Breeding and Genomics Approaches to Increase Crop Yield under Drought Stress in Climate Change Scenario

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Abstract

Climate change has been a threat to food security challenges. Climate change presents an additional stress on food security challenges as it affects production of food in many ways. 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. Huge data are being derived from genome wide selection (GWS) studies at the transcriptomics, proteomics and metabolomics levels, but how efficiently to explore and exploit these data to extract the essential functional pathways or networks for genetic improvement of drought resistance remains a significant challenge.

Keywords: Breeding approaches; Genomics; Crop yield; Drought tolerance; Climate change

Abbreviation

GS: Genomic selection; GWS: Genome wide selection; QTL: Quantitative Trait Loci; CGs: Candidate genes; AM: Association mapping; LD: Linkage Disequilibrium; GEBV: Genomic Estimated Breeding Value

Introduction

One of the major challenges we are facing is to feed ten billion people by 2050 while at the same time reduce the impact of environment on global food production. Agriculture in itself contributes to the anthropogenic 30% of the greenhouse gas emissions [1-3]. Several mitigation measures should be made available within agriculture for the reduced emission of greenhouse gases. The major challenge of having to increase food production in regard to nutrient deficiencies, increased water scarcity, and uncertainty due to predicted climatic changes. One of the serious consequences of climate change is drought [4,5].

Various abiotic and biotic stresses arise due to climate change in the climate pattern which might affect the productivity of cereal. Abiotic stress is quite prominent today. C_3 and C_4 carbon fixation are biochemical mechanisms in plants that fix CO_2 to make carbohydrates through photosynthesis. C_4 plants will respond less to increased CO_2 levels) [6,7]. Drought is caused by high temperatures, which effect photosynthetic rates, and further reducing crop yields. Drought is becoming a serious problem as a result of climate change. Drought tolerance is found out to be a complex trait meaning that it is controlled by many genes with small effect [8-10]. Genomics along with functional biology are good approaches for genetic dissection of drought tolerance. To understand the drought response mechanisms by a plant, we need to understand the genetic basis and physiology. Here, we will stress on the progress which has been made for accurate phenotyping as well as genomic assisted breeding. Studies involved in understanding the mutagenic nature of complex traits like drought tolerance have also been focused [11-14].

Climate is Changing Globally

The impact of this change will have serious effects, including reduced crop yield but also change in vegetation in many areas in the world. Agriculture also has a serious indirect effect on climate change [15,16]. Climate change may have beneficial as well as detrimental effects for agriculture. With the virtually certain likelihood of warmer and more frequent hot days and nights, there are projected to be increased insect outbreaks impacting agriculture, forestry and ecosystems. Changes in climate models and growing population is threatening food security as well as agricultural productivity [16-19]. Climate

change is one of the major reason for the worldwide occurrence of most osmotic stresses and drought being one of them.

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 [20-22]. Maintaining field stress at the flowering time is the key for rapid improvement of breeding and selection strategies which has been successful up to some extent; it requires large investments in land, labor and capital for effective screening of the progenies as well [23,24].

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 [25-27]. 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 [28-30].

Identification of Genes Associated with Drought Tolerance

With the success of genome sequencing technologies, several genome sequence for various 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 [31,32]. 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 [33,34]. 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 Varshney et al. (**Figure 1**) [35].
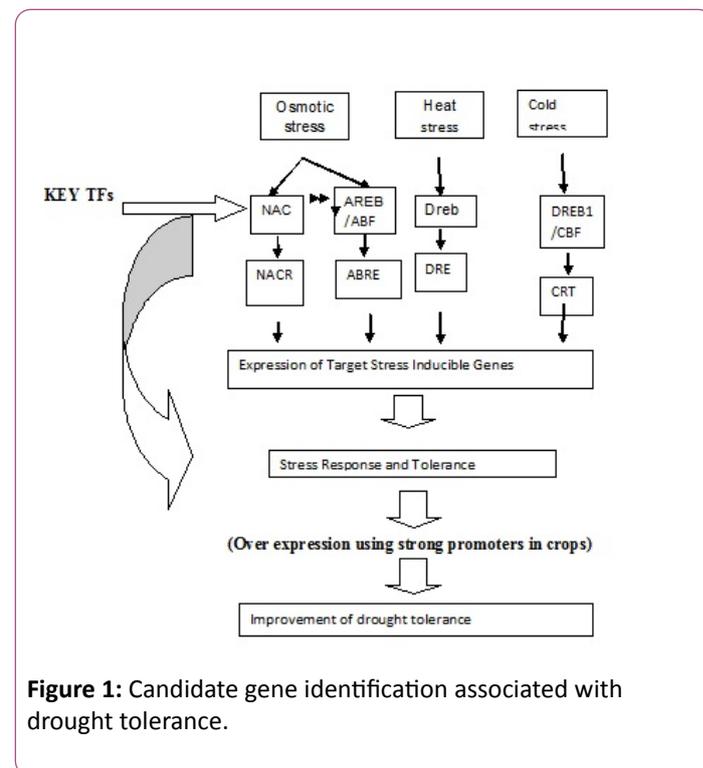
Figure 1: Candidate gene identification associated with drought tolerance.

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 [36]. 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. Several genes and proteins that were thought to be involved in drought resistance mechanism and the genes with no described homolog were identified through differential gene expression approach (**Table 1**) [37,38].

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

Gene	Mechanism of tolerance
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 with stress-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

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 use/deployment in breeding programmes for the development of stress tolerance lines/varieties which nowadays is made possible by the use of molecular markers whose function is to detect the location of drought tolerant genes [39,40].

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 F1 generations; it is possible to recover the recurrent parent genotype using only two or three backcrosses [41-43]. 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 [44,45].

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 is used which is one of the population improvement method aiming at increasing the frequency of desirable alleles is used [46,47].

Here the selection strategy involves F2 population and thus it increases the frequency of F2 alleles i.e. maximum in segregating generation, wherein marker selection is done in every step followed by repeated rounds of intermating and thus aid in selecting phenotypically outstanding lines [48-50]. Marker assisted recurrent selection is being used in many crops like maize and sorghum (**Table 2**).

Table 2: Examples of MAS Selection in crop plants.

Crop	Trait improved	No of genes /Qtl transferred
Rice	Yield and quality	Multiple Qtl
	Leaf width and grain number	2Major Qtl
Wheat	Root penetration ability	13 Qtl
	Grain yield under 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

Genome-Wide Selection (GWS)/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 [51,52]. 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 [53,54]. 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 [55-57].

Genetic Engineering of Crop Plants for Drought Tolerance: Role of Transcription Factors

Stress response may involve stress-responsive and stress-tolerant genes. Functions like plant adaptation are played by different TFs which are capable of regulating several downstream genes essential for protection against drought [58,59]. TFs are potential candidate genes for developing drought tolerant plants and regulators of various genes that help the plants to effectively sustain drought stress. Molecular tools facilitate the identification of such important genes controlling traits related to drought tolerance. Apart from the general regulatory mechanisms, TFs are recorded to be regulated by miRNAs and vice versa. This phenomenon of TFs and miRNAs can possibly mediate drought tolerance without disturbing the phenology modifications. Clear understanding of genetic network between the miRNAs and its associated TFs could be a valuable area to identify appropriate TF regulating drought tolerance [60,61]. Thus detailed studies of differential expression of miRNAs and their targets in TFs overexpressing plant model under drought stress are ultimately necessary. Different crops such as rice, wheat, soybean and maize were engineered with this stress responsible TF to increase their tolerance to drought. The transgenic approach using drought stress associated TF could cover a wide range of genes and help the growing population to meet food demands. TFs are potential candidate genes for developing drought tolerant plants and regulators of various genes that help the plants to effectively sustain drought stress. Molecular tools facilitate the identification of such important genes controlling traits related to drought tolerance [62-65]. Apart from the general regulatory mechanisms, TFs are recorded to be regulated by miRNAs and vice versa. This phenomenon of TFs and miRNAs can possibly mediate drought tolerance without disturbing the phenology modifications. Clear understanding of genetic network between the miRNAs and its associated TFs could be a valuable area to identify appropriate TF regulating drought tolerance. Thus detailed studies of differential expression of miRNAs and their targets in TFs overexpressing plant model under drought stress are ultimately necessary [66,67]. Different crops such as rice, wheat, soybean, and maize were engineered with this stress responsible TF to increase their tolerance to drought. The transgenic approach using drought stress associated TF could cover a wide range of genes and help the growing population to meet food demands [68,69].

The gene encoding cold shock protein B (CspB) from Bacillus subtilis – a soil bacterium–was used for genetic engineering MON 87460 maize. CspB allows the transgenic maize plant to react more quickly to drought, slowing its growth and conserving water, thereby making water available for key plant functions after the onset of drought stress [70,71]. Based on this transgenic event, the DroughtGard™ hybrid maize was bred and released for farming in the USA in 2013.13 Prior to the release, the company facilitated 250 large-scale on-farm trials (on about 4000 ha) of DroughtGard™ in the western half of the US Great Plains [72]. Under stress, a DroughtGard™ hybrid used 261 mm of water from the soil while the control used 338 mm of water from the soil; i.e., their WUE rates (or the amount of water for producing 1 bushel or 25.4 kg of maize grains) were 0.59 and 0.44, respectively. At harvest, DroughtGard™ had more grain yield than non-transgenic maize hybrids with enhanced WUE (up to 0.4 t ha^{-1} greater in some locations of the western Great Plains) [73,74]. In theory, DroughtGard™ can save about 2.5 mm of water inputs per hectare; i.e., 5 trillion liters of water, which translates to providing, water to the US city of Denver (Colorado) for one month.

Conclusion and Future Thrust

With increasing temperature and greenhouse gases the soil moisture as well as due to less water status in the soil is proving fatal to crop productivity. 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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