

Review Article

A Review on Molecular Approaches in Breeding for Abiotic Stress Tolerance

R. R. Dhutmal*, S. P. Mehtre and A. G. Mundhe

Sorghum Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth,
Parbhani-431401 (M.S.), India

*Corresponding author

ABSTRACT

Effective identification of stress resistance traits, characterization of their genetic complexity and identification of genes in a wide array of both wild and cultivated genotypes are the important steps in identifying the gene sources. Genomic technologies coupled with advanced bioinformatic analysis of lab and field data allows complex traits of agronomic importance with a multitude of interconnected pathways and processes to be studied at gene level (Casu *et al.*, 2001). It was possible to analyse many genes encoding proteins involved directly or indirectly in response against abiotic stresses. These data would be useful for functional genomics studies as well as for breeding programmes through marker assisted selection (MAS) and genetic engineering using transgenic technology. Functional genomic analysis on these genes involved in abiotic stress would enable dissection of the complex traits into component characters and would offer sources for various parameters in breeding abiotic stress tolerance. Recent reports of tight linkages between the candidate genes and the QTLs conferring abiotic stress resistance are encouraging (Francai *et al.*, 2005). For breeders, the knowledge on the precise location of the QTLs that have large effects, have application for integration in MAS. Identification of genes/ promoters would facilitate their transfer through genetic transformation. In future all these developments are expected to address the problem of abiotic stresses through the integration of breeding and molecular techniques.

Keywords

Molecular approaches,
Breeding,
Abiotic stress tolerance

Introduction

Abiotic stresses such as drought, high temperature, salinity, cold and frost reduce crop productivity. It has been estimated that crops attain only about 25% of their potential yield because of the detrimental effects of environmental stress (Boyer, 1982). The abiotic stresses are location specific, exhibiting variation in frequency, intensity and duration. Stresses can occur at any stage of plant growth and development, thus illustrating the dynamic nature of crop plants and their productivity. Because of the large impact that stresses have on crop

yields, many plant breeders also target increased resistance to stress as a major route to crop improvement. One difficulty in breeding for stress tolerance per se is that genetic advancement is often evaluated in terms of yield performance.

Drought

Drought is the primary abiotic stress causing not only differences between the mean yield and the potential yield, but also causing yield instability. It has been predicted that in

the coming years, rainfall patterns might shift due to an increase of the global temperature caused by burning of fossil and corresponding increase in atmospheric dioxides (Guido and Paul, 1994).

Consequently, farming communities in the Northern hemisphere could become increasingly dependent on drought tolerant varieties (Sorresls *et al.*, 2000).

Salinity

Salinity is another major constraint in sugarcane productivity affecting germination, tillering, growth, yield and quality. Chlorides, sulphates and bicarbonates of sodium, calcium and magnesium contribute in varying degrees of soil salinity.

Cold/Frost

Cold is another important environmental stress affecting plant growth and crop productivity. Chilling (low temperatures above 0° C) and freezing (temperatures below 0° C inducing extracellular ice formation) limit the geographical distribution and growing season of many crops and cause significant crop losses (Xin and Browse, 2000).

The most appropriate strategy would be to evaluate the available germplasm for their cold tolerance and winter sprouting ability and then to use them as parents to generate progenies for selection under natural conditions of the particular region.

Waterlogging

Waterlogging stress is by the deficiency of oxygen required for root respiration following the replacement of air in the soil with water.

Genetics of abiotic stress tolerance

Abiotic stress is a complex trait involving a battery of genes and regulatory elements. With the progress made in the fields of molecular biology, genetics and physiology, genetic improvement for the trait has been substantial in recent years in crops, like rice. Even polypliod crops can get benefit of the recent developments in the field of molecular biology and biotechnology to address these problems. The effective identification of useful stress resistance trait is the first step in the breeding process.

The genomes of species that have evolved in environments with extreme condition are likely to contain genes that confer stress resistance. It is important to search for resistance traits and genes in a wide array of wild and domesticated genotypes.

QTL approach

A QTL (Quantitative Trait Locus) is defined as of the genome that is associated with an effect on a quantitative trait. The term QTL was coined by Geldman (1971). Inheritance of quantitative traits refers to the inheritance of a phenotypic characteristic that varies in degree and can be attributed to the interactions between two or more genes and their environment (also called polygenic inheritance).

Though not necessarily genes themselves, quantitative trait loci (QTLs) are stretches of DNA that are closely linked to the genes that underlie the trait in question. QTLs can be molecularly identified (for example, with PCR) to help map regions of the genome that contain genes involved in specifying a quantitative trait. This can be an early step in identifying and sequencing these genes. Abiotic stress tolerance is generally a quantitative character governed by QTLs.

QTL mapping is the statistical study of the alleles that occur in a locus and the phenotypes (physical forms or traits) that they produce. To begin, a set of genetic markers must be developed for the species in question and then to identify the marker that is significantly more likely to co-occur with the trait through statistical association. Analysis generally reveals regions of DNA that are very close to the genes in question rather than finding the specific gene in question. When a QTL is found, often it is not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene. Where such linkage occurs, the marker locus and the QTL will co-segregate.

For organisms whose genomes are known, one might now try to exclude genes in the identified region whose function is known with some certainty not to be connected with the trait in question. If the genome is not available, it may be an option to sequence the identified region and determine the putative functions of genes by their similarity to genes with known function, usually in other genomes.

Requirements for QTL mapping

A suitable mapping population generated from phenotypically contrasting parents.

A saturated linkage map based on molecular markers.

A reliable phenotypic screening of mapping population.

Appropriate statistical packages to analyze genotypic information in combination with phenotypic information for QTL detection.

In several species, genetic maps have allowed to identify chromosomal regions

controlling some traits related to stress response. Screening of segregating populations of graminaceous crops like rice, maize, sorghum, wheat and pearl millet have been studied for different quantitative characters such as phenology, root characters, abscisic acid accumulation, photosynthesis parameters, stay green characters, water use efficiency, osmotic adjustment parameters have resulted in the identification of the governing QTLs. Over 50% of the putative QTLs associated with the root characters mapped to the same chromosomal regions as QTLs influencing drought avoidance in the field experiments (McCouch and Doerge, 1995). Subsequently several QTLs have been identified in the model crops of rice. Research is underway to determine the consistency of the above QTLs in different genetic backgrounds and their associations with plant performance under field conditions.

Indicator traits and need of special experimental designs

Indicator traits for drought resistance can provide convenient selection criteria for breeding programs. In maize the silk-tassel interval was identified as highly indicative secondary trait for drought-resistant breeding. In crops like sugarcane, drought tolerance is associated with many different morphological and physiological traits or responses including stomatal regulation, root morphology and depth, osmotic adjustment, antioxidant capacity, membrane thermo stability, maintenance of photosynthesis, leaf rolling etc. (Nguyen, 2000). Genetic dissection of complex traits like drought resistance could be achieved with special experimental designs. In particular, the plant-wise drought treatment protocol may provide a generally useful method for independent evaluation of the individual components contributing to drought

resistance in rice as well as in other species. The genetic basis of each component could be characterized by further resolving the component into individual QTL that could be used either in breeding programs by marker-assisted selection or as the starting point for gene identification using various approaches. When using QTL data in marker-assisted selection (Mas) it is better to take into account the size and effects of QTL regions, as well as economic thresholds that must be targeted.

Gene identification

Expressed Sequence Tags (EST) is an important storehouse of expressed genes. The use of EST technology permits the rapid identification of new coding regions in plant genes, especially those whose isolation would otherwise be difficult or impossible. Plant biologists can directly use knowledge about proteins and genes from non-plant sources. Expressed Sequence Tags (EST) is an important storehouse of expressed genes. An example is the sugarcane EST genome Project- SUCEST carried out by a consortium of 25 Brazilian Laboratories (<http://succest.lbi.ic.unicamp.br/en/>).

Millions of sugarcane ESTs sequences were obtained from the cDNA libraries derived from different sugarcane tissues, organs and growth conditions. Filters containing EST cDNA clones are constructed and probed with radiolabelled DNA that is hybridized to the surface bound DNA. Signal variations with significantly higher intensities in the arrays were considered to determine the threshold for changes in gene expression that could be attributed to the stress treatment. A high correlation between the replicate experiments can identify the stress inducible genes. Drought/ cold responsible sugarcane ESTs include xanthine dehydrogenase (XDH), ocs element binding

factor I genes, ABI interacting proteins, protein kinase, CBF protein, cold associated transcriptional control mechanisms i.e. HAT (Histone Acetyl transferase complexes) and cellulose synthase (Noguiria *et al.*, 2003).

Transcriptomics and proteomics

A more comprehensive approach to study stress tolerance has been using transcriptomics and proteomics. Several of the ESTs encode proteins with a wide range of functions including transcription, signaling amino acid metabolism, defense development and water status. These results suggested that several metabolic processes including perception of stress signals and region of gene expression repressed during cold stress. The macro array data thus obtained can be validated by RNA dot blotting and gene expression profiles obtained using macro arrays and RNA gel blots can be compared.

Protein spot intensities by 2-D gel electrophoresis can be quantified and mapped as protein quantity loci (PQL), de Vienne (1999) and Prioul *et al.*, 1999) identified candidate genes that corresponded to QTLs for xylem sap, ABA content, leaf senescence, ADP glucose pyrophosphorylase, invertase and sucrose phosphate synthase.

Comparative genetics

Genome analysis of rice, maize, wheat, barley sorghum, foxtail millet and sugarcane demonstrated that gene content and order are highly conserved at both the map and megabase levels between different species within the grass family, but the amount and organization of repetitive sequences have diverged considerably (Devos and Gale, 1977). Comparative genetics research has the general goal of estimating similarity at

some level of organization. Comparative maps allow transfer of information about genetic control of traits from species with small diploid genomes, such as rice (*Oryza sativa* L.), to species with more complex genomic structures (increased repetitive DNA, polyploidy). Because of the size and complexity of the genome, it may not be appropriate to sequence the entire genomes. However, alternative strategies involve identification of gene-rich regions and comparison of the genome structure and genetic colinearity with other member of the tribe. A comparative approach to this problem will increase the number of available markers in any grass crop and will be useful for construction of a framework map of conserved regions in the genomes of the Gramineae family. Comparative mapping is bringing scientists together who would otherwise have little in common. International cross-species collaboration has been proposed for the Gramineae. The International Grass Genome Integration (IGGI) Program uses rice as the central genome for comparative mapping. Anchor probes will link genomes to amp across species. Anchor probes are currently available for wheat vs.rice comparisons. Significant progress in identifying genes and rapidly transferring biochemical and physiological information is expected when mutants are mapped.

High density molecular genetic maps coupled with the development of BAC and YAC libraries have led to the isolation of several rice genes (Wang *et al.*, 1999; Hayano – Saito, 2000). Rice has become a model system for genomics research due to the comparatively smaller size of the genome and synteny of its genome with other members of the grass family.

An extensive Data mining in the EST data bank can find homologs of stress inducible

sequence data base containing the stress inducible gene reported in the literature and present in the gene bank databases. With BlastN search, these protein sequences were used as drivers to identify putative assembled sequences.

All these studies have enabled identification of three categories of genetic elements

Genes with up-regulated expression in response to stress,

Genes that protect the plants from stress

Promoters that regulate gene expression under stress.

Genes with up-regulated expression

Two productive approaches to establishing the responses of plants to drought involve studying the candidate genes and differential screening. Comparing the expression of genes such as the enzymes in drought-induced metabolic pathways under drought verses non-drought conditions can provide useful information. Differential screening could identify many genes encoding proteins of known function.

Drought and cold responsive candidate genes

A candidate gene is a gene, located in a chromosome region suspected of being involved in the expression of a trait such as a disease, whose protein product suggests that it could be the gene in question. A candidate gene can also be identified by association within the phenotype and by linkage analysis to a region of the genome.

Candidate genes are potentially an extremely useful source of markers for marker assisted selection because they may

be the genes responsible for expression of the trait. If so, there will be no recombination between the candidate gene and the trait and thus is the perfect indirect selection tool. Further research to determine the association of the trait and the presence of the gene is still a useful marker, otherwise, this would show whether the gene is implicated in the expression of the trait, in which case the gene could provide valuable information on the underlying genetics, biochemistry and physiology of the trait itself (CSIRO project report, 2004).

Candidate gene analysis starts with selection of some target genes abased on the biochemical pathway.

A drought related EST databases; microarray analysis and mutagenesis approach yielded a large number of valuable candidate genes for verification of their association with drought tolerant traits (Nyugen, 2000; Gorantal *et al.*, 2005). Another approach was searching of orthologues in existing literature and databases on drought related abiotic stress genes. After genetic mapping is accomplished, ESTs that map very close to the trait QTLs can be targeted for further candidate gene analysis.

The potential candidate genes which are expected for enhancing tolerance to drought and salinity are:

Genes encoding stress induced proteins such as LEA (late embryogenesis abundant proteins), RAB protein (response to ABA), dehydrin proteins and heat shock proteins (HSP).

Genes encoding enzymes for the synthesis of osmolytes which regulate the osmotic pressure through osmotic adjustment.

Genes encoding enzymes or proteins involved in scavenging the reduced oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH) and H_2O_2 .

Genes encoding proteins involved in ion transport and ion homeostatis and Ca^{2+} ATPase.

One of the most interesting researches in this direction is the role of dehydration responsive transcription factors (DREB) which medilate transcription of several genes in response to water stress. DREB genes encode transcription factors that bind to the cis acting promoter element (DRE) of stress- related genes and turn on their expression (Smirnoff and Bryant, 1999).

This binding initiates synthesis of gene products implicated in plant acclimatization response to low temperature and water stress (Ingraham and Batels, 1996).

Over-expression of a fusion of a DRE-containing promoter from a dehydration induced gene (rd 29A) with a DREB gene (DREB 1A) in Arabidopsis resulted in marked increase towards tolerance to water stress and salinity (kasuga *et al.*, 1999).

The result of differential screening suggested the existence of two separate signal transduction pathways and ABA-responsive and ABA-independent pathway (Nordin *et al.*, 1991). Dehydrins (late embryogenesis abundant) are produced in response to dehydration, low temperature etc. These could stabilize macromolecules / protect membranes against chilling damages.

ESTs encoding proteins that have been shown to be directly involved in chilling and freezing tolerance includes WCROR4106, WCOR 413, Dehydrin 2, Barley ABA

inducible protein, thaumatin like protein, glucanase like protein and chitinase like protein.

PR proteins with antifreeze activity can have direct effects on the stability of cellular membranes and reduce chilling injury (Kuramae *et al.*, 2004).

Genes that protect plants from stress

Genes that have been isolated and tested as drought tolerance genes include alanine aminotransferase, D-myo-inositol methyl transferase, fructan etc. and transgenics for improved drought tolerance with these genes have been developed in tobacco and rice (having HVAI gene).

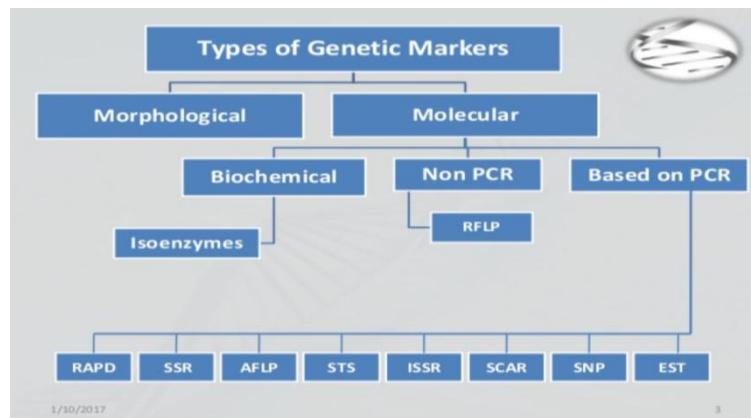
Regulatory element for regulation of gene expression under stress

Regulatory elements that are responsive to environmental signals and lead to specific gene expression are important components of stress tolerance. Rab 16B (from rice), Em (wheat), Rd22, Rd 29A (Arabidopsis) are some of the promoters identified in response to drought stress.

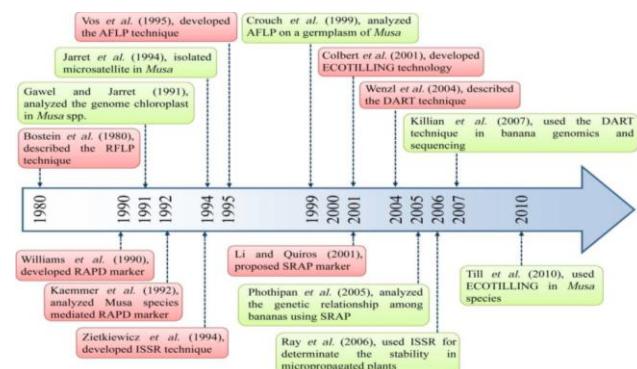
These results clearly show that a thorough analysis of the physiological events during stress and their genetical control is essential to categories the genes that are regulatory,

Candidate genes that can serve as markers for the physiological stage of the plant, genes that produce secondary stress-induced metabolites.

Genomics



QTL approach



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