

BIOTECHNOLOGICAL APPROACHES FOR THE DEVELOPMENT OF SALT AND COLD TOLERANCE IN CROP PLANT

Abstract

Salt and cold tolerant plants have a strong antioxidant defence system that includes enzymes such as superoxide dismutase, catalase, and peroxidases that scavenge ROS and protect cells from oxidative stress. Some salt-tolerant plants can transport potassium (K⁺) ions preferentially over sodium ions into the root cells. This helps maintain a favorable potassium-to-sodium ratio, which is crucial for cellular function and osmotic balance. Quantitative trait loci are chromosomal areas linked to specific quantitative features, such as salt tolerance, and can be discovered using molecular technology. Cold-responsive gene regulation involves the activation or repression of particular genes in response to low temperature (cold) stress in plants. The promoters of some genes that are susceptible to cold contain the CRT sequence element, It is bound by CBF transcription factors and triggers transcription. Therefore, over-expression of one CBF gene causes the production of several genes that are susceptible to cold. The C-repeat element and low temperature responsive element are common regulatory elements found in the promoters of all genes associated to cold tolerance. In this chapter we can concluded that biotechnology or genomic approaches are beneficial for salt and cold tolerance, by which we can generate the tolerant genotypes.

Keyword: Salt tolerant gene ,CBF gene, Cold reponsive gene, QTL, MAS

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I. INTRODUCTION

The primary target of genomics is identification and characterization of genes and gene products. Starting point of genomics may be a whole genome sequence, from which gene and protein structures can be predicted by computational and bioinformatics approaches or genes can be identified step starting from identification of a phenotype by induction and detection of mutation, construction of a genetic map demarcating the position of the character, physical location, identification of the gene, cloning and characterization of the gene or genome sequence by sequencing followed by expression analysis to correlate the phenotypic expression. In genomics, Transcriptomics is a field of molecular biology that focuses on studying the complete set of RNA molecules transcribed from the genome of an organism, often referred to as the transcriptome. The transcriptome represents all the RNA molecules present in a cell or tissue at a specific point in time, including messenger RNA (mRNA), non-coding RNA, and other functional RNAs. Transcriptomics also covers the study of non-coding RNAs, which are RNA molecules that do not encode proteins. These include microRNAs, long non-coding RNAs (lncRNAs), and small nuclear RNAs (snRNAs), among others. Non-coding RNAs play crucial roles in various cellular processes, such as gene regulation and epigenetic control. The transcriptome can provide insights into the regulatory mechanisms controlling gene expression. This includes identifying transcription factors and other regulatory elements that influence which genes are turned on or off in response to specific conditions. transcriptomics plays a crucial role in advancing our understanding of gene regulation, cellular processes, and the molecular mechanisms underlying various biological phenomena. It relies on techniques such as RNA sequencing (RNA-seq) and microarray analysis to generate comprehensive profiles of RNA molecules in a sample. The detection of groups of genes that show altered expression patterns under certain experimental settings or at various phases of development provides a means of identifying likely multi gene clusters involved in a particular response or developmental stage. Thus, the technology is one of the most effective tools for allele discovery. However, It has a broader applicability in development. of Tolerance crop. In Transcriptomics some technologies are utilised for gene expression analysis like Expressed sequence tag collections (EST), Serial analysis of gene expression (SAGE), Microarrays and Gene chips and massively parallel signature sequencing. This chapter will look at various ways to increase crop plant output by improving their tolerance to salt and cold shocks. In his chapter we will also look at the number of example of how this technology can be used to genetically improve agricultural productivity.

II. RESISTANCE TO SALT STRESS

Resistance to salt stress, also known as salinity resistance, is the ability of an organism, particularly plants, to survive and grow under conditions of elevated soil salinity. High salt levels in the soil can be detrimental to plants, as it interferes with water uptake and ion balance, leading to cellular damage and reduced growth. Salt stress is a significant abiotic stress factor that affects agriculture, particularly in regions with poor drainage or where irrigation water has a high salt content.

Improving salt stress tolerance in crops is of great importance for sustainable agriculture in salt-affected regions. Plant breeding programs and biotechnological approaches, such as MAS and genetic engineering, are being employed to develop salt-

resistant crop varieties that can thrive in saline soils and contribute to food security in challenging environments.

Salt tolerance is the difference in how different genotypes of a species respond to the same tissue salt concentration in terms of various life processes. There is a lot of evidence to suggest that genotypes can tolerate different amounts of salt in their tissues. However, halophytes' cellular and enzyme functions are just as salt-sensitive as glycophytes'. When saline fields are reclaimed by applying gypsum, the majority of crops are planted there, but yields are often low in the first three to four years. Therefore, boosting crops' resistance to salt may significantly aid in raising food output for India's expanding population. As a result, in addition to reducing the demand for chemical amendment input, the growth of salt-resistant types can play a significant role in the rehabilitation of such areas.

1. Salt Resistant Mechanism: Salt resistant mechanisms at the physiological level in plants involve various adaptations that help maintain cellular homeostasis, water balance, and overall plant function under high salinity conditions. These systems are designed to reduce the harmful consequences of excessive salts (sodium and chloride ions) and osmotic stress generated by a water-ion imbalance. Salt-tolerant plants transport excess salts to specific cellular compartments, such as the vacuoles, where they are sequestered and stored, reducing their harmful effects on essential cellular processes. This compartmentalization prevents harmful ions from accumulating in the cytoplasm and maintains ion homeostasis. High salt concentrations in the soil create an osmotic imbalance, leading to water loss from the plant cells. Salt-tolerant plants counteract this osmotic stress by accumulating compatible solutes or osmolytes include amino acids like proline, betaine, taurine, myo-inositol, and various sugar derivatives, which help maintain cellular turgor and retain water within the cells. Salt stress can cause the production of reactive oxygen species (ROS), which can cause oxidative damage to biological components.. Salt-tolerant plants have a strong antioxidant defence system that includes enzymes such as superoxide dismutase, catalase, and peroxidases that scavenge ROS and protect cells from oxidative stress. Some salt-tolerant plants can transport potassium (K⁺) ions preferentially over sodium ions into the root cells. This helps maintain a favorable potassium-to-sodium ratio, which is crucial for cellular function and osmotic balance. Plant hormones, such as abscisic acid (ABA), play a important role in regulating responses to salt stress. ABA helps in stomatal closure to minimize water loss and initiates the expression of stress-responsive alleles.

These physiological mechanisms collectively contribute to the salt resistant of plants, enabling them to adapt and survive in saline environments. Understanding and harnessing these mechanisms through breeding and Biotechnological techniques can lead to the production of salt-tolerant crop types capable of thriving in salt-affected soils and contributing to sustainable agriculture in difficult regions.

As salt buildup affects large areas of irrigated land, salt tolerance is increasingly becoming a major priority for crop improvement. Additionally, the need for land has necessitated thinking about the possibilities of cultivating crops in more salty circumstances with lower quality water. Osmotic stress is caused by salty circumstances, which inhibit water intake by roots and water outflow from cells. However, by impeding protein synthesis, photosynthesis, and enzymes that are vulnerable, the accumulation of

Na⁺ and Cl⁻ ions in the cytoplasm has the potential to be directly damaging (Estan, M.T. et al., 2005). As a result, Methods for engineering water stress resistant by producing suitable solutes may provide protection against the osmotic effect of salinity but not against ion toxicity. There might also be a need for further strategies to reduce the hazardous effects of particular ions. Strategies for engineering salt tolerance have been developed by evaluating the reactions to salt stress of plants that are susceptible to high salt conditions (glycophytes) with those of plants that can resist high salt conditions (halophytes). Typically, glycophytes accumulate osmoprotectants in response to salt stress, whereas halophytes use particular methods to counteract the harmful effects of Na⁺ and Cl⁻ ions. Plants, on the other hand, tend to regulate Na⁺ ions out of the cell, whilst other cells with large vacuoles may act as sinks for the buildup of excess sodium via transit into the vacuole. Some halophytes produce salt through specialised glands on the surface of their leaves.

Intense scientific efforts have been directed towards elucidating the physiological basis of tolerance in higher plants (Cuartero, J., et al., 2008; Afzal et al., 2022a and 2022b). Tolerance to salt stress is a complex process that occurs at both the plant and cellular levels. For the selection of high tolerant cultivars, certain physiological markers that are extremely specialised for saline conditions were identified and used often. These factors included low Na⁺/K⁺ ratio, preferential Na⁺ accumulation in older leaves, strong Cl⁻ uptake, low K⁺ uptake, and Na⁺ transport to shoot (Sharma and Goyal, 2003).

One method for improving salt resistance is to emulate the mechanisms used by halophytes to transport Na⁺ ions out of the cytoplasm. In order to put this into effect, the mechanisms of ion transport out of the cytoplasm must be considered. The initial transgenic studies looked at Na⁺ ion transport into the vacuole. Because it is working against a concentration gradient, its transport requires energy input. To do this, a proton pump that transports H⁺ ions in the opposite direction is coupled to the transport protein. It is widely understood that the *Arabidopsis* vacuolar Na⁺/H⁺ antiport protein AtNHX couples to proton pumps such as AVP1, a vacuolar H⁺ translocating pyrophosphatase. AtNHX1 and AVP1 have been compared as a rotating door and an energy source for the door, respectively. Therefore, to improve the flow of traffic over the membrane, one may either add additional doors or give the current doors more energy to spin more quickly.

The first method, transformation with the *Arabidopsis* AtNHX1 antiport protein gene, was successful in engineering salt resistant in tomato plants (Yokoi et al., 2002). NaCl caused a rise in AtNHX1 steady state transcript levels, indicating that osmotic stress is more common than ionic stress in the up-regulation of AtNHX1 transcripts (Zhu 2002). To increase salt tolerance, NHX antiporters have been highly expressed (Wu et al., 2004). When salt tolerance has been studied, *Arabidopsis* genes were also transferred into maize and wheat plants, resulting in better grain yields and improved salt tolerance. For instance, rice modified with the OsNHX1 gene demonstrated enhanced growth and buildup of biomass when exposed to salt stress. (Wu *et al.*, 2005, Chen *et al.*, 2007).

It has been proposed to over-express the allele encoding AVP1, which was firstly tested in *Arabidopsis*, in order to enhance the vacuole's H⁺ pumping potential and hence its capacity to transfer salt (Gaxiola et al., 2001). Since the changed ion stability allowed the flora to hold extra water, this has improved the experimental plants' resistance to both

drought and salt. Similar outcomes have been attained by overexpressing the homologues from *Triticum aestivum* (TVP1) and *Thellungiella halophila* (TsVP) in tobacco and *Arabidopsis*, respectively (Gao et al., 2006; Brini et al., 2007).

- 2. Salt Tolerance QTL Mapping:** A QTL is thus an area of a chromosome where it is thought that functionally different alleles will segregate and have a large impact on a quantitative feature. Molecular marker and phenotypic data from a large segregating population must be statistically analysed for QTL mapping in order to identify the markers where genetic variation connects with the quantitative trait phenotype.

Quantitative trait loci are chromosomal areas linked to specific quantitative features, such as salt tolerance, and can be discovered using molecular technology. Few researchers have documented the QTL for salt damage at the early embryo stage in rice (Prasad et al., 2000).

III. COLD STRESS TOLERANCE

The capacity of various plants to tolerate cold and freezing temperatures varies greatly (Figure 1). The majority of tropical plants can not withstand frigid temperatures. On the other hand, depending on the species, any temperature plants can tolerate a range of subfreezing temperatures from -5 to -30°C . Even lower stand temperatures are frequently encountered by plants from colder climates. It is commonly understood that plants benefit from a period of cold acclimation at a low but non-freezing temperature, they will be better equipped to handle cold or freezing stress. For instance, wheat plants growing at typical mild temperatures die from freezing at -5°C , but after a time of acclimatisation to the cold, when the plant grow at temperatures below 10°C , they can survive freezing temperatures as low as -20°C .

Different plants have different tolerances to cold or freezing temperatures, and plant breeders have been selectively breeding for this feature for ages. However, traditional breeding has not significantly improved the major crop species' ability to withstand cold over the past 20 years, which has led to the quest for molecular remedies to this issue.

Studying the mechanisms that some plant species use to survive freezing has been one strategy. Plants release a number of cold-induced proteins during the acclimatisation stage that are thought to contribute to the development of cold resistance. In various plant species, about 50 cold-induced proteins have been discovered. These can be divided into a less number of categories, but they all have the quality of being very hydrophilic in common. Numerous them also feature repeating themes in their comparatively straightforward amino acid compositions. These families include proteins known as late embryogenesis abundant (LAE) proteins, which seem to act as a barrier against damage during seed desiccation. According to their expression patterns, a class of genes known as cold responsive genes encodes additional groups of proteins. Although the precise role of these cold-induced genes is unknown, it has been hypothesised that they may directly increase freezing tolerance by reducing the potentially harmful consequences of dehydration brought on by freezing. Therefore, a potential method for targeted engineering of cold or freezing stress tolerance is the over expression or ectopic production of this cold-induced protein.

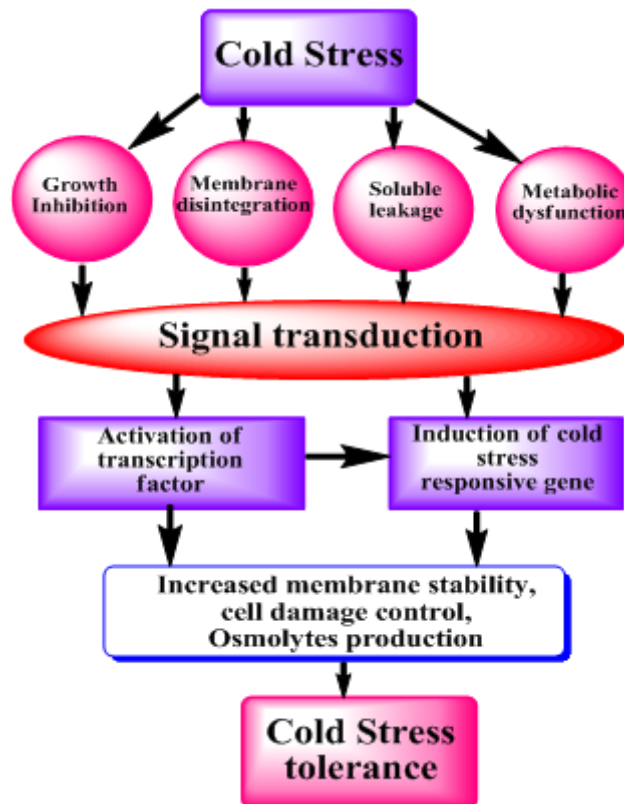


Figure 1: Cold Stress Response in Plant

There are various examples of transgenic plants expressing cold-induced proteins. For example, constitutive expression of the tiny, hydrophilic, chloroplast specific COR protein COR 15a in *Arabidopsis* improved the freezing durability of chloroplast frozen in situ and protoplast frozen in vitro. The survival of frozen plants, however, is unaffected by COR 15a expression. This observation can be explained by the fact that all of the cold-induced proteins are necessary to fully protect the cell and that they may be directed towards variously vulnerable cell components. It follows that many COR genes would need to be added to a transgenic crop in order to significantly improve cold tolerance.

Following the discovery that several different Cold tolerance-related genes are genes that play a crucial role in enabling organisms to survive and thrive in cold environments by helping them adapt to the stresses associated with low temperatures. These genes are involved in various physiological and molecular processes that collectively contribute to cold tolerance. They can be found in a wide range of organisms, including plants, animals, and microorganisms. Cold Acclimation gene (COR) are often found in plants and are induced when plants are exposed to low temperatures. They encode proteins that help protect plant cells from damage caused by cold stress. Examples include proteins that scavenge reactive oxygen species (ROS) and chaperone proteins that assist in maintaining proper protein folding. Dehydrins are a class of proteins found in plants that accumulate in response to cold stress. They are thought to help protect plant cells by stabilizing cellular structures and membranes during freezing. It was also discovered that cold induced transcription factors regulate the expression of other cold tolerance-related genes. They act as master regulators of

the cold response, coordinating the activation of various stress response pathways. In order to induce this complete collection of COR cold tolerance genes, the technique is to increase the expression of the CBF1 gene. There have been created transgenic Arabidopsis plants containing a 35S promoter, CBF1 gene construct. These plants have been shown to be freezing resistant in the absence of prior cold acclimation and to express a number of COR genes. Transgenic plants overexpressing the COR15a protein, used as a control, were discovered to be less freeze resistant than the CBF1 plants. In a comparable experiment, it was shown that various stress responses are interconnected. Plants with improved tolerance for drought, salt, and frost were produced when the expression of a CBFF1 homologue, the DRE binding protein DREB1A, was regulated by a stress-induced promoter in transgenic Arabidopsis.

1. Transcriptional Regulation by Low Temperature Signaling Pathway: The transcriptional regulation of genes in response to low temperature, or cold, is mediated by a complex signaling pathway known as the cold signaling pathway. This pathway allows plants to sense changes in temperature and activate specific transcription factors and other regulatory proteins that control the expression of cold-responsive genes. The activation of these genes helps the plant acclimate to cold conditions and enhances its cold tolerance.

The promoters of some genes that are susceptible to cold contain the CRT sequence element, It is bound by CBF transcription factors and triggers transcription. Therefore, over-expression of one CBF gene causes the production of several genes that are susceptible to cold.

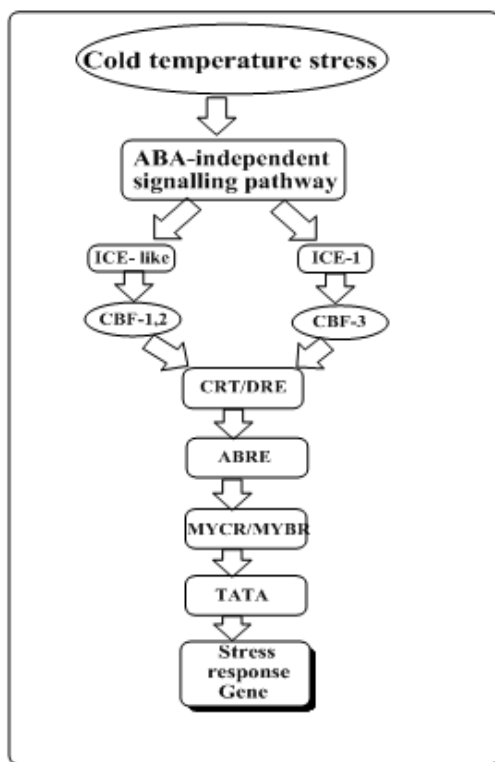


Figure 2: Signal Transduction Pathway for the Regulation of Cold Stress Response Gene Expression

However, the CCGAC motif is not present in the promoters of all cold-induced genes. Despite the fact that low temperature-induced gene expression, which is controlled by the CRT element, appears to be well conserved in plants. Figure 2 depicts the pathway of low temperature-induced gene expression. This pathway demonstrates how a streamlined signal transduction cascade controls a stress-induced gene's promoter elements. This diagram makes it obvious that the CRT element responds to two signalling channels. These signal transduction pathways, which are ABA independent, are ICE1 and ICE-like protein. The CBF gene is activated by ABA independent signalling. The basic zipper proteins AREB/ABF and the MYC/MYB transcription factors, which cooperatively bind to the MYCR/MYBR regions found in several ABA responsive genes, are two further sets of transcription factors that ABA also promotes the activation.

- 2. Cold Responsive Gene Regulation:** Cold-responsive gene regulation involves the activation or repression of particular genes in response to low temperature (cold) stress in plants. This regulatory process is crucial for plants to adapt and survive under cold conditions and involves various molecular mechanisms. The process begins with the perception of low temperature by temperature sensors or receptors in the plant cells. The mechanisms of temperature sensing are not fully understood, but changes in membrane fluidity and protein conformation are believed to play a role.

Cold stress triggers a cascade of signaling events that ultimately lead to the activation of specific transcription factors. One of the key transcription factor families involved in cold response is the CBF/DREB family. These transcription factors become activated upon exposure to low temperature and bind to particular DNA regions in the promoters of cold-responsive genes known as C-repeat/dehydration-responsive elements (CRT/DRE).

CBF/DREB1 transcription factors bind to CRT/DRE sites in the promoters of cold-responsive genes once activated. This binding activates the transcription of these genes, initiating the synthesis of cold-responsive gene products. The activated transcription factors, such as CBF/DREB1, induce the expression of a set of cold-responsive genes. These genes encode various cold tolerance proteins, including Cold-Regulated (COR) proteins, Dehydrins, and Late Embryogenesis Abundant (LEA) proteins. The products of cold-responsive genes, such as COR proteins, Dehydrins, and LEA proteins, are essential for protecting plant cells from cold-induced damage. They stabilize cellular structures, prevent ice crystal formation, and maintain cellular function under cold stress.

The promoter sequences of several cold-induced COR genes were studied and compared. The promoters of multiple different COR genes share a common regulatory region known as the C-repeat (CRT) or low temperature response element (LTRE), which is five nucleotides long and has a consensus sequence of CCGAC. This characteristic, known as the dehydration responsive element (DRE), had previously been linked to drought tolerance. The CBF (C-repeat binding factor) transcription factor is a key regulatory protein that plays a central role in cold stress response in plants. It is part of a larger family of transcription factors known as the AP2/ERF (APETALA2/Ethylene Response Factor) family. CBF transcription factors are particularly well-studied in the context of cold acclimation, which is the process by which plants become more cold-

tolerant in response to low temperatures. The structure of CBF1 in Figure 3 depicts the nuclear localization sequence, DNA binding domain, and an acidic region that may be involved in interactions with other proteins. Cold acclimation causes CBF1 expression, which then causes the expression of the COR genes. The COR regulon has been used to refer to this collection of genes that share a common regulation mechanism.

The regulation of CBF transcription factors involves a complex signaling pathway. The perception of cold stress leads to the activation of various components, including ICE1 (Inducer of CBF Expression 1) and other proteins, which ultimately results in the activation of CBF genes. CBF transcription factors control the expression of numerous downstream genes that are involved in cold acclimation. These genes encode proteins such as dehydrins, chaperones, antifreeze proteins, and enzymes involved in osmolyte biosynthesis. The products of these genes collectively contribute to the plant's ability to withstand freezing temperatures and other cold-related stresses. CBF transcription factors act as transcriptional activators. Once they bind to the CRT/DRE motifs in the promoter regions of target genes, they recruit other proteins and enzymes that facilitate the transcription of these genes into mRNA molecules.

However, not all cold-induced genes have the CCGAC element in their promoters, despite the fact that low temperature-induced gene expression, mediated by the CRT element, appears to be highly preserved in plants. Plants appear to have additional low temperature gene expression pathways that are not regulated by CRT/CBF, and the sequence element CCGAAA has been shown to confer low temperature inducibility on some genes.

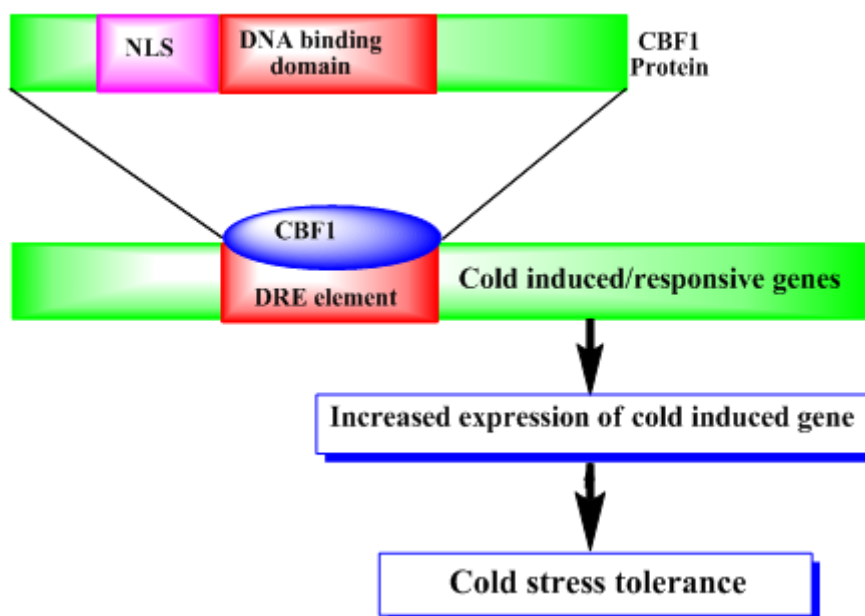


Figure 3: A number of cold reaction genes include the DRE element in their promoters, which is bound by transcription of the CBF family, which stimulates transcription upregulation of a single CBF gene, which induces the production of multiple cold sensitive genes. NLS (Nuclear localization signal)

Numerous genes that are induced by various conditions have been discovered through genetic engineering and molecular research. Numerous functional protein-coding stress inducible genes have been employed to increase stress tolerance. Several reviews have been published for the stress tolerance (Christensen and Feldmann 2007; Umezawa *et al.* 2006, Valliyodan and Nguyen 2006, Ahmed 2021). Transgenic plants that can withstand low temperatures have been created using a variety of transcriptional activators, including DREB1/CBF (Kasuga *et al.* 1999; Liu *et al.* 1998; kreps, 2002) that trigger the stress sensitive genes (Zhang, 2003). In many plant species, including rice (Dubouzet *et al.*, 2003; Ito *et al.*, 2006), pepper (Hwang *et al.*, 2005), chickpea (mantra *et al.*, 2007), and potato (Rensink *et al.*, 2005), the DREB/CBF genes have been successfully exploited to develop low temperature stress tolerance.

A crucial first step is to look for genes related to cold tolerance. Numerous experiments have been conducted to determine how well plants can withstand cold stress. The C-repeat element and low temperature responsive element are common regulatory elements found in the promoters of all genes associated to cold tolerance. provides a list of genes and transcription factors that are enhancing cold tolerance in various plants.

IV. CONCLUSION

In this chapter, we investigated the impact of climatic variables such as cold and salt on agricultural plants, emphasising the importance of both stresses in determining the substantial yearly changes in crop output. Genomics, the study of structure and function of the genome is, therefore, a subject that extends genetics from mere phenotype analysis to holistic analysis of the whole genome. The science of genomics has immense implications in every sphere of biological science from evolution to enhance crop yield. In fact it is expanding so rapidly and generating new information every moment that a new data is generated and reported almost every minute.

Plant fundamental biology under abiotic stress conditions is also understood via molecular genetics. The descriptive power of molecular analysis with the crop grown under stress circumstances, however, may be greatly increased using a novel method derived from functional genomics. The goal of molecular mapping is to create a map with a fine enough scale to identify the precise position of the genes crucial for defining key agronomic features.

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