**Application of Biotechnological Tools for Improving Heat and Drought Tolerance in Crops**

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6. **Introduction**

Biotechnology plays a crucial role in addressing abiotic stress in various organisms, particularly in the agricultural sector. Abiotic stresses include factors such as temperature extremes (heat and cold stress), drought, salinity, flooding, and heavy metal toxicity. These stresses can significantly impact crop productivity and limit the growth and survival of various plants and microorganisms. Different approaches are used in biotechnology to increase crop output and quality. We will talk about how crop plants are affected by environmental factors like high temperatures and water availability in this chapter. The two main environmental factors affecting crop productivity are heat and drought stress. According to Amit Kumar and R.S. Sengar (2013), both types of stress have an impact on the physiological, biochemical, and developmental processes of crops. **Fig 1.1** displays several physical pressures that could subject plants to two abiotic stresses and negatively impact their quality and output. The image also highlights the fact that most abiotic stimuli cause oxidative stress by producing free radicals and reactive oxygen species either directly or indirectly.



**Fig:1 The figure shows the heat and drought stresses on plant growth and development.**

Table 1.1 compares the impacts of biotic and abiotic pressures (weed, pest, and disease effects) on crop output. The stark contrast between crop yields on average and record yields is among the first things to be observed. These numbers make it very evident that abiotic stress is responsible for a large portion of the variance between record yield and average yield. As a result, the yield of wheat varies so much from year to year due to environmental factors that the average production is only 13% of the maximum. Contrarily, biotic stresses are controlled in industrialised farming so that they typically have a smaller yearly yield reduction than the most detrimental abiotic stresses. Therefore, increasing crops' resistance to abiotic stressors may help them maintain growth and development throughout typical oscillations in unfavourable conditions, protecting them from the significant swings in yield that occur from one year to the next.

**Table: 1.1 Average and record yields of some major crops**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Crop** | **Record yield**  **(Kg/ha)** | **Average Yield (kg/ha)** | **Average yield (% of record yield)** | **Average losses**  **(% of record yield)** | |
| **Biotic** | **Abiotic** |
| Wheat | 14500 | 1880 | 13.0 | 5.0 | 82.1 |
| Barley | 11400 | 2050 | 18.0 | 6.7 | 75.4 |
| Soybean | 7390 | 1610 | 21.8 | 9.0 | 69.3 |
| corn | 19300 | 4600 | 23.8 | 10.1 | 65.8 |

(Source: Bray *et al*. 2000)

Long-term environmental pressures on crop plants are projected to grow as a result of the predicted ozone layer depletion and climate changes brought on by global warming, making it even more important to create stress-tolerant crop types. Furthermore, there is pressure to expand the area of crop production into conditions (high temperature) that are not ideal for the growth of key crops. Therefore, one of the main goals of plant biotechnology and one that is anticipated to become more crucial is the production of stress-tolerant plants.

1. **Nature of Abiotic stress**

Abiotic stress refers to environmental factors or conditions that can adversely affect the growth, development, and survival of living organisms, particularly plants. Unlike biotic stresses caused by living organisms such as pests and pathogens, abiotic stresses are non-living factors that impact various organisms, including plants, animals, and microorganisms. These stresses can occur naturally or may be exacerbated by human activities. Abiotic stress can vary in intensity, duration, and geographical distribution. In many cases, organisms have evolved mechanisms to cope with certain levels of stress, and some species may be naturally more tolerant to specific abiotic stressors. However, with the increasing impact of climate change and human activities on the environment, abiotic stress events are becoming more frequent and severe, posing significant challenges for agriculture, forestry, and ecological systems. Biotechnological approaches, as mentioned in the previous response, are one of the strategies employed to mitigate the adverse effects of abiotic stress and enhance the resilience of organisms in such conditions.

It is important to first try to define stress in respect to plant physiology before talking about stress tolerance. Plants are susceptible to a wide range of environmental changes. Because of their sessile growth habit, plants are unable to employ many of the methods utilised by animals to mitigate the consequences of these changes. Because of this, plants rely heavily on internal processes to adapt to changes in their environment. Since plants can adapt to natural variation due to their flexibility, not all of these changes stress them out. As a result, plants have evolved to survive in a changing environment, and they can respond to typical exterior changes by changing internally without harming their ability to grow and develop. Only acute or persistent environmental extremes can result in environmental stress that has the ability to harm the plant physically.

In both natural and agricultural environments, plants commonly experience abiotic stresses such as excessive salt levels, toxicity, harsh temperatures, and poor water availability. In many instances, different types of abiotic stress provide a challenge to plants in tandem. For instance, extreme heat and water scarcity may be made worse by mineral toxins that limit root growth. Abiotic stressors are thought to lower yields to less than half of what would be feasible under optimum growth conditions across a variety of cropping systems worldwide (Boyer, 1982). Abiotic stress reactions in plants are intricate morphological and physiological occurrences, particularly in response to water shortage and excessive salt levels (wang et al., 2003). Changes in extracellular solute concentrations lead to osmotic stress and ultimately insufficient water availability at the cellular level. This water loss lowers turgor pressure and raises intracellular solute concentrations, which puts stress on membranes and macromolecules. Since abiotic stressors have the ability to diminish agricultural yields by 70%, they pose a significant obstacle in our quest for sustainable food production. Photosynthesis is hampered by acute water shortage (Gallagher et al., 1975). According to Parry (1990), changes in regional spatial and temporal temperature patterns would have a significant impact on agricultural production since temperature affects both plant growth and development (Porter and Moot, 1998).

It might be assumed that a broad variety of various tactics would be needed to engineer specific types of stress given the variety of abiotic pressures to which plants are exposed. The heat and drought-related pressures will be the main focus of this chapter. These water shortage-related damages are brought on by a variety of environmental factors, such as drought, heat, and cold.

1. **Tolerance to drought stress**

Tolerance to drought stress is the ability of an organism, typically plants, to survive and maintain relatively normal physiological functions and growth under conditions of water scarcity. Drought stress is a significant abiotic stress factor that can severely impact plant growth, development, and productivity. Plants have evolved various strategies to cope with and tolerate drought stress, and these mechanisms can vary among different species and even within different cultivars of the same species.

Drought tolerance is a complex trait influenced by both genetic and environmental factors. Plant breeders and biotechnologists work to identify and incorporate drought-tolerance-related genes and traits into crop plants through genetic engineering, marker-assisted breeding, and other advanced techniques to develop drought-resistant varieties that can sustain agriculture in water-limited regions.

In many parts of the world, agriculture is a major consumer of water. In the near future, water will become an even more precious resource due to rising aridity and an expanding population. Drought, which results in sub-optimal water availability for unconstrained plant development and transpiration, is a significant barrier to agricultural productivity (Boyer, 1982; Delmer, 2005). One of the most frequent environmental disturbances, drought alters gene expression and metabolism in plants, affecting their growth and development. It is a constant factor in the decline of agricultural production in many developing nations and a sporadic factor in wealthy nations (Ceccarelli and Grando, 1996). In India, 10% of the entire cultivable area is experiencing severe drought, making up 29% of the total (Anonymous, 2003).

High temperatures, intense irradiance, the inability of the soil to allow root penetration, and low water potential all offer stressors on crop plants cultivated under dry conditions. One of the main causes of numerous wheat yield losses on marginal land and a large reduction in yields in temperate regions is drought (Morris et al., 1991; Trethowan et al., 2001). The most significant abiotic stress limiting wheat output globally is drought (Chaves, 2003; Aberkane, 2021).

Arabidopsis, despite not being a crop plant, has been crucial in the understanding of the fundamental mechanisms underpinning stress tolerance, and the knowledge gained has somewhat been applied to significant food plants. Many of the genes that are understood to play a role in stress tolerance were first extracted from Arabidopsis. The potential of this approach is typically constrained by the tendency of cell systems to restore homeostasis, which controls two general strategies for the metabolic engineering of abiotic stress tolerance: increased production of specific desired compounds or reduction in the enzymatic step (Fig.1.2). A viable tool to break through species' reproductive isolation and make use of advantageous foreign genes is the transgenic approach.

Genetic study has shown numerous gene loci that regulate abiotic stress tolerance in plants. Many of the genes that regulate agronomically significant features, however, still need to be found and altered in order to produce new varieties with desired qualities. There is proof that abiotic stress tolerance is increased in transgenic plants whose expression of a single gene has been altered.



**Fig. 1.2 Methods to increase plant stress tolerance**

**3.1 Trait Associated with drought Stress**

Several traits are associated with drought stress tolerance in plants. These traits enable plants to withstand and survive water scarcity, ensuring their growth and productivity even under challenging environmental conditions. Plant breeders and researchers often focus on these traits when developing drought-tolerant varieties. The complicated process of a plant's reaction to drought stress is influenced by a variety of component reactions, each of which reacts differently to the severity and length of water deficiencies while also interacting with one another. It is one of the most frequent environmental stresses that alters gene expression and metabolism in plants, affecting their growth and development.

According to genomic research, drought activates a number of genes with various functions. The buildup of the hormone ABA triggers the expression of the majority of these genes. According to research (Ingram and Bartel, 1996; Shinozaki et al., 2003), ABA is accumulated 40 times greater during drought than under regulated conditions. It has been demonstrated that the hormone is key in promoting tolerance to the stress of drought. As a result, it was believed that ABA played a crucial function in regulating genes under water stress.

Under conditions of water deficiency, increased biosynthesis may lead to an accumulation of ABA (Bray, 1997). In response to numerous stimuli, including as dryness, low temperature, and hypoxia, ABA is synthesised in the root and shoot. 9-cis epoxycarotenoid dioxygenase (NCED), an enzyme that transforms the epoxy carotenoid precursor to xanthonin in the plastids, catalyses the crucial stage of ABA synthesis in roots and leaves (Qin and Zeevaart, 1999). Cytosolic enzymes then transform xanthonin to ABA via abscisic aldehyde. In cowpeas, tomatoes, beans, and maize, the NCED gene has been cloned, and it has been discovered that drought induces the promoter in the roots and leaves (Taylor et al., 2000; Thompson et al., 2000).

The availability of Arabidopsis mutants for certain features paved the way for genetic analysis of biochemical processes. Several genes were activated when ABA deficient and ABA insensitive (abi) mutants were under drought stress. This implied that these genes were expressed via a different route from ABA. Indeed, before the accumulation of ABA in water-stressed pea leaves, changes in the poly (A) RNA population were seen.

When dealing with rd clones in Arabidopsis thaliana, Yamaguchi-shinozaki et al. (1992) discovered that desiccation shock powerfully and swiftly generated the rd29 cDNA. This finding raised the possibility that rd29 has at least two cis-acting components. One contributed to the delayed response that is associated with ABA, but the other worked without it. A 8,048 bp long area of the Arabidopsis genome was where rd29 were found in tandem, according to genomic clone analysis.

**3.2 Marker assisted Selection for Drought stress**

Marker-assisted selection (MAS) is a powerful technique used in plant breeding to expedite the selection of desirable traits, such as drought stress tolerance, with increased efficiency and accuracy. MAS relies on the identification of genetic markers closely linked to the target trait of interest. These markers are then used to track the presence of the trait during the breeding process, allowing breeders to select plants with the desired trait more effectively.By using Marker-assisted selection, breeders can streamline the development of drought-tolerant varieties, significantly reducing the time and resources required compared to conventional breeding methods. This approach can ultimately contribute to the production of more resilient crops capable of thriving in water-limited environments and ensuring food security in the face of climate change and water scarcity.

In a breeding approach known as "marker assisted selection," the preferred allele from the donor source is monitored for integration using markers that are closely connected to the gene of interest rather than the gene itself. Numerous biological and economic factors will affect the precise application of genetic diagnostics in plant breeding, but the key determinant will be the cost-effectiveness of diagnostic technology that is compatible with the goals.

Genetic maps, molecular markers connected to agronomic features, high throughput, automated diagnostic technique, and a change in breeding practise that fully utilises the knowledge offered by such diagnostic assays are all necessary components of molecular breeding. The molecular markers' inherent repeatability, position on the map, and association with economically significant quantitative and qualitative qualities all affect how well they predict outcomes in MAS. A close relationship between qualitative features and a molecular marker may be advantageous for marker assisted selection to maximise the benefits of selection. The establishment of inbred lines and populations may be possible with MAS. By using map-based cloning techniques, QTLs and single genes that have been sufficiently mapped can be separated. The precision of the phenotypic classification of trait expression and the strength of the association between the markers and the traits of interest will determine how efficient any MAS is.

Using an adequate mapping population of plants, molecular markers are utilised to create precisely spaced genetic maps of a specific genome (Sehgal et al., 2008; Kumar 1999; Kumar 2023). The discovery of systemic linkage groups and individual chromosome maps in several taxonomic groups of crop plants, such as the Gramineae, has been strengthened by the use of molecular markers. This is especially true when repetitive DNA is removed and the maps for single copy sequences are compared. This demonstrates that even among crops as disparate as wheat and rice, the genes we are interested in are essentially the same in both species and their placements on the genetic maps are quite comparable. The majority of these maps were created using RFLP (Restriction fragment length polymorphism) markers, and more recently, different PCR-based markers have been used for the construction of genetic maps (Gupta and Rustogi 2004; Sehgal et al. 2008). These markers include SSR (Simple Sequence Repeats), RAPD (Random amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphis

* 1. **Candidate gene related to drought stress**

The main methods used to evaluate plant responses to drought in the past were growth analysis, in vitro assessment of enzyme, metabolite, and gene expression patterns, and leaf to atmosphere gas exchange. The use of genetic markers, genomics, and post-genomic methods has been the first molecular strategy to aid breeders in their efforts to promote drought tolerance (Nguyen et al., 2004; Lancers et al., 2004). A candidate gene is a gene that exhibits molecular polymorphisms, is genetically linked to a quantitative trait locus, or is statistically linked to the trait of drought tolerance. On the basis of their roles, the candidate genes can be split into two groups: those that protect cells from stress, such as osmotic stress, and those that control the expression of other genes that respond to stress.

A number of such genes have been identified, isolated, cloned and expressed in plants, which are potential source or resistance to drought stress. In **table 1.2** some of the candidate genes have been identified for drought tolerance gathered from literature survey.

**Table-1.2 List of Candidate gene for drought tolerance**

|  |  |  |  |
| --- | --- | --- | --- |
| **Candidate gene** | **Gene Product** | **Function** | **References** |
| *DREB* | Drought responsive element (DRE) binding factor | Transcription factor | Oh *et al.,* (2005)  Ito *et al.,* (2006) |
| *ERA1* | Farnesyl-transferase | Negative regulator of ABA sensing | Wang *et al.* (2005) |
| *Mn-SOD* | Mn-superoxide dismutase | ROS-scavenging Proteins | McKersie *et al.,* (1996) |
| *AVP1* | Vacuolar H+ phyrophosphatase | Ion transport | Park *et al.,* (2005) |
| *HVA1* | LEA Proteins | Protective Proteins | Xiao *et al.,*(2007) |
| *P5CS* | Pyrroline-5-carboxylate synthetase | Proline synthesis | Zhu *et al.,* (1998) |
| *ABF3* | ABA-responsive element | Transcription factor | Kang *et al.,* (2002) |
| *PARP* | Poly ADP-ribose polymerase | ROS (reactive oxygen species)-scavenging mechanism | De Block *et al.,*(2005) |
| *LTP* | Lipid transfer protein | Membrane biogenesis | Vinod *et al.*, (2006) |
| *NADP-Me* | NADP-malic enzyme | Carbon metabolism | Laporte *et al.,* (2002) |
| *MAPK* | Mitogen activated protein kinase | Signaling factor | Shou *et al.,* (2004) |
| *HRD* | Hardy protein | Transcription factor | Karaba *et al.,* (2007) |
| *AtNF-YB1* | NF-YB protein | Transcription factor | Nelson *et al.,* (2007) |
| *LFY* | Leafy protein | Transcription factor | Tuyen and Prasad., (2008) |
| *SRK2C* | Protein kinase | Signaling factor | Umezawa *et al.,*(2004) |

Groups of transcription factors that are controlled by abiotic stress in Arabidopsis were identified by Chen et al. in 2002. These transcription factors' intricate expression patterns imply that a highly complex gene regulation network regulates stress tolerance and resistance at the transcriptional level. Numerous candidate genes encoding transcription factors have been discovered using genome-wide transcriptome analysis and are either activated or repressed in response to environmental challenges (Chen, M. et al., 2007).

Twelve genes were identified as cold and drought target genes belonging to the DREB 1 transcription factor family through the transcript profiling (1300 genes) of Arabidopsis plants over expressing the gene encoding for dehydration response element binding protein (DREB 1a) (Seki et al., 2000). According to Shinozaki and Yamaguchi-Shinozaki (2007), genes involved in mechanisms to prevent water loss, safeguard cellular machinery, and repair damage are among those that are activated by drought. In various plant species, including Arabidopsis, the DREB 2 genes activated by drought stress have been found to confer drought tolerance. (Sakuma *et al.,* 2002), rice (Dubozet *et al.,* 2003), Wheat (Egawa *et al.,* 2006), maize (Qin *et al.,* 2007) Barley DRFs (Xue *et al.,* 2004).

**3.4 QTL Mapping for drought Tolerance**

Quantitative Trait Locus (QTL) mapping is a powerful technique used to identify genomic regions associated with quantitative traits, such as drought tolerance, in plants. QTL mapping allows researchers to understand the genetic basis of complex traits and can provide valuable information for breeding drought-tolerant crop varieties. QTL mapping for drought tolerance can significantly contribute to the development of improved crop varieties that can withstand water-limited conditions, thereby enhancing food security and agricultural sustainability in regions prone to drought stress.

Polygenes control quantitative traits, which are significantly influenced by the environment. In contrast to the discrete variation that is typical of qualitative qualities, it exhibits continuous variation as a result. Polygenes are those genes that affect a trait in a variety of ways, each of which has a minor but cumulative effect. One or more polygenes involved in determining a quantitative characteristic can be found at a location on a chromosome known as a quantitative trait locus.

Since sparse marker maps severely restrict the efficacy of QTL mapping, many DNA marker maps are not sufficiently dense to perform good QTL mapping. The size of the mapping population is necessary to identify QTLs with small effects, and the statistical significance cutoff determines the biological significance of the discovered QTLs. Environmental variables and genetic background strongly influence QTL mapping results; as a result, some QTLs may be evident in some settings but not in others. Analysing gene x gene and gene x environment interactions is one of the most effective uses of QTL mapping, however to fully analyse a system, numerous big, time-consuming studies are needed.

In order to map QTLs, DNA markers from every region of the genome were examined for their potential association with QTLs. A appropriate mapping population's individuals are examined in terms of their DNA marker genotypes, phenotypes, and the desired trait. The Individuals are divided into classes for each DNA marker based on marker genotypes. These markers are being utilised to find quantitative trait loci (QTL) related to drought and their effective transfer into commercially developed rice, wheat, maize, millet, and barley crop varieties. For the main cereal species, accurate genetic maps based on molecular marker technologies are already available (Snape et al., 2005; Langridge et al., 2006). QTL analysis is a crucial tool in the identification of genetic markers to support breeding efforts in crop species with vast, complicated genomes. Because the genome of wheat is polyploid, this method is challenging. In addition, rice, maize, and barley have low levels of polymorphism but are straightforward (Snape et al., 2005).

Numerous QTLs associated with the field assessment of stress tolerance have been extensively analysed in studies on the abiotic stress tolerance of cereals (Langridge et al., 2006). By creating high density genome linkage maps for crops like wheat, the advent and development of molecular markers in quantitative genetics substantially improves the study of complex quantitatively inherited traits (Xiao et al., 1996). The discovery and genetic mapping of loci influencing complex traits like grain yield and its contributing traits, which have enormous importance in plant breeding, are thus made possible by the use of molecular markers and interval mapping, which is implied to be a strong strategy (Broman et al., 1999). A lack of water during a drought may restrict a plant's growth and have an impact on how different plant parts develop. Recent developments in plant genomics have identified a large number of genes related to water stress that may be advantageous as well as methods for gene overexpression or silencing. Furthermore, they are transferable to next generations when introduced into transgenic plants with the proper promoters (Delmer, 2005; Ma S Bohnert, 2007).

According to a recent study by Tuberosa and Saliva (2007), genomics-based approaches give us access to agronomically desirable alleles that are located at quantitative trait loci (QTLs) that influence these responses, allowing us to more effectively increase the drought tolerance and yield of crops in water-limited environments. Three QTLs for heat tolerance, one each on chromosome arms 2DL (Mohammadi et al., 2008a), IBS and 5BL (Mohammadi et al., 2008b), and one QTL for drought tolerance were found on chromosome arm 7AL (Quarrie et al., 2005), and their linked SSR markers were used to validate them for use in MAS.

1. **Tolerance to heat stress**

Tolerance to heat stress is the ability of an organism, such as plants, animals, or microorganisms, to withstand and maintain normal physiological functions and growth under elevated temperatures. Heat stress can have detrimental effects on living organisms, leading to cellular damage, reduced growth, and even death if not adequately tolerated.

Plants, in particular, are highly susceptible to heat stress, which can adversely impact agricultural productivity and food security. However, certain plant species and cultivars have evolved various strategies to cope with heat stress and maintain their growth and reproduction even under high-temperature conditions.

Heat stress tolerance is a complex trait influenced by both genetic and environmental factors. Plant breeders and researchers are actively working to understand the underlying genetic mechanisms and identify heat tolerance-related genes to develop heat-resistant crop varieties. Advances in biotechnology, such as genetic engineering and marker-assisted breeding, offer promising avenues to enhance heat stress tolerance in crops, contributing to agricultural resilience in the face of climate change and global warming.

Heat shock proteins (HSPs), which may be grouped into five classes and are all highly conserved in prokaryotes and eukaryotes, are known to be produced in response to heat stress in a variety of organisms. These four are divided into the HSP 100, HSP 90, HSP 70, and HSP 60 classes based on their size, and it appears that each of their members serves as a molecular chaperone. Some of them participate in typical protein synthesis and folding and are expressed constitutively. By safeguarding or refolding denatured proteins, those brought on by heat seem to be engaged in reducing the effects of heat stress. Their expression is brought on by heat treatment, and in some circumstances, it is linked to the development of thermotolerance. Although the function of the fifth class of tiny HSPs, which are particularly prevalent in plants, is not yet known.

Individual HSPs have been made into plants to improve heat tolerance, in a manner similar to techniques for engineering cold tolerance. It is also known that a heat shock factor coordinates the quick heat shock reaction. Although this protein is constitutively produced, it normally resides as a monomer coupled to an HSP70 protein. Upon heating, attaches to a heat shock element shared by the HSP gene promoters. Five to seven repeats of the sequence NGAAN are found in the promoter near the TATA box.

There was no effect on thermotolerance when the AtHSF 1 gene was overexpressed in Arabidopsis because the transcription factor was inactive. The N or C terminus of the gus, however, cannot fuse with AtHSF1. A fusion protein created by a reporter gene had the ability to trimerize in the absence of heat. By introducing this fusion protein into Arabidopsis, scientists were able to create transgenic plants that express HSPs naturally and have improved thermotolerance without the need for prior heat treatment.

**4.1 Marker Assisted Selection for heat stress**

Molecular markers have been used in cereals for MAS for biotic challenges, abiotic stresses, and quality attributes as well as genotype identification, mapping, and tagging of valuable genes. Numerous genes have been identified and mapped for these features; tagged, cloned, and linked markers have also been created. In order to create genotypes with resistance, those have been successfully used in markers-assisted breeding programmes.

This method involves choosing suitable plants in the segregating generations by using molecular markers linked to significant agronomic features. This is especially beneficial for features for which standard plant breeding methods are either difficult or inefficient in terms of cost or time. Due to the realisation that increasing traits like water and nutrient use efficiency and tolerance to a variety of other abiotic stresses would be necessary to increase the productivity of all major food crops in order to meet the demands of future food and nutritional security, this has taken on significance in recent years.

One must be knowledgeable about either the marker trait association, as determined by linkage-based QTL interval mapping and LD-based association mapping, or the genomic estimated breeding values of individual markers, as calculated in the case of genomic selection, in order to meet the demands of molecular breeding. Numerous changes to the fundamental strategy that was initially offered have been suggested after extensive discussion of the advantages and limitations of these three distinct approaches.

The two most popular molecular methods for increasing plant stress tolerance are marker assisted selection and genetic editing. Since the development of molecular markers, allelic variation among the genes underlying characteristics can now be precisely and effectively recognised, making MAS a crucial part of the emerging field of molecular breeding (Mohammadi et al., 2008b). For the purpose of mapping the QTL implicated in stress tolerance, many markers, including RAPDs, AFLPs, RFLPs, and SSRs, have been described for diverse abiotic stresses (Korzun et al., 1999). However, only a small amount of study has been done to pinpoint genetic markers linked to heat tolerance in various plant species.

**4.2 Candidate gene Related to heat Stress**

Heat tolerance is expected to improve significantly if data on the presence of genetic variation for the traits associated with heat tolerance in crops is generated and these traits are tagged and mapped with an appropriate marker that can be used in marker assisted breeding. In order to produce plants with increased abiotic stress resistance, it has been reported that marker assisted selection (MAS) methods have substantially contributed in understanding the genetic basis of plant stress tolerance.

The production of thermotolerant crops has utilised a variety of transgenic techniques (Rodriguez, M. et al., 2005). Heat tolerance crops have been developed using a variety of heat shock proteins (HSPs). Many researchers looked at the degree of heat shock protein expression by altering the transcription factor (HSF), which is in charge of producing Arabidopsis that can withstand high temperatures under stress. A number of these genes, which may serve as a source of resistance to drought stress, have been found, isolated, cloned, and expressed in plants. Some of the putative genes for heat tolerance have been retrieved through a literature review and are shown in **Table 1.3.** A candidate gene is a gene that exhibits molecular polymorphisms, is genetically linked to a quantitative trait locus, or is statistically linked to the thermotolerance trait.

**Table-1.3 List of Candidate gene for Heat tolerance**

|  |  |  |
| --- | --- | --- |
| **Candidate gene** | **Function of Candidate gene** | **Reference** |
| *HSP 101* | Synthesis of Heat Shock Protein for Temperature Tolerance | ( Queitsch *et al.,*2000) |
| *APX 1* | H2O2 detoxification and conferred heat  Tolerance | (Shi WM 2001 *et al.,*2001) |
| *HSP 70* | Synthesis of Heat Shock Protein for Temperature Tolerance | (Montero-Barrientos *et al.,*2010) |
| *Cod A* | Glycine betaine systhesis for tolerance to temperature tolerance | (Alia HH *et al.,* 1998, Ayed *et al.,* 2021) |
| *HSP 17.7* | Synthesis of sHSP | (Malik M.K. *et al.,*1999) |
| *FAD 7* | Enhanced the level of Unsaturated fatty acid and provide temperature tolerance | (Sohn, SO *et al.,* 2007) |
| *AtPARP2* | Increased tolerance to heat stress | (Vanderauwera S et al.,2007) |
| *TLHS1* | Synthesis of Class I HSP | (Park, S.M *et al.,* 2002) |
| *AtHSF 1* | HSF fuse with β-glucuronidase and such modification will increase HSP production | (Lee JH *et al.,* 1995) |
| *ANP 1* | H2O2 responsive MAPK kinase kinase  (MAPKKK) production to protect against the  lethality in HT | (Kovtun Y *et al.,* 2000) |
| *MT-sHSP* | Molecular Chaperone | (Sanmiya K *et al.,* 2004) |
| *Dnak 1* | Tolerance to temperature | (Ono K *et al.,* 2001) |
| *BADH* | Over production of GB osmolyte that will  enhance the heat tolerance | (Salvucci ME *et al.,* 2004) |

**4.3 QTL mapping for heat tolerance**

One goal of molecular mapping is to create a map with a fine enough scale to identify the precise position of the genes involved in defining key agronomic features. Numerous of these qualities are characterised as quantitative, meaning that various environmental and genetic factors influence them. However, by assessing the association between the value of the quantitative trait and the allelic states at associated genetic markers, it is possible to map genes that have a significant impact on quantitative traits. A chromosomal region where there is thought to be a fair possibility that functionally distinct alleles segregate and have a meaningful impact on a quantitative characteristic is called a quantitative trait locus (QTL). To map QTLs, it is necessary to statistically analyse phenotypic and molecular marker data from a large population that segregates in order to identify the markers whose allelic polymorphism correlates with the phenotype of the quantitative trait. The gene is located within a chromosome area of roughly 10–30 cM, which may contain several hundred genes, according to this original QTL mapping, which is rough. Positional cloning and association mapping are two strategies that can be used to pinpoint the gene responsible for the quantitative trait.

In order to connect this map position to the DNA sequence and bring the QTL to a much finer resolution, more processes are needed for positional cloning. Crossing virtually isogenic lines where the sole allelic variation is found in the small region of the coarsely mapped QTL is one tactic. The QTL is described in the resulting populations, and a fine map with a more accurate cM distance between the QTL and nearby molecular markers can be created. This is only currently achievable for plants whose genomes have previously been, or are now being, sequenced. It is relatively simple to do this when there are numerous polymorphic markers mapped in the area. It may then be able to identify the gene from among the candidate genes in the site by identifying the mutation responsible for the QTL effect. At this point, the markers closest to the QTL are utilised to attach the genetic map to the physical map. Alternately, it could be necessary to overexpress or downregulate the gene to test each anticipated coding sequence in the area for functionality.

It has been difficult to pinpoint genetic markers in wheat and other crop species that are connected to high temperature tolerance. For a marker aided breeding programme to be successful, reliable marker trait associations are required (Kato et al., 2000). Quantitative trait locus mapping is the most efficient method for establishing these linkages (Patterson, 1998). Many researchers (Mohammadi et al., 2008 a; Mason et al., 2010; Pinto et al., 2010; Barakat et al., 2011 and Tiwari et al., 2013) have reported QTLs for heat tolerance. Currently, breeders frequently test their material by sowing their seeds later than usual, knowing that this would expose the plants to high temperatures during grain filling. Following this, selection is frequently based on yield performance. The CIMMYT strategy to generate high yielding early maturing wheat lines is promising for south Asia and Mexico, according to recent findings, which also demonstrate that simultaneous augmentation of yield potential and heat tolerance is feasible (Mondal et al., 2013). The ability to use marker-assisted selection to develop wheat with high temperature tolerance has been made possible by the identification of QTLs for heat tolerance (Paliwal et al., 2012).

1. **Conclusion**

In this chapter, we learned that the two main environmental factors that have an impact on the physiological and biochemical processes of plants are heat stress and drought stress. These environmental elements have an impact on crop development, yield, and growth. The purpose of this chapter is to inform readers that biotechnology is one of the finest methods for increasing crops' output by strengthening their capacity to withstand heat and drought stress. Molecular genetics is utilised in biotechnology to comprehend the underlying biology of plants under abiotic stress conditions. While molecular mapping aims to create a map with a fine enough scale to identify the precise location of genes that are crucial for defining essential agronomic aspects. However, by assessing the association between the value of the quantitative trait and the allelic states at associated genetic markers, it is possible to map genes that have a significant impact on quantitative traits. Recombinant Inbreed Line (RIL) production from parents is necessary for QTL mapping, which is closely related to resistance to abiotic stress. These populations will be useful in identifying the QTL's chromosomal location. This chapter's overarching conclusion is that heat and drought conditions cause complicated reactions in plants, and that the best defence may require a number of genes.

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