# Biotechnological Tools for Enhancing Abiotic Stress Tolerance in Plant

**Chapter** · March 2018

DOI: 10.1007/978-981-10-6934-5_8

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# Biotechnological Tools for Enhancing Abiotic Stress Tolerance in Plant

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## Abstract

Environmental abiotic stresses, for example, high temperatures, low water accessibility, mineral insufficiency, high salt levels, and lethality, are serious threats to the harvest survival which someway influence the harvest yield. A few traditional strategies are used for sustainable harvest efficiency; however, with the expanding abiotic stress because of changing climatic conditions and enhancing pressure of populace, the conventional procedures of overcoming abiotic stress are not ready to meet the demands. 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 harvest yield and quality. This chapter concentrates on the traditional and new enhanced biotechnological strategies for the betterment of abiotic stress tolerance in plants.

## Keywords

Abiotic stress · Candidate gene · HSPs · MAS · QTL mapping

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R. S. Sengar, A. Singh (eds.), *Eco-friendly Agro-biological Techniques for Enhancing Crop Productivity*, https://doi.org/10.1007/978-981-10-6934-5_8

## 1 Introduction

Biotechnology is the best way 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 focus on the impact of environmental conditions such as extreme temperature, salinity on crop plants, and water accessibility. Figure 1 shows a number of physical factors that may impose an abiotic stress on plants and adversely affect their quality and yield. These stresses also affect physiological, biochemical, and developmental processes of crop (Amit kumar and Sengar 2013). These include a number that can be gathered together as temperature stresses (heat, chilling, and freezing) which in turn belong to a bigger subgroup that can be arranged as stresses that result in water shortfall. The figure likewise accentuates the point that most abiotic stresses straightforwardly or indirectly lead to the generation of free radicals, creating oxidative stress and reactive oxygen species.

The impact of abiotic stresses on crop yield compared with biotic stresses (weed, pest, and disease effects) is shown in Table 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 1 year to the next produces such a variation in yield for wheat in which 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

**Fig. 1** Types of environmental stress

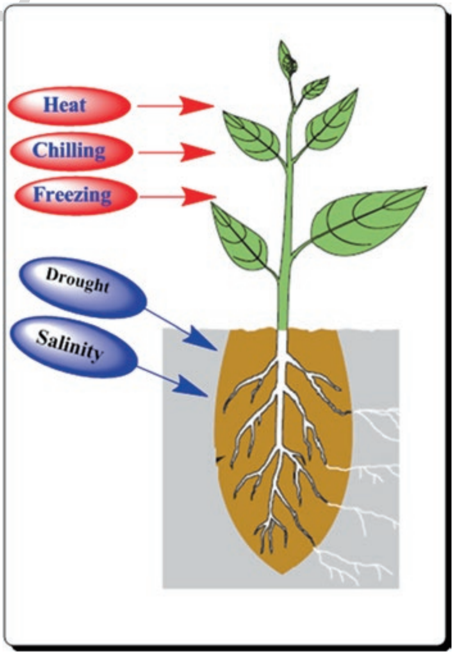

**Table 1** Average and record yields of some major crops

| Crop | Record yield (kg/ha) | Average yield (kg/ha) | Average yield (% of record yield) | Average losses (% of record yield) | |
|---|---|---|---|---|---|
| | | | | Biotic | Abiotic |
| Wheat | 14,500 | 1880 | 13.0 | 5.0 | 82.1 |
| Barley | 11,400 | 2050 | 18.0 | 6.7 | 75.4 |
| Soybean | 7390 | 1610 | 21.8 | 9.0 | 69.3 |
| Corn | 19,300 | 4600 | 23.8 | 10.1 | 65.8 |

Source: Bray et al. (2000)

stable proportion, which is generally less than 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 1 year to the next.

In the long time, the predicted depletion of the ozone layer and climate changes related with a worldwide temperature alteration are probably going to add to the burden of environmental stresses on harvest plants and enhance the imperative to prepare stress-tolerant varieties. Moreover, there is increasing pressure to extend the area of harvest cultivation to environments that are not ideal for the development of significant harvests (high-salt condition). The preparation of stress-tolerant plant is therefore a major aim of agricultural biotechnology and one that is likely to become increasingly important.

## 2 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 can adapt to typical variety by virtue of their plasticity. Consequently, plants are adjusted to work in a fluctuating environment, and normal outside changes are countered by inward change without detriment to development and advancement. 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, for example, high temperatures, low water accessibility, high salt levels, mineral insufficiency, and toxicity, are regularly skirmish by plants in both natural and agriculture frameworks. By and large, a few classes of abiotic stress challenge plants in blend; for instance, high temperature and shortage of water can be exacerbated by mineral toxicities that constrain root development. Over a range of cropping frameworks around the globe, abiotic stresses are estimated to shorten yields not as much as a half of that possible under perfect development conditions (Boyer 1982).

Abiotic stress responses, particularly to water inadequacy and high level of salts, are unpredictable physiological and morphological phenomena in plants (Wang et al. 2003). At the cellular level, alterations in extracellular solute concentrations cause osmotic stress and finally inadequate water availability. This water deficiency causes an abatement in turgor pressure and an expansion in concentration of intracellular solutes, which gives a strain on layers and macromolecules.

Abiotic stresses exhibit a big challenge in our journey for sustainable food generation as these may lessen the potential yields by 70% in cultivates. Intense water insufficiency hinders photosynthesis (Gallagher et al. 1975). Changes in the worldwide atmosphere, remarkably in territorial spatial and temporal temperature designs, are anticipated to have vital outcomes for crop production (Parry and Duinder 1990); both plant development and improvement are influenced 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 variety of different stresses. These damages result from water deficit caused by various distinctive environmental conditions, including temperature, cold, drought, and salinity.

## 3 Tolerance to Drought Stress

Agriculture is a major client of water assets in numerous regions of the world. With expanding aridity and a developing populace, water will turn significantly scarcer in the future. Suboptimal accessibility of water for unlimited plant development and transpiration, i.e., drought, is a noteworthy constraint to agriculture yield (Boyer 1982 and Delmer 2005). Drought is a standout among the most well-known environmental stresses that influence development and advancement of plants through shifts in metabolism and expression of gene. It is changeless to agriculture creation in many developing nations and periodic reason for losses of agriculture production in created 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).

Harvest plants developed under dry spell conditions are presented to a mix of stresses that are attributable from high temperatures, over the top irradiance, and soil resistance to root infiltration and low water potential. Drought is one of the main factors adding to a few yield losses of wheat developed in minimal land and to essentially diminish yields in temperate area (Morris et al. 1991; Trethowan et al. 2001). Drought is the most genuine abiotic stress restricting wheat production in various parts of the world (Chaves et al. 2003).

In spite of the fact that it is not a crop plant, *Arabidopsis* has played an important role in the explanation of the essential procedures underlying stress tolerance, and the information acquired has been transferred to a specific degree to imperative food

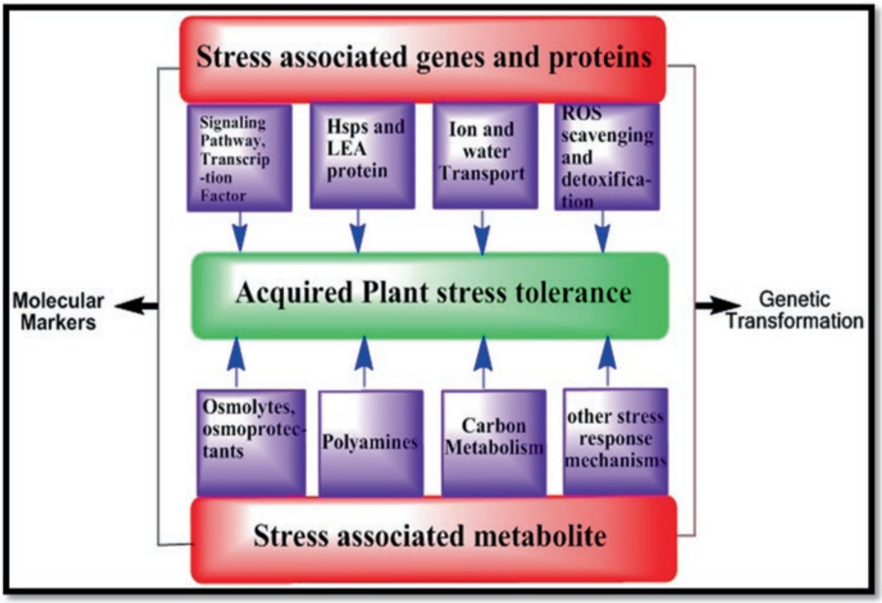

**Fig. 2** Approaches to develop stress tolerance plant

plants. Different types of the gene known to be required in stress resistance have been isolated at first from *Arabidopsis*. Two general procedures for the metabolic engineering of abiotic stress resistance have been proposed which expanded production of particular specific desired compounds, or the decrease in the enzymatic step is typically managed by the tendency of cell frameworks to reestablish homeostasis, thus limiting the potential of this approach (Fig. 2). The transgenic approach is a helpful innovation to beat reproductive isolation among species and use useful exotic genes.

Numerous loci for gene that control tolerance to abiotic stress in plants have been recognized by hereditary investigation. However, numerous genes that control agronomically imperative characteristics remain to be recognized and changed to create new varieties with desirable traits. There is a confirmation that transgenic plants in which the expression of a solitary gene has been changed have improved resistance to abiotic stress.

## 3.1 Trait Associated with Drought Stress

Plant reaction to drought stress is a complex procedure molded by various segment reactions that both interact and vary in their individual reactions to the intensity and span of water deficiencies. It is a standout among the most widely recognized environmental stresses that influence development and advancement of plants through modifications in metabolism and gene expression.

Genomic studies have demonstrated that few genes with different capacities are induced by drought. Expression of the greater part of these genes is induced by the collection of hormone ABA. It has been discovered that ABA is amassed 40 times more in drought over the controlled condition (Ingram and Bartel 1996; Shinozaki et al. 2003). The hormone has appeared to assume essential role in imparting resistance against drought stress. Subsequently, the part of ABA in controlling genes under water stress was thought 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 and Thompson et al. 2000).

Aggregation of ABA under water deficit may come about because of increased biosynthesis (Bray 1997). ABA is synthesized in root and shoot in light of different stresses including drought, low temperature, and hypoxia. The key stride of ABA synthesis in roots and leaves is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED), a protein that changes over the epoxy-carotenoid precursor to xanthonin in the plastids (Qin and Zeevaart 1999). Xanthonin is then changed over to ABA by cytosolic proteins by means of abscisic aldehyde. The NCED gene has been cloned in tomatoes, cowpeas, maize, and beans and appeared to have a promoter that is induced by dry season 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 of 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 suggested that *rd29* could have no less than two cis-acting components. One was involved in ABA-associated slow response and the other functioned independently of ABA. Genomic clone investigation demonstrated that rd29 were situated in tandem in an 8048-bp-long region of *Arabidopsis* genome.

## 3.2 Marker-Assisted Selection for Drought Stress

Marker-assisted selection (MAS) is the breeding technique in which determination for a gene depends on molecular markers firmly connected to the gene of interest instead of the gene itself, and the markers are utilized to screen the joining of the desirable allele from the contributor source. The correct route in which hereditary diagnostics are applied in plant breeding will be impacted by numerous considerations, both biological and economic, yet the accessibility of cost-effective diagnostic technology that is good with the goals will be the main factor.

Molecular breeding requires the following innovation: hereditary maps, molecular markers connected to agronomic attributes, high throughput, automated diagnostic procedure, and an adjustment in breeding practice that takes full favorable position of the information given by such indicative examines. The prescient estimation of molecular markers utilized as a part of MAS relies upon their inherent repeatability, position of map, and linkage with economically imperative qualitative and quantitative attributes. The presence of a tight linkage between qualitative characteristics and molecular marker might be helpful in MAS to increase gain from selection. MAS may have potential in populace and inbreed line advancement. Whenever QTLs and single gene are satisfactorily mapped, they can be isolated by map-based cloning methodologies. The viability of any MAS will rely upon the precision of the phenotypic order of attribute expression and the level of linkage between the markers and traits of interest.

Molecular marker is utilized to develop firmly separated genetic maps of a specific genome utilizing an appropriate mapping populace of plants (Sehgal et al. 2008; Kumar 1999). Molecular marker mapping has reinforced our realization that, in a few taxonomic groups of yield plants, e.g., the Gramineae, which share the similar fundamental chromosome number, the linkage group and the individual chromosome maps look fundamentally the same, i.e., they are syntenic, especially when we take out the repetitive DNA and analyze the maps for single duplicate sequences. This implies that even between crops as differing as wheat and rice, the genes we are occupied with are fundamentally the same in both species, and their areas in the hereditary maps are very much alike. The greater part of these maps has been made utilizing RFLP (restriction fragment length polymorphism) markers, and more recently, different PCR-based markers are utilized for the development of genetic map (Gupta and Rustogi 2004; Sehgal et al. 2008), for example, SSR (simple sequence repeats), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), STS (Sequence Tagged Sites), and so on permit loci controlling quality related to stress tolerance to be recognized and mapped in the genome.

## 3.3 Candidate Gene Related to Drought Stress

Conventional assessment of plant reactions to drought has depended chiefly on leaf to atmosphere gas exchange, development investigation, and in vitro assurance of enzymes, metabolites, and gene expression patterns. The main molecular way to help breeders in their endeavors to expand drought tolerance has been with molecular marker, genomics, and post-genomic techniques (Nguyen and Blum 2004; Lancers et al. 2004). Candidate gene can be characterized as the gene demonstrating molecular polymorphisms and hereditarily related with QTL or statistically connected with drought tolerance trait. The candidate genes can be partitioned into two classes on the premise of their functions: in the first category, gene qualities are

**Table 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) and Ito et al. (2006) |
| ERA1 | Farnesyltransferase | Negative regulator of ABA sensing | Wang et al. (2005) |
| Mn-SOD | Mn-superoxide dismutase | ROS-scavenging proteins | McKersie et al. (1996) |
| AVP1 | Vacuolar $H^+$ pyrophosphatase | 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) |

required in cell assurance during stress, for example, osmotic, and, second, genes are included in the direction of different genes for the stress responses.

Various such genes have been recognized, separated, cloned, and expressed in plants, which are potential sources or resistant to drought stress. In Table 2 a portion of the candidate genes have been recognized for drought tolerance accumulated from literature survey.

Chen et al. (2002) recognized groups of transcription components directed by the abiotic stress in *Arabidopsis*. The expression patterns of these transcription components are extremely complex which propose that stress tolerance and resistance are controlled at the transcriptional level by an amazingly multifaceted gene regulatory system. Genome-wide transcriptome investigation has distinguished several candidate genes encoding transcription components which are induced or repressed by ecological stresses (Chen et al. 2007a, b).

In *Arabidopsis* plants, transcript profiling (1300 genes) for overexpressing the gene encoding for dehydration-responsive element binding protein (DREB 1a) distinguished 12 gene as cold and drought season target gene having a place with DREB 1 transcription factor family (Seki et al. 2000). The gene enacted by drought incorporates those included in mechanism to maintain a strategic distance from water misfortune, secure the cellular machinery, and repair damage (Shinozaki and Yamaguchi-Shinozaki 2007). The DREB 2 genes, incited by drought stress, have been accounted for to give drought tolerance in a few plant species, for example, *Arabidopsis* (Sakuma et al. 2002), rice (Dubozet et al. 2003), wheat (Egawa et al. 2006), maize (Qin et al. 2007), and barley DRFs (Xue and Loveridge 2004).

## 3.4 QTL Mapping for Drought Tolerance

Quantitative trait is represented by polygenes and is markedly influenced by the environment. Thus, it demonstrates a constant variation rather than the discrete variation that is characteristic of qualitative traits. Polygenes are those genes that have little however combined impact on the concerned traits, and a few polygenes influence a single trait. A quantitative trait locus is a position in a chromosome that contains at least one polygenes involved in the assurance of a quantitative trait.

Many of DNA marker maps are not adequately thick to accomplish satisfactory QTL mapping, since inadequate marker maps extremely confine the power of QTL mapping. The mapping populace must be relatively huge in order to identify QTLs having minor impacts, and the biological relevance of the revealed QTLs relies upon the cutoff decided for statistical significance. In QTL mapping, natural variables and genetic background have a marked effect on the outcomes; as a result, some QTLs may be detectable in some but not in other environments. A standout among the most capable applications of QTL mapping is to analyze gene x gene and gene x environment interactions, yet this requires some extensive, tedious trials to allow a thorough investigation of a system.

QTL mapping involved testing DNA markers all through the genome for the probability that they are related with a QTL. Individuals in a suitable mapping populace are investigated regarding DNA marker genotypes and the phenotypes and the phenotype of interest. For every DNA marker, the individuals are split into classes as indicated by marker genotypes. These markers are being utilized to distinguish drought-related quantitative trait loci and their productive transfer into economically developed crop varieties of rice, wheat, maize, and millet. Great hereditary maps in view of molecular marker technologies are presently accessible for large cereal species (Snape et al. 2005; Langridge et al. 2006). In cultivated species with huge, complex genomes, QTL investigation is a vital tool in the recognizable proof of genetic markers to help breeding efforts. This approach is complicated in wheat on account of the polyploidy nature of the genome. Furthermore, the low levels of polymorphism, however, is straightforward in rice, barley, and maize (Snape et al. 2005)

Studies on the abiotic stress resistance of cereals incorporate the broad investigation of QTLs linked to the field assessment of stress tolerance (Langridge et al. 2006). Approach and improvement of molecular markers in quantitative genetics greatly incredibly encourage the investigation of complex quantitatively acquired inherited traits by the development of high-density genome linkage maps for harvests, for example, wheat (Xiao et al. 1996). This infers the utilization of molecular markers, and interim mapping is an intense approach, which allows the distinguishing proof and genetic mapping of loci controlling complex traits like grain yield and its contributing traits having extraordinary significance in plant breeding (Broman and Speed 1999). Lacking accessibility of water during drought may constrain the size of the plant and furthermore influence the improvement of different plant parts. Recent advances in plant genomics have prompted the identification of a vast number of potentially beneficial water stress-related gene, in addition to innovations for gene overexpression or silencing. Also, these can be brought into transgenic plants under the control of proper promoters and are transmitted to subsequent generations (Delmer 2005; Ma and Bohnert 2007).

Recently, Tuberosa and Saliva (2007) detailed that genomic-based methodologies give access to agronomically attractive alleles present at quantitative trait loci (QTLs) that influence such responses, accordingly enabling us to enhance the drought resistance and yield of crops in submerged restricted conditions more effectively. QTLs for drought tolerance, one each situated on chromosome 7AL (Quarrie et al. 2005), and three QTLs for heat resistance, one each situated on chromosome arms 2DL (Mohammadi et al. 2008a), IBS, and 5BL (Mohammadi et al. 2008b) and their linked SSR markers, were utilized for validation for their inevitable exploitation during amid MAS.

## 4 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 coordinated 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. 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 overexpressed in *Arabidopsis*, the translation factor was not dynamic, and there was no impact on thermotolerance. Nonetheless, combination of AtHSF1 to the N or C end of the gus A reporter gene created a fusion protein that could trimerize without heat. Transformation of this fusion protein into *Arabidopsis* created transgenic plants that expressed HSPs constitutively and showed upgraded thermotolerance without requiring earlier 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 utilize genes, and MAS in cereals for biotic stresses, abiotic stresses, and quality traits. Many genes for those traits have been mapped, tagged, and cloned, and linked markers have been created. Those have been successfully used in marker-assisted breeding program 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.

MAS and hereditary engineering are two most basic molecular methodologies for enhancing stress resistance in plants. With the appearance of molecular markers, MAS has turned into a fundamental component of new discipline named as molecular breeding with the assistance of which allelic variation among the genes' basic traits can be accurately and proficiently detected (Mohammadi et al. 2008b). Various markers, for example, RAPDs, SSRs, RFLPs, and AFLPs, have been accounted for different abiotic stresses for accomplishing mapping of the QTL involved in stress tolerance (Korzun et al. 1999). Relatively, however, limited research has been directed to recognize genetic markers related with heat resistance in various 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 related with heat resistance in crops, and tagging and mapping of these traits with an appropriate marker can be utilized in marker-assisted breeding. It has been observed that marker-assisted selection (MAS) approaches have contributed enormously to a superior comprehension of the hereditary bases of plant stress tolerance, which prompted the improvement of plants with enhanced resistance of abiotic stress.

A few transgenic approaches have been utilized for the advancement of heat tolerance crops (Rodriguez et al. 2005). Many heat shock proteins (HSPs) have been utilized for the advancement of thermotolerance crops. Many scientists studied the expression level of HSPs by making an improvement in the heat shock factor (HSF) which is responsible for the production of high-temperature stress-tolerant *Arabidopsis*. Various such genes have been recognized, isolated, cloned, and expressed in plants, which are potential sources or resistant to drought stress. In Table 3 some of the candidate genes have been identified for heat tolerance gathered from literature survey. Candidate gene can be defined as the gene indicating molecular polymorphisms and genetically related with QTL or measurably associated with thermoresistance trait.

**Table 3** List of candidate gene for heat tolerance

| Candidate gene | Function of candidate gene | References |
|---|---|---|
| HSP 101 | Synthesis of heat shock protein for temperature tolerance | Queitsch et al. (2000) |
| APX 1 | H2O2 detoxification and conferred heat tolerance | Shi et al. (2001) |
| HSP 70 | Synthesis of heat shock protein for temperature tolerance | Montero-Barrientos et al. (2010) |
| Cod A | Glycine betaine synthesis for tolerance to temperature tolerance | Alia et al. (1998) |
| HSP 17.7 | Synthesis of sHSP | Malik et al. (1999) |
| FAD 7 | Enhanced the level of unsaturated fatty acid and provide temperature tolerance | Sohn and Back (2007) |
| AtPARP2 | Increased tolerance to heat stress | Vanderauwera et al. (2007) |
| TLHS1 | Synthesis of class I HSP | Park and Hong (2002) |
| AtHSF 1 | HSF fused with β-glucuronidase and such modification will increase HSP production | Lee et al. (1995) |
| ANP 1 | H2O2-responsive MAPK kinase kinase (MAPKKK) production to protect against the lethality in HT | Kovtun et al. (2000) |
| MT-sHSP | Molecular chaperone | Sanmiya et al. (2004) |
| Dnak 1 | Tolerance to temperature | Ono et al. (2001) |
| BADH | Overproduction of GB osmolyte that will enhance the heat tolerance | Salvucci and Crafts-Brandner(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 impacted by various hereditary and environmental factors. However, it is possible to map genes that have majorly affected quantitative traits by assessing the correlation between estimation of the quantitative trait and the allelic states at linked hereditary markers. A QTL (quantitative trait locus) is therefore a chromosomal location where there is considered to be a reasonable probability that practically unique alleles segregate and cause huge impacts on a quantitative trait. QTL mapping requires a statistical analysis of molecular marker and phenotypic information from an extensive segregating populace 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 relates 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 as a mendelized and fine map with more precise centimeter distance between the QTLs, 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 downregulating the gene.

To date, the level of accomplishment in distinguishing hereditary markers related with high-temperature tolerance in wheat, and to be sure other crop species, has been limited. Dependable marker trait associations are essential for a viable marker-assisted breeding program (Kato et al. 2000), and these are most successfully settled by means of quantitative trait locus mapping (Patterson 1998). QTLs for heat resistance have been reported by various researchers (Mohammadi et al. 2008a; Mason et al. 2010; Pinto et al. 2010; Barakat et al. 2011; Tiwari et al. 2013). At present, the good breeders' technique is to challenge the material by sowing late, in the information that this will expose the plants to high temperatures amid grain filling; after this, determination is regularly based on yield performance. Recent outcomes demonstrate that synchronous improvement of yield potential and thermotolerance is possible and that the CIMMYT strategy to grow high-yielding early developing wheat lines is promising for South Asia and Mexico (Mondal et al. 2013). The recognizable proof of QTLs for heat tolerance has given a chance to deploy MAS for the improvement of the high-temperature resistance wheat (Paliwal et al. 2012).

## 5 Tolerance to Salt Stress

Salt resistance might be characterized as a differential impact on different life processes of a similar tissue concentration of salt in different genotypes of a species. There is considerable evidence that genotypes differ in resistance to a similar amount of salt in their tissues. However, the enzyme and cellular processes of halophytes are as sensitive to salt as those of glycophytes. Most of the crops grow under the saline environment by which yields are generally poor in the initial 3–4 years of the reclamation of saline lands through application of gypsum. Therefore, improving salt tolerance of crops may help to a great extent in increasing food production of the growing population of India. Therefore, cultivation of salt-tolerant varieties can play important role in rehabilitation of such lands besides reducing the requirement of input in the form of chemical amendment.

### 5.1 Salt Tolerance Mechanism at Physiological Levels

Salt tolerance is increasingly becoming a major target for crop improvement as substantial areas of irrigated land are damaged by the accumulation of salt. Furthermore, the pressure for land has made it necessary to consider the possibility of growing crops in more saline conditions, with poorer quality water. As described above, saline conditions lead to osmotic stress by preventing water uptake by the roots and water efflux from the cells. However, the accumulation of $Na^+$ and $Cl^-$ ions in the cytoplasm may also have direct toxic effects by inhibiting protein synthesis, photosynthesis, and susceptible enzymes (Estan et al. 2005). Thus, strategies for engineering water stress tolerance via the production of compatible solutes may provide protection against the osmotic effect of saline conditions, but not against ion toxicity. Additional approaches to minimize the toxic effects of specific ions may also be required. Strategies for engineering salt tolerance have been developed by comparing the response to salt stress of plants that are sensitive to high-salt conditions (glycophytes) with that of plants that can tolerate high-salt conditions (halophytes). As a general rule, glycophytes respond to salt stress by accumulating osmoprotectants, whereas halophytes employ specific mechanisms to avoid the toxic effects of $Na^+$ and $Cl^-$ ions. Some halophytes actually excrete salt via specialized glands on their leaf surfaces, but it is more common for plants to regulate $Na^+$ ions out of the cell, whereas other cells with large vacuoles may act as sinks for the accumulation of excess sodium by transport into the vacuole.

Resistance to salt stress is an unpredictable phenomenon at both the entire plant level and the cellular level, and serious research endeavors have concentrated on understanding the physiological basis of resistance in higher plants (Cuartero et al. 2008). Different physiological parameters which are highly specific for saline condition were identified and used regularly for the selection of high-tolerant varieties. Some of these parameters were $Na^+$ transport to shoot, preferential accumulation of Na in older leaves, high $Cl^-$ uptake, lower $K^+$ uptake, and low $Na^+/K^+$ ratio (Sharma and Goyal 2003)

One approach to enhancing salt tolerance would be to copy the strategies used by halophytes to transport $Na^+$ ions out of the cytoplasm. In order to put this into practice, it is necessary to consider the mechanisms of ion transport out of the cytoplasm. The first transgenic experiments considered transport of $Na^+$ ions into the vacuole. Since this transport is working against a concentration gradient, it requires the input of energy. This is achieved by coupling the transport protein to a proton pump, transporting $H^+$ ions in the opposite direction. The vacuolar $Na^+/H^+$ antiporter protein AtNHX of *Arabidopsis* has been studied extensively and is known to be coupled to proton pumps such as AVP1, a vacuolar $H^+$-translocating pyrophosphatase. An analogy that has been used is to compare AtNHX1 with a revolving door and AVP1 as providing the energy for the door to spin. To increase the traffic through the membrane, one could therefore either increase the number of doors or provide more energy for the existing doors to spin faster.

The first approach was effectively used to engineer salt resistance in tomato plants by transformation with the *Arabidopsis* AtNHX1 antiporter protein gene (Yokoi et al. 2002). AtNHX1 enduring state transcript levels were expanded in response to NaCl, recommending that AtNHX1 transcript upregulation is not particular to ionic stress but rather is regular to osmotic stress (Zhu 2001). High expression of NHX antiporters has been utilized to enhance salt resistance (Wu et al. 2004). The *Arabidopsis* gene has also been transformed into maize and wheat plants, prompting to increased salt resistance, and improved grain yields in salt tolerance have been investigated. For example, rice changed with the OsNHX1 gene demonstrated enhanced development and accumulation of biomass under salt stress conditions (Wu et al. 2005, Chen et al. 2007a, b).

More recently, the effects of introducing a plasma membrane $Na^+/H^+$ antiporter to extrude $Na^+$ ions from the plant cells have been investigated. Overexpression of the *Arabidopsis* plasma membrane $Na^+/H^+$ antiporter AtSOS1 gene in transgenic *Arabidopsis* improved the salt tolerance of callus cultures and reduced the levels of $Na^+$ ions in the xylem of transgenic plants (Shi et al. 2003). Expression of the fission yeast SOD2 gene in *Arabidopsis* improved seed germination and seedling salt tolerance (Zhao et al. 2006). Transformation of rice with the *E.coli nhaA* plasma membrane $Na^+/H^+$ antiporter improved germination rate, growth, and yield under conditions of both salt and drought stress (Wu et al. 2005). Interestingly, expression of *nhaA* also increased the levels of proline in the transgenic rice, suggesting that enhanced osmoregulation might in plat be due to activation of proline biosynthesis.

Another approach to enhancing vacuolar transport has been to overexpress the gene encoding *AVP1*, initially in *Arabidopsis*, to increase the proton pumping potential of the vacuole and hence its ability to transport sodium (Gaxiola et al. 2001). This has improved not only the salt tolerance of these experimental plants but also the drought tolerance, since the altered ion balance has enabled the plants to retain more water. Similar results have been obtained by overexpressing the homologues from *Thellungiella halophila* (*TsVP*) in tobacco (Gao et al. 2006) and *Triticum aestivum* (*TVP1*) also in *Arabidopsis* (Brini et al. 2007).

### 5.2 QTL Mapping for Salt Tolerance

QTL is therefore a chromosomal location where there is considered to be a reasonable probability that functionally different alleles segregate and cause significant effect 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 and phenotypic data from a large segregating population to determine those markers where allelic polymorphism correlates with the quantitative trait phenotype.

Molecular technology is a new technology for analyzed the quantitative trait such as salt tolerance and detect the chromosomal location, which is associated with such character, known as Quantitative trait loci. Few scientists have been reported that the QTL for salinity damage at early embryo stage in rice (Prasad et al. 2000).

## 6 Tolerance to Cold Stress

Diverse plants differ enormously in their capacity to withstand cold and freezing temperatures (Fig. 3). Most tropical plants have essentially no ability to survive chilling conditions. Furthermore, plants can survive a scope of chilling temperatures from −5 to −30 °C relying on the species. Plants from colder districts routinely withstand temperatures even lower than this. It is realized that plants are better ready to withstand colder or chilling stress in the event that they initially experience a time of chilly acclimation, at a low however nonchilling temperature. For instance, wheat plants developed at ordinary warm temperature are killed by chilling at −5 °C; however, after a time of cold acclimation when the plant develops at temperatures underneath 10 °C, they can survive chilling temperature down to −20 °C.

Plants differ in their ability to withstand cold or freezing condition, and cold tolerance is one of the traits that plant breeders have selected for over many centuries. However, there has been little improvement in the cold tolerance of major crop species over the past two decades by conventional breeding, prompting the search for molecular solutions to this problem.

One approach has been to study the mechanisms of freezing resistance that exist in some plant species. Amid the time of acclimation, plants produce various chilly instigated proteins that are expected to assume a part in the ensuing cold tolerance. Around 50 frosty prompted proteins have been recognized in various plant species. These fall into few groups, but they all share the property of being to a high degree hydrophilic. A significant number of them additionally have relatively basic amino acid composition, with current motifs. Some of these groups had already been distinguished as late embryogenesis abundant (LEA) proteins, which seem to play a

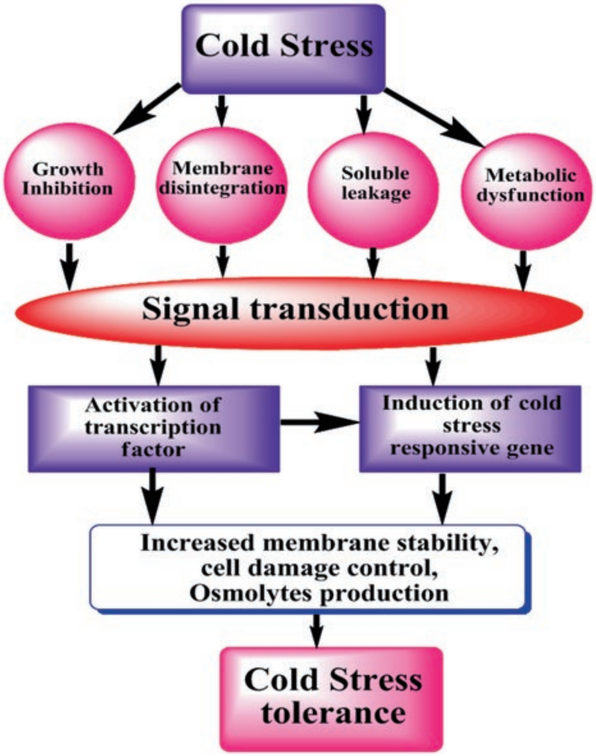

**Fig. 3** Cold stress response in plant

defensive role amid seed desiccation. Different groups of proteins encoded a class of genes assigned as chill-responsive gene as per their patterns of expression. The exact function of these chill prompted genes is not yet known, but rather it has been hypothesized that they may contribute specifically to chilling tolerance by mitigating the possibly harming impacts of lack of hydration related with freezing. Overexpression of this chilly prompted protein could in this way be a possible route to be particular technology of cold or chilling stress resistance.

There is some case of the expression of chilly induced proteins in transgenic plants. For instance, constitutive expression of the little, hydrophilic, chloroplast-targeted COR protein COR 15a in *Arabidopsis* enhanced the chilling resistance of chloroplast frozen in situ or protoplast frozen in vitro. In any case, COR 15a expression has no perceptible impact on the survival of frozen plants. One clarification for this observation is that the cold-prompted proteins might be focused to various vulnerable cell segments and that they are altogether required to provide full protection to the cell. By implication, many COR genes would need to be changed into a transgenic crop to get in appreciable change in cold resistance.

One answer for the issue of engineering a multigene trait has risen after the recognition that few distinctive chill resistance-related genes contain a comparative regulatory element in their promoter: the C-repeat (CRT) element/low-temperature response element (LTRE)/lack of hydration- or dehydration-responsive element (DRE). Besides, it has been discovered that the transcription factor CBF1 ties to the CRT/DRE/LTRE element and activates expression of this gathering of genes, which contain the COR regulon. Along these lines, the procedure is to overexpress the CBF1 gene, prompting the acceptance of this whole group of COR cold resistance gene. In transgenic, *Arabidopsis* plants conveying a 35S promoter, CBF1 gene constructs have been developed. These plants express various COR genes without chill acclimation and have been appeared to be cold resistance without earlier cold acclimation. As a control, transgenic plants overexpressing an individual COR protein, COR15a, were observed to be less cold tolerant than the CBF1 plants. The interrelated nature of various stress reactions was shown in a similar investigation. The expression of a CBFF1 homologue and DRE restricting protein DREB1A under the control of a stress-induced promoter in transgenic *Arabidopsis* brought about plants that had enhanced drought, salt, and cold resistance.

## 6.1 Cold-Responsive Gene Regulation

Some of the cold-induced COR genes have been characterized and the sequence of their promoter compared. One of the features of several different COR genes is that their promoters share a common regulatory element termed the C-repeat (CRT) or low-temperature response element (LTRE), which is five nucleotide long and has a consensus sequence of CCGAC. This element had already been linked to drought resistance and termed the dehydration-responsive element (DRE). The CRT/LTRE/DRE is bound by a CBF transcription factor, CBF1. The structure of CBF1 is shown in Fig. 4, indicating the nuclear localization sequence, DNA-binding domain, and an acidic region that may be involved in interactions with other protein CBF1 expressions which are induced by cold acclimation and lead to the expression of the COR genes. This group of genes, sharing a common regulatory mechanism, has been termed the COR regulon.

CBF1 is an individual from a small gene family member; CBF2 and CBF3 and also transcription factors and expression of all three CBF genes are induced rapidly by low temperatures. In addition, CBF3 overexpression results in several biochemical changes related with cold acclimation, such as elevated levels of compatible osmolyte, proline, and soluble sugar.

Although low-temperature-induced gene expression, mediated by the CRT element, appears to be well conserved in plants, not all cold-induced genes have the CCGAC element in their promoters. Other pathways of low-temperature gene expression, not mediated through CRT/CBF, appear to be present in plants, and another sequence element, CCGAAA, has been identified as conferring low-temperature inducibility in some genes.

Genetic engineering and molecular studies have recognized numerous genes which are incited by many stresses. Several stress-inducible genes encoding useful protein have been utilized for the improvement of stress resistance. Most of the

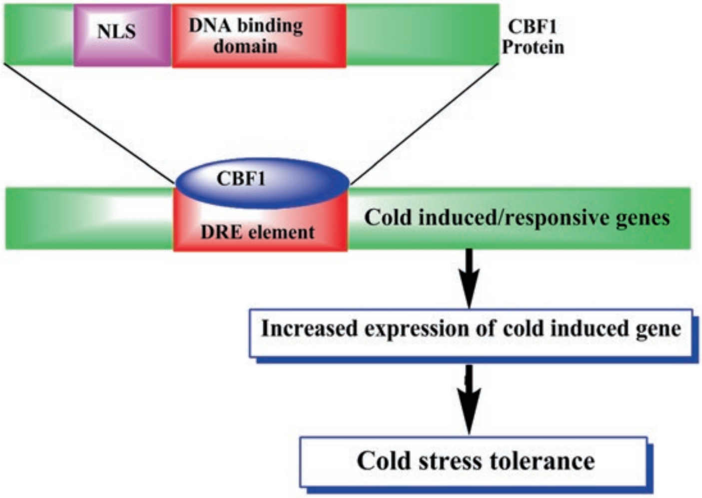

**Fig. 4** Cold-responsive gene contains CBF1 transcription factor and drought-responsive element (DRE) by which increased the expression of cold-induced gene

reviews have been published on stress resistance (Christensen and Feldmann 2007; Umezawa et al. 2006; Valliyodan and Nguyen 2006). Numerous transcriptional activators, for example, DREB1/CBF (Kasuga et al. 1999; Liu et al. 1998; Kreps et al. 2002), which incite the stress-responsive gene, have been used to deliver the low-temperature-tolerant transgenic plants (Zhang 2003). The DREB/CBF qualities have been effectively used to engineer low-temperature stress resistance in different plant species, for example, rice (Dubouzet et al. 2003; Ito et al. 2006), pepper (Hwang et al. 2005), chickpea (Mantri et al. 2007), and potato (Rensink et al. 2005).

Screening for gene required in cold resistance is a critical introductory step. Vast quantities of studies have been accomplished for chilly stress resistance in plants. All the cold resistance-related genes contain a comparative regulatory element in their promoters, the C-repeat component and low-temperature-responsive component. A rundown of genes and transcription factor which are enhancing the chill resistance in various plants is given in Table 4.

## 7 Conclusion

In this chapter, we studied the effect of environmental stress, for example, temperature, water, cold, and salt, on cultivated plants and also highlighted the importance of abiotic stress in determining the large annual fluctuations in crop yield. Molecular genetics is used for the understanding of the underlying biology of plants under abiotic stress condition. 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. Many of these traits are described as quantitative; that is, they are

**Table 4** List of plant genes conferring increased cold tolerance

| Genes | Plant | Mode of action | References |
|---|---|---|---|
| *SICZFP1* | *Arabidopsis* | Regulating cold-responsive gene | Zhang (Zhang 2010) |
| *OrbHLH001* | *Arabidopsis* | Involved in metabolic regulation or ionic homeostasis pathways in stress | Li et al. (2010) |
| *Osmyb4* | *Osteospermum ecklonis*, *apple*, *Arabidopsis* | Transcription factor | Laura et al. (2010) |
| *OsLTP* | *Phalaenopsis amabilis* | Increased accumulation of total soluble sugar, proline, antioxidant superoxide dismutase | Qin et al. (2011) |
| *OsSPX1* | *Arabidopsis and tobacco* | Accumulation of proline and sugar | Zhao et al. (2009) |
| *OsDREB1D* | *Arabidopsis* | Transcription factor | Zhang et al. (2009) |
| *OsiSAP8* | *Tobacco* | Cytoplasmic zinc finger protein that is involved in the signal transduction | Kanneganti and Gupta (2008) |
| *OsDREB1F* | *Arabidopsis* | Transcription factor | Wang et al. (2008) |
| *Cat* | *Wheat* | Use for the catalase | Matsumura et al. (2002) |
| *GS2* | *Rice* | Glutamine synthase | Hoshida et al. (2000) |
| *P35S-ZFP245* | *Rice* | Accumulation of proline, activation of the pyrroline-5-carboxylate synthetase and proline transporter genes, and enhancement of the ROS-scavenging enzymes | Huang et al. (2009) |
| *OsP5CS2* | *Rice* | Accumulation of proline | Hur et al. (2004) |

influenced by different heredity and environmental factors. However, it is possible to map genes that have a large effect on quantitative traits by evaluating the correlation between the value of quantitative trait and the allelic states at linked heredity markers. QTL mapping is essential for the development of recombinant inbred line (RIL) from parents, which is wildly related to resistance to abiotic stress. These populace will help to detect the QTL in chromosome region.

Although crops experience a number of different abiotic stresses, several of these cause the same two major problems: water-deficit and oxidative stress. Two general strategies for engineering tolerance to abiotic stresses in plants are therefore possible. Some measure of tolerance to water-deficit stress can be provided by the synthesis of compatible solutes. On the other hand, expressing enzymes involved in protection against ROS can combat oxidative stress.

In some cases, single-gene mechanisms for tolerating specific stresses can be deployed (e.g., salt stress and cold stress). However, the overriding theme from this chapter is that abiotic stresses induce complex reaction from plants and that optimal protection may well involve several genes.

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