0038-7

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of *DREB* transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:263–1274. https://doi.org/10.1007/s00299-006-0204-8

Agarwal P, Agarwal PK, Joshi AJ, Sopory SK, Reddy MK (2010) Overexpression of *PgDREB2A* transcription factor enhances abiotic stress. Mol Biol Rep 37:1125–1135. https://doi.org/10.1007/s11033-009-9885-8

Agarwal PK, Gupta K, Lopato S, Agarwal P (2017) Dehydration responsive element binding transcription factors and their applications for the engineering of stress tolerance. J Exp Bot 68(9):2135–2148. https://doi.org/10.1093/jxb/erx118

Akhtar M, Jaiswal A, Taj G, Jaiswal JP, Qureshi MI, Singh NK (2012) DREB1/CBF transcription factors: their structure, function and role in abiotic stress tolerance in plants. J Genet 91(3):385–395. https://doi.org/10.1007/s12041-012-0201-3

Al-Abed D, Madasamy P, Talla R, Goldman S, Rudrabhatla S (2007) Genetic engineering of maize with the *Arabidopsis* DREB1A/CBF3 gene using split-seed explants. Crop Sci 47:2390–2401. https://doi.org/10.2135/cropsci2006.11.0712

Anbazhagan K, Bhatnagar-Mathur P, Sharma KK, Baddam R, Kavi Kishor PB, Vadez V (2015a) Changes in timing of water uptake and phenology favours yield gain in terminal water stressed chickpea *AtDREB1A* transgenics. Funct Plant Biol 42:84–94. https://doi.org/10.1071/FP14115

Anbazhagan K, Bhatnagar-Mathur P, Vadez V, Dumbala SR, Kavi Kishor PB, Sharma KK (2015b) *DREB1A* overexpression in transgenic chickpea alters key traits influencing plant water budget across water regimes. Plant Cell Rep 34:199–210. https://doi.org/10.1007/s00299-014-1699-z

Ban Q, Liu G, Wang Y (2011) A DREB gene from *Limonium bicolour* mediates molecular and physiological responses to copper stress in transgenic tobacco. J Plant Physiol 168(5):449–458. https://doi.org/10.1016/j.jplph.2010.08.013

Bhatnagar-Mathur P, Devi MJ, Reddy DS, Lavanya M, Vadez V, Serraj R, Yamaguchi-Shinozaki K, Sharma KK (2007) Stress-inducible expression of *AtDREB1A* in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. Plant Cell Rep 26:2071–2082. https://doi.org/10.1007/s00299-007-0406-8

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27:411–424. https://doi.org/10.1007/s00299-007-0474-9

Bhatnagar-Mathur P, Devi MJ, Vadez V, Sharma KK (2009) Differential antioxidative responses in transgenic peanut bear no relationship to their superior transpiration efficiency under drought stress. J Plant Physiol 166:1207–1217. https://doi.org/10.1016/j.jplph.2009.01.001

Bhatnagar-Mathur P, Rao JS, Vadez V, Sharma KK (2010) Transgenic strategies for improved drought tolerance in legumes of semi-arid tropics. J Crop Improv 24(1):92–111. https://doi.org/10.1080/15427520903337095

Bhatnagar-Mathur P, Rao JS, Vadez V, Dumbala SR, Rathore A, Yamaguchi-Shinozaki K, Sharma KK (2014) Transgenic peanut overexpressing the *DREB1A* transcription factor has higher yields under drought stress. Mol Breed 33:327–340. https://doi.org/10.1007/s11032-013-9952-7

Bosamia TC, Mishra GP, Radhakrishnan T, Dobaria JR (2015) Novel and stress relevant EST derived SSR markers developed and validated in peanut. PLoS ONE 10(6):e0129127. https://doi.org/10.1371/journal.pone.0129127

Bouaziz D, Pirrello J, Ben Amor H, Hammami A, Charfeddine M, Dhieb A, Bouzayen M, Gargouri-Bouzid R (2012) Ectopic expression of dehydration responsive element binding proteins (*StDREB2*) confers higher tolerance to salt stress in potato. Plant Physiol Biochem 60:98–108. https://doi.org/10.1016/j.plaphy.2012.07.029

Bouaziz D, Pirrello J, Charfeddine M, Hammami A, Jbir R, Dhieb A, Bouzayen M, Gargouri-Bouzid R (2013) Overexpression of StDREB1 transcription factor increases tolerance to salt in transgenic potato plants. Mol Biotechnol 54(3):803–817. https://doi.org/10.1007/s12033-012-9628-2

Charfeddine M, Bouaziz D, Charfeddine S, Hammami A, Ellouz ON, Bouzid RG (2015) Overexpression of dehydration-responsive element-binding 1 protein (DREB1) in transgenic *Solanum tuberosum* enhances tolerance to biotic stress. Plant Biotechnol Rep 9:79–88. https://doi.org/10.1007/s11816-015-0345-8

Chen JR, Lü JJ, Rong L, Xiong XY, Wang TX, Chen SY, Guo LB, Wang HF (2010) DREB1C from *Medicago truncatula* enhances freezing tolerance in transgenic *M. truncatula* and china rose (*Rosa chinensis* Jacq.). Plant Growth Reg 60(3):199–211. https://doi.org/10.1007/s10725-009-9434-4

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK (2003) ICE1, a regulator of cold induced transcriptome and freezing tolerance in *Arabidopsis*. Genes Dev 17:1043–1054. https://doi.org/10.1101/gad.1077503

Datta K, Baisakh N, Ganguly M, Krishnan S, Shinozaki KY, Datta SK (2012) Overexpression of *Arabidopsis* and rice stress genes inducible transcription factor confers drought and salinity tolerance to rice. Plant Biotechnol J 10:579–586. https://doi.org/10.1111/j.1467-7652.2012.00688.x

de Paiva Rolla AA, de Fátima Corrêa Carvalho J, Fuganti-Pagliarini R, Engels C, do Rio A, Marin SRR, de Oliveira MCN, Beneventi MA, Marcelino-Guimarães FC, Farias JRB, Neumaier N, Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL (2014) Phenotyping soybean plants transformed with rd29A: AtDREB1A for drought tolerance in the greenhouse and field. Transgen Res 23(1):75–87. https://doi.org/10.1007/s11248-013-9723-6

Devi MJ, Bhatnagar-Mathur P, Sharma KK, Serraj R, Anwar SY, Vadez V (2011) Relationships between transpiration efficiency and its surrogate traits in the *rd29A*:*DREB1A* transgenic lines of groundnut. J Agron Crop Sci 197(4):249–328. https://doi.org/10.1111/j.1439-037X.2011.00464.x

Dong C, Ma Y, Wisniewski M, Cheng ZM (2017) Meta-analysis of the effect of overexpression of CBF/DREB family genes on drought stress response. Environ Exp Bot 142:1–4. https://doi.org/10.1016/j.envexpbot.2017.07.014

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29(1):185–212. https://doi.org/10.1051/agro:2008021

Gao SQ, Chen M, Xia LQ, Xiu HJ, Xu ZS, Li LC, Zhao CP, Cheng XG, Ma YZ (2009) A cotton (*Gossypium hirsutum*) DRE-binding transcription factor gene, *GhDREB*, confers enhanced tolerance to drought, high salt, and freezing stresses in transgenic wheat. Plant Cell Rep 28:301–311. https://doi.org/10.1007/s00299-008-0623-9

Gutha LR, Reddy AR (2008) Rice *DREB1B* promoter shows distinct stress- specific responses, and the overexpression of cDNA in tobacco confers improved abiotic and biotic stress tolerance. Plant Mol Biol 68:533–555. https://doi.org/10.1007/s11103-008-9391-8

Hackenberg M, Shi B-J, Gustafson P, Langridge P (2012) A transgenic transcription factor (TaDREB3) in barley affects the expression of microRNAs and other small non-coding RNAs. PLoS ONE 7(8):e42030. https://doi.org/10.1371/journal.pone.0042030

Hong B, Ma C, Yang Y, Wang T, Yamaguchi-Shinozaki K, Gao J (2009) Over-expression of *AtDREB1A* in chrysanthemum enhances tolerance to heat stress. Plant Mol Biol 70:231–240. https://doi.org/10.1007/s11103-009-9468-z

Jagana SR, Vadez V, Bhatnagar-Mathur P, Narasu ML, Sharma KK (2012) 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 10:1–7

Jiang Q, Hu Z, Zhang H, Ma Y (2014) Overexpression of *GmDREB1*improves salt tolerance in transgenic wheat and leaf protein response to high salinity. Crop J 2:120–131. https://doi.org/10.1016/j.cj.2014.02.003

Jiang Q, Sun X, Niu F, Hu Z, Chen R, Zhang H (2017) *GmDREB1* overexpression affects the expression of microRNAs in GM wheat seeds. PLoS ONE 12(5):e0175924. https://doi.org/10.1371/journal.pone.0175924

Jin T, Chang Q, Li W, Yin D, Li Z, Wang D, Liu B, Liu L (2010) Stress-inducible expression of *GmDREB1* conferred salt tolerance in transgenic alfalfa. Plant Cell Tissue Organ Cult 100:219–227. https://doi.org/10.1007/s11240-009-9628-5

Jin R, Kim BH, Ji CY, Kim HS, Li HM, Kwak SS (2017) Overexpressing IbCBF3 increases low temperature and drought stress tolerance in transgenic sweetpotato. Plant Physiol Biochem 118:45–54. https://doi.org/10.1016/j.plaphy.2017.06.002

Lata C, Bhutty S, Bahadur RP, Majee M, Prasad M (2011) Association of an SNP in a novel *DREB2*-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot 62(10):3387–3401. https://doi.org/10.1093/jxb/err016

Lee H, Xiong L, Gong Z, Ishitani M, Stevenson B, Zhu JK (2001) The *Arabidopsis* HOS1 gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. Genes Dev 15:912–924. https://doi.org/10.1101/gad.866801

Li X, Cheng X, Liu J, Zeng H, Han L, Tang W (2011) Heterologous expression of the *Arabidopsis DREB1A/CBF3* gene enhances drought and freezing tolerance in transgenic *Lolium perenne* plants. Plant Biotech Rep 5(1):61–69. https://doi.org/10.1007/s11816-010-0157-9

Li J, Sima W, Ouyang B, Wang T, Ziaf K, Luo Z, Liu L, Li H, Chen M, Huang Y, Feng Y (2012) Tomato SlDREB gene restricts leaf expansion and internode elongation by downregulating key genes for gibberellin biosynthesis. J Exp Bot 63(18):6407–6420. https://doi.org/10.1093/jxb/ers295

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively in *Arabidopsis*. Plant Cell 10(8):1391–1406. https://doi.org/10.1105/tpc.10.8.1391

Liu Y, Zhang M-H, Yu Y-B, Sun Z, Zhen Z, Gao X-J (2014) Event-specific qualitative and quantitative detection in transgenic soybean OsDREB3 based on the 5′ flanking sequence. Food Biotechnol 28(1):63–78. https://doi.org/10.1080/08905436.2013.871289

Mishra GP, Singh B, Seth T, Singh AK, Halder J, Krishnan N, Tiwari SK, Singh PM (2017) Biotechnological advancements and Begomovirus management in okra (*Abelmoschus esculentus* L.): status and perspectives. Front Plant Sci 8:360. https://doi.org/10.3389/fpls.2017.00360

Morran S, Eini O, Pyvovarenko T, Parent B, Singh R, Ismagul A, Eliby S, Shirley N, Langridge P, Lopato S (2011) Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. Plant Biotechnol J 9:230–249. https://doi.org/10.1111/j.1467-7652.2010.00547.x

Movahedi S, Sayed TBE, Alizade H, Ghobadi C, Yamchi A, Khaksar G (2012) Constitutive expression of *Arabidopsis DREB1B* in transgenic potato enhances drought and freezing tolerance. Biol Plantarum 56(1):37–42. https://doi.org/10.1007/s10535-012-0013-6

Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T (2003) Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of *Arabidopsis* rd29A gene in response to dehydration and high salinity stresses. Plant J 34:137–148. https://doi.org/10.1046/j.1365-313X.2003.01708.x

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005) *Arabidopsis CBF3/DREB1A* and *ABF3* in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351. https://doi.org/10.1104/pp.104.059147

Oh SJ, Kwon C-W, Choi D-W, Song SI, Kim JK (2007) Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. Plant Biotechnol J 5:646–656. https://doi.org/10.1111/j.1467-7652.2007.00272.x

Oraby H, Ahmad R (2012) Physiological and biochemical changes of *CBF3* transgenic oat in response to salinity stress. Plant Sci 185–186:331–339. https://doi.org/10.1016/j.plantsci.2012.01.003

Patel KG, Mandaliya VB, Mishra GP, Dobaria JR, Radhakrishnan T (2016) Transgenic peanut overexpressing *mtlD* gene confers enhanced salinity-stress tolerance via mannitol accumulation and differential antioxidative responses. Acta Physiol Plant 38:181. https://doi.org/10.1007/s11738-016-2200-0

Patel K, Thankappan R, Mishra GP, Mandalia V, Kumar A, Dobaria JR (2017) 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. https://doi.org/10.3389/fpls.2017.01881

Paul S, Gayen D, Datta SK, Datta K (2015) Dissecting root proteome of transgenic rice cultivars unravels metabolic alterations and accumulation of novel stress responsive proteins under drought stress. Plant Sci 234:133–143. https://doi.org/10.1016/j.plantsci.2015.02.006

Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D (2004) Stress-induced expression in wheat of the *Arabidopsis thaliana DREB1A* gene delays water stress symptoms under greenhouse conditions. Genome 47:493–500. https://doi.org/10.1139/g03-140

Pruthvi V, Narasimhan R, Nataraja KN (2014) Simultaneous expression of abiotic stress responsive transcription factors, AtDREB2A, AtHB7and AtABF3 improves salinity and drought tolerance in peanut (*Arachis hypogaea* L.). PLoS ONE 9(12):e111152. https://doi.org/10.1371/journal.pone.011115

Rai GK, Rai NP, Rathaur S, Kumar S, Major S (2013) Expression of rd29A:AtDREB1A/*CBF3* in tomato alleviates drought-induced oxidative stress by regulating key enzymatic and non-enzymatic antioxidants. Plant Physiol Biochem 69:90–100. https://doi.org/10.1016/j.plaphy.2013.05.002

Rao ES, Kadirvel P, Symonds RC, Geethanjali S, Thontadarya RN, Ebert AW (2015) Variations in *DREB1A* and VP1.1 genes show association with salt tolerance traits in wild tomato (*Solanum pimpinellifolium*). PLoS ONE 10(7):e0132535. https://doi.org/10.1371/journal.pone.0132535

Ravikumar G, Manimaran P, Voleti SR, Subrahmanyam D, Sundaram RM, Bansal KC, Viraktamath BC, Balachandran SM (2014) Stress-inducible expression of *AtDREB1A* transcription factor greatly improves drought stress tolerance in transgenic indica rice. Transgen Res 23(3):421–439. https://doi.org/10.1007/s11248-013-9776-6

Reis RR, da Cunha BADB, Martinsa PK, Martinsa MTB, Alekcevetch JC, Chalfun-Júnior A, Andrade AC, Ribeiro AP, Qind F, Mizoie J, Yamaguchi-Shinozaki K, Nakashima K, Carvalho JDFC, de Sousa CAF, Nepomucenof AL, Kobayashi AK, Molinari HBC (2014) Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. Plant Sci 221:59–68. https://doi.org/10.1016/j.plantsci.2014.02.003

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006) Dual function of an *Arabidopsis* transcription factor *DREB2A* in water-stress-responsive and heat-stress-responsive gene expression. Proc Natl Acad Sci USA 103(49):18822–18827. https://doi.org/10.1073/pnas.0605639103

Sarkar T (2014) Development of transgenic resistance to abiotic stress in groundnut using *AtDREB1A* gene through *Agrobacterium* mediated genetic transformation. Ph.D. Thesis, Saurashtra University, Rajkot, Gujarat, India

Sarkar T, Radhakrishnan T, Kumar A, Mishra GP, Dobaria JR (2014) Heterologus expression of *AtDREB1A* gene in transgenic peanut conferred tolerance to drought and salinity stresses. PLoS ONE 9(12):e110507. https://doi.org/10.1371/journal.pone.0110507

Sarkar T, Radhakrishnan T, Kumar A, Mishra GP, Dobaria JR (2016) Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (*Arachis hypogaea* L.) conferred tolerance to soil-moisture deficit stress. Front Plant Sci 7:935. https://doi.org/10.3389/fpls.2016.00935

Sarkar T, Mogili T, Sivaprasad V (2017) Improvement of abiotic stress adaptive traits in mulberry (*Morus* spp.): an update on biotechnological interventions. 3 Biotech 7:214. https://doi.org/10.1007/s13205-017-0829-z

Schramm F, Larkindale J, Kiehlmann E, Ganguli A, Englich G, Elizabeth Vierling E, Koskull-Doäring PV (2008) A cascade of transcription factor DREB2A and heat stress transcription factor *HsfA3* regulates the heat stress response of *Arabidopsis*. Plant J 53:264–274. https://doi.org/10.1111/j.1365-313X.2007.03334.x

Shah SH, Ali S, Jan SA, Jalal-Ud-Din Ali GM (2015) Piercing and incubation method of in planta transformation producing stable transgenic plants by overexpressing *DREB1A* gene in tomato (*Solanum lycopersicum* Mill.). Plant Cell Tissue Organ Cult 120:1139–1157. https://doi.org/10.1007/s11240-014-0670-6

Shavrukov Y, Baho M, Lopato S, Langridge P (2016) The TaDREB*3* transgene transferred by conventional crossings to different genetic backgrounds of bread wheat improves drought tolerance. Plant Biotechnol J 14:313–322. https://doi.org/10.1111/pbi.12385

Tang Y, Liu K, Zhang J, Li X, Xu K, Zhang Y, Qi J, Yu D, Wang J, Lil C (2017) *JcDREB2*, a physic nut *AP2/ERF* gene, alters plant growth and salinity stress responses in transgenic rice. Front Plant Sci 8:306. https://doi.org/10.3389/fpls.2017.00306

Tsutsui T, Kato W, Asada Y, Sako K, Sato T, Sonoda Y, Kidokoro S, Yamaguchi-Shinozaki K, Tamaoki M, Arakawa K, Ichikawa T, Nakazawa M, Seki M, Shinozaki K, Matsui M, Ikeda A, Yamaguchi J (2009) DEAR1, a transcriptional repressor of DREB protein that mediates plant defense and freezing stress responses in *Arabidopsis*. J Plant Res 122:633–643. https://doi.org/10.1007/s10265-009-0252-6

Vadez V, Rao S, Sharma KK, Bhatnagar-Mathur P, Jyotsna DM (2007) DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit. J SAT Agric Res 5(1):1–5

Wang F, Gao ZH, Qiao YS, Mi L, Li JF, Zhang Z, Lin Z-L, Gu X-B (2014) *RDREB1BI* gene expression driven by the stress-induced promoter RD29A enhances tolerance to cold stress in benihope strawberry. In: ISHS acta horticulturae 1049: VII international strawberry symposium. https://doi.org/10.17660/actahortic.2014.1049.159

Wang H, Wang H, Shao H, Tang X (2016) Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. Front Plant Sci 7:67. https://doi.org/10.3389/fpls.2016.00067

Wei S, Sun Z, Han L, Yu L (2006) Transgenic tobacco plants over-expressing *Arabidopsis* transcriptional factor CBF1 show morphological and biochemical characteristics associated with cold tolerance. Asian J Plant Sci 5:932–939. https://doi.org/10.3923/ajps.2006.932.939

Wei T, Deng K, Gao Y, Liu Y, Yang M, Zhang L, Zheng X, Wang C, Song W, Chen C, Zhang Y (2016) *Arabidopsis* DREB1B in transgenic *Salvia miltiorrhiza* increased tolerance to drought stress without stunting growth. Plant Physiol Biochem 104:17–28. https://doi.org/10.1016/j.plaphy.2016.03.003

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6:251–264. https://doi.org/10.1105/tpc.6.2.251

Yang JS, Wang R, Meng JJ, Bi YP, Xu PL, Guo F, Wan SB, He QW, Li XG (2010) Overexpression of *Arabidopsis CBF1* gene in transgenic tobacco alleviates photoinhibition of PSII and PSI during chilling stress under low irradiance. J Plant Physiol 167(7):534–539. https://doi.org/10.1016/j.jplph.2009.11.005

Yang S, Tang XF, Ma NN, Wang LY, Meng QW (2011a) Heterology expression of the sweet pepper CBF3 gene confers elevated tolerance to chilling stress in transgenic tobacco. J Plant Physiol 168(15):1804–1812. https://doi.org/10.1016/j.jplph.2011.05.017

Yang W, Liu XD, Chi XJ, Wu CA, Li YZ, Song LL, Liu XM, Wang YF, Wang FW, Zhang C, Liu Y (2011b) Dwarf apple MbDREB1 enhances plant tolerance to low temperature, drought, and salt stress via both ABA-dependent and ABA-independent pathways. Planta 233(2):219–229. https://doi.org/10.1007/s00425-010-1279-6

Zhang S, Li N, Gao F, Yang A, Zhang J (2010) Over-expression of TsCBF gene confers improved drought tolerance in transgenic maize. Mol Breed 26:455–465. https://doi.org/10.1007/s11032-009-9385-5

Zhang X-X, Tang Y-J, Ma O-B, Yang C-Y, Mu Y-H, Suo H-C et al (2013) OsDREB2A, a rice transcription factor, significantly affects salt tolerance in transgenic soybean. PLoS ONE 8(12):e83011. https://doi.org/10.1371/journal.pone.0083011

Zhao L, Hu Y, Chong K, Wang T (2010) ARAG1, an ABA-responsive DREB gene, plays a role in seed germination and drought tolerance of rice. Ann Bot 105:401–409. https://doi.org/10.1093/aob/mcp303

Zhuang J, Wang F, Xu Z-S, Xiong A-S (2015) Microarray analysis of different expression profiles between wild-type and transgenic rice seedlings overexpression *OsDREB1BI* gene. Biologia 70(6):760–770. https://doi.org/10.1515/biolog-2015-0092

Zong J-M, Li X-W, Zhou Y-H, Wang F-W, Wang N, Dong Y-Y, Yuan Y-X, Chen H, Liu X-M, Yao N, Li H-Y (2016) The AaDREB1 transcription factor from the cold-tolerant plant *Adonis amurensis* enhances abiotic stress tolerance in transgenic plant. Int J Mol Sci 17:611. https://doi.org/10.3390/ijms17040611