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

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6. **Introduction**

Biotechnology is critical in managing abiotic stress in diverse organisms, particularly in agriculture. Abiotic stresses include factors i.e, 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 discuss about how crop plants are affected by environmental factors like high temperatures and water availability. The two main abiotic stresses 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 figure also emphasises the fact that most abiotic stimuli produce oxidative stress by directly or indirectly creating free radicals and reactive oxygen species.

**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 thirteen percent of the maximum. Contrarily, biotic stresses are controlled in mechanized 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 Record yield, Average and Averate losses 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 O3 layer depletion and environmental changes brought on by global warming, making it even more important to create stress-tolerant crop types. Furthermore, there is pressure to push agricultural output into situations (high temperatures) that are not ideal for critical crop growth. As a result, the creation of stress-tolerant plants is expected to become a more prominent goal of plant biotechnology.

1. **Nature of Abiotic stress**

Abiotic stress refers to environmental factors or conditions that can adversely affect the expansion, growth, and survival of living organisms, particularly plants. Abiotic stresses, in contrast to biotic stresses brought on by living species like viruses and pests, have an impact on a variety of organisms, including plants, animals, and microbes. 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.

Before discussing stress tolerance, it is crucial to attempt to define stress in terms of plant physiology. A variety of environmental variables can affect plants. 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 the concentration of extracellular solutes cause osmotic stress, which ultimately results in 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).

Given the wide range of abiotic stresses to which plants are subjected, it might be predicted that a wide number of strategies would be required to engineer particular forms of stress. The heat and drought-related pressures will be the core center of attention of this chapter. These water shortage-related damages are brought on by a variety of clinatic factors, i.e., drought, heat, and cold.

1. **Tolerance to drought stress**

Tolerance to drought stress is the ability of plants to withstand and adapt to periods of limited water availability. Drought stress occurs when the demand for water exceeds its availability, leading to dehydration and physiological changes in plants. Tolerance to drought stress involves various mechanisms that allow plants to survive and maintain their functions even under water-deficient conditions. Drought tolerance involves the activation of stress-responsive genes. Transcription factors like DREB/CBF (Dehydration-Responsive Element Binding/C-repeat binding factor) play a role in activating genes involved in stress response, osmotic adjustment, and protective proteins. Drought stress can lead to the production of reactive oxygen species (ROS) that damage cells. Drought-tolerant plants have efficient antioxidant systems, including enzymes like superoxide dismutase and catalase, to neutralize ROS and prevent oxidative damage. In response to drought, plants close their stomata (tiny pores on leaves) to reduce water loss through transpiration. Drought-tolerant plants are often more efficient at regulating stomatal closure and preventing excess water loss. Drought-tolerant plants may adjust their photosynthetic rate to conserve water. They may switch to more water-efficient photosynthetic pathways or reduce the number of open photosynthetic centers.

Drought resistant is a composite trait influenced by both genetic and climatic 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, Water is largely consumed by agriculture. 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 Farmable land 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 resistance, 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 ability of this strategy to increase abiotic stress tolerance is typically limited by the tendency of cell systems to restore homeostasis, which regulates one of two general strategies for metabolic engineering of abiotic stress tolerance: increased production of specific desired compounds or decreased 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.

Numerous gene loci that control abiotic stress tolerance in plants have been identified through genetic research. Many of the genes that regulate agronomically significant features, however, still need to be found and altered in order to produce new cultivars with desired qualities. There is proof that abiotic stress tolerance is increased in genetic modified plants whose expression of a single gene has been altered.



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

**3.1 Trait related with drought Stress**

Several traits are related with drought stress resistant 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 climatic 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 is a type of carotenoid-derived compound that plays a crucial role in the plant's response to abiotic stress, particularly drought stress. Carotenoids are naturally occurring pigments found in plants and other organisms, serving various functions including photosynthesis and protection against oxidative stress. 9-cis epoxycarotenoids are involved in the biosynthesis of abscisic acid (ABA), a key hormone that regulates plant responses to environmental stresses (Qin and Zeevaart, 1999). 9-cis epoxycarotenoid is an intermediate compound in the biosynthesis of abscisic acid. This process begins with the cleavage of a carotenoid precursor (violaxanthin) to produce xanthoxin. Xanthoxin is then further converted to ABA through several enzymatic steps, with 9-cis epoxycarotenoid acting as a pivotal intermediate in this pathway. (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 lacking and ABA insensate (abi) mutants were under drought stress. This implied that these genes were expressed via a different route from ABA. In fact, alterations in the poly (A) RNA population were noticed prior to the deposition of ABA in water-stressed pea leaves.

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 breeding technique used in agriculture and genetics to enhance the efficiency of selecting desired traits in plants, animals, or other organisms. It involves the use of molecular markers—specifically identifiable genetic sequences that are linked to or associated with target traits—to aid in the selection of individuals with desired traits more accurately and rapidly than traditional breeding methods. Markers are often associated with traits that are not easily observable in early stages of development. MAS enables breeders to select for these traits even before they become visually evident. Phenotypic traits can be influenced by environmental factors, leading to variability in selection. MAS focuses on genetic markers, reducing the impact of environmental influences. MAS can be used for complex traits influenced by multiple genes. It allows breeders to simultaneously select for several traits 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. 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.

A change in breeding practises that fully leverages the knowledge provided by such diagnostic assays, high throughput, automated diagnostic technology, and molecular markers coupled to agronomic aspects are all essential elements of molecular breeding. How successfully the molecular markers predict outcomes in MAS depends on their inherent repeatability, location on the map, and relationship with economically significant quantitative and qualitative traits. 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. How effective any MAS is will depend on the accuracy of the phenotypic classification of trait expression and the strength of the link between the markers and the traits of interest.

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 particularly evident when the maps for single copy sequences are compared after repetitive DNA has been eliminated. This shows that the genes we are interested in are largely the same in both species and their locations on the genetic maps are relatively comparable, even in crops as different as wheat and rice. Restrictions fragment length polymorphism (RFLP) markers made up the majority of these maps, but more recently, PCR-based markers have also been utilised to build genetic maps (Gupta and Rustogi 2004; Sehgal et al. 2008). These markers include AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeats).

* 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 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 candidate genes for drought tolerance have been found in table 1.2 based on a literature review.

**Table-1.2 List of potential gene for drought Resistance**

|  |  |  |  |
| --- | --- | --- | --- |
| **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).

Through the transcript profiling (1300 genes) of Arabidopsis plants over expressing the gene encoding for dehydration response element binding protein (DREB 1a), twelve genes were discovered to be cold and drought target genes belonging to the DREB 1 transcription factor family (Seki et al., 2000). Activated genes include those engaged in mechanisms to stop water loss, protect cellular machinery, and repair damage, according to Shinozaki and Yamaguchi-Shinozaki (2007). In various plant species, including Arabidopsis, DREB genes are transcription factors, meaning they regulate the expression of other genes. When a plant is exposed to drought conditions or other stressors, DREB genes are activated, leading to the activation of stress-responsive genes that help the plant adapt and survive in challenging conditions. These genes are of particular interest in agricultural research, as understanding and manipulating their activity could potentially lead to the development of more drought-resistant crops, which could be crucial for maintaining food security in regions affected by water scarcity. (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.

Many DNA marker maps are too thick to perform effective QTL mapping because sparse marker maps substantially limit its effectiveness. 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).

Studies on the abiotic stress tolerance of cereals (Langridge et al., 2006) have thoroughly investigated a number of QTLs related to the field assessment of stress tolerance. The introduction and use of molecular markers in quantitative genetics significantly improves the study of complex quantitatively inherited traits by enabling the creation of high density genome linkage maps for crops like wheat (Xiao et al., 1996). Thus, the use of molecular markers and interval mapping, which is implied to be a strong strategy, is made possible for the discovery and genetic mapping of loci influencing complex traits like grain yield and its contributing traits, which have enormous importance in plant breeding (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 high temperature treatment, and in various 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.

Similar to methods for engineering cold tolerance, specific HSPs have been incorporated into plants to enhance high temperature 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.

The transcription factor was inactive, hence overexpression of the AtHSF 1 gene in Arabidopsis had no impact on thermotolerance. 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 genetic modified plants that express HSPs naturally and have improved heat resistant without the need for prior heat treatment.

**4.1 Marker Assisted Selection for heat stress**

In cereals, molecular markers have been applied to qualitative qualities, abiotic pressures, and biotic challenges as well as to genotype identify, map, and tag important genes. For these traits, several genes have been found and mapped, and tagged, cloned, and linked markers have also been developed. These have been successfully utilised in markers-assisted breeding efforts to develop genotypes with resistance.

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 resistance to a variety of other environmental stresses would be essential to increase the production of all major food crops in order to meet the demands of future food and nutritsional security, this has taken on significance in recent years.

Marker-trait association, often referred to as "marker-trait linkage" or "genetic marker-trait association," is a concept used in genetics and genomics to describe the relationship between specific genetic markers and traits or characteristics of interest in organisms, such as plants, animals, or humans.

Genetic markers are specific DNA sequences that are easily identifiable and can be used to track the inheritance of genes or genomic regions. These markers serve as signposts on the genome, helping researchers locate and study genes associated with particular traits. Traits can encompass a wide range of characteristics, including disease susceptibility, physical attributes, behavior, or responses to environmental factors.

The advent of advanced genomic technologies has greatly accelerated the process of identifying marker-trait associations. Techniques like genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping allow researchers to analyze large sets of genetic markers and their association with traits across populations. These approaches have enabled the identification of specific genes or genomic regions responsible for variations in traits.

It's worth noting that while marker-trait association can provide valuable insights into genetic relationships, it doesn't always establish causation. Correlation between a marker and a trait doesn't necessarily mean that the marker directly causes the trait; it could be in linkage with another nearby gene responsible for the trait. Further functional studies are often needed to confirm causative relationships.

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**

If data on the presence of genetic diversity for the traits linked with heat tolerance in crops are generated and these qualities are tagged and mapped with an appropriate marker that can be used in marker aided breeding, heat tolerance is projected to considerably increase. It has been claimed that marker assisted selection (MAS) techniques have significantly contributed to understanding the genetic basis of plant stress tolerance, enabling the development of plants with higher abiotic stress resistance.

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**

Quantitative Trait Locus (QTL) mapping for heat tolerance is a technique used in genetics and genomics to identify regions of a genome that are associated with variations in heat tolerance traits in organisms. Heat tolerance is an important trait in various contexts, including agriculture, where it affects crop productivity, and in understanding the impacts of climate change on different species. 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. The trait of interest (heat tolerance in this case) needs to be accurately measured or quantified. This could involve assessing the response of individuals to heat stress in controlled environments or field conditions. Traits related to heat tolerance might include survival rate, growth rate, photosynthetic efficiency, or physiological responses like heat shock protein expression.

Statistical methods are then used to identify regions of the genome (QTLs) that are associated with variations in the trait. This is done by looking for correlations between marker genotypes and trait phenotypes across the population. The significance of these correlations is determined through statistical tests. QTLs that are identified in the initial analysis need to be validated. This can involve further experiments or studies to confirm that the identified genomic regions consistently influence the trait of interest. If the QTL regions are large, researchers may conduct further analysis to narrow down the regions and identify specific candidate genes that might be responsible for the observed heat tolerance variation. QTL mapping for heat tolerance is crucial for breeding heat-tolerant varieties of crops, understanding the genetic basis of thermal adaptation in different organisms, and mitigating the impacts of climate change. It's important to note that this process can be complex and resource-intensive, requiring expertise in genetics, genomics, statistics, and bioinformatics.

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 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, it is possible to map genes that significantly influence quantitative traits by evaluating the connection between the value of the quantitative trait and the allelic states at related genetic markers. Recombinant Inbreed Line (RIL) production from parents is necessary for QTL mapping, which is closely related to tolerance to environmental 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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