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

**Amit Kumar, Vishal Kumar, Anuj Yadav**

Department of Agriculture, Faculty of Science,

Swami Vivekanand Subharti University, Meerut-250005 (UP)

Email- [amit.agbiotech1582@gmail.com](mailto:amit.agbiotech1582@gmail.com)

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6. **Introduction**

Biotechnology is the best ways by which the productivity of crops can be improved by enhancing their ability to resist or tolerate biotic and abiotic stresses. In biotechnology different strategies are involved for the improvement of crop yield and quality. In this chapter, we will discuss the effects of environmental conditions such as high temperature and water availability on crop plants**.** Heat and drought stress are the two main environmental factors which influencing crop productivity. Both these stress affect on physiological, Biochemical and developmental processes of crop (Amit kumar and R.S.Sengar., 2013). **Fig 1.1** shows a number of physical stresses that may impose two abiotic stresses on plants and adversely affect their quality and yield. The figure also emphasizes the point that most abiotic stresses directly or indirectly lead to the production of free radicals and reactive oxygen species, creating oxidative stress.



**Fig:1 The figure shows the heat and drought stresses on plant growth and development. The figure emphasizes the point that virtually both stresses result in the production of reactive oxygen species (ROS) and this create oxidative stress.**

The impact of abiotic stresses on crop yield compared with biotic stresses (weed, pest and disease effects) is shown in **Table 1.1**. One of the first things to notice is the large difference between the average yields of crops and the record yields. It is clear from these data that the major difference between record yield and average yield is accounted for by abiotic stress. Thus, the variation in environmental conditions from one year to the next produces such a variation in yield for wheat that the average yield is only 13% of the maximum. In contrast, the control of biotic stresses in industrialized farming is such that they tend to reduce the annual yield by a fairly stable proportion, which are generally less that the most adverse abiotic stresses. Improving the tolerance of crops to abiotic stresses could therefore enable them to maintain growth and development during the normal fluctuations of adverse conditions, and consequently buffer crops against the large swings in yield experienced 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)

In the long term, the predicted depletion of the ozone layer and climate changes associated with global warming are likely to add to the burden of environmental stresses on crop plants, and increase the imperative to develop stress tolerant varieties. Furthermore, there is increasing pressure to extend the area of crop cultivation to environments that are not optimal for the growth of major crops (high temperature condition). The development of stress tolerant plants is therefore a major aim of plant Biotechnology, and one that is likely to become increasingly important.

1. **Nature of Abiotic stress**

When discussing the subject of stress tolerance, it is necessary first to try to define stress in relation to plant physiology. Plants are subject to many types of fluctuation in the physical environment. Many of the strategies used by animals to avoid the effects of these fluctuations are not available to plants, because of the sessile nature of their growth habit. Plants therefore depend largely upon internal mechanisms for tolerating variations in the external environment. Not all such fluctuations present a stress to plants, since they are able to cope with normal variation by virtue of their plasticity. Thus, plants are adapted to function in a fluctuating environment and normal external changes are countered by internal change without detriment to growth and development. It is only acute or chronic extremes of environmental condition that lead to environmental stress that has the potential to cause physical damage to the plant.

Abiotic stresses such as extreme temperatures, low water availability, high salt levels, mineral deficiency and toxicity are frequently encountered by plants in both natural and agricultural systems. In many cases, several classes of abiotic stress challenge plants in combination for example, high temperature and scarcity of water can be exacerbated by mineral toxicities that constrain root growth. Across a range of cropping systems around the world, abiotic stresses are estimated to reduce yields to less than a half of that possible under ideal growth conditions (Boyer, 1982). Abiotic stress reactions, especially to water deficiency and high level of salts, are complex morphological and physiological phenomena in plants (wang *et al.,* 2003). At the cellular level, alterations in extra cellular solute concentrations cause osmotic stress and finally inadequate water availability. This water loss causes a decrease in turgor pressure and an increase in concentrations of intracellular solutes, which imparts a strain on membranes and macromolecules.

Abiotic stresses present a major challenge in our quest for sustainable food production as these may reduce the potential yields by 70% in crops. Acute water deficiency impairs photosynthesis (Gallagher *et al.,* 1975). Changes in the global climate, notably in regional spatial and temporal temperature patterns are predicted to have important consequences for crop production (Parry., 1990), both plant growth and development are affected by temperature (Porter and Moot., 1998).

Given the range of abiotic stresses to which plants are exposed, it might be thought that a wide range of different strategies would be required to engineer particular types of stress. This chapter will concentrate on the heat and drought stresses. These damage that result from water deficit caused by a number of different environmental conditions, including drought, heat and cold.

1. **Tolerance to drought stress**

Agriculture is a major user of water resources in many regions of the world. Which increasing aridity and a growing population, water will become an even scarcer commodity in the near future. Sub optimal availability of water for unrestricted plant growth and transpiration, i.e. drought, is a major limitation to agricultural production (Boyer, 1982; Delmer, 2005). Drought is one of the most common environmental stresses that affect growth and development of plants through alternations in metabolism and gene expression. It is a permanent to agriculture production in many developing countries and occasional cause of losses of agriculture production in developed ones (Ceccarelli and Grando., 1996). In India, 29 percent of the total cultivable area faces drought condition out of which 10 percent is under severe drought (Anonymous, 2003).

Crop plants grown under drought conditions are exposed to a combination of stresses that are attributable to high temperatures, excessive irradiance, soil resistance to root penetration and low water potential. Drought is one of the major factor contributing to several yield losses of wheat grown in marginal land and to significantly reduce yields in temperate areas (Morris *et al.,* 1991; Trethowan *et al.,* 2001). Drought is the most serious abiotic stress limiting wheat production in different parts of the world (Chaves, 2003; Aberkane, 2021).

Although not a crop plant, Arabidopsis has played a vital role in the elucidation of the basic processes underlying stress tolerance and the knowledge obtained has been transferred to a certain degree to important food plants. Many of the genes known to be involved in stress tolerance have been isolated initially from Arabidopsis. Two general strategies for the metabolic engineering of abiotic stress tolerance have been proposed which increased production of specific desired compounds or reduction in the enzymatic step is usually regulated by the tendency of cell systems to restore homeostasis, thus limiting the potential of this approach (**Fig.1.2**). Transgenic approach is a useful technology to overcome reproductive isolation among species and utilize beneficial exotic genes.

Many loci for gene that control tolerance to abiotic stress in plants have been identified by genetic analysis. However, many genes that control agronomically important traits remain to be identified and modified to generate new varieties with desirable traits remain to be identified and modified to generate new varieties with desirable traits. There is evidence that transgenic plants in which the expression of a single gene has been modified have enhanced tolerance to abiotic stress.



**Fig. 1.2 Approaches to develop stress tolerance plant**

**3.1 Trait Associated with drought Stress**

Plant response to drought stress is a complex process conditioned by a number of component responses that both interact and differ in their individual responses to the intensity and duration of water deficits. It is one of the most common environmental stresses that affect growth and development of plants through alterations in metabolism and gene expression.

Genomic studies have shown that several genes with various functions are induced by drought. Expression of most of these genes is induced by the accumulation of hormone ABA. It has been found that ABA is accumulated 40 times more in drought over the controlled condition (Ingram and Bartel, 1996; Shinozaki *et al.,* 2003). The hormone has been shown to play important role in imparting tolerance against drought stress. Hence, the role of ABA in controlling genes under water stress was assumed to be critical.

Accumulation of ABA under water deficit may result from enhanced biosynthesis (Bray, 1997). ABA is synthesized in root and shoot in response to various stresses including drought, low temperature and hypoxia. The key step of ABA synthesis in roots and leaves is catalyzed by 9-cis epoxycarotenoid dioxygenase (NCED), an enzyme that converts the epoxy carotenoid precursor to xanthonin in the plastids (Qin and Zeevaart, 1999). Xanthonin is then converted to ABA by cytosolic enzymes via abscisic aldehyde. The NCED gene has been cloned in cowpeas, tomatoes, beans and maize and shown to have a promoter that is induced by drought in roots and leaves (Taylor *et al.,* 2000; Thompson *et al.,* 2000).

Availability of Arabidopsis mutants for defined traits opened paths to dissect biochemical processes at genetic level. When ABA deficient and ABA insensitive (abi) mutants were subjected to drought stress, several genes were induced. This suggested that expression of these genes followed an ABA independent pathway. Indeed, alterations in poly (A) RNA population were observed prior to accumulation off ABA in water stressed pea leaves.

While working with *rd* clones in *Arabidopsis thaliana*, Yamaguchi-shinozaki *et al.* (1992), reported that *rd29* cDNA was induced very quickly and strongly by desiccation stress. This observation was suggested that *rd29* could have at least two *cis*-acting elements. One was involved in ABA-associated slow response and the other functioned independently of ABA. Genomic clone analysis showed that *rd29* were located in tandem in a 8,048 bp long region of Arabidopsis genome.

**3.2 Marker assisted Selection for Drought stress**

Marker assisted selection is the breeding strategy in which selection for a gene is based on molecular markers closely linked to the gene of interest rather than the gene itself, and the markers are used to monitor the incorporation of the desirable allele from the donor source. The exact way in which genetic diagnostics are applied in plant breeding will be influenced by many considerations, both biological and economic, but the availability of cost effective diagnostic technology that is compatible with the objectives will be the main factor.

Molecular breeding requires the following technology: genetic maps, molecular markers linked to agronomic traits, high throughput, automated diagnostic technique and a modification in breeding practice that takes full advantage of the information provided by such diagnostic assays. The predictive value of molecular markers used in MAS depends on their inherent repeatability, map position and linkage with economically important quantitative and qualitative traits. The presence of a tight linkage between qualitative traits and molecular marker may be useful in marker assisted selection to increase gain from selection. MAS may have potential in population and inbreed line development. When QTLs and single gene and adequately mapped, they can be isolated by map based cloning strategies. The effectiveness of any MAS will depend on the accuracy of the phenotypic classification of trait expression and the degree of linkage between the markers and traits of interest.

Molecular marker is used to construct closely spaced genetic maps of a particular genome using an appropriate mapping population of plants (Sehgal *et al.,* 2008; Kumar 1999; Kumar 2023). Molecular marker mapping has strengthened our realization that, in several taxonomic groups off crop plants *e.g.,* the Gramineae, that share the same basic chromosome number, the linkage groups and the individual chromosome maps look very similar, *i.e.,* they are systemic, particularly when we take out the repetitive DNA and compare the maps for single copy sequences. This means that even between crops as diverse as wheat and rice the genes we are interested in are basically the same in both species and their locations in the genetic maps are very similar. Most of these maps have been made using RFLP (Restriction fragment length polymorphism) markers and more recently different PCR based marker are used for the construction of genetic map (Gupta and Rustogi 2004; Sehgal *et al.* 2008) such as SSR (Simple Sequence Repeats), RAPD (Random amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), STS (Sequence Sequence Repeats) etc. allow loci controlling trait related to stress tolerance to be identified and mapped in the genome.

**3.3 Candidate gene related to drought stress**

Traditional evaluation of plant responses to drought has relied mainly on leaf to atmosphere gas exchange, growth analysis and in vitro determination of enzymes, metabolites, and gene expression patterns. The first molecular approach to help breeders in their efforts to increase drought tolerance has been with molecular marker, genomics and post genomics strategies (Nguyen *et al*.,2004 and Lancers *et al*., 2004) Candidate gene can be defined as the genes showing molecular polymorphisms and genetically associated with QTL or statistically associated with drought tolerance trait. The candidate genes can be divided into two categories on the basis of their functions, in the first categories genes involved in cell protection during stress such as osmotic and second, genes which are involved in the regulation of other genes for the stresses responses.

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) |

Chen *et al.,* (2002) identified groups of transcription factors regulated by the abiotic stress in Arabidopsis. The expression patterns of these transcription factors are highly complex which suggest that stress tolerance and resistance are controlled at the transcriptional level by an extremely intricate gene regulatory network. Genome wide transcriptome analysis has identified hundreds of candidate genes encoding transcription factors are induced or repressed by environmental stresses (Chen, M *et al.,* 2007).

The transcript profiling (1300 genes) of Arabidopsis plants over expressing the gene encoding for dehydration responsive element binding protein (DREB 1a) identified 12 genes as cold and drought target genes belonging to DREB 1 transcription factors family (Seki et al., 2000). The genes activated by drought include those involved I mechanism to avoid water loss, protect the cellular machinery and repair damage (Shinozaki and Yamaguchi-Shinozaki, 2007). The DREB 2 genes induced by drought stress, have been reported to confer drought tolerance in several plat species such as Arabidopsis (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 is governed by polygenes and is markedly affected by the environment. As a result, it shows a continuous variation as opposed to the discrete variation that is characteristic of qualitative traits. Polygenes are those genes that have small but cumulative effect on the concerned trait, and several polygenes affect a single trait. A quantitative trait locus is a position in a chromosome that contains one or more polygenes involved in the determination of a quantitative trait.

Many of DNA marker maps are not sufficiently dense to achieve satisfactory QTL mapping, since sparse marker maps severely limit the power of QTL mapping. The mapping population must be relatively large in order to detect QTLs having minor effects, and the biological relevance of the uncovered QTLs depends on the cut-off chosen for statistical significance. In QTL mapping, environmental factors and genetic background have a marked impact on the results; as a result, some QTLs may be detectable in some but not in other environments. One of the most powerful applications of QTL mapping is to analyze gene x gene and gene x environment interactions, but this requires many large, time consuming experiments to allow a thorough analysis of a system.

QTL mapping involved testing DNA markers throughout the genome for the likelihood that they are associated with a QTL. Individual in a suitable mapping population are analyzed in terms of DNA marker genotypes and the phenotypes and the phenotype of interest. For each DNA marker, the Individuals are split into classes according to marker genotypes. These markers are being used to identify drought related quantitative trait loci and their efficient transfer into commercially grown crop varieties of rice, wheat, maize, millet and barley. Good genetic maps based on molecular marker technologies are now available for major cereal species (Snape *et al.,* 2005; Langridge *et al.,* 2006). In crop species with large, complex genomes, QTL analysis is an important tool in the identification of genetic markers to assist breeding efforts. This approach is complicated in wheat because of the polyploidy nature of the genome. And the low levels of polymorphism, but is straight forward in rice, maize and barley (Snape *et al.,* 2005)

Studies on the abiotic stress tolerance of cereals include the extensive analysis of QTLs linked to the field evaluation of stress tolerance (Langridge *et al.,* 2006). Advent and development of molecular markers in quantitative genetics greatly facilitates the study of complex quantitatively inherited traits by the construction of high density genome linkage maps for crops such as wheat (Xiao *et al.,* 1996). This implies that the use of molecular markers and interval mapping is a powerful approach, which permits the identification and genetic mapping of loci controlling complex traits like grain yield and its contributing traits having great importance in plant breeding (Broman *et al.,* 1999). Inadequate availability of water during drought may limit the size of the plant and also affect the development of various plant parts. Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water stress related genes, plus technologies for gene over expression or silencing. Moreover, these can be introduced into transgenic plants under the control of appropriate promoters and are transmitted to subsequent generations (Delmer, 2005; Ma S Bohnert, 2007).

Recently, Tuberosa and Saliva (2007) reported that genomics based approaches provide access to Agronomically desirable alleles present at quantitative trait loci (QTLs) that affect such responses, thus enabling us to improve the drought tolerance and yield of crops under water limited conditions more effectively. QTLs for drought tolerance, one each located on chromosome 7AL (Quarrie *et al.,* 2005) and three QTLs for heat tolerance, one each located on chromosome arms 2DL (Mohammadi *et al.,* 2008a), IBS and 5BL (Mohammadi *et al.,* 2008b) and their linked SSR markers were used for validation for their eventual exploitation during MAS.

1. **Tolerance to heat stress**

For many years it has been known that heat stress applied to a wide range of organisms induces a specific set of heat shock proteins (*HSPs*); they fall into five classes four of which are highly conserved in prokaryotes and eukaryotes. These four are categorized according to size as the *HSP 100, HSP 90, HSP 70* and *HSP 60* classes whose members appear to function as molecular chaperones. Some of them are expressed constitutively and are involved in normal protein synthesis and folding. Those induced by heat appear to be involved in countering the effects of heat stress by protecting or refolding denatured proteins. Their expression is induced by heat treatment and, in some cases, can be correlated with the acquisition of thermotolerance. The fifth group of several classes of small HSPs is particularly abundant in plants, but their function is not yet clear.

In a way analogous to strategies for engineering cold tolerance, individual *HSPs* have been transformed into plants to enhance heat tolerance. However, it is also known that the rapid heat shock response is co-ordinated by a heat shock factor. This protein is expressed constitutively, but in normal conditions exists as a monomer bound to one of the *HSP70* proteins. Upon heat binds to a heat shock element common to the promoters of *HSP* genes. Sequence NGAAN; five to seven of these repeats occur in the promoter close to the TATA box.

When the *AtHSF 1* gene was over expressed in Arabidopsis, the transcription factor was not active, and there was no effect on thermotolerance. However, fusion of *AtHSF1* to the N or C terminus of the *gus A* reporter gene produced a fusion protein that was able to trimerize in the absence of heat. Transformation of this fusion protein into Arabidopsis produced transgenic plants that expressed *HSPs* constitutively and demonstrated enhanced thermotolerance without requiring prior heat treatment.

**4.1 Marker Assisted Selection for heat stress**

Molecular marker have been applied in quantification of genetic diversity, genotype identification, mapping and tagging of useful genes and marker assisted selection in cereals for biotic stresses, abiotic stresses and quality traits. Many genes for those traits have been mapped; tagged, cloned and linked markers have been developed. Those have been successfully used in markers assisted breeding programme to develop genotypes with resistance.

This approach involves the use of molecular markers associated with important agronomic traits for selection of desirable plants in the segregating generations. This is particularly desirable for traits, for which selection through conventional method of plant breeding is either difficult or cost/time ineffective. This has assumed significance in recent years due to the realization that improvement of traits like water use efficiency and nutrient use efficiency along with tolerance to a number of other abiotic stresses would be necessary to augment the productivity of all major food crops, as is required to meet the future demands of food and nutritional security.

In order to meet the demands of molecular breeding, one needs to have the knowledge either about the marker trait association, as determined in case of linkage based QTL interval mapping, and LD based association mapping, or about the genomic estimated breeding values of individual markers as worked out in case of genomic selection. The merits and limitation of these three different approaches have been widely discussed, and improvements in the basic proposed initially have been suggested.

Marker assisted selection and genetic engineering are two most common molecular approaches for improving stress tolerance in plants. With the advent of molecular markers, MAS has become an essential component of new discipline termed as molecular breeding with the help of which allelic variation among the genes underlying traits can be precisely and efficiently detected (Mohammadi *et al.,* 2008b). Numerous markers such as RAPDs, AFLPs, RFLPs and SSRs have been reported for various abiotics stress for achieving mapping of the QTL involved in stress tolerance (Korzun *et al.,*1999). Comparatively, however, limited research has been conducted to identify genetic markers associated with heat tolerance in different plant species.

**4.2 Candidate gene Related to heat Stress**

The enhancement of heat tolerance is expected to be much higher if information is generated about the presence of genetic variation for the traits associated with heat tolerance in crops, and tagging and mapping of these traits with a suitable marker that can be used in marker assisted breeding. It has been observed that marker assisted selection (MAS) approaches has contributed greatly to a better understanding of the genetic bases of plant stress tolerance, which led to the development of plants with enhanced tolerance of abiotic stress.

Several Transgenic approaches have been used for the development of thermotolerance crops (Rodriguez,M *et al*.,2005). Many heat shock proteins (*HSPs*) have been used for the development heat tolerance crops. Many scientist studied that the expression level of heat shock proteins by making a change in the transcription factor (*HSF*) which is responsible for the production of high temperature stress tolerant *Arabidopsis*. 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 1.3** some of the candidate genes have been identified for heat tolerance gathered from literature survey. Candidate gene can be defined as the genes showing molecular polymorphisms and genetically associated with QTL or statistically associated with thermo tolerance 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 of the aims of molecular mapping is to produce a sufficiently fine scale map to pinpoint the location of genes that play a role in determining important agronomic traits. Many of these traits are described as quantitative; that is they are influenced by multiple genetic and environment factors. However, it is possible to map genes that have a major effect on quantitative traits by evaluating the correlation between the value of the quantitative trait and the allelic states at linked genetic markers. A quantitative trait locus (QTL) is therefore a chromosomal location where there is considered to be a reasonable probability that functionally different alleles segregate and cause significant effects on a quantitative trait. QTL mapping requires a statistical analysis of molecular marker and phenotypic data from a large segregating population to determine those markers where allelic polymorphism correlates with the quantitative trait phenotype. This primary QTL mapping is coarse and locates the gene within a chromosome region of approximately 10-30 cM, which could contain several hundred genes. To identify the gene involved in the quantitative trait, two methods are available: positional cloning and association mapping.

Positional cloning requires further steps to the QTL to a much finer resolution and relate this map position to the DNA sequence. One strategy is to cross nearly isogenic lines in which the only allelic variation occurs in the short region of the coarse mapped QTL. In the resulting populations, the QTL is described a mendelized and fine map with more precise cM distance between the QTL and adjacent molecular markers can be produced. This is relatively straightforward when there are many polymorphic markers mapped in the region, but this is currently possible only for those plants whose genome has already been, or is in the process of being, sequenced. At this stage, the markers closest to the QTL are used to anchor the genetic map to the physical map and it may then be possible to determine the gene responsible from candidate gene in the location by identifying the mutation responsible for the QTL effect. Alternatively, it may be necessary to test each predicted coding sequence in the region functionally, by overexpressing or down regulating the gene.

To date, the level of success in identifying genetic markers associated with high temperature tolerance in wheat, and indeed other crop species, has been limited. Reliable marker trait associations are a prerequisite for an effective marker assisted breeding program (Kato *et al.,* 2000), and these are most effectively established via quantitative trait locus mapping (Patterson, 1998). QTLs fro heat tolerance has been reported by a number of worker (Mohammadi *et al.,* 2008 a; Mason *et al.,* 2010; Pinto *et al.,* 2010; Barakat *et al.,* 2011 and Tiwari *et al.,* 2013). At present, the typical breeders’ strategy is to challenge the material by sowing late, in the knowledge that this will expose the plants to high temperatures during grain filling; after this, selection is commonly based on yield performance. Recent results show that simultaneous enhancement of yield potential and heat tolerance is possible and that the CIMMYT stragety to develop high yielding early maturing wheat lines is promising for south Asia and Mexico (Mondal *et al.,* 2013). The identification of QTLs for heat tolerance has provided a opportunity to deploy marker assisted selection for the improvement of the high temperature tolerance wheat (Paliwal *et al.,* 2012).

1. **Conclusion**

In this chapter we studied that the Heat and drought stresses are the two major environmental factor which affect the physiological and Biochemical process of plant. These environmental factors affect the crop growth, crop yield and developmental process. The objective of this chapter is to provide information that Biotechnology is the best ways by which the productivity of crops can be improved by enhancing their ability to resist or tolerate heat and drought stress. In the biotechnology, Molecular genetics used for the understanding of underlying biology of plants under abiotic stress condition. Whereas, molecular mapping is to produce a sufficiently fine scale map to pinpoint the location of genes that play a key role in determining important agronomic traits. However, it is possible to map genes that have a major effect on quantitative traits by evaluating the correlation between the value of quantitative trait and the allelic states at linked genetic markers. QTL mapping requires for the development of recombinant Inbreed line (RIL) from parents, which is wildly related to tolerance to abiotic stress. These populations will help to locate the QTL in chromosome region. The overall conclusion from this chapter is that heat and drought stresses induce complex reaction from plants, and that optimal protection may well involve several genes.

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