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REVIEW ARTICLE

Engineering crop plants against abiotic stress: Current achievements and prospects

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Abstract

The growth, development and productivity of crop plants is negatively influenced by abiotic stresses like drought, salinity, heat and chilling leading to significant losses in crop yield. The modern technology of genetic engineering has enabled the scientists to move genes from distant sources into crop plants to develop resistance against insect pests, weeds and invading pathogens, some of them have already been commercialized. Similarly, efforts have been made to develop crop plants with enhanced tolerance against drought, salinity and chilling and waterlogging stress. Engineering crops against abiotic stresses has always been a challenge as this character is controlled by multigenes. The stress signaling and regulatory pathways have been elucidated using advanced molecular approaches and genes encoding tolerance to drought, salinity and chilling stress are being introduced in crop plants of economic importance using transformational technologies. The present review focuses the recent advances made in the development of transgenic crop plants of commercial importance with enhanced tolerance to abiotic stress; also the future prospects of stress tolerant crops have also been discussed.

Key words: Crop productivity, Desired trait, Genetic transformation, Stress physiology

Introduction

An external factor incurring negative influence on the plant can be defined as stress Levitt (1972). The major stresses that affect plant growth are water deficiency, chilling and freezing, heat and salinity stress. Drought and salinity are expected to cause serious salinization of more than 50% of all arable lands by the year 2050 as they are already widespread in many regions (Ashraf, 1994). Among the various abiotic stress conditions, water deficiency is the most devastating factor (Araus et al., 2002). Drought is severely affecting grain production and quality, and situation is becoming more serious with increasing global climate change (HongBo et al., 2006). It negatively impacts on growth and production of crops worldwide, induces a range of physiological and biochemical responses in plants.

Plants are constantly exposed to stress conditions under both natural and agricultural field conditions (Zhu, 2002; Mahmoud and Narisawa, 2013). In extreme conditions, some of the stresses like air temperature may damage plants within minutes while some take couple of days to weeks or months (e.g. soil water) even months (mineral nutrients) to become stressful (Boyer et al., 1982; Salisbury and Ross, 1989). Under stress condition, a plant generally adapts its metabolism and responds to various physiological and biochemical changes. The and major physiological processes including nitrogen fixation, respiration and photosynthesis and carbohydrates, metabolism of genetic material, proteins and lipids are affected (Smirnoff and Cumbes, 1989). Seed germination, maturation, senescence stages of plant growth are also affected differentially (Leprince et al., 1993; Gagliardi et al., 1995).

The conventional breeding has contributed significantly in the development of drought tolerant high yielding crop varieties since centuries, however the pace to develop new crop cultivars has been relatively slow; limitation of fertility barriers that allowing only plants of the same, or closely related species for hybridization. The advent of genetic engineering approaches enabled the researchers to overcome such problem (Bakhsh et al., 2009; Khan et al., 2011). Currently, the

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researchers have understood the changes in cellular, biochemical and molecular process under stress condition that laid a solid background to engineer crop varieties with enhances stress tolerance using different plant transformation approaches (Bhatnagar-Mathur et al., 2008).

The development of technologies for tissue culture and plant transformation resulted in development of transgenic crops that opened a window of successes for plant biotechnologist. In 1980s, the production of novel chimeric genes further expanded plant transgenic technologies (Fraley et al., 1983), plant expression vectors (Hoekema et al., 1983 Bevan, 1984), systems of DNA transfer (Draper et al., 1982; Fromm et al., 1985; Sanford et al., 1987), coupled with plant regeneration and transformation methodologies (Zambryski et al., 1983; Shimamoto et al., 1989; Gordon-Kamm et al., 1990). Figure 1 describes briefly the schematic depiction of signal transduction pathways between stress signal perception and gene expression, also cis- and trans-elements involved in stress responsive gene expression.

The various transformation methods available can be categorized in direct and indirect transformation methods. Direct methods do not use bacterial cells as mediators and where the most often applied direct methods are micro projectile bombardment or protoplast transformation. The indirect methods are based on the introduction of a binary vector carrying gene of interest into plant cell via agrobacteria, *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. Though all transformation methods have advantages that are unique to each of them, transformation using Agrobacterium and micro projectile bombardment are currently the most extensively used methods (Dai et al., 2001). Among others, the most often used are infiltration, silicon carbide fiber mediated transformation, electroporation of cells and tissues, microinjection, liposome-mediated transformation and transformation via the pollen-tube pathway (Zhu et al., 1993; Vasil, 1994; Songstad et al., 1995; Newell, 2000; Patnaik and Khurana, 2001; Rao et al., 2009).

Many genes induced under stress circumstances have been identified, characterized, cloned, and used as candidate genes to transform a crop plant encoding stress-related protein. The proteins having a role in abiotic stress tolerance, such as LEA (late embryogenesis abundant proteins), and other important enzymes encoding for osmolytes production, and regulatory proteins that are involved in regulation of signal transduction or stress responsive gene expression, like transcription factors (Shinozaki and Yamaguchi-Shinozaki, 2007). The present review focuses the current achievements of developing transgenic crops plants with improved tolerance against abiotic stresses. Earlier reviews contain limited information about field crops engineered with abiotic stress related genes; here we have focused on the endeavors achieved in crop plants of economic importance, also the abiotic stress tolerant crops tested in field conditions have also been discussed. Although numerous studies have been conducted and bulk of literature is available, but only the transgenic crops with significant and promising tolerance have been reported. Furthermore, the future prospects of stress-tolerant crops have also been discussed.

Crop Plants with Stress Related Genes

Transgenic techniques are being used to introduce biotic and abiotic stress related genes in crop plants (Ashraf et al., 2008). Plant responses under abiotic stresses are complex involving number of genes with additive effects, hence prospects of improve stress tolerance are not very bright. In spite of it, researchers have exerted efforts during last decade to generate transgenic crops with improved tolerance against abiotic stresses. Some of the transgenic crops have been enlisted in Table 1. The major emphasis has been to introduce gene encoding compatible organic osmolytes, heat shock proteins, plant growth regulators, late embryogenesis abundant proteins and transcription factors responsible in activating gene expression (Ashraf et al., 2010).

Many genes play an important role in the synthesis of osmoprotectants in stress tolerant plant like proline, glycinebetaine and polyamines, mannitol, trehalose and galactinol which accumulate during osmotic adjustment (Vincour and Altman, 2005). Now it is well evident that these organic solutes play an important role in induction of drought tolerance (Ashraf and Foolad, 2007). Certain major crop plants such as rice, soybean, and potato lack significant amounts of betaines or any other osmoprotectant (reviewed by McNeil et al., 1999; Bhatnagar-Mathur et al., 2008); therefore, osmoregulatory genes have been first target of the researchers to introduce in crops. Also the overexpression of the genes encoding synthesis of organic solutes has been carried out. Some of the crops engineered with these osmoprotectants have been summarized in Table 1. Among many organic osmolytes having substantial role in stress tolerance, glycine betaine (GB) is richly synthesized under dehydration stress (Mansour, 2000; Mohanty et al., 2002; Yang et al., 2003; Ashraf and Foolad, 2007).

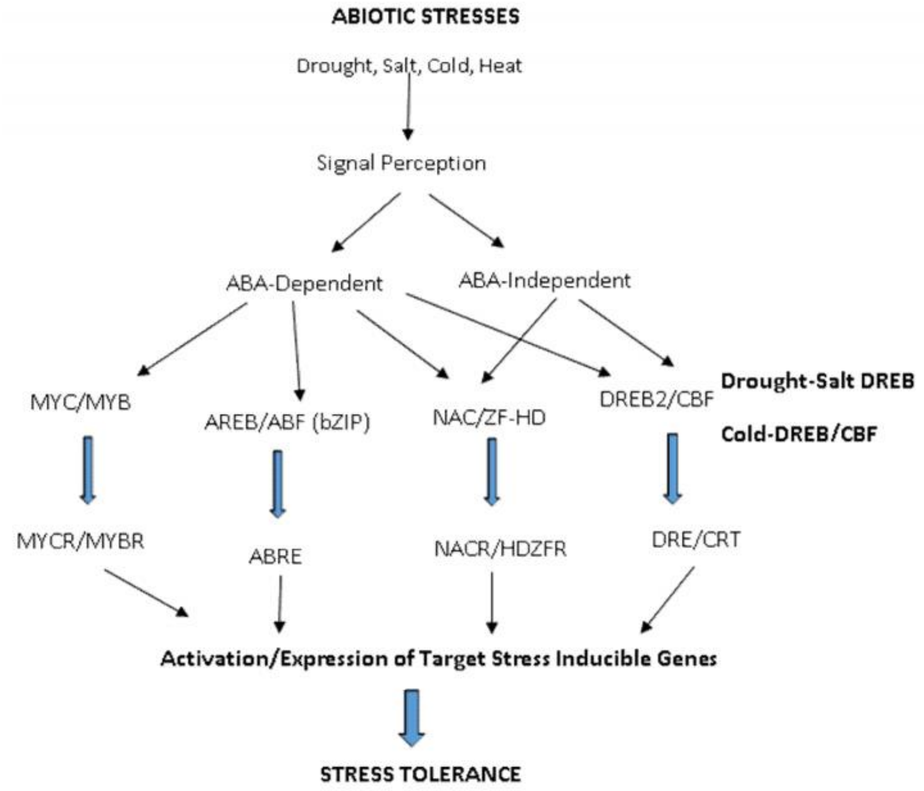

Figure 1: A diagrammatic illustration of signal transduction pathways between stress signal recognition and gene Expression; also the cis- and trans- acting elements involved in abiotic stress responsive gene expression, as previosuly described by Lata et al. (2011). Dehydration results in induction of ABA biosynthesis that further activates two regulatory ABA-dependent gene expressions; bZIP/ABRE and the MYC/MYB. DREBs are the members of the ERF family of transcription factors and follow ABA-independent signal transduction pathway. DRE: drought responsive element, ABRE: abscisic acid responsive binding element, MYBRS: MYB recognition site, MYCRS: MYC recognition site, bZIP: basic-domain leucine-zipper.

Organic Osmolytes

Choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) are the important enzymes that are required in the production of glycine betaine in higher plants. Tobacco transgenic lines overexpressing CMO were reported (Shen et al., 2002; Zhang et al., 2008). An increased glycine betaine accumulation was recorded under water deficient conditions; ultimately leading to enhance drought stress. Quan et al. (2004) transformed maize inbred line DH4866 with *E. coli* betA gene encoding choline dehydrogenase. Transgenic plants were reported to have higher level of glycine betaine and showed more drought as compared to control plants. Recently, Zhang et al. (2009) introduced AhCMO into a Chinese cotton cultivar SM3 via Agrobacterium-mediated transformation. Transgenic lines of cotton developed were subjected to evaluate salinity tolerance in both green house and field conditions. They reported that seedlings of the cotton line have 26 and 131% more glycine betaine when compared to control plants using normal and salt-stress. The salt stress condition applied was as 150 mmol l^{-1} NaCl. An improved salt tolerance was recorded in cotton transgenic lines. Transgenic lines having betaA gene from *E. coli* had enhanced tolerance to chilling temperature (Zhang et al., 2012). Rice has also been engineered to accumulate more levels of glycine betaine with improved tolerance against drought, salt and chilling stresses respectively (Su et al., 2006; Mohanty et al., 2002; Shiraswa et al., 2006). Ahmad et al. (2008) reported the development of transgenic potato lines with improved tolerance against oxidative, drought and salt stresses using choline oxidase gene (COX). Using the same approach, Brassica and tomato have also been engineered to encode resistance against chilling, drought and salt stresses respectively (Parsad et al., 2000; Park et al., 2007). Like glycine betaine, proline is another significant organic osmolyte having an important role in stress tolerance. A key enzyme for the proline biosynthesis is pyrroline-5-carboxylate synthetase (P5CR). The genes encoding this enzyme has been engineered in number of crops like tobacco (Gubis et al., 2007) petunia (Yamada et al., 2005) and soybean (Ronde et al., 2004). The transgenic lines developed of tobacco, petunia and soybean showed increased accumulation of proline and thus enhanced drought tolerance.

Another significant osmoprotectant, mannitol, has also been transformed in crops as it encodes stress tolerance. Genes (e.g. mtID) involved in biosynthesis of mannitol have been overexpressed for this purpose. Abebe et al. (2003) developed wheat plants overexpressed with ecotypic expression of the mt1D. An enhanced tolerance to both drought and salt stressed has been documented. However, there are very less report of transgenic crops with mannitol encoding genes; may be lack of relationship of mannitol overexpression with drought tolerance.

There are also many reports utilizing trehalose (a potential osmoprotectant) to protect plants against stresses. The crops plants have been engineered with the key enzyme involved in trehalose biosynthesis (Table 1). Romero et al. (1997) and later on Karim et al. (2007) developed transgenic tobacco lines harbouring gene TPS1 showed enhanced drought tolerance. Likewise, drought tolerant rice was developed by introducing a chimeric gene Ubi1::TPSP (Jang et al., 2003). Transgenic rice had an increased level of trehalose and hence stress tolerance but abnormal phenotype was observed under normal growth conditions because of continuous accumulation of trehalose, a non reducing sugar. Cortina and Culiáñez-Macià (2005) developed transgenic tomato using yeast TPS1 gene, transgenic lines showed more sugar and starch contents as compared to control plants, also an enhanced improvement was recorded against oxidative, drought and salinity stress. It is also important to mention here that transgenic lines showed pleiotropic effects showing dark green leaves, thick roots, erected branches and root development. A stress inducible promoter was used to drive the expression of *E. coli* trehalose biosynthesis genes (otsA and otsB) as fusion gene (Garg et al., 2002) that resulted in normal phenotype and had an improved tolerance against drought stress.

Regulatory Genes

The various stress-related genes, metabolites such as abscisic acid (ABA) or osmotically active compounds, and some other specific proteins are trigged under stress condition (Shinozaki and Yamaguchi-Shinozaki 2007). The numerous genes induced under drought conditions have been identified, characterized, cloned, and overexpressed to induce tolerant in crop plants. ABA is a significant player that in involved plant growth and development when plants are subjected to stressed condition (Xiong et al., 2001; Xiong and Zhu 2003). The genes contributing to ABA biosynthesis are well characterized and introduced in *Arabidopsis thaliana* and and less studies are available in other crop plants. Jeanneau et al. (2002) reported the development of transgenic maize lines with increased photosynthetic capacity and water use efficiency and with high biomass under stressed conditions. Yue et al. (2012), very recently, overexpressed Arabidopsis LOS5 gene in cotton variety. The developed lines of transgenic cotton had increased endogenous ABA (25%) and proline (20%) when compared with the control plants. In addition to it, increased activity antioxidants (superoxide dismutase, peroxidase, and ascorbate peroxidase) was also recorded; leading ultimately to better drought tolerance response.

The molybdenum cofactor sulphurase (encoded by LOS5/ABA3 gene) is engaged in aldehyde oxidase activity in Arabidopsis, which further induces ABA biosynthesis indirectly and increases stress tolerance. Li et al. (2013) overexpressed LOS5/ABA3 in soybean (*Glycine max* L.) under the control of a super constitutive 35S promoter. Expression of LOS5/ABA3 in transgenic plants was up-regulated by drought stress, which led to increased aldehyde oxidase (AO) activity and then a significant increase in ABA accumulation. The reduced water loss was recorded in transgenic soybean under drought stress regime. Most importantly, transgenic soybean plants showed at least 21% higher yield than that of wide-type plants under drought stress conditions in the field conditions.

Table 1. Selected examples of introduction of organic osmolytes in crop plants using genetic engineering Approaches.

Crop	Gene introduced	Character/Trait improved	Reference
Glycine betaine			
Brassica juncea L.	codA	Tolerance to salt stress conditions was reported	Parsad et al., 2000
Oryza sativa L.	codA	Transgenic lines showed improved tolerance to salinity	Mohanty et al., 2002
Nicotiana tobaccum L.	CMO	An improved tolerance to drought was recorded by the accumulation of glycine betaine	Shen et al., 2002; Zhang et al., 2008
Zea Mays L.	betA	The higher level of glycine betaine accumulated and showed more drought as compared to control plants	Quan et al., 2004
Oryza sativa L.	COX	Transgenic plants were tolerance tos aline conditions	Su et al., 2006
Oryza sativa L.	CMO	Enhanced tolerance to salt stress and temperature stress in the seedling stage	Shirasawa et al., 2006
Lycopersicon esculentum Mill	codA	Chloroplastic GB accumulation further raised the level of stress tolerance in tomato plants	Park et al., 2007
Solanum tuberosum L.	COX	Improved tolerance against oxidative, drought and salt stresses	Ahmad et al., 2008
Gossypium hirsutum L.	AhCMO	Transgenic lines accumulated 26 and 131% more glycine betaine and showed enhanced salt stress	Zhang et al., 2009
Gossypium hirsutum L.	betA	Enhanced tolerance to chilling temperature was recorded	Zhang et al., 2012
Proline			
Glycine max L.	----	Photosynthetic response of transgenic soybean during heat and drought stress was reported	Ronde et al., 2004
Petunia hybrida	----	The transgenic lines tolerated 14 dsyd of drought stress	Yamada et al., 2005
Nicotiana tobaccum L.	P5CR	Transgenic tobacco plants showed tolerance to drought with more accumulated protein levels	Gubis et al., 2007 Popisilova et al., 2011
Mannitol and Trehalose			
Nicotiana tobaccum L.	TPS1	Transgenic tobacco lines transformed with TPSI showed improved drought tolerance	Romero et al., 1997 Karim et al., 2007
Oryza sativa L.	otsA and ots B	A stress inducible promoter drived expression otsA and otsB with improved tolerance against drought stress	Garg et al., 2002
Triticum aestivum L.	mtlD	Tolerance to both drought and salt stress has been documented in wheat transgenic lines	Abebe et al., 2003
Oryza sativa L.	Ubi1::TPS1	Drought tolerant rice transgenic lines were generated	Jang et al., 2003
Lycopersicon esculentum	Yeast TPS1	More sugar and starch contents; enhanced tolerance of lines against oxidative, drought and salinity stress	Cortina and Culiáñez-Macià (2005)

Late Embryogenesis Abundant Proteins

LEA (late embryogenesis abundant) proteins are also believed to have vital role in stress tolerance as these protein help to maintain structure of cellular membranes, ionic balance, water binding and acts as molecular chaperons under drought stress conditions gets accumulated in plants under stress (Babu et al., 2004; Gosal et al., 2009). The researchers have engineered LEA genes in crop plants to encode drought tolerance (Table 2). Cheng et al. (2002) engineered PMA1959 and PMA80 LEA protein genes in rice. PMA1959 and PMA80 LEA encode a group of 1LEA and 2LEA proteins respectively. Likewise, HVA1 (encoding group 3 LEA protein) from barley was transformed in rice by Xu et al. (1996) and in wheat by Sivamani et al. (2000). These transgenic lines of rice and wheat showed increased tolerance to drought stress as it was evident from the data. The LEA gene from *Brassica napus* (ME-lea N4) was genetically transformed in lettuce and chinese cabbage (Park et al., 2005a; 2005b). They used 35S CaMV promoter to drive the expression of LEA gene. An increased tolerance to both salinity and drought was well documented. LEA protein gene OsLEA 3-1 has been transformed in rice under stress inducible promoter successfully. The transgenic lines of rice were further evaluated in field condition harboring LEA protein gene and showed improved tolerance against drought stress (Xiao et al., 2007). Literature available regarding the use of LEA proteins in stress tolerance seems quite promising.

Heat Shock Proteins

The heat shock proteins abbreviated as HSP are gene families found in plants usually when subjected drought stressed condition (Joshi and Nguyen, 1996). The role of HSPs has been well established now and these are considered to have an important role in protein–protein interactions i.e. protein folding, assisting in correct formation, and prevention of undesired protein aggregation. The heat shock proteins are produced by plant cells as a normal function, however, more heat shock proteins are necessary under stressed conditions as aggregation and denaturation of proteins is increased (Cooper, 1997). Zhu and Bresan (1997) reported that increased synthesis of heat shock proteins is significant to cope plant during the osmotic stress.

Keeping in view the established role of heat shock protein genes in enhancing stress tolerance, molecular chaperon activity has been over expressed in crop plants (Li et al., 2003; Katiyar-Agarwal et al., 2003). There have been reports suggesting on positive correlation between the accumulated levels of HSPs and response to stress condition (Sun et al., 2001; Wang et al. 2005). A comprehensive review article written by Grover et al. (2013) discusses the types of heat shock families and also enlists the crop plants that have been transformed with heat shock protein genes. In addition to model plants, crop plants like tomato (Neta-sharir et al., 2005) rice (Uchida et al., 2008; Qi et al., 2011) tobacco (Uchida et al., 2008; Sun et al., 2012) soybean (Xhu et al., 2006) and cotton (Maqbool et al., 2010) have been reported in the literature. Neta-Sharir et al. (2005) overexpressed HSP21 in tomato under 35S CaMV promoter and observed protected photosystem II (PSII) from temperature dependent oxidative stress.

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Maqbool et al. (2007) reported GHSP26, small heat shock protein which functions as a drought-induced gene in *Gossypium arboreum* L. The GHSP26 protein gene was later on overexpressed in *Gosypium hirsutum* L. (Maqbool et al., 2010). Transgenic plant showed enhanced tolerance against water stress. The transgenic cotton lines harboring GHSP26 were analyzed in successive progenies and transgenic plants harboring GHSP26 tolerated 10–12 days of drought stress as compared to control plants (Shamim et al., 2013).

Detoxifying Genes

The drought stress in plants results in enhanced accumulation of ROS (reactive oxygen species) which causes an oxidative stress. Naturally, the plants can develop various types of antioxidants. The researchers have targeted various gene encoding antioxidants to induce stress tolerance in plants (Pastori and Foyer, 2002; Sunkar et al., 2006). The gene encoding superoxide dismutase (SOD) was transformed in alfaalfa, potato and rice respectively (McKersie et al., 1996, 1997; Perl et al., 1993; Wang et al., 2005). Badawai et al. (2004) introduced Arabidopsis gene encoding ascorbate peroxidase in tobacco chloroplasts and achieved enhanced tolerance to osmotic stress induced by polyethylene glycol. Alvarez Viveros et al. (2013) reported the effects of overexpressing glyoxalase I and glyoxalase II genes in transgenic tomato (Solanum lycopersicum Mill.). The transgenic lines overexpressing GlyI and GlyII showed reduced lipid peroxidation and the production of H2O2 in leaf tissues under high NaCl concentration (800 mM). The significant decrease in chlorophyll a+b content in wild-type (WT) was also recorded when compared with transgenic lines. Hence, it was concluded that over expressing GlyI and GlyII led to salt stress tolerance in transgenic tomato by decreasing oxidative stress. The physiological role of TomloxD (13-lipoxygenase) was investigated by Hu et al. (2013). The transgenic tomato plants over expressing TomloxD CaMV 35S promoter increased lipoxygenase activity and content of endogenous jasmonic acid (JA). They found increased expression levels of defense genes LeHSP90, LePR1, LePR6 and LeZAT in the transformants as compared to non-transformed plants. The transgenic tomato plants harboring TomloxD were more tolerant to *Cladosporium fulvum* and high temperature stress showing tomloxD gene has as potential candidate in engineering crop plants against biotic as well abiotic stress stresses.

Table 2. Selected examples of late embryogenesis abundant proteins and transcription factors (regulatory proteins) in crop plants.

Crop	Gene introduced	Character/trait improved	Reference
Late embryogenesis abundant			
Oryza sativa L.	HVA1	Barley HVA1 encoded increased tolerance to drought stress	Xu et al., 1996
Triticum aestivum L.	HVA1	Wheat transgenic lines harboring barley HVA1 showed drought tolerance	Siwamani et al., 2000
Oryza sativa L.	PMA1959 and PMA80 LEA	The accumulated level of wheat LEA genes, PMA80 and PMA1959, enhanced dehydration tolerance of transgenic rice	Cheng et al., 2002
Lactuca sativa L.	ME-lea N4	*Brassica napus* ME-lea N4 enhanced drought and salt tolerance in transgenic lettuce plants.	Park et al., 2005a
Brassica campestris L.	ME-lea N4	Transgenic plants of Chinese cabbage showed increase tolerance to both salt and drought stress	Park et al., 2005b
Oryza sativa L.	OsLEA 3-1	Overexpression of OsLEA 3-1 gene under stress inducible promoter in transgenic rice lines resulted in increased tolerance to drought stress under field conditions	Xiao et al., 2007
Transcription factors			
Oryza sativa L.	OsDREB	Over expression of OsDREB in rice resulted in gene activations and regulation involved in drought-, high-salt- and cold-responsive gene expression	Dubouzet et al., 2003
Triticum aestivum L.	AtDREB	Wheat cultivar transformed with atDREB1A under stress inducible promoter (rd29A) showed increase drought tolerance	Pellegrineschi et al., 2004
Oryza sativa L.	AtDREB	Expression DREB1A /(CBF3) in transgenic rice showed enhanced tolerance to salinity and drought	Oh et al., 2005
Zea Mays L.	ZmDREB2A	Overexpression of ZmDREB2A in Maize under stress inducible as well as constitutive promoter encoded stress tolerance	Qin et al., 2007
Nicotiana tobaccum L.	GhDREB1	Transgenic tobacco overexpressing GhDREB1 showed significant chilling tolerance compared wild-type plants.	Shan et al., 2007
Arachis hypogaea L.	AtDREB1	Transgenic peanut overexpressing AtDREB1A under stress-inducible promoter showed increased stress tolerance, accumulated level of proline and antioxidants	Bhatnagar-Mathur et al. (2009
Oryza sativa L.	AP37	AP37 in rice field conditions was reported to increase yield by 16% to 57% over controls under severe drought conditions, being potential candidate gene for drought tolerance.	Kim and Kim, 2009
Arachis hypogaea L.	AtDREB1	Transgenic peanut harboring DREB1transcription factor in field conditions showed up to 24 % increase in yield in four drought trials under natural field conditions as compared to control variety	Bhatnagar-Mathur et al., 2014

Transcription Factors

Transcription factors are the proteins that bind DNA specifically, transcribe and regulate genes. Knowing the importance of gene regulations by transcription factors in plants, researchers have made continuous efforts to identify, characterize, clone and transform different transcription factors involved in regulation of stress related genes (Table 2). Vinocur and Altman (2005), in a review article, described several identified transcription factors that can regulate gene expression under stress conditions. A number of transcription factors have also been reported by Gosal et al. (2009). With the first report of Jaglo-Ottosen et al. (1998) and Liu et al. (1998) that showed up-regulation of many genes in transgenic DREB1/CBF Arabidopsis against drought, salinity, freezing etc, dehydration-responsive element-binding factors (DREB) have attracted the attention of plant scientist to induce stress tolerance in higher crop plants.

A wheat cultivar was transformed with Arabidopsis DREB1A under stress inducible promoter (rd29A). The transgenic wheat lines showed increased drought tolerance (Pellegrineschi et al., 2004) while the expression Arabidopsis DREB1A /(CBF3) in transgenic rice plants under 35S CaMV promoter also showed enhanced tolerance to salinity and drought (Oh et al., 2005). Overexpression of four rice CBF/DREB1A orthologs exhibited tolerance to salinity, drought and freezing as well (Dubouzet et al. 2003). Maize has also been overexpressed successfully with ZmDREB2A under stress inducible as well as constitutive promoter to encode stress tolerance (Qin et al., 2007). Bhatnagar-Mathur et al. (2009) produced transgenic peanut that overexpressed AtDREB1A under stress-inducible promoter.

The transgenic plants showed increased stress tolerance, accumulated levels of proline and antioxidants (ascorbate peroxidase, superoxide dismutase and glutathione reductase) was also recorded. Transgenic tobacco lines overexpressing GhDREB1 from cotton (*Gossypium hirsutum* L.) exhibited stronger chilling tolerance than wild-type plants (Shan et al., 2007) also reviewed by Amudha et al. (2011). In addition to DREB, some other transcription factors have also been discovered to regulate gene in plant stress responses against abiotic stress and are well described by Oh et al. (2009). These transcription factors have also been utilized to induce tolerance in plant against abiotic stress.

Kim and Kim (2009) introduced transcription factor AP37 in rice and evaluated transgenes in field conditions and reported increased grain yield by 16% to 57% over controls under severe drought conditions. They concluded AP37 gene has the potential to enhance drought tolerance with normal phenotypes. James (2013) reported the plantation of first commercial drought tolerant maize in United States on an area of 50,000 hectares cultivated in drought-prone Corn Belt.

The SNAC1 gene (NAC superfamily of transcription factors) identified from rice was overexpressed in cotton cultivar YZ1 via Agrobacterium mediated transformation. SNAC1-overexpressing cotton plants showed significant improvement in tolerance to both drought and salt stresses in the greenhouse conditions. In the presence of 250 mM NaCl hydroponic growth conditions, transgenic plants exhibited vigorous growth (especially root development) when compared to wild type plants. The transpiration rate of transgenic cotton plants significantly decreased in comparison to the wild-type during drought and salt treatments, but the photosynthesis rate maintained the same at the flowering stage in the transgenic plants (Liu et al. 2014). Transgenic plants also showed increased number of bolls. Recently, Bhatnagar-Mathur and colleagues have reported evaluation of transgenic peanut harboring DREB1A transcription factor in field conditions and reported upto 24 % increase in yield in four drought trials under natural field conditions as compared to control variety (Bhatnagar-Mathur et al., 2014).

Waterlogging

Waterlogging is one of the major harmful abiotic stresses and results in considerable crop losses. Genetic engineering is considered an effective technology of developing crops for waterlogged/submerged conditions. Until now, engineering of genes associated with waterlogging stress has been challenging and considered as limiting factor (Mukhopadhyay et al., 1997). Scientists have focused on carbohydrate metabolism as reduced O2 supply hinders normal respiration process resulting in lesser ATP synthesis. Mukhopadhyay et al. (1997) and Minhas and Gover (1999) reported that respiration pathway switches to fermentation pathway from oxidation during oxygen-deprived (anaerobiosis) condition. This inclusion of ethanolic fermentation pathway is considered to be an important component of responses that are educed in rice against flooding stress and in other plants as well. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes are involved in ethanolic fermentation (pyruvate to ethanol). Grover at al., (1995, 1997 and 1999) cloned pdc1, pdc2, and pdc3 gene under the control of three different promoters (35S CaMV, actin-1 and anoxia induced 6X ARE) and regenerated large number of rice transgenic plants. Quimio et al. (2000) in IRRI developed rice using one of the above mentioned construct (pdc1-S under control of actin-1) with enhanced metabolic capacity under anaerobiosis conferring submergence tolerance. Tillers of transformed rice plants exhibited higher pdc activities and ethanol production as compared to non-transformed plants. Consequently, ethanol production of tillers of transgenic plants was positively correlated with survival after submergence. The researchers at CSIRO have also made considerable progress to change ADH levels in rice (Dennis et al. 2000). Efforts are going on around the world to develop water logging tolerant crops but till date limited information is available and no any remarkable achievement has been reported.

Stacked Genes to address Abiotic Stress

Abiotic stress factors (Salt, drought, cold and extreme temperatures) severely limit crop yield. The modern approaches in crop engineering have accelerated the process of developing stress tolerant crop varieties. Abiotic stress tolerance has repeatedly been achieved in plants by the transfer of a single gene (Muthurajan and Balasubramanian, 2009). As abiotic stress tolerance of plants is a very complex trait and involves multiple physiological and biochemical processes. Thus, the improvement of plant stress tolerance should involve pyramiding of multiple genes. Therefore, the generation of transgenic plants by introducing two or more foreign genes has become one of the important goal of plant biologist to combat abiotic stresses (Gouiaa et al., 2012).

Wei et al. (2011) developed maize expressing betA (encoding choline dehydrogenase from *Escherichia coli*) and TsVP (encoding V-H+ -PPase from *Thellungiella halophila*) using conventional cross hybridization technique. Transgenes were evaluated successfully for the integration and expression of both genes using standard molecular techniques of PCR, southern blotting and real-time RT-PCR. Furthermore, higher glycinebetaine contents and H+ -PPase activity was found in transgenic maize plants as compared to parental lines. Under drought stress treatments, the pyramided maize plants also exhibited higher relative water content, greater solute accumulation and lower cell damage. The transgenic plants also exhibited more vigorous growth (with less retardation), shorter anthesis-silking period and increase in yield compared to parental lines and non wild type. Co-expression of multiple, effective genes in transgene can effectively lead to increased abiotic stress tolerance.

The co-expression of ApGSMT2 (glycine sarcosine methyltransferase) and ApDMT2 (glycine sarcosine methyltransferase) in maize has been reported to be an effective approach for enhancing abiotic stress tolerance in maize breeding programmes. He et al. (2013) reported genetically engineered maize plants with an enhanced ability to synthesise glycinebetaine (GB) by introducing two genes ApGSMT2 and ApDMT2 from the bacterium Aphanothece halophytica. The increased expression levels of ApGSMT2 and ApDMT2 under drought conditions resulted in GB accumulation in the leaves of transgenic maize plants and conferred improved drought tolerance. Under drought conditions, the transgenic plants showed an increased accumulation of sugars and free amino acids, greater chlorophyll content, a higher photosynthesis rate and biomass, and lower malondialdehyde and electrolyte leakage compared to the wild-type.

Two wheat genes encoding two important vacuolar ion transporters, Na+/H+ antiporter (TNHXS1) and H+-pyrophosphatase (TVP1), were linked via IRES (the internal ribosome entry site from the 5′UTR of the heat-shock protein of tobacco gene NtHSF-1) to generate the bicistronic constructTNHXS1-IRES-TVP1. Molecular analysis of transgenic tobacco plants exhibited proper integration of the TNHXS1-IRES-TVP1 construct in plant genome and the production of the full-length bicistronic mRNA from the 35S promoter. Under salt treatment, transgenic plants harboring TNHXS1-IRES-TVP1 displayed better performance than either of the single gene-transformed line or wild type plants. IRES system can co-ordinate the expression of two important abiotic stress-tolerance genes is a valuable tool for obtaining transgenic plants with improved salt tolerance (Gouiaa et al., 2012). Nguyen et al. (2013) showed effectiveness of co-expression of two heterologous abiotic stress tolerant genes (HVA1 and mtlD) in maize (*Zea mays* L.) to confers drought and salt tolerance along with an Increase in Crop Biomass. Plants expressing combination of HVA1+mtlD in fourth generation showed higher leaf relative water content and greater plant survival as compared with their single transgene transgenic plants and with their control plants under drought stress. Similarly, transgenic plants showed higher fresh and dry shoot and dry root matter compared to single transgene or their control plants under salt stress treatment. The area of developing abiotic stress tolerant plants is gaining momentum among researchers worldwide.

Conclusion and Prospects

The breeding of crop plants against abiotic stresses has been challenging for the breeders as the stress tolerance is a multiply genic character in contrary to plant resistance against biotic stress that is most dependent on monogenic trait. The different approaches have been adopted by researchers to induce stress tolerance in plants. Firstly, the genes with known enzymatic activity encoding single metabolite were initially introduced in plants. This approach has not been sustainable because of the fact that stress tolerance involves many genes simultaneously. However, currently regulatory proteins of stress induced genes are being introduced in plants. These regulatory proteins encode stress inducible transcription factors which further regulate many genes simultaneously involved in stress response of a plant. On basis of scientific literature available, one can conclude that this approach has offered new ways to increased tolerance towards multiple stresses mainly including drought, salinity, chilling etc.

Development of stress tolerant varieties is important to ensure food supply in order to meet the challenges with increasing population in the world. Serious efforts have been made and still going on to engineer crop plants with genes encoding tolerance against drought, salinity, heat, waterlogging and chilling stresses. As discussed in the article, genes involved in the synthesis of organic osmolytes, antioxidants, late embryogenesis abundant proteins, plant growth regulators, antioxidants and transcription factors (regulatory proteins) have been introduced in various crop plants successfully. Abiotic stress tolerance has been achieved in plants by the transfer of a single gene. Abiotic stress tolerance in plants is an intricate character trait and involves multiple physiological and biochemical processes. Efforts are going on to develop transgenic plants by pyramiding two or more foreign genes.

The field evaluation of stress tolerant crops have been reported which is remarkable achievement in this direction. Field testing of stress tolerant transgenic is going on in various part of the world. The first commercial drought tolerant maize in United States has been planted on an area of 50,000 hectares in drought-prone Corn Belt. Some of the other abiotic stress tolerant plants (peanut, rice, maize and soybean) have been evaluated in field conditions on pilot scales, the need of the hour is to expand the field evaluations at larger scale to determine the potential of these transgenic crops against abiotic stresses. It is important to note that these studies have been conducted in laboratory or in green house condition under applied/controlled stress conditions. It will be interesting to evaluate these crops how they behave in field conditions. Taken together of rice, peanut, soybean and maize examples, we can say it an important breakthrough as we are well aware of the losses incurred by abiotic stress in crops. The economic benefits of insect and herbicides resistance crops have revolutioned agriculture; we can expect more achievements by plant scientist in the future by creating a new generation of biotech crops be commercialized.

Author contributions

T. H. collected literature in a chronological order. A. B. wrote the main manuscript and analyzed it critically to present it in its current form.

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