Chapter

Heterologous Expression of Genes in Plants for Abiotic Stresses

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Abstract

Abiotic stresses are considered to be the major factors causing a decrease in crop yield globally, these stresses include high and low temperature, salinity, drought, and light stress etc. To overcome the consistent food demand for the ever-growing population, various genes from micro-organisms and non-plant sources have been expressed in transgenic plants to improve their tolerance against abiotic stresses. Gene expression in transgenic plants through conventional methods are time-consuming and laborious that's why advanced genetic engineering methods for example *Agrobacterium*-mediated transformation and biolistic methods are more accurate, useful, and less time-consuming. This review provides an insight into various bacterial genes for example *mtID*, *codA*, *betA*, *ADH*, *IPT*, *DRNF1* and *ggpPS*, etc. that have been successfully expressed in transgenic plants against various abiotic stress for stress tolerance enhancement and crop yield improvement which exhibited good encouraging results. Genes from yeast (*Saccharomyces cerevisiae*) have been introduced in transgenic plants against drought and salinity stress. All these genes expressed from non-plant sources in plants can be very helpful to enhance crops for better yield productivity in the future to meet the demands of the consistently rising population of the world.

Keywords: abiotic stresses, heterologous expression, bacterial, yeast, fish and insect genes

1. Introduction

Plant stress is a condition in which plant growing in an unfavorable condition that mainly causes growth problems, deficiencies in crop yields, and even death when the stress-causing factors cross the limit that plants can tolerate [1]. It refers to external environmental conditions that adversely affect the overall growth, progress, or production of plants [2].

There are two types of stresses to which plants are subjected that is abiotic stress and biotic stress. The crop loss worldwide is mainly due to abiotic stress which consists of drought, cold, salinity, high environmental temperature, and radiation, etc. [3]. While biotic stresses are the attack of various pathogens on plants including bacteria, fungi, herbivores, and nematodes) etc. [4]. Due to the sessile nature

of plants they cannot avoid these environmental factors but develop several mechanisms to tackle these abiotic and biotic stresses for their survival and environmental adaptation.

1.1 Abiotic stress mechanism in plants

Plants usually sense the environmental stress and then stimulate appropriate suitable response takes place, cell surface receives the stimuli and the transformation to the transcriptional system in the nucleus takes place via various pathways that help in transduction, make plants resistant to various environmental stress by the activation of molecular, biochemical, and physiological suitable response [5]. The first line of defense of plants is situated in roots to overcome abiotic stress. If the plant growing in the soil is healthy and there is biological diversity the chances of survival against the abiotic stress of the plant will be high. High salinity affects the growth and development of plants. The disruption of (Na^+) and (K^+) ratio in the cytoplasm is mainly the primary response shown by the plants against stress. Living microorganisms need to ensure effective growth and generate an effective environmental response, this especially very important in plants because of their immobility and encountering large changes/alterations in temperature, humidity, light, and availability of nutrients in the environment. Massive agricultural losses happen due to environmental stresses [6, 7] and the improvement of crop resistance is a major goal for crop programs.

A genetic locus that keeps productivity maintained even in serious conditions are situated within the germplasm of existing crops, their relative species that are earlier adopted to severe environments. Selective breeding in combination with other loci has improved crops yield in extremely challenging environmental conditions throughout agricultural history. An efficient advanced paradigm is the precise selection of genetic factors of stress adaptation that have been in nature for years and passes on by plants to their higher verities [8]. Abiotic stress causes biosynthetic capacity and nutrient decrease which leads to inhibition in plant growth and has been further elaborated by various researchers in their work by knowing the response to abiotic stress through various signaling pathways involving several genes, mechanism of post-transcriptional modification, and proteins. Those pathways are MAPK, ABF/bZIP, Ca2+-CBL-CIPK, and CBF/DREB which enables much stress responding transcription factors to initiate downstream signals needed for abiotic stress defense [9]. These signaling pathways can predict the effects generated by abiotic stress to control growth and plant adaptation. Recently genes have been identified which control plant growth during stress conditions for example molecular mechanism which controls leaf progress and growth under drought conditions relates both transcriptional signals to the circadian clock. Importantly (ERFs), ERF2 and ERF8 related to ethylene response factors showed to affect leaf in drought and wet conditions [10].

Abscisic acid plays a huge role in helping plants for their environmental adaptation against cold, drought, alteration in temperature, salinity, and wounding [11]. During extreme environmental conditions, the level of Abscisic acid goes up through the ABA biosynthesis process. High-level ABA combines with receptor for the initiation of signal transduction which leads to the cellular response to stress [12]. Various mechanisms that help in the protection of plant survival against abiotic stress are very much important, yet they are activated at the cost of plant growth and its productivity which is essential for agriculture. Recent studies in molecular genetics help us to

understand the basis of abiotic stress tolerance [13]. **Figure 1** illustrates the various signaling pathways involved in abiotic stress mechanisms in plants [9].

1.2 Abiotic stresses (factors) that affect plants

1.2.1 Temperature

Temperature is a very important abiotic stress factor that affects plant from seed germination to reproduction [14]. Significant temperature changes can lead to permanent disturbance in the plant cycle which even leads to death. It causes plant stress by two means; extremely cold and hot temperature, severe cold conditions below the optimum temperature can cause physical and mechanical changes to the plant and leads to severe cell disruption [15]. In various areas extremely low temperature causes agricultural crop productivity and affects the cultivation process [16]. While due to uncontrolled rise in temperature affects the rate of photosynthesis, water availability to plants, and fruit ripening. Due to climatic changes an appreciable rise in temperature in the coming times will cause rainfall reduction, alteration in wind speed, and snow leads to less growing plant season and eventually will harm crop production and quality [17]. The effects of verglas/frost and high temperature have been evaluated recently on the production of Wheat (*Triticum aestivum L*), fruitless plants and

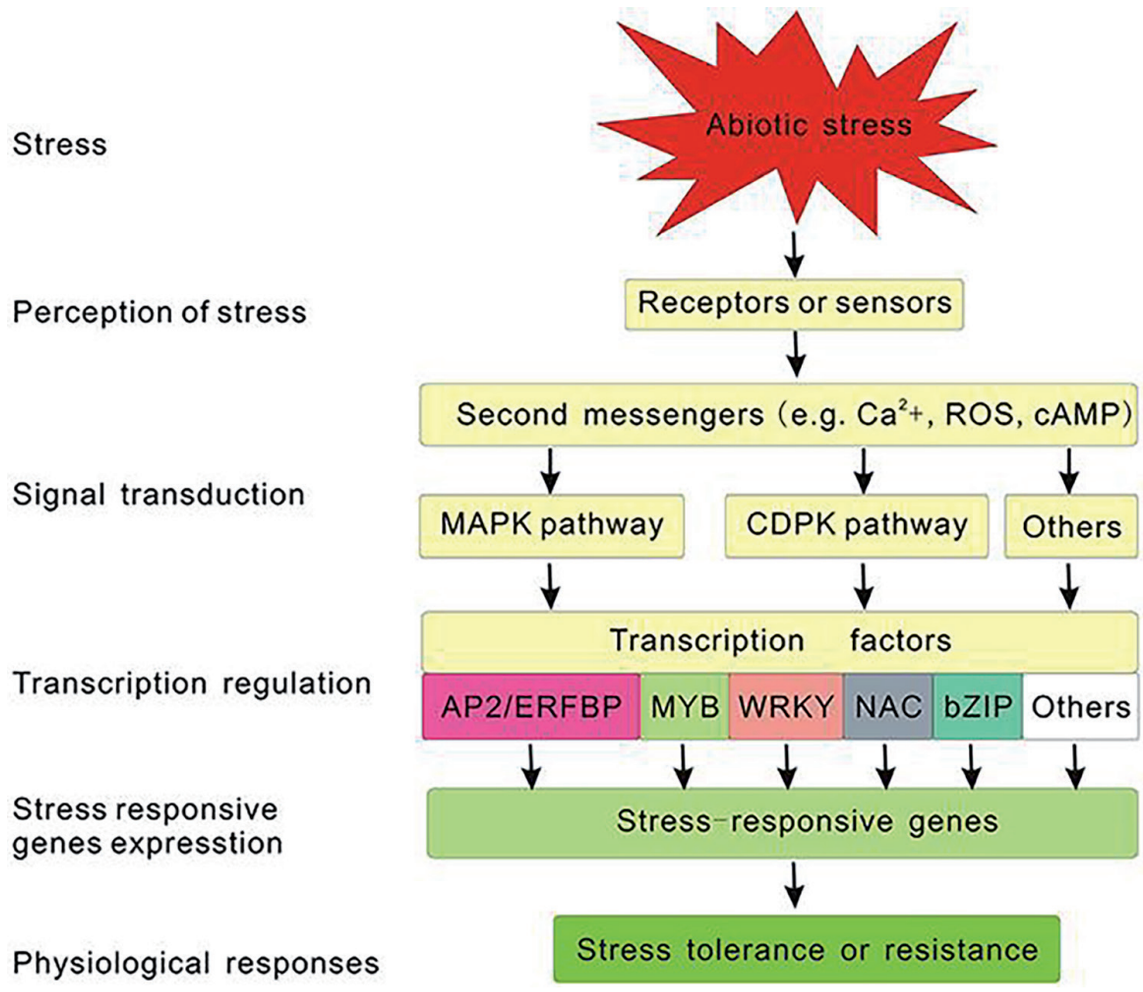

Figure 1.
Illustration of various pathways involved in abiotic stress mechanism in plants [9].

termination of matured grains was due to frost while extreme temperature caused a decrease in grains number during the filling grain period [18]. These noteworthy effects due to extreme climatic changes in crop production will result in food insecurity and crop production trends in the future [19].

1.2.2 Drought/water stress

To obtain maximum crops yield globally drought or water stress is a very important factor it affects plants in many ways; during the growth phase, water stress decreases leaf expansion development, photosynthetic process, the height of the plant, and the overall area of leaf. The early symptoms caused by drought stress are leaf rolling and dryness of leaf tip, cell elongation is seriously affected by drought stress water scarcity blocks stomata, and reduces transpiration [20]. It has a huge negative impact on plant growth and the potential quality of yield in the agricultural system. *Miscanthus* has very good potential for the production of biofuel it was observed after an experiment that drought the weight of the plant significantly by about 45% and cell wall composition and biomass were affected by drought stress during the plant growth phase [21]. The availability of water to plants is very necessary however waterlogging in the area surrounding the roots can be very damaging it can cause lack of oxygen and even death of the plant due to its lethality [22]. The transfer of free oxygen exchange between the soil and atmosphere is caused by water stress suffocation [23]. Waterlogging is often caused by floods, heavy rain, and snow in winter, such soils have limited or lack of oxygen due to less gas exchange [24].

1.2.3 Light stress

For plants, the energy production process through photosynthesis sunlight plays an important role. Plants adapt themselves to change in light which alter considerably at various times. That is why plants can develop certain mechanisms that help in maximum use of existing light during irradiance state while other mechanisms to escape the long-term sunlight exposure [25]. As a result of low light or reduction in solar energy significant decrease happens in metabolic rate which leads to a reduction in crop yields and lower growth rates. An increase in reactive oxygen species (ROS) and photo-damage is caused by prolonged exposure of plants to sunlight [26].

1.2.4 Salinity stress on plants

Soil salinity is considered as one of the major abiotic stress affecting the performance of crop plants adversely around the globe, it can create a cluster of diverse interactions that harms the nutrition uptake, metabolic process, and plant vulnerability to various biotic stresses as well [27]. Minerals and nutrients present in the soil have valuable importance but the unwanted existence of salts results in extreme ionic and osmotic stress in plants [22]. The cations present in inorganic soils or water includes potassium (K^+), magnesium (Mg^+), calcium (Ca^+), and sodium (Na^+) while the important anions are NO_3^-, HCO_3^-, SO_4^{2-}, Cl^-, and CO_2^{-3} other components include SiO_2, Al^{3+}, Sr^{2+}, B, Mo, and Ba^{2+} [28]. Enzymes inactivation, cell death, and subsequently whole plant can diminish due to high salinity [29]. Salinity stress in plants leads to a huge decrease in dry and fresh weight obtained from stem, roots, and leaves [30]. An excessive amount of salt increases osmotic pressure in plants which reduces the chances of minerals like (K^+ Ca^{2+}) and nutrient uptake for

survival, such primary effects leads to secondary effects as a non-proper expansion of cell, decrease in membrane function and a significant decrease in cytosol metabolic activity [5]. According to FAO world's 6% of the land is affected by salt. **Table 1** shows the distribution of salt-affected land around the world.

2. Heterologous expression of genes in plants for abiotic stresses tolerance

During the past two decades the use of recombinant DNA technologies, the methods of gene transfer, and tissue culture techniques have improved the transformation and transgenics in many varieties of crop production in agriculture. Transformation techniques provide larger accessibility to the pool of genes as compared to conventional methods because the genes are inserted from bacteria, animals, viruses, yeast, fungi, and even from various synthetic chemicals prepared in the laboratory (Chahal and Gosal 2002). Various methods are used for genetic transformation of crop plants, Biolistic bombardment, and *Agrobacterium*-mediated are the most common methods used for gene transfer in plants [31].

2.1 Genetic engineering through bacterial genes in plants against abiotic stresses

Cloned genes insertion has produced transgenics against abiotic stresses in plants [32]. Many bacterial genes have been expressed in plants to confer abiotic stresses like salinity, drought, temperature, cold, and light stress. Those bacterial genes include *mtID* which is expressed in several transgenic crops like tomato (*Lycopersicon esculentum* L. *var. Pusa Uphar*) was transformed against salinity, high and cold temperature and drought stress by the insertion of *mtID* gene [33] Wheat (*Triticum aestivum* L.) was transformed by the expression of this gene against salinity and waterlogging stress, as a result, the transgenic plants showed good resistance than WT non-transformed plants [34]. Tobacco plants were transformed by the expression of this gene against various abiotic stresses and the results were very improved in comparison to wild type plants [35]. Finger millet (*Eleusine coracana*) is a major food crop consumed and cultivated around the globe has been genetically transformed by the expression of this bacterial gene against drought and salinity [36]. Peanut (*Arachis hypogaea* L.) has been genetically transformed by the insertion of bacterial *mtID* gene against salinity and drought stress [37]. Moreover Indica rice

Regions	Total area (Mha)	Saline soil (Mha)	Percent %	Sodic soil (Mha)	Percent%
Africa	1899	39	2	34	1.3
Asia, the Pacific and Australia	3107	195	6.3	249	8
Europe	2011	7	0.3	73	3.6
Latin America	2039	61	3	51	2.5
Near East	1802	92	5.1	14	0.8
North America	1929	5	0.2	15	0.8
Total	**12,781**	**397**	**3.1**	**434**	**3.4**

Table 1.
Various parts of the world affected by salinity stress [5].

was transformed through the above-mentioned gene to improve its productivity in drought and saline environment [37]. Another Bacterial gene codA which has been isolated from (*Arthrobacter globiformis)* coding for choline oxidase and expressed Arabidopsis thaliana to improve its resistance to salinity, freezing, and high temperature [38]. The same bacterial *codA gene* was expressed in tomato (*Lycopersicon escuelentum*) to enhance its tolerance against high temperature, chilling, and drought stress [39]. Other Bacterial gene *IPT i*solated from *Agrobacterium tumefaciens* and expressed for the enhancement of various crop plants to improve their tolerance against various abiotic stresses like sugarcane (*Saccharum* spp.) cv. enhancement against cold stress [40] the same gene was inserted in rice for its enhancement against drought stress and increase in crop yield as well [41]. ADH *sysr1* gene of *cyanobacteria* was expressed in tobacco plants for salt tolerance improvement and that showed encouraging results in comparison to wild type plants [42]. *IPT* gene was also introduced in canola (*Brassica napus* L.) plants for delayed leaf senescence leading to crop improvement in drought stress [43]. The expression of Bacterial *ggpPS* gene isolated from (*Azotobacter vinelandii*) for glucosyl glycerol biosynthesis confers salt and drought stress tolerance in *Arabidopsis thaliana* [44]. Gene encoding for bacterial chaperons have been inserted in various transgenic plants for the improvement of tolerance to abiotic stresses successfully [45].

Abiotic stress tolerance related genes from micro-organisms are considered to be very valuable for the production of transgenic plants. A cyanobacterium (*Nostoc flagelliforme*) that can tolerate water deficit conditions is proved to be very useful prokaryotic organism for gene isolation. Salt tolerant gene *DRNF1* having P-loop NTPase (nucleoside-triphosphatase) domain, has been expressed in *Arabidopsis thaliana* the results indicated improvement in the growth of shoots and seed germination in saline conditions [46].

2.1.1 The expression of mtID bacterial gene for the improvement of various abiotic stresses in plants

To improve abiotic stress tolerance in transformed tomato plants a bacterial mannitol-1-phosphate dehydrogenase (*mtID*) gene was inserted through Agrobacterium-mediated method supported by CaMV35S promoter, Rt PCR, and southern blotting analysis was used for the confirmation of transient integration, reverse transcription (RT)-PCR and direct activity of mtID gene was analyzed for the confirmation of transgene expression [33]. Transgenic tomato plants upon exposure to low temperature round about 4°C in a cold chamber survived for almost 2 days as compared to untransformed plants that were not able to survive and their death accrued slowly. During the exposure of transgenic tomato plants to chilling effect showed a significant decrease in electrolyte leakage in the plant membrane, when they are exposed to stress the leakage starts to the surrounding environment, and the damage caused to the cell with hardiness can be identified through the leaked contents conductivity in water by the comparison of injured and non-injured plants [47] while an increase to lipid peroxidation [48], antioxidant enzymes [49] and relative water content [50] as compared to the non-transformed plants. Drought stress was tested through polyethylene glycol in the medium, and salinity by the content of sodium chloride (NaCl) showed a greater response to these stress than non-transformed plants. By the indication of all these observations, it was clear that the introduction of bacterial (mtID) gene in tomato showed a good response to abiotic stress than transformed plants. As **Figure 2** shows the various transformation phases of tomato [33].

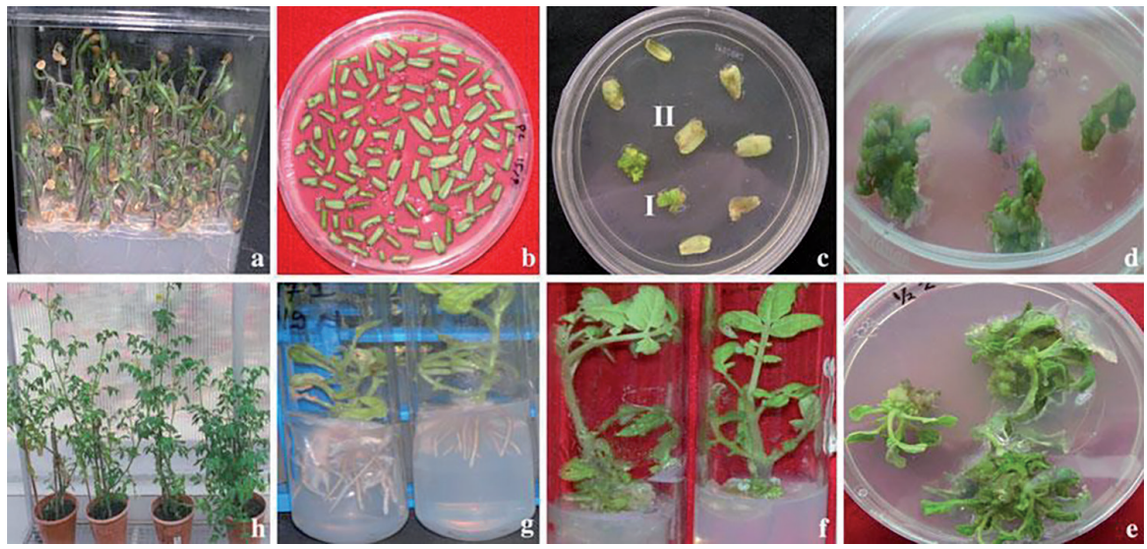

Figure 2.
*Different tomato (*Lycopersicon esculentum L. *var.* Pusa Uphar*) transformation phases [33].*

The above work has shown that the accumulation of mannitol in several transgenic plants can improve plant tolerance against abiotic stresses. At the cellular level due to *mtID* gene insertion from *E. coli* [51] has been reported for the protection of Wheat (*Triticum aestivum* L.) against the adverse impact of waterlogging and soil salinity. Through the exposure of calli to polyethylene glycol ranging from 8000 mm of NaCl. The stress in the T2 plant was caused by the addition of a large amount of water and 150 mm of NaCl to the nutrition medium, the fresh weight of *mtID* calli was decreased by about 40% in the existence of PEG and 37% during salt stress, no effect was observed in the growth of *+mtID* callus, while in the plants of *–mtID* the content of fresh and dry weight, height of the plant, and leaf areas was decreased by about 70%, 56%, 40%, and 45% in a comparison with 40%, 8%, 18% and 29% comprehensively with that of *+mtID* plants. Salinity stress decreased shoots of fresh and dry weight, changes in the height of plants were observed, and the leaf area was reduced by 77%, 73%, 25%, and 36% in *-mtID* plants as compared to 50%, 30%, 12%, and 20% in plants having *+mtID*. As a result, no effect was seen on the growth of *mtID* callus and transgenic wheat plants showed significant tolerance against salinity and waterlogging stress due to the insertion of this bacterial gene [34].

For expression in higher plants against abiotic stress a bacterial gene that codes for mannitol-l phosphate dehydrogenase, *mtID* was inserted stably in tobacco plants which translated in tobacco through a functional enzyme, led to the accumulation of mannitol, which was identified and detected through NMR and mass spectrometry, the concentration of mannitol increased by d 6 jumol/g (fresh weight) in roots and leaves of the transgenic tobacco while these sugar were not detected in wild or untransformed tobacco plants that were passed through the same treatment. This study could help us in understanding sugar role of alcohol in the enhancement of plant tolerance against abiotic stresses in higher plants comprehensively [35].

In Asia, Nepal, India, and almost 25 countries of Africa, finger millet (*Eleusine coracana*) is cultivated and consumed as a major crop for food, it covers more than 12% of the world's millet cultivating area [52, 53]. It also has better nutritional properties and ingredients than wheat and other major crops [54, 55]. It is very vulnerable to various abiotic stresses like drought and salinity in fields during

the early stages of seed germination and the development of seedlings, therefore it is very important to make finger millet plants resistant to drought, salinity, and oxidative stress. Proper radical scavenging capability and cell protection by osmotic modification during several abiotic stresses are major mechanisms in plants, mannitol is an osmolyte [56] that helps in the neutralization of various free hydroxyl radicals produced due to abiotic stresses and decreases stress disruption in various plant species. Through *Agrobacterium*-mediated transformation, the biosynthetic pathway gene from bacteria mannitol-1-phosphate dehydrogenase (*mtID*) was expressed in finger millet plants to understand the performance of transgenic plant upon their exposure to drought and salinity stress simultaneously. The results obtained through these experiments showed that transgenic finger millet had better performance in saline and drought stresses as compared to wild type plants [36].

Peanut (*Arachis hypogaea L.*) is an important crop grain cultivated in tropical and sub-tropical zones in about 21–24 M ha production areas, it is generally grown in rain-fed areas where drought is a major crop decreasing factor occurring in semiarid lands that cover 70% of the peanut cultivation areas [57] one of the many strategies is to genetically transform peanut plants that can resist in drought conditions [34]. Plant breeding through conventional methods is time-consuming with lesser success and far more laborious, while genetic engineering techniques show great potential to develop peanuts plants that can tolerate drought conditions. Plants have developed several mechanisms against drought stress for their survival [58]. To overcome drought stress in peanuts bacterial *mtID* genes were expressed through CaMV35S promotor using *Agrobacterium tumefaciens*-mediated transformation. The transformed plants showed significant resistance to water deficit conditions as a result of mannitol accumulation during these experiments, **Figure 3** illustrates the whole process [37].

The accumulation of mannitol an osmolyte plays an important role in abiotic stress. So, through the insertion of *mtID* gene from *E. coli* for the improvement of *Indica* Basmati rice against salinity and drought stress, by agrobacterium-mediated transformation, many putative transformed plants were generated. Transgene existence was confirmed in early transformed plants by PCR through *mtID* and hygromycin phosphotransferase, the transgenic lines showed better performance against salinity and drought stress as compared to wild type plants [59].

2.1.2 RNA chaperones genes of bacteria confer abiotic stresses in transgenic plants

With a consistent increase in the world's population, constant supply of food demands, and a decrease in water shortage alongside cultivating land, it is necessary

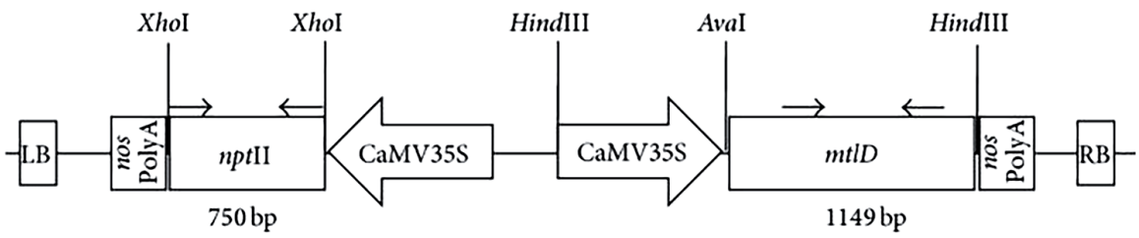

Figure 3.
Schematic illustration of the T-DNA section of pCAMBIA 1380 binary plasmid used for transformation of deembryonated cotyledons with Agrobacterium tumefaciens strain LBA 4404. The location of the primers used in PCR assays is shown by arrows on the top of the mtID gene. LB, left T-DNA border sequence; RB, right border sequence; 35S, CaMV35S promoter; and mtID, mannitol-1-phosphate dehydrogenase [37].

to transform crops like rice that can grow in salt-affected areas [60]. High saline condition seriously affects the growth of rice like leaf expansion ability, root, and shoot formation [61]. The decrease in leaf expansion occurs in rice due to the low rate of osmotic turgor pressure under saline and cold conditions [62]. Results obtained from various research studies have shown that chloroplast and mitochondria in rice plants are seriously affected by salt and chilling stress [63, 64]. During abiotic stress conditions, bacterial RNA chaperones play a major role in stable messenger RNA expression, in salinity stress these bacterial genes develop transgenic rice plants that can tolerate even cold stress apart from salinity [60].

Drought is the major factor that causes crop yield reduction globally leading to socioeconomic complications. During an estimation, it was observed that a 40% loss in Maize crop is caused by drought stress alone in North America annually [65]. Maize crops are vulnerable to drought stress through-out their growing stages, effects of stresses that initiate during the flower development phases either before the start of floral events or post pollination results in a significant reduction of crop yields at the end of the season [66, 67]. In 2013 the first drought-resistant maize crop was genetically transformed by the expression of bacterial genes that codes for chaperonin showed significant improvement in resistance to water deficit stress [45].

The expression of bacterial CSPs (cold shock proteins) exhibited improvement against cold stress in transgenic *Arabidopsis thaliana* seedlings cultivated at very low temperatures on standard agar media in Petri dishes as illustrated in **Figure 4**. The tests were conducted of the transformed Arabidopsis seedlings having *CspA* and *CspB* to check the improvement for cold stress using non-transformed seedling as controls. The seedlings were exposed to low temperatures for 6 weeks and the results suggested improve tolerance against cold stress in comparison to non-transformed wild type plants [45].

2.1.3 The expression of ADH (alcohol dehydrogenase gene) *isolated from* cyanobacteria Synechocystis *sp. improves salt tolerance in tobacco plants*

A gene PCC 6906 (*sysr1*) from *synechocystis* that shows a good response to salt was engineered and stably inserted in higher developed tobacco plants. The tolerance response of the gene *sysr1* (An ADH superfamily member) was investigated through quantitative real-time PCR, gas chromatography-mass spectrometry, and bioassays. The tobacco plants having *ADH* showed considerably improved tolerance to salt stress, besides that the activity of many

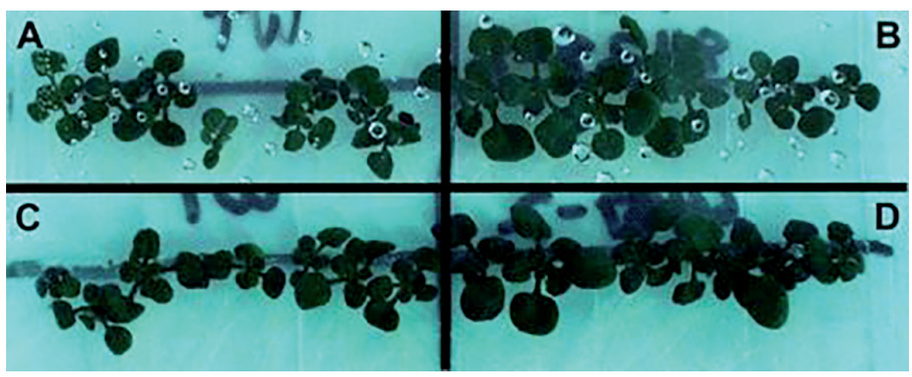

Figure 4.
Demonstration of Arabidopsis thaliana seedlings exposed to cold temperatures for 6 weeks [45].

stress-responsive genes was up-regulated and enhanced due to the expression of (*sysr1*). The results suggested that the expression of *ADH* genes could significantly improve transgenic tobacco plants against salt stress through genetic engineering techniques in the future. **Figure 5** shows the identification of *sysr1* gene plants in three transgenic lines (1, 4 and 7) [42].

2.1.4 Bacterial codA *gene enhances tolerance against various abiotic stresses in plants*

Arabidopsis thaliana was genetically transformed by a gene isolated from *Arthrobacter globiformis* that codes for choline oxidase, an enzyme used for the synthesis of glycine betaine from choline, which remarkably improved cold or freezing stress in plants. Moreover, the photosynthesis machinery was more resistant to freezing/cold stress than non-transformed plants. These results also indicated the accumulation of glycine betaine in transformed plants, enhanced their ability to extremely cold temperatures [68].

In the control of RNA CaMV35S promotor, *A. thaliana* was genetically transformed by *codA* gene of bacterial *Arthrobacter globiform* coding for choline oxidase. Subsequently, the accumulation of increased glycinebetaine occurred in the seeds of transformed plants. The transformation of *codA* gene significantly boosted

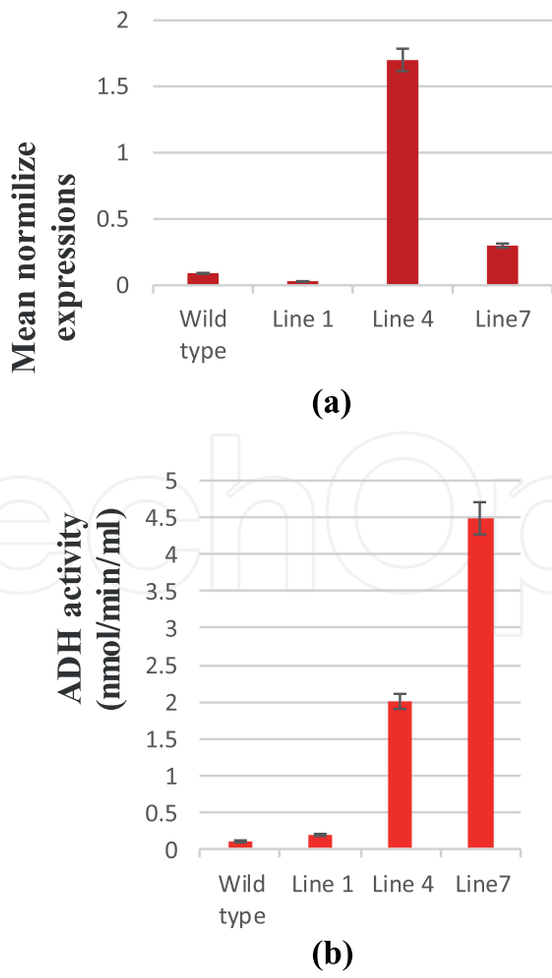

Figure 5.
Identification of transgenic plants with sysr1 gene. (a) Results obtained from the analysis of quantitative real-time PCR in three transformed lines (lines 1, 4 and 7) used for the assays of salt tolerance. (b) the ADH activity of leaf of transgenic tobacco plants with the control [42].

the ability of plants to high-temperature stress in the period of seed germination and the growth phase of young seedlings. The level of improvement of the resistance to high temperature was evaluated with the extent of the expression of choline oxidase and the accumulation of glycinebetaine in transgenic plants [69].

Tomato (*Lycopersicon escuelentum*) was genetically transformed by the introduction of *codA* gene from *Arthrobacter globiform* bacterium for choline oxidase that had been allowed to target both mitochondria and cytosol. The accumulation of glycinbetaine was detected in the seeds of transformed plants by about 1 μmol g^{-1} dry weight while no accumulation of glycinbetaine was seen in wild type/non-transformed plants. The transformed codA seeds germinated at fast speed during high temperatures. After heat stress, the content of small mitochondrial heat shock proteins, 70 heat shock proteins, and cognate 70 were much increased in transformed seeds during the heat stress phase than non-transformed seeds. Cognat 70 (HSP70) accumulation was more obvious in codA transgenic seeds than non-transgenic seeds. The results suggested that the transformation of tomato seeds with codA gene showed improved tolerance to a higher temperature in tomato plants [70].

Genetically transformed tomato (*Lycopersicon escuelentum*) plants which can synthesize glycinbetain was produced by the introduction of the bacterial *codA* gene. The expression of the gene was examined through RT-PCR analysis and in combination with RNA blotting hybridization. During the seed germination phase, the transformed plants exhibited greater tolerance to salt stress following the growth of young seedlings as well. The insertion of *codA* gene resulted in high-stress resistance ability in leaves and overall plants. Results from the experiments revealed that the developed leaves of *codA gene* transformed plants showed more water content, chlorophyll content, and enhanced proline levels in comparison with non-transformed plants during salinity and water stress [39].

They are vulnerable to chilling stress because of lower glycinbetaine synthesis ability. The cold temperature lower than 10°C causes severe injuries to tomato plants leading to lesser yield production. Bacterial *codA* gene has been introduced into the genome of the tomato by targeting chloroplast. The transformed plants expressed this gene and synthesize choline, by the accumulation of glycinbetain in leaves and the formation of shoots up to 0.3 and 0.2 μm/g fresh weight. The chloroplast of transgenic plants contained 86% of glycinbatain, in different developmental stages during the seed sprouting and fruit production process, the glycinbetain containing plants were more resistant to chilling stress than their wild types, 10–30% increase was seen in fruit production on average during abiotic stress, thus the introduction of GB biosynthesis pathways is an important strategy against chilling stress in tomato plants [71].

A. thaliana was genetically transformed through the bacterial *codA* gene which encodes for choline oxidase. The photosynthetic activity examined for chlorophyll fluorescence of transgenic plants were more resistant to light stress than non-transformed wild type plants. This improvement in resistance to light stress was eventually because of the high speed of the recovery process of the photosystem II complex from the photo-inactive stage. It showed that in vivo production of glycinbetain and no changes in the lipids membrane or H_2O_2 level, it ensured the protection of photosystem II complex in transgenic plants from the possible damage due to light stress [38].

2.1.5 The expression of IPT *gene against various abiotic stresses in plants*

To increase the cold stress tolerance, *IPT* gene was introduced in sugarcane (*Saccharum* spp.) cv. RB855536, in the control of a promoter (AtCOR15a), through

biolistic, non-biological transformation method. The leaves extracted from genetically transformed plants showed good resistance and decrease leaf senescence upon their exposure to low temperature as compared to wild-type control plants. Improved enhancement against cold stress was seen due to the expression of this gene in non-acclimatized plants when the transgenic plants were exposed to extremely cold temperatures. The chlorophyll content of leaf was 31% more than non-transformed plants. A decrease in malondialdehyde level and the leakage of electrolyte showed lesser damage caused by chilling stress in transgenic plants. So, stress-inducible promoter *COR15a* used in the insertion of the *IPT* gene in transgenic plants shown no adverse effect while improving them against cold stress [40].

To delay the process of leaf senescence would allow capturing sunlight for longer periods, which leads to photosynthetic improvement and its contribution to plant growth and enhanced seed yield. Moreover, delayed senescence would allow the slow degeneration of source tissues so that the metabolites, proteins, nutrients could be slowly and gradually released to the sink tissues. Increase in plant potential biomass, maintenance of photosynthetic process, the higher influx of nitrate, increase in the life of flowers after harvesting, improved drought resistance, and greater seeds yield are the benefits of delayed leaf senescence [72, 73]. Cytokinin; a plant hormone that plays an important role in the process of cell division, cell growth, and differentiation, and it influences various developmental and phycological characteristics in plants ranging from seed germination, the flowering period of the plant, apical dominance, developmental process of flowering, fruits and leaf senescence [74, 75]. In various plants the role of a plant hormone cytokinin in delaying leaf senescence has been reported by [73, 76–78]. A gene *IPT* isolated from *Agrobacterium tumefaciens* has been inserted in several plants to enhance cytokinin level as this gene synthesize the rate-limiting step in cytokinin. Canola plants were transformed by *IPT* gene in combination with *AtMYB32* promoter, the insertion of *IPT* gene in transformants caused delayed leaf senescence cultivated under control condition and various field experiments at two separate geographical areas for one season. As a result, the transformed Canola (*Brassica napus* L.) plants maintained high chlorophyll content for a longer period and an increase in seed yield under drought and irrigated conditions was observed as compared to wild type non-transformed plants. In comparison to control plants, all of the seed quality parameters and oleic acid content in transformed plants were exactly similar, as a result of the experiments, it was concluded that the introduction of bacterial *IPT* gene can significantly improve crop yield and seed quality under irrigated and drought stress conditions in various plants [43].

In rain-fed areas drought is a major hindrance to rice crop productivity [79, 80]. To fulfill the constant demand of rice by 2030 a remarkable increase by almost 35% in yield is necessary [81] that is why the development of transgenic rice to drought stress and improved productivity is an important challenge, various studies have indicated that the expression of bacterial *IPT* gene using different promotors could help in delaying leaf senescence to improve crop productivity under drought stress conditions [82] In tobacco plants *IPT* gene was introduced under the control of senescence-related receptor [83] and a promotor to induce stress exhibited enhancement in photosynthetic capacity leading to improvement in drought tolerance in tobacco plants [73]. Moreover, transgenic rice plants resistant to drought stress were produced by the insertion of *IPT* gene under the control of P_{SARK} a stress-inducible promoter. The plants were tested against drought stress tolerance at two yield sensitive developmental phases; pre and post-anthesis. During both treatments, the transformed rice

plants showed remarkable resistance to drought stress and an increase in yield grain as compared to non-transformed wild type control plants [41].

2.1.6 The expression of bacterial ggpPS *gene isolated from* Azotobacter vinelandii *for glucosyl glycerol biosynthesis confers salt and drought stress tolerance in transgenic plant*

Various organisms generally accumulate compatible solutes to show response against salt and drought stress, which includes heterotrophic and cyanobacteria which shows resistance to salty environment and produces glucosyl glycerol as their major compound for protection. To know the potential of glucosyl glycerol to enhance salt resistance in higher plants, a gene *ggpPS* that codes combinedly for GG-phosphate synthase/phosphatase was isolated through PCR from the chromosomal DNA of the cells treated with lysozyme from a heterotrophic (*proteobacterium A. vinelandii*) and introduced into model plant *A. thaliana*. The high accumulation of glucosyl glycerol was observed due to the expression of this gene. In various growth experiments, three separate Arabidopsis lines were tested that showed varied glucosyl glycerol levels. Plants having a low level of glucosyl glycerol within leaves showed no changes in growth development in the control condition, rather an improvement to salt tolerance. While plants having a very low or higher glucosyl glycerol content exhibited growth delay and no enhancement of salt resistance was observed, the results suggested that the suitable solute synthesis has a positive impact on the stress tolerance of plants as long as the accumulation extent does not interfere adversely with the metabolic process of plants [44].

2.1.7 The expression of bacterial betA *gene confers abiotic stress tolerance in transgenic plants*

Drought stress exists in most of the areas where sugarcane is grown and cultivated, which has no support of irrigation system and has lower rainfall. To know psychological and biochemical mechanisms better, underlying plants response to water deficit stress, have been overcome by the development of drought-resistant plants through biotechnological techniques. To tackle water stress plants use various strategies like variations in gene expression and the accumulation of compatible solutes for survival and growth. A bacterial gene *betA* that codes for *CDH* choline dehydrogenase has been effectively expressed in sugarcane to produce drought resistant plants. The function of *CDH* is the conversion of choline in betaine aldehyde that is then transformed into glycinebetaine GB, the expression of *betA* gene improves the level of glycinbetaine that act as an osmoprotectant and help in the acclimatization of sugarcane in water deficit stress, the drought-resistant sugarcane was first developed by Ajinomoto Company in Tokyo [84].

Transgenic cotton (*Gossypium hirsutum* L.) was genetically transformed by the expression of a bacterial *betA* gene from *E. coli* for the enhancement of glycinbetaine, its accumulation was identified at three stages. Five lines expressing this gene showed significant improvement to drought stress than wild type non-transformed plants from seedlings to flowering plants. The five transgenic lines showed better relative water content, a decrease in the leakage of ions, and less malondialdehyde content in comparison to wild-type plants. The glyycinbetaine content was positively related with water deficit tolerance in water stress, the results indicated that the expression of the *betA* gene not only provide protection to cell membrane against drought stress but also act in the osmotic adjustment in transgenic cotton plants, more importantly,

line 4 among five lines showed a significant increase in cotton seed yield after exposure to drought stress which will help a great deal in cotton production in future [85].

The similar *betA* gene of *E. coli* was expressed through *Agrobacterium*-mediated transformation in maize to improve its tolerance against cold or chilling stress, five transgenic lines were tested in which four lines exhibited a higher level of glycinebetaine than WT plants. At lower temperatures 10 and 15°C three transformed lines showed an increase in germination stages, as identified through the progress of germination and presented lower inhibition in the speed of shoot growth in seedlings than non-transformed lines. Upon exposure to chilling stress the tolerance of transgenic plants was significantly improved in cell membrane injury, the level of damage caused by cold stress, survival rate, and photosynthetic capacity in transgenic lines than WT plants [86].

Tobacco plants were also genetically transformed by the expression of this gene from *E. coli* and improvement in glycinbetaine was observed leading to improvement in the resistance of transgenic plants to chilling and salinity stress than wild-type plants [87, 88].

3. Gene expression from yeast (*Saccharomyces cerevisiae*) in plants against abiotic stresses tolerance

Just like the above bacterial genes expression in plants to improve their tolerance against abiotic stresses, yeast genes have also been introduced in transgenic plants to enhance their tolerance, *TPSI* gene of *Saccharomyces cerevisiae* has been expressed in transgenic tobacco against salt and drought stress and the results were very much better than WT plants [89] *HAL1* gene was expressed in tomato against salt tolerance and the results showed better improvement in comparison to wild type plants [90] similarly *HAL1* and *HAL3* genes were introduced in *A. thaliana* for its enhancement against saline stress and the transgenic plants exhibited much better tolerance than wild type non transformed lines [90], the procedures of the expression of these genes have been discussed below.

3.1 Insertion of a yeast gene *TPSI* in transgenic tobacco plants against drought and salt stress

A gene trehalose-6-phosphate synthase from yeast was introduced in tobacco plants by the control of Cauliflower mosaic virus (CaMV35S) regulation sequence. *Agrobacterium*-mediated transformation method was used for the introduction of a gene into the genomic DNA of tobacco (*Nicotiana tabacum* L) plants. The accumulation of trehalose was found in transgenic plants through ion-exchange chromatography in combination with ampometry detection procedure. The disaccharide that was non-reducing accumulated almost 0.17 per gram of fresh weight in leaf extracts of the transformants. The plants with trehalose accumulation had various changes in phenotypes like dwarfness, pointed or lancet leaves pattern, and decrease in sucrose level. Moreover, the expression of *TPS1* gene in tobacco plants showed significant tolerance to drought and salt stress as illustrated in **Figure 6** [91].

3.2 The role of yeast *HAL1*, and *HAL3* genes against salt tolerance in plants

To overcome salinity stress in *Arabidopsis thaliana*, the yeast genes *HAL1* and *HAL3* were introduced under the control of 35S promoter via the

Figure 6.
Drought tolerance in transgenic tobacco plants by the overexpression of the TPSI gene from yeast. The left 2 rows consist of non-transformed control plants while the right two rows contain the transgenic homozygous plants. No water has been given to all plants for almost 15 days. The results obtained are similar by exposure to drought stress with 400 mM NaCl. The visible better changes can be seen in transgenic plants with TPSI gene [91].

Agrobacterium-mediated method. Almost 33 plants showing resistance to kanamycin were obtained from 70,000 plus seeds. Southern blotting analysis showed that *HAL1* and *HAL3* genes were introduced into all the genomes of the transgenic plants. The copy number of the yeast gene in all plants was in the range of 1–3 by the confirmation of southern blotting analysis, there was no difference in the phenotype of the transgenic plants compared to wild ones. Most of the transformants were self-pollinated, the progenies of transformants and non-transform *A. thaliana* plants were observed through different experiments for gene expression to know the salt resistance. The measurement of (K^+) and (Na^+) showed that the transgenic plants accumulated fewer (Na^+) as compared to the control lines. In light of several tests, it was observed that the introduction of yeast *HAL1* gene exhibited more resistance to saline soil in comparison to non-transform plants [92].

In past, remarkable advancements have been made in the identification and isolation of various genes which could be used in the process of abiotic stress protection in plants. It is hard to believe that a single gene insertion would make a dramatic improvement to salt stress directly producing a fresh salt-resistant transgenic plant that could be enough for breeding purpose point of view. Yeast *HAL1* gene was introduced in tomato (*Lycopersicon esculentum*) through a well-modified plasmid containing the elements of enhancer and salt resistance was evaluated in transgenic plants from progenies. The result showed that transgenic lines having one copy of the *HAL1* gene had higher salt tolerance than non-transformed plants [90].

For the production of transformed watermelon plants, and adjusted *agrobacterium* mediated protocols were maintained. The efficient transformation rate was 2.8–5.3% in the cultivars. Yeast *HAL1* gene under the control of 35S cauliflower mosaic virus having a double sequenced enhancer was cloned in pBiN19 plasmid. RNa4 from Alfalfa mosaic virus was used alongside 35S. The vector was introduced in the LBA4404 strain of *agrobacterium tumefaciens* for the inoculation of watermelon cotyledon explants. PCR and Southern hybridization analysis were used for the assessment of the *HAL1* gene in new transformants. Improved elongation of leaves and new roots emergence was seen in plantlets in culture media having NaCl. It was

observed that the *HAL1* gene as a molecular tool for genetic engineering could be very useful to protect crop plants in the future [93].

3.3 *HAL1* gene mode of action in (*Saccharomyces cerevisiae*)

Yeast (*S. cerevisiae*) *HAL1* gene was initially found in the screening process for various genes that could be expressed in various plasmid copies that improve saline resistance in yeast (*S. cerevisiae*). It codes for a soluble protein in the cytoplasm, even though there is no significant information available about this gene, still it is a major affective ions regulator during the homeostatic process, slight expression of its promotor generally have an impact on the potassium levels inside the cells [94]. However, a significant expression by strong promotor had an impact on (K^+) and (Na^+) homeostasis [95]. The expression of the *HAL1* gene decreases the loss of (K^+) from cells affected by salt stress a phenomenon initiated through an unknown K^+ efflux system. The cells with *HAL1* contain a high level of potassium in cells, and a low level of sodium within the cells, and an increased K^+/Na^+ ratio as compared to control cells the last one indicating the enhancement in salt tolerance [96]. Currently, it is not known how a protein product from the cytoplasm of the *HAL1* gene can control the transportation of sodium and potassium efflux. Besides the lack of information available about this process *HAL1* gene possess a high capability to improve salt tolerance of various plants, and it was selected in the first trials for expression of genes in transgenic plants [89].

4. Anti-freeze proteins

During the study on fishes in the waters of temperate oceans proteins that act as antifreeze elements were found, in winter the temperature of these waters reaches (−1.9°C) but fishes under these waters still survive. NaCl is the most common electrolyte in blood serum of most species, but to inhibit freezing environment it only helps in 40–50% of the examined freezing point depression [97] the other substances due to which freezing point depression occurred were marked as proteins and glycoproteins [98–100] the molecular masses of antifreeze-glycoproteins ranges from 2.6 to 34 kD. They consist of tripeptide repeats (A l a-A l a-T h r) along with the moiety of disaccharide (-Naga-Gal) having the residue of threonyl [101].

4.1 AFP gene mechanism of action

Many researchers have studied the ant-freeze protein from winter flounder because of their small size and are very effective for structural mechanism requirements, there are some changes in the size and AFP amino acid composition which depends on the isolation technique from the serum of the fish [102]. Through southern blot and restriction maps of genomic clones analysis, the pattern of antifreeze protein multigene family was observed in winter flounder [103]. Most of them are equal in number to 40 AFP genes in this fish are present in 7–8 kbp DNA elements which act like tandem repeats, in every repeat, there is 1 kbp long AFP gene having same transcription shape and orientation, they also have some restriction site polymorphism ability even though the repeats are homologous. When winter flounder genomic DNA goes through the digestion phase mainly by Restriction endonuclease which normally does not cut inside the repeats, many of the AFP genes goes to 40 kbp

long fragments that represent five or more repeats in tandem as clusters. After the digestion of genomic DNA, these genes reside in the fragments of extremely high mol. Weight indicating the groups of clusters in the genome [104]. By the combination of protein and DNA sequencing methods, the precursor of amino acid in the second AFP protein B gene has been observed in winter flounder. The precursor containing 82 amino acid residues is only different in three main sites to AFP, A gene that acts in the process of substitution, various other changes, all are grouped inside the DNA that codes for the mature portion of protein. In the process of post-transcriptional modification, the c-terminal glycine residue removal takes place [105].

4.2 The introduction of fish antifreeze AFP gene in transgenic plants

The quality of fruits and vegetables can be compromised by adverse effects due to the formation of ice crystals inside the frozen tissues. At lower concentrations, some proteins from the blood of fishes have shown the ability to help in the inhibition of ice crystals formation. To know whether the expression of certain genes improves freezing properties of the plant tissues, the transgenic tomato and tobacco have been produced by the expression of anti-freeze gene *AFA3* were introduced at higher steady mRNA levels in the leaves of transgenic plants but no crystals inhibition was observed in tissues extracts. As a result of these experiments, ice crystal inhibition was seen in transformed tomato and tobacco plant tissues [106]. The freezing rate and temperature of the storage site are the two factors that influence ice formation in frozen fruits and vegetables, when plants tissues gradually freeze the large and randomly distributed extracellular gaps are filled by ice crystals in comparison to small intracellular and extracellular gaps which freezes rapidly during storage temperature changes lead to large shaped ice crystals and reorganization of ice in food [107].

AFP genes isolated from fish and insects are more useful in the inhibition of frost or crystal formation in several crop plants. AFPs isolated from insects and then their expression in plants against freezing stress are much better than those of fish because of their survival ability in freezing temperatures. AFPs can decrease water freezing level (thermal hysteresis) has generated the phenomenon that the damage could be avoided by those plants which are much more sensitive to frost at the end of autumn and the start of spring due to the expression of higher activity genes coding antifreeze proteins allowing them to be unfrozen in extremely cold and freezing temperatures. During the last two decades, the effectiveness of this idea has been conducted in several different research studies that produce transgenic plants by the expression of various AFPs. Earlier the anti-freezing proteins isolated from fish were used in these studies but later on, as AFPs of insects with high levels of anti-freezing activity were discovered and now being used for plant transformation studies as a choice. A chemically synthesized antifreeze gene from winter flounder fish was introduced through the *Agrobacterium*-mediated transformation method in potato *solanum Tuberosum L. cv.* which decreased the electrolyte leakage from the leaves at freezing temperature [108].

Spring wheat which is vulnerable to the damage caused by frost can also be transformed to show tolerance to frost by the expression of winter flounder gene AFPs in the cytoplasm and apoplast of the plant where ice formation leads to damage at the cellular level. The transformed wheat lines which were targeted by apoplast antifreeze proteins showed the highest anti-freezing activity and exhibited remarkable protection against frost at very lower temperatures [109].

Various marine species survive in extremely cold seawater below the freezing point temperature of their non-protected blood serum by producing anti-freezing proteins and glycoproteins [110, 111]. These proteins and glycoproteins have subsequently been considered for the neutralization of ice nucleator agents [112] to protect the cell from ice crystallization potential damage by hypothermic temperatures [113]. The introduction of these proteins in transgenic plants has been a very important tool for increasing their cold stress tolerance against freezing temperatures. In early work, an AFP gene that codes for alanine-rich, α-helical Type I AFP from winter flounder fish was introduced into tobacco plants through the *Agrobacterium*-mediated transformation method. The transformed plants produced antifreeze proteins mRNA and upon exposure to cold showed the accumulation of AFP to a detectable extent. The observation from the results was that fish antifreeze gene could be very useful in protecting plants from cold and freezing stress [114].

An anti-freezing gene (IIA7 cDNA) was isolated from a fish winter flounder *Pseudopleuronectes americanus* which can survive below the freezing temperature point under cold sea waters, which encodes for 91 amino acid and then proceeded to a mature protein of 53 amino acids. Only mature antifreeze proteins are encoded by this gene, a start methionine was also cloned alongside a plasmid that allowed improved expression from a double cauliflower mosaic virus CaMV 35S promotor. A binary vector pMON200 and intermediate vector pBI121 was used for the subcloning of anti-freezing protein. Various Kanamycin resistant seedlings were tested against the frost tolerance more than 30% of plants survived as compared to the control wild type plants these results confirmed that these genes can help in the resistance to frost in tobacco plants [115].

4.3 Transformation of plants with insects AFPs

The first transgenic plants were produced by the expression of insect AFPs [116], a chemically synthesized gene based on the anti-freezing proteins from an insect *Choristoneura fumiferana*, was introduced into (*Nicotiana Tubaccum*) tobacco plants through Cauliflower mosaic virus 35S promoter. The transformation success was determined by the levels of properly shaped transcripts through real-time PCR, in crude leaf homogenates the recrystallization inhibition activity and the apoplast plant extracts, and the most importantly the degree of water freezing point 0.37% in the apoplast liquid [117].

Transgenic *A. thaliana* was produced by the gene isolated from an insect (*Dendroides canadensis*), AFPs were introduced by *agrobacterium* mediated transformation. The AFP genes simultaneously with and without peptide signals sequence were expressed in transgenic plants. The thermal hysteresis activity showed the existence of active AFPs in proteins isolated from plants that expressed both proteins and were found in fluids of leaf apoplast of plants expressing AFPs alongside signal peptide. The transformed lines did not show any enhancement to survive in freezing temperatures in comparison to wild type plants, however, when cooled under four different stages the transformed lines containing active AFPs apoplast fluid froze at significantly low temperatures in comparison to wild type, especially when there was no intrinsic nucleation [118].

To illustrate the activity of AFPs from beetle (*Microdera punctipennis*) from the deserts of Xinjiang *China,* for freezing stress resistance in plants the *MpAFP149* gene, alongside the signal peptide sequence used for the secretion of *MpAFP149* into the

apoplast gaps in the control of cauliflower virus 35S was expressed in tobacco plants through *Agrobacterium tumefaciens* transformation method. The transformants were determined by reverse transcription-polymerase chain reaction analysis of leaf fragments, and those plants having higher transcripts contents were identified for further experiments and analysis. The introduced AFPs were restricted to cell walls of transformants by the use of immune-gold label procedure, and the existence of AFPs in apoplast liquid was indicated by western blot. The inhibition of crystal formation and thermal hysteresis tests to observe the expressed AFPs active state were not done. However, it could be expected that a slight extent of activity existed, the resistance to freez stress of transformed plants near to non-transformed plants was identified through ion-exchange chromatography technique, ion leakage, and malondialdehyde (membrane lipid peroxidase product) measurement release leading to the plant exposure to −1°C for varied periods for 72 h. Upon exposure to −1°C for 2 and 3 days, non-transformed plants were observed to be more adversely affected than the transformed, as by the assessment of more wilted leaves in them. The transgenic plants seemed fully recovered after 1 day at 25–28°C while the non-transformed plants appeared stressed by the indication of wilted leaves, which was obvious even after 5 days of recovery time. After 1 day at −1°C lower ion leakage and malondialdehyde level was observed in transformed and wild type plants but the level increased significantly after 2–3 days at the same temperature. Therefore the AFPs genes protected transgenic tobacco plants from frost stress [119].

The AFPs synthesized from Spruce budworm (*C. fumiferana*) an insect in the *Choristoneura* genus and introduced into *A. thaliana* by plant codon and a peptide with PR-signal of tobacco, the expression vector in plants had a synthesize gene of AFP with double 35S promotor. The transgenic lines showing the high content of anti-freezing protein transcript were selected based on RT-PCR of total RNA from *Arabidopsis* leaves. After 3 weeks growth progress was determined at 23°C under the condition of extended photoperiod, wild and transformed plants were moved to 4°C for 48 h (at long and short photoperiods simultaneously) and further exposed to a very low temperature of −20°C for 30 min. The plants were then maintained at 4°C at night and transferred back to the facility of growth chamber having 23°C temperature. Through visual inspection, the death of most wild-type plants in comparison to the survival of most transformed plants was observed, although the exact numbers were not determined. The transformed lines having a high level of *AFP* transcript showed better survival ability in comparison with wild type plants that exhibited very poor survival capabilities. The rise in electrolyte leakage and malondialdehyde content was observed in all plants upon their exposure to cold treatment, but the levels were much higher in wild type than transgenic plants. The results showed that the expression of AFPs gene from Spruce budworm (*C. fumiferana*) in transgenic *A. thaliana* plants increased their tolerance to freezing temperatures and helped in the removal of injuries [120].

The lists of genes that have been expressed in plants for abiotic stresses tolerance improvement are shown in **Tables 2** and **3**.

Several genes have been expressed in transgenic plants from bacteria for abiotic stresses tolerance that exhibited good results in many transgenic plants for example tomato, tobacco, finger millet, peanut, potato, *A. thaliana*, wheat etc. are shown in the following **Table 2**.

Other genes from insects, fish, and yeast have been introduced in transgenic plants that exhibited better tolerance against various abiotic stresses are shown in **Table 3**.

Gene	Origin	Plant	Abiotic stress	Reference
mtID	*Escherichia coli*	Tomato	Cold and drought	[33]
mtID	*E. coli*	Wheat	Salinity and flooding	[34]
mtID	*E. coli*	Finger millet	Drought, salinity	[36]
ADH	*Cyanobacteria Synechocystis* sp	Tobacco	Salinity	[42]
CodA	*Arthrobacter globiformis*	*Arabidopsis thaliana*	Cold, light stress, and high temperatures	[68]
CodA	*A. globiformis*	Tomato	Chilling and high temperatures	[70]
IPT	*Agrobacterium tumefaciens*	sugarcane	Cold	[40]
ggpPS	*Azotobacter vinelandii*	*A. thaliana*	Salinity and drought	[44]
BetA	*E. coli*	Sugarcane	Drought	[84]
BetA	*E. coli*	Maize	Chilling	[86]
DRNF1	*Nostoc flagelliforme*	*Arabidopsis Thaliana*	Salinity	[46]
IPT	*A. tumefaciens*	Canola	Drought	[43]

Table 2.
Bacterial genes expressed in plants for abiotic stresses tolerance.

Genes	Origin	Plants	Abiotic stress	Reference
TPS1	*Saccharomes cerevisiae*	Tobacco	Drought and salinity	[89]
HAL1, *HAL3*	*Saccharomyces cerevisiae*	*Arabidopsis thaliana*	Salinity	[92]
HAL1	Yeast	Tomato	Salinity	[90]
AFA3	Winter flounder	Tomato	Freezing stress	[106]
AFA5	Winter flounder	Potato	Frost	[108]
IIA7	*Pseudopleuronectes americanus*	Tobacco	Freezing stress	[115]
MpAFP149	*Microdera punctipennis*	Tobacco	Frost	[119]

Table 3.
List of heterologous expression of genes in transgenic plants for abiotic stresses tolerance from yeast, fish and insects.

5. Conclusion

In this study, the use of various genes isolated from non-plant sources have been expressed in plants for improving their tolerance against abiotic stresses that adversely affect plant growth, and crop yield productivity are reviewed comprehensively. Gene expression in transgenic plants through conventional methods are time consuming and laborious that is why advanced genetic engineering methods for example *Agrobacterium*-mediated transformation and biolistic methods are more accurate, useful, and less time consuming. This review of the chapter provides an extensive insight into various bacterial genes for example *mtID*, *codA*, *betA*, *ADH*, *IPT*, *DRNF1* and *ggpPS*, etc. that have been successfully expressed

in transgenic plants against various abiotic stresses for stress tolerance enhancement and crop yield improvement which exhibited good encouraging results. Genes from yeast (*Saccharomyces cerevisiae*) have been introduced in transgenic plants against drought and salinity stress, other genes isolated from fish for example *AFA3* and *AFA5* which codes for anti-freezing proteins improve transgenic plants against frost stress. Genes from insects have also been inserted in plants to improve their resistance. According to the available literature, several genes isolated from bacteria, yeast, fish, and insets have been expressed in transgenic plants for their enhancement against high and low temperatures, drought, light, and salinity stress. Various research studies have been conducted to improve transgenic plants for the fulfillment of the constant demands of the ever-increasing population. Further work can be done in the future to enhance crop and transgenic plants through new sophisticated technologies. The above mentioned genes can be tested on various other crops to improve their resistance, better yield productivity, longevity in shelf life and enhanced resistance against abiotic and biotic stresses.

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Abbreviations

Ala	alanine
ABA	abscisic acid
ADH	alcohol dehydrogenase
AFPs	anti-freeze proteins
Al_3^+	aluminum ion

B	boron
Ba_2^+	barium
Ca^+	calcium
CaMV	cauliflower mosaic virus
CaMV35S	cauliflower mosaic virus regulatory sequence
Cl	chlorine
CO_2^{-3}	carbonate
CodA	choline oxidase gene
CSPs	cold shock proteins
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
FAO	food and agriculture organization
GG	glycinbetaine
ggpPS	geranylgeranyl diphosphate synthase
HAL1	yeast gene
HCO_3	bicarbonate
IPT gene	isopentenyltransferase gene

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References

[1] Mosa KA, Ismail A, Helmy M. Omics and System Biology Approaches in Plant Stress Research. Plant Stress Tolerance. Cham: Springer; 2017. pp. 21-34

[2] Parween A, Singh V, Bajpai M. Abiotic stress and red clover: A less explored area of research. Advances in Research. 2020;**21**(6):1-5

[3] Kanojia A, Dijkwel PP. Abiotic Stress Responses are Governed by Reactive Oxygen Species and Age. 2018. pp. 295-326

[4] Pedrol N, González L, Reigosa MJ. Allelopathy and abiotic stress. Allelopathy. Dordrecht: Springer; 2006. pp. 171-209

[5] Gull A, Lone AA, Wani NUI. Biotic and Abiotic Stresses in Plants. Abiotic and Biotic Stress in Plants. London, IntechOpen; 2019

[6] Charles H, Godfray J, Beddington JR, Crute IR, Haddad L, Lawrence D, et al. Food security: The challenge of feeding 9 billion people. Science. 2010;**327**(5967):812-818

[7] Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K. Effects of abiotic stress on plants: A systems biology perspective. BMC Plant Biology Journal. 2011;**11**(1):163

[8] Mickelbart MV, Hasegawa PM, Bailey-Serres J. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nature Reviews Genetics. 2015;**16**(4):237-251

[9] Wang H, Wang H, Shao H, Tang X. Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. Frontiers in Plant Science. 2016;**7**:67

[10] Dubois M, Claeys H, Van den Broeck L, Inzé D. Time of day determines Arabidopsis transcriptome and growth dynamics under mild drought. Plant, Cell & Environment. 2017;**40**(2):180-189

[11] Sah SK, Reddy KR, Li J. Abscisic acid and abiotic stress tolerance in crop plants. Frontiers in Plant Science. 2016;**7**:571

[12] Ng LM, Melcher K, Teh BT, Xu HE. Abscisic acid perception and signaling: Structural mechanisms and applications. Acta Pharmacologica Sinica. 2014;**35**(5):567-584

[13] Bechtold U, Field B. Molecular Mechanisms Controlling Plant Growth during Abiotic Stress. Oxford University: Press UK; 2018

[14] Kranner I, Minibayeva FV, Beckett RP, Seal CE. What is stress? Concepts, definitions and applications in seed science. New Phytologist. 2010;**188**(3):655-673

[15] Olien C, Smith MN. Ice adhesions in relation to freeze stress. Plant Physiology. 1977;**60**(4):499-503

[16] Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World Journal of Microbiology and Biotechnology. 2011;**27**(5):1231-1240

[17] Naglaa A, Moses V, Prakash C. Climate change effects on abiotic stress in plants. GM Crop Food. 2014;**5**:77-80

[18] Barlow K, Christy B, O'leary G, Riffkin P, Nuttall J. Simulating the impact of extreme heat and frost events on

wheat crop production: A review. Field Crops Research. 2015;**171**:109-119

[19] Djanaguiraman M, Perumal R, Ciampitti I, Gupta S, Prasad P. Quantifying pearl millet response to high-temperature stress: Thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. Plant, Cell & Environment. 2018;**41**(5):993-1007

[20] Kallarackal J, Milburn J, Baker D. Water relations of the Banana. III. Effects of controlled water stress on water potential, transpiration, photosynthesis and leaf growth. Functional Plant Biology. 1990;**17**(1):79-90

[21] Van der Weijde T, Huxley LM, Hawkins S, Sembiring EH, Farrar K, Dolstra O, et al. Impact of drought stress on growth and quality of miscanthus for biofuel production. GCB Bioenergy. 2017;**9**(4):770-782

[22] Sazzad K. Exploring Plant Tolerance to Biotic and Abiotic Stresses. Uppsala: Swedish University of Agricultural Sciences; 2007

[23] Drew MC. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annual Review of Plant Biology. 1997;**48**(1):223-250

[24] Jackson M, Colmer T. Response and adaptation by plants to flooding stress. Annals of Botany. 2005;**96**(4):501-505

[25] Adamiec M, Drath M, Jackowski G. Redox state of plastoquinone pool regulates expression of Arabidopsis thaliana genes in response to elevated irradiance. Acta Biochemical Polonica. 2008;**55**(1):161-174

[26] Barta C, Kálai T, Hideg K, Vass I, Hideg É. Differences in the ROS-generating efficacy of various ultraviolet wavelengths in detached spinach leaves. Functional Plant Biology. 2004;**31**(1):23-28

[27] Safdar H, Amin A, Shafiq Y, Ali A, Yasin R, Shoukat A, et al. A review: Impact of salinity on plant growth. Natural Science. 2019;**17**(1):34-40

[28] Manchanda G, Garg N. Salinity and its effects on the functional biology of legumes. Acta Physiologiae Plantarum. 2008;**30**(5):595-618

[29] HanumanthaRao B, Nair RM, Nayyar H. Salinity and high temperature tolerance in mungbean [Vigna radiata (L.) Wilczek] from a physiological perspective. Frontiers in Plant Science. 2016;**7**:957

[30] Qados AMA. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). Journal of the Saudi Society of Agricultural Sciences. 2011;**10**(1):7-15

[31] Mubeen H, Naqvi R, Masood A, Shoaib M, Raza S. Gene transformation: Methods, uses and applications. Journal of Pharmaceutical and Biological Sciences. 2016;**4**(2):54

[32] Altieri MA. The ecological impacts of transgenic crops on agroecosystem health. Ecosystem Health. 2000;**6**(1):13-23

[33] Khare N, Goyary D, Singh NK, Shah P, Rathore M, Anandhan S, et al. Transgenic tomato cv. Pusa Uphar expressing a bacterial mannitol-1-phosphate dehydrogenase gene confers abiotic stress tolerance. Plant Cell, Tissue and Organ Culture (PCTOC). 2010;**103**(2):267-277

[34] Abebe T, Guenzi AC, Martin B, Cushman JC. Tolerance of mannitol-accumulating transgenic wheat to water

stress and salinity. Plant Physiology. 2003;**131**(4):1748-1755

[35] Tarczynski MC, Jensen RG, Bohnert HJ. Expression of a bacterial *mtlD* gene in transgenic tobacco leads to production and accumulation of mannitol. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**(7):2600-2604

[36] Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-Kumar M, Udayakumar M. Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS One. 2014;**9**(6):e99110

[37] Bhauso TD, Radhakrishnan T, Kumar A, Mishra GP, Dobaria JR, Patel K, et al. Overexpression of bacterial mtlD gene in peanut improves drought tolerance through accumulation of mannitol. The Scientific World Journal. 2014;**2014**

[38] Kondo Y, Sakamoto A, Nonaka H, Hayashi H, Saradhi PP, Chen TH, et al. Enhanced tolerance to light stress of transgenic Arabidopsis plants that express the codA gene for a bacterial choline oxidase. Plant Molecular Biology. 1999;**40**(2):279-288

[39] Goel D, Singh AK, Yadav V, Babbar SB, Murata N, Bansal KC. Transformation of tomato with a bacterial *codA* gene enhances tolerance to salt and water stresses. Journal of Plant Physiology. 2011;**168**(11):1286-1294

[40] Belintani N, Guerzoni J, Moreira R, Vieira L. Improving low-temperature tolerance in sugarcane by expressing the *IPT* gene under a cold-inducible promoter. Biologia Plantarum. 2012;**56**(1):71-77

[41] Peleg Z, Reguera M, Tumimbang E, Walia H, Blumwald E. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. Plant Biotechnology Journal. 2011;**9**(7):747-758

[42] Yi SY, Ku SS, Sim H-J, Kim S-K, Park JH, Lyu JI, et al. An alcohol dehydrogenase gene from Synechocystis sp. confers salt tolerance in transgenic tobacco. Frontiers in Plant Science. 2017;**8**:1965

[43] Kant S, Burch D, Badenhorst P, Palanisamy R, Mason J, Spangenberg G. Regulated expression of a cytokinin biosynthesis gene *IPT* delays leaf senescence and improves yield under rainfed and irrigated conditions in canola (*Brassica napus* L.). PLoS One. 2015;**10**(1):e0116349

[44] Klähn S, Marquardt DM, Rollwitz I, Hagemann M. Expression of the *ggpPS* gene for glucosylglycerol biosynthesis from Azotobacter vinelandii improves the salt tolerance of *Arabidopsis thaliana*. Journal of Experimental Botany. 2009;**60**(6):1679-1689

[45] Castiglioni P, Warner D, Bensen RJ, Anstrom DC, Harrison J, Stoecker M, et al. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. Plant Physiology. 2008;**147**(2):446-455

[46] Cui L, Liu Y, Yang Y, Ye S, Luo H, Qiu B, et al. The drnf1 gene from the drought-adapted cyanobacterium Nostoc flagelliforme improved salt tolerance in transgenic Synechocystis and Arabidopsis plant. Genes. 2018;**9**(9):441

[47] Mattsson A. Predicting field performance using seedling quality assessment. New Forests. 1997;**13**(1-3):227-252

[48] Cheng L, Zou Y, Ding S, Zhang J, Yu X, Cao J, et al. Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. Journal of Integrative Plant Biology. 2009;**51**(5):489-499

[49] Almeselmani M, Deshmukh P, Sairam R, Kushwaha S, Singh T. Protective role of antioxidant enzymes under high temperature stress. Plant Science. 2006;**171**(3):382-388

[50] Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, et al. Drought-and salt-tolerant plants result from overexpression of the AVP1 H+-pump. Proceedings of the National Academy of Sciences. 2001;**98**(20):11444-11449

[51] Davis T, Yamada M, Elgort M, Saier MH Jr. Nucleotide sequence of the mannitol (mtl) operon in *Escherichia coli*. Molecular Microbiology. 1988;**2**(3):405-412

[52] Gowda B, Halaswamy B, Seetharam A, Virk D, Witcombe J. Participatory approach in varietal improvement: A case study in finger millet in India. Current Science. 2000:366-368

[53] Hamza NB. Progress and prospects of millet research in Sudan. In: Tadele Z, editor. 2009. pp. 260-260

[54] Mbithi-Mwikya S, Van Camp J, Mamiro PR, Ooghe W, Kolsteren P, Huyghebaert A. Evaluation of the nutritional characteristics of a finger millet based complementary food. Journal of Agricultural Food Chemistry. 2002;**50**(10):3030-3036

[55] Oghbaei M, Prakash J. Bioaccessible nutrients and bioactive components from fortified products prepared using finger millet (Eleusine coracana). Journal of Science Food Agriculture. 2012;**92**(11):2281-2290

[56] Bhatt D, Negi M, Sharma P, Saxena SC, Dobriyal AK, Arora S. Responses to drought-induced oxidative stress in five finger millet varieties differing in their geographical distribution. Physiology and Molecular Biology of Plants. 2011;**17**(4):347-353

[57] Reddy T, Reddy V, Anbumozhi V. Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: A critical review. Plant Growth Regulation. 2003;**41**(1):75-88

[58] Ravi K, Vadez V, Isobe S, Mir RR, Guo Y, Nigam SN, et al. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theoritical Application of Genetics. 2011;**122**(6):1119-1132

[59] Pujni D, Chaudhary A, Rajam M. Increased tolerance to salinity and drought in transgenic Indica rice by mannitol accumulation. Journal of Plant Biochemistry and Biotechnology. 2013;**16**

[60] Ganie SA, Ahammed GJ, Wani SH. Vascular plant one zinc-finger (VOZ) transcription factors: Novel regulators of abiotic stress tolerance in rice (*Oryza sativa* L.). Genetic Resources and Crop Evolution. 2020;**67**(4):799-807

[61] Hussain S, Zhang J-h, Zhong C, Zhu L-f, Cao X-c, Yu S-m, et al. Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. Journal of Integrative Agriculture. 2017;**16**(11):2357-2374

[62] Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D,

Balestrazzi A. Seed priming: State of the art and new perspectives. Plant Cell Reports. 2015;**34**(8):1281-1293

[63] Matsumuro T, Miyake H, Takeoka Y. Salinity-induced ultrastructural alterations in leaf cells of Rice (*Oryza sativa* L.). Plant Production Science. 2000;**3**:422-429

[64] Sun J, Zheng T, Yu J, Wu T, Wang X, Chen G, et al. TSV, a putative plastidic oxidoreductase, protects rice chloroplasts from cold stress during development by interacting with plastidic thioredoxin Z. New Phytologist. 2017;**215**(1):240-255

[65] Boyer JS. Plant productivity and the environment. Science. 1982;**218**(4571):443-448

[66] Boyer J, Westgate M. Grain yields with limited water. Journal of Experimental Botany. 2004;**55**(407):2385-2394

[67] Claassen M, Shaw RH. Water deficit effects on corn. II. Grain components 1. Agronomy Journal. 1970;**62**(5):652-655

[68] Sakamoto A, Valverde R, Alia T, Chen H, Murata N. Transformation of Arabidopsis with the codA gene for choline oxidase enhances freezing tolerance of plants. Plant Journal. 2000;**22**(5):449-453

[69] Alia H, Hayashi AS, Murata N. Enhancement of the tolerance of Arabidopsis to high temperatures by genetic engineering of the synthesis of glycinebetaine. Plant Journal. 1998;**16**(2):155-161

[70] Li S, Li F, Wang J, Zhang W, Meng Q, Chen TH, et al. Glycinebetaine enhances the tolerance of tomato plants to high temperature during germination of seeds and growth of seedlings. Plant Cell Environmental Biology. 2011;**34**(11):1931-1943

[71] Park EJ, Jeknić Z, Sakamoto A, DeNoma J, Yuwansiri R, Murata N, et al. Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. Plant Journal. 2004;**40**(4):474-487

[72] Chang H, Jones ML, Banowetz GM, Clark DG. Overproduction of cytokinins in petunia flowers transformed with PSAG12-IPT delays corolla senescence and decreases sensitivity to ethylene. Plant Physiology. 2003;**132**(4):2174-2183

[73] Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, et al. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proceedings of the National Academy of Sciences. 2007;**104**(49):19631-19636

[74] Haberer G, Kieber JJ. Cytokinins. New insights into a classic phytohormone. Plant Physiology. 2002;**128**(2):354-362

[75] Miller CO, Skoog F, Okumura F, Von Saltza M, Strong F. Isolation, structure and synthesis of kinetin, a substance promoting cell division1, 2. Journal of the American Chemical Society. 1956;**78**(7):1375-1380

[76] Gan S, Amasino RM. Inhibition of leaf senescence by autoregulated production of cytokinin. Science. 1995;**270**(5244):1986-1988

[77] Qin H, Gu Q, Zhang J, Sun L, Kuppu S, Zhang Y, et al. Regulated expression of an isopentenyltransferase gene (IPT) in peanut significantly improves drought tolerance and increases yield under field conditions. Plant and Cell Physiology. 2011;**52**(11):1904-1914

[78] Robson PR, Donnison IS, Wang K, Frame B, Pegg SE, Thomas A, et al. Leaf senescence is delayed in maize expressing the agrobacterium IPT gene under the

control of a novel maize senescence-enhanced promoter. Plant Biotechnology Journal. 2004;**2**(2):101-112

[79] Farooq M, Wahid A, Basra S. Improving water relations and gas exchange with brassinosteroids in rice under drought stress. Journal of Agronomy and Crop Science. 2009;**195**(4):262-269

[80] Serraj R, Kumar A, McNally K, Slamet-Loedin I, Bruskiewich R, Mauleon R, et al. Improvement of drought resistance in rice. Advances in Agronomy. 2009;**103**:41-99

[81] Bouman B, Humphreys E, Tuong T, Barker R. Rice and water. Advances in Agronomy. 2007;**92**:187-237

[82] Ma Q-H. Genetic engineering of cytokinins and their application to agriculture. Critical Reviews in Biotechnology. 2008;**28**(3):213-232

[83] Hajouj T, Michelis R, Gepstein S. Cloning and characterization of a receptor-like protein kinase gene associated with senescence. Plant Physiology. 2000;**124**(3):1305-1314

[84] Sugiharto B. Biotechnology of drought-tolerant sugarcane. Sugarcane: Technology and Research. 16 May 2018;**138**

[85] Lv S, Yang A, Zhang K, Wang L, Zhang J. Increase of glycinebetaine synthesis improves drought tolerance in cotton. Molecular Breeding. 2007;**20**(3):233-248

[86] Quan R, Shang M, Zhang H, Zhao Y, Zhang J. Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize. Plant Science. 2004;**166**(1):141-149

[87] Holmström KO, Somersalo S, Mandal A, Palva TE, Welin B. Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. Journal of Experimental Botany. 2000;**51**(343):177-185

[88] Yilmaz JL, Bülow L. Enhanced stress tolerance in *Escherichia coli* and *Nicotiana tabacum* expressing a betaine aldehyde dehydrogenase/choline dehydrogenase fusion protein. Biotechnology Progress. 2002;**18**(6):1176-1182

[89] Serrano R, Culiañz-Maciá FA, Moreno V. Genetic engineering of salt and drought tolerance with yeast regulatory genes. Scientia Horticulturae. 1998;**78**(1-4):261-269

[90] Gisbert C, Rus AM, Bolarín MC, López-Coronado JM, Arrillaga I, Montesinos C, et al. The yeast *HAL1* gene improves salt tolerance of transgenic tomato. Plant Physiology. 2000;**123**(1):393-402

[91] Romero C, Bellés JM, Vayá JL, Serrano R, Culiáñez-Macià FA. Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: Pleiotropic phenotypes include drought tolerance. Planta. 1997;**201**(3):293-297

[92] Yang SX, Zhao YX, Zhang Q, He YK, Zhang H, Luo D. HAL1 mediate salt adaptation in *Arabidopsis thaliana*. Cell Research. 2001;**11**(2):142-148

[93] Ellul P, Ríos G, Atarés A, Roig LA, Serrano R, Moreno V. The expression of the Saccharomyces cerevisiae *HAL1* gene increases salt tolerance in transgenic watermelon [Citrullus lanatus (Thunb.) Matsun. & Nakai.]. Theoretical and Applied Genetics. 2003;**107**(3):462-469

[94] Gaxiola R, De Larrinoa IF, Villalba JM, Serrano R. A novel and conserved salt-induced protein is an important determinant of salt

tolerance in yeast. The EMBO Journal. 1992;**11**(9):3157-3164

[95] Rios G, Ferrando A, Serrano R. Mechanisms of salt tolerance conferred by overexpression of the HAL1 gene in Saccharomyces cerevisiae. Yeast. 1997;**13**(6):515-528

[96] Serrano R. Salt tolerance in plants and microorganisms: Toxicity targets and defense responses. International Review of Cytology. 1996;**165**:1-52

[97] DeVries A. Role of glycopeptides and peptides in inhibition of crystallization of water in polar fishes. Philosophical Transactions of the Royal Society of London B, Biological Sciences. 1984;**304**(1121):575-588

[98] Burcham TS, Osuga DT, Chino H, Feeney RE. Analysis of antifreeze glycoproteins in fish serum. Analytical Biochemistry. 1984;**139**(1):197-204

[99] DeVries AL. [21] antifreeze glycopeptides and peptides: Interactions with ice and water. Methods in Enzymology. 1986;**127**:293-303

[100] Mantel LH, Farmer LL. Osmotic and ionic regulation. Internal Anatomy and Physiological Regulation. 1983;**5**:53-161

[101] DeVries AL, Komatsu SK, Feeney RE. Chemical and physical properties of freezing point-depressing glycoproteins from Antarctic fishes. Journal of Biological Chemistry. 1970;**245**(11):2901-2908

[102] Scott GK, Davies PL, Shears MA, Fletcher GL. Structural variations in the alanine-rich antifreeze proteins of the pleuronectinae. European Journal of Biochemistry. 1987;**168**(3):629-633

[103] Gourlie B, Lin Y, Price J, DeVries A, Powers D, Huang R. Winter flounder antifreeze proteins: A multigene family. Journal of Biological Chemistry. 1984;**259**(23):14960-14965

[104] Scott GK, Hew CL, Davies PL. Antifreeze protein genes are tandemly linked and clustered in the genome of the winter flounder. Proceedings of the National Academy of Sciences. 1985;**82**(9):2613-2617

[105] Pickett M, Scott G, Davies P, Wang N, Joshi S, Hew C. Sequence of an antifreeze protein precursor. European Journal of Biochemistry. 1984;**143**(1):35-38

[106] Hightower R, Baden C, Penzes E, Lund P, Dunsmuir P. Expression of antifreeze proteins in transgenic plants. Plant Molecular Biology. 1991;**17**(5):1013-1021

[107] Joslyn M. The freezing of fruits and vegetables. In: Meryman HT, editor. Cryobiology. 1966:565-607

[108] Wallis JG, Wang H, Guerra DJ. Expression of a synthetic antifreeze protein in potato reduces electrolyte release at freezing temperatures. Plant Molecular Biology. 1997;**35**(3):323-330

[109] Khanna HK, Daggard GE. Targeted expression of redesigned and codon optimised synthetic gene leads to recrystallisation inhibition and reduced electrolyte leakage in spring wheat at sub-zero temperatures. Plant Cell Reports. 2006;**25**(12):1336-1346

[110] Davies PL, Hew CL, Fletcher GL. Fish antifreeze proteins: Physiology and evolutionary biology. Canadian Journal of Zoology. 1988;**66**(12):2611-2617

[111] DeVries A. Antifreeze peptides and glycopeptides in cold-water fishes. Annual Review of Physiology. 1983;**45**(1):245-260

[112] Parody-Morreale A, Murphy KP, Di Cera E, Fall R, DeVries AL, Gill SJ. Inhibition of bacterial ice nucleators by fish antifreeze glycoproteins. Nature. 1988;**333**(6175):782-783

[113] Rubinsky B, Arav A, Fletcher G. Hypothermic protection—A fundamental property of "antifreeze" proteins. Biochemical and Biophysical Research Communications. 1991;**180**(2):566-571

[114] Kenward KD, Altschuler M, Hildebrand D, Davies PL. Accumulation of type I fish antifreeze protein in transgenic tobacco is cold-specific. Plant Molecular Biology. 1993;**23**(2):377-385

[115] Lee J-S. The Frost Tolerance of Tobacco Plants Transformed with the Gene Encoding the Antifreeze Protein from Winter Flounder. Louisiana State University and Agricultural & Mechanical College; 1991

[116] Holmberg N, Farrés J, Bailey JE, Kallio PT. Targeted expression of a synthetic codon optimized gene, encoding the spruce budworm antifreeze protein, leads to accumulation of antifreeze activity in the apoplasts of transgenic tobacco. Gene. 2001;**275**(1):115-124

[117] Tyshenko MG, Doucet D, Davies PL, Walker VK. The antifreeze potential of the spruce budworm thermal hysteresis protein. Nature Biotechnology. 1997;**15**(9):887-890

[118] Huang T, Nicodemus J, Zarka DG, Thomashow MF, Wisniewski M, Duman JG. Expression of an insect (*Dendroides canadensis*) antifreeze protein in *Arabidopsis thaliana* results in a decrease in plant freezing temperature. Plant Molecular Biology. 2002;**50**(3):333-344

[119] Wang Y, Qiu L, Dai C, Wang J, Luo J, Zhang F, et al. Expression of insect (Microdera puntipennis dzungarica) antifreeze protein MpAFP149 confers the cold tolerance to transgenic tobacco. Plant Cell Reports. 2008;**27**(8):1349

[120] Zhu B, Xiong A-S, Peng R-H, Xu J, Jin X-F, Meng X-R, et al. Over-expression of ThpI from *Choristoneura fumiferana* enhances tolerance to cold in Arabidopsis. Molecular Biology Reports. 2010;**37**(2): 961