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GENOMICS APPROACHES TO MITIGATE THE IMPACT OF DROUGHT AND HEAT STRESS ON YIELD PROCESSES IN CROP PLANTS

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ABSTRACT

A comprehensive understanding of the effect of drought and heat stress will be critical in evaluating the impact of climate change and climate variability on crop production. Abiotic stress tolerance influence an array of processes including physiological, growth, developmental, yield, and quality of crop. The objective of this review is to provide an overview of the influences of stresses on the above processes independently and in combination and advances to combat abiotic stress tolerance. Our review suggests a clear need of information on interactive effects of stresses particularly of drought and heat stress which mostly occur in combination. Among various stresses, drought is a big concern and rising at an alarming rate with climate change. Scientific approaches are being tried to understand the mechanisms of drought stress. The emergence of new molecular biology approaches and new sequencing as well as phenotyping platforms good research progress has been made in regard to drought and drought resistance mechanisms via identification of quantitative trait loci or genes responsible for drought tolerance mechanisms through Qtl mapping, Family based Qtl mapping, Linkage disequilibrium, Structural and Functional genomics approaches. Genome wide selection methods have been used for the current world concern of drought which will eventually lead to climate resilient crops and will solve the problem of food insecurity in near future.

Key words : *Drought, heat, genome selection, functional genomics, linkage disequilibrium.*

Reproduction is highly phasic, with each phase showing susceptibility to drought and heat stress. Early reproductive processes particularly those of micro- and mega-sporogenesis, pollen and stigma viability, anthesis, pollination, pollen tube growth, fertilization, and early embryo development are all highly susceptible to drought and/or heat stress. Failure of any of these processes decreases fertilization or increases early embryo abortion, leading to lower number of seeds or grains, thus limiting crop yield. Stress just before anthesis and at anthesis caused significant increase in floral abortion and lower seed numbers in, wheat *Triticum aestivum* L., (1), rice (2). Most of the reproductive abortion in legumes occurs after fertilization during the early stages of embryo development. Drought stress during early stages of embryo development increased the rate of abortion. It is important to know if the abortion is related to whole-plant signaling system involving hormones (particularly ABA). Drought imposed at flowering can also decrease photosynthetic rates and thus decrease the amount of photosynthates allocated to floral organs, causing increased abortion.

The plant hormone ABA signals drought stress and increased concentrations of ABA in the root is observed under drought stress and may help maintain root growth and increases root hydraulic conductivity, which can lead to increase in water uptake and postpone development of water stress. Studies indicate that a stress induced hormone, ABA, plays an important role in signal transduction of stress from the whole plant (places where drought stress is sensed) to the remote plant parts such as developing embryos (where stress is not sensed) leading to sterility or abortion. ABA is transported in the xylem from roots to shoots, where it can causes stomatal closure, decrease leaf expansion and thus preventing the dehydration of leaf tissues. Exposure to heat stress during flowering results in pollen sterility and loss of seed-set in legumes (groundnut; (3) dry bean, *Phaseolus vulgaris* L., (3) cowpea, *Vigna unguiculata* (L.) (4) and cereals (rice; (5); wheat. Lower seed-set under heat stress can be caused either by poor anther dehiscence, hence low numbers of germinating pollen grains on the stigma (1) or because of decreased pollen viability (Gross). Pollen is known to be relatively more sensitive to heat stress conditions.

Recent studies suggest that there is a quantitative response to heat stress (temperature) between fertility and tissue temperature for both rice (5) and peanut (3) above a particular threshold temperature. There was a strong negative linear relation between pollen production and cumulative temperature >34°C in peanut (Fig.-1) (3) and between spikelet fertility and cumulative temperature >33°C in rice (Fig.-2) (5).

A modified bilinear model best described the response of pollen germination and pollen tube growth to temperature in peanut (6), cotton, *Gossypium* spp., (7), and soybean (4). Exposure to as short as 1 h to temperature > 37°C during flowering decreases seed-set

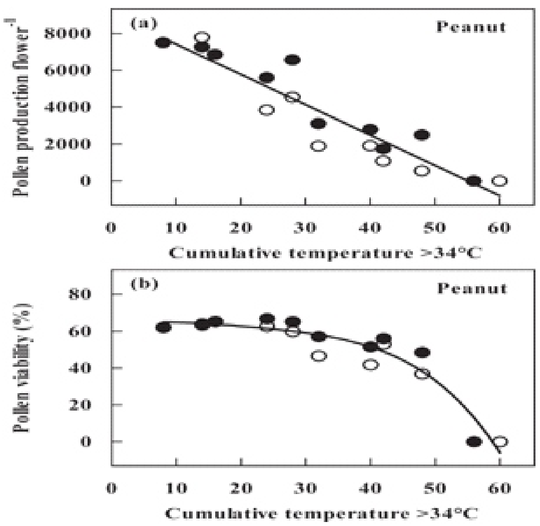

Fig.-1 : Relationship between accumulated hourly temperature and pollen production and (b) pollen viability in peanut.

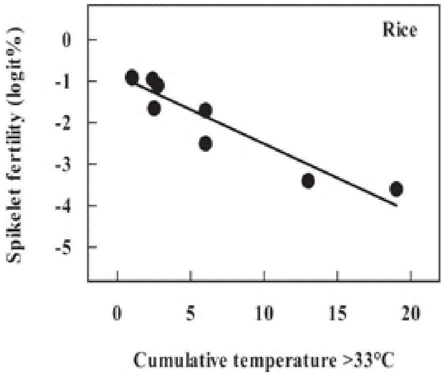

Fig.-2 : Relationship between accumulated hourly temperature and spikelet fertility in rice.

(2). Similarly exposure to temperature >33°C for first half of the day (6 h after anthesis) was enough to decrease pollen viability and thus seed-set in peanut (Fig.-3) (3) and yield (8).

Drought stress later during the reproductive development (after fertilization) decreases seed size rather than seed number. Seed size is the final component of yield. Seed size is largely dependent on the availability of photosynthetic reserves that are either currently available or those that can be moved from other parts into the grains. Drought and heat stress can negatively influence leaf area production and also green leaf area duration, thus decreasing the available photosynthates to seeds, ultimately influencing grain size and yield. High temperature can also directly influence the seed-filling rate or seed-filling duration, influencing the seed size and yield (8) without negatively influencing the leaf photosynthesis and total leaf area. Genotypes selected for rapid ear growth under well-watered conditions were shown to maintain relatively higher seed-set under drought conditions at flowering.

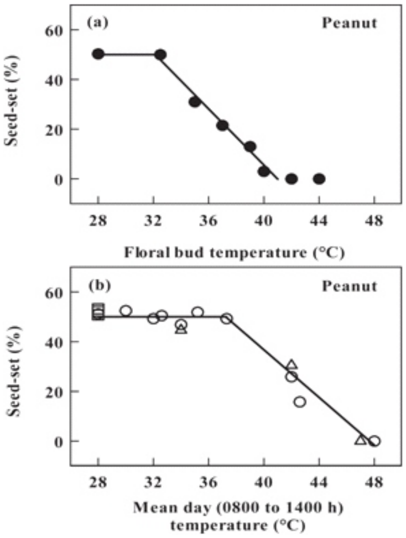

Fig.-3 : Loss in seed-set (%) in peanut when exposed to heat stress at flowering and (b) when exposed to heat stress during first 6 h (0800–1400 h) of the day.

Conventional Breeding for Resistance to Drought Stress : Greenhouse or field studies of plants are undertaken for the selection of drought adaptive traits. Selection in field is possible only in the environment having less rainfall i.e. less than 100 mm which mainly depends on irrigation because less soil moisture in the area. Maintaining field stress at the flowering time is the key for rapid improvement of breeding and selection strategies. It requires large investments in land, labor and capital for effective screening of the progenies as well.

QTL Mapping Studies for Drought Adaptive Traits : In various findings and other genetic studies it has been found that tolerance to stresses like drought is controlled by a large number of genes or Qtls with small effects. Genomics and functional biology technologies are new tools for understanding this complex nature of drought. There are many drawbacks of traditional QTL mapping like developing mapping population, indentifying markers which are polymorphic, genotyping, construction of genetic maps, Phenotyping as well as merging of both phenotypes and genotypes. In order to avoid such drawbacks "linkage disequilibrium mapping" or association genetic studies has been suggested. It is one of the methods of mapping Qtl which takes advantage of historic linkage disequilibrium to form a link between phenotype and genotype. The process involves: (1) Selecting a large number of individual from natural diverse population, (2) Phenotyping, (3) Candidate gene sequencing, (4) Framing population structure, kinship, phenotypic and genotyping correlation for linkage disequilibrium analysis.

Identification of Genes Associated with Drought Tolerance : With the success of genome sequencing technologies, several genome sequence for various

Table-1 : Identification of drought responsive genes via functional genomics and transcriptomics.

OSPFA-DSP1 (tyrosine kinase)	Negatively regulates drought stress responses
Rice OsSDIR1	Enhancer for drought and salt tolerance
Tomato ethylene factor (ERF) Protein TSRF1	Improved osmotic and drought tolerance in rice seedling
Rice DREB2A gene	TF/Regulatory control
OSNAC1	Regulatory control
ZMNF-YB2	Transcription factor
Os LEA-3-1	Osmoprotectant
DREB (1A)	Regulatory control
HVA (1)	Protective Proteins
mTLd	Mannitol as osmoprotectant
P5CS	Osmoprotectant
Sorghum SbDREB2 gene withstress-induced promoter CaMV35S or rd29A	Over expression of OsDREB2A significantly enhanced drought and salt tolerance of transgenic rice
ZAT10	Zinc finger
TAPIMP1	Transcription factor
HVCBF4	Transcription factor
HARDY	Transcription factor
TPSP	Osmotic adjustment
CIPK	Protein kinase
ZFP522	C_2H_2 Motif
NPK1	Map kinase

model plants and major crop plants like rice, maize and wheat is now becoming available which will facilitate the identification of genes involved in drought tolerance. Genome annotation task and functional biology in major crop species help in recognizing the CGs for drought tolerance. The genes involved in the drought tolerance mechanism also called as candidate genes may involve the regulation of certain transcription factors and protein kinases during expression of tolerance towards drought. DREB, bZIP, MYB are some of the main factors involved and are used in certain molecular breeding platforms. Before the deployment of some candidate genes they need to be validated which can be done through association analysis, proteomics studies and other reverse genetic tools like Tilling and Eco Tilling. Certain approaches are further discussed by (9) (Fig.-1).

Functional Studies and Transcriptomics : In order to understand the complex nature of drought tolerance various functional biology studies as well as expression profiling studies of genes are performed. Functional studies and proteome analysis are used to study various molecular responses to drought stress. Expressed sequence tags are currently being used in the candidate based selection. Scientists are able to find various genes which responses to drought stress and also at the same time aid marker development process. Another approach for identification of drought responsive genes is differential gene expression. Genes with no described homolog were identified through differential gene expression approach.

Marker Aided Selection for Genetic Improvement of Drought Tolerance in Crops : Once we have identified the genes responsible for drought tolerance, the next step is their deployment in breeding programmes for the development of stress tolerance lines/ varieties.

Marker-Assisted Backcrossing (MABC) Method for Drought Tolerance : Marker-Assisted Backcrossing (MABC) is the most promising approach which makes use of the markers for identifying and selecting genes for drought tolerance. MABC is an efficient tool by which using large population sizes (400 or more plants) for the backcross F_1 generations; it is possible to recover the recurrent parent genotype using only two or three backcrosses. Many abiotic stresses like drought tolerant high yielding varieties have been developed in the shortest time span. In major crops like wheat, rice this strategy has been commonly used. Near isogenic lines (NILs) were developed by introgression of three root QTLs from CT9993, an upland japonica into IR20, a lowland indica cultivar using this approach.

Marker-Assisted Recurrent Selection (MARS) : In order to overcome some drawbacks of MABC especially when many genes with minor effect are involved in a complexity of a trait, the approach of recurrent selection method aiming at increasing the frequency of desirable alleles is used. Here the selection strategy involves F_2 population and thus it increases the frequency of F_2 alleles i.e. maximum in segregating generation, where in marker

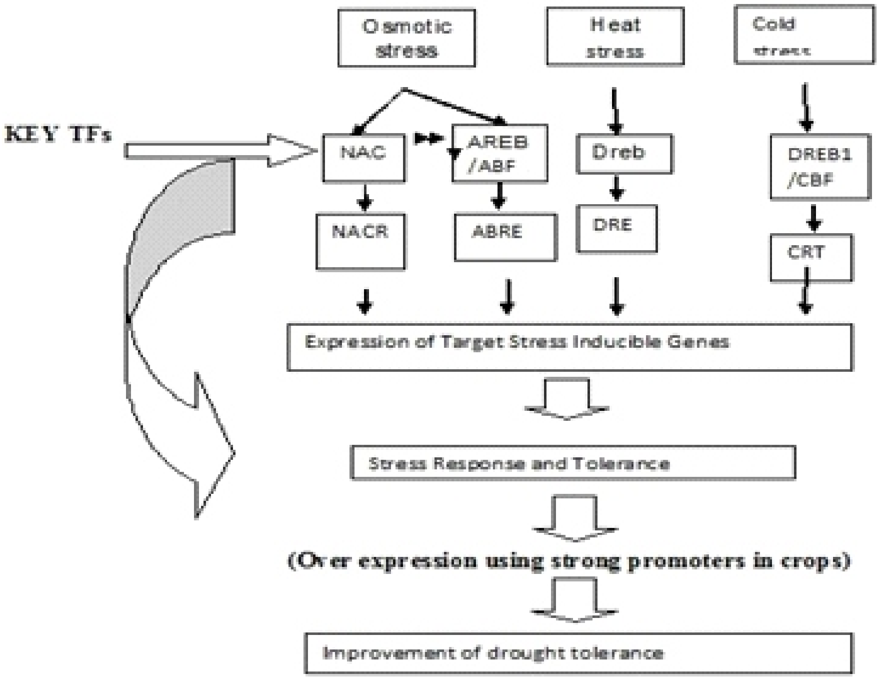

Fig.-1 : Candidate gene identification associated with drought tolerance.

selection is done in every step followed by repeated rounds of inter-mating and thus aid in selecting phenotypically outstanding lines. Marker assisted recurrent selection is being used in many crops like maize and sorghum.

Table-2 : Examples of Mass selection in crop plants.

Crop	Trait improved	No of genes /Qtl transferred
Rice	Yield and quality	Multiple Qtl
	Leaf width and grain number	2 Major Qtl
Wheat	Root penetration ability	13 Qtl
	Grainyieldunder multi-environment	1 Major Qtl
Maize	Yield and agronomic traits	14 Qtls
	Root architecture traits	15 Qtls
Cotton	DR related trait	7 Qtl
Common bean	DR related trait	Multiple Qtl
Pearl millet	Terminal	A Major Qtl
Soybean	Seed yield	4 Major Qtls
Chickpea	Root traits	71 Major Qtls
Pigeon pea	Fertility Restoration and Earliness	4 Major Qtls

Genomic Selection : It is a type of marker aided selection which makes use of genetic markers covering the whole markers (high density markers) so that all genes/Qtls are in linkage disequilibrium with at least one the marker. It is a novel method of improving quantitative traits like drought resistance. In this genomic prediction is based on both genotypic as well as phenotypic data for further increase in the prediction accuracies of breeding and genotypic values. Genomic selection uses two types of population: a training population and a validation/ breeding population. The training set is where the marker effects are estimated; marker effects are estimated based on the training set using certain statistical methods to incorporate this information; the genomic breeding value or genetic values of new genotypes are predicted based only on the marker effect. The validation set contains the selection candidates (derived from the reference population) that have been genotyped (but not phenotyped) and selected based on marker effects estimated in the training set.

CONCLUSION AND FUTURE THRUST

We have to look for an integrated approach i.e. both conventional and molecular approach which will play an important role in understanding the mechanism of drought resistance. Approaches like marker aided backcrossing is not yet proven that strong but when it comes to pyramiding multiple Qtl/genes into a single cultivar for complex abiotic stresses like heat stress, drought MARS and GS have been very effective. Functional genomics made it possible conduct high-throughput sequencing, genotyping and resequencing, which aid in identifying the genes that show response to drought stress tolerance. Most genetic and molecular studies of DR have focused on the aboveground parts of plants, the underground parts have received much less attention because of difficult phenotyping and huge data sets from genome-wide studies and also the data sets to extract the essential functional pathways or networks for genetic improvement of drought resistance remains a significant challenge. Most important challenge is how to efficiently explore the large data sets derived from genomics, expression profiling, proteomics and metabolomics. Integration of physiology, genomics and breeding is carried out orphan crops for security of food in many developing countries.

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