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Breeding And Genetic Engineering Of Drought-Resistant Crops

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ABSTRACT: Drought resistance (DR) being a complex traits triggers a wide array of physiological responses in plants thus affecting the activity of various genes. Plants on the other hand respond to the ever changing environment in a complex way that allows them to react to different conditions at a given time. Therefore, the genetic control of tolerance to abiotic stress is not only complex but also influenced by other environmental factors at various stages of plant growth. Plants use multiple ways to respond to drought stress and have adapted to drought through physiological and morphological changes which are controlled by a cascade of diverse signals. Plant response to drought is also accompanied by activation of genes which are involved in both perceptions of drought stress and transmission of the stress signals. Other genes regulate transduction of stress signal and modulate gene expression. This paper highlights potential improvement of DR of crops in field trials though the biochemical and molecular basis though stress adaptation remains the major challenges for the genetic improvement of DR.
Key words: Photosynthesis, Tolerant, Stress, Transcription, Abiotic, Antioxidants

INTRODUCTION

Adverse environmental factors are examples of the most severe constraints to agriculture not only to developing countries but to the world at large. Water scarcity being one of the factors contributes to the highest percentage death rate of different crops. Climate changes together with increasing population are some of the factors which contribute to water scarcity and thus the importance of water use efficiency (WUE) improvement in agriculture. This requires the development of crop varieties with increased tolerance to drought through conventional breeding and genetic engineering methods thus meeting global food demands with less water.

Drought resistance (DR) being a complex traits triggers a wide array of physiological responses in plants which affects the activity of various genes. Plants respond to the ever changing environment in a complex way that allows them to react to different conditions at a given time. Therefore, the genetic control of tolerance to abiotic stress is not only complex but also influenced by other environmental factors at various stages of plant growth.

Plants use multiple ways to respond to drought stress and have adapted to drought through physiological and morphological changes which are controlled by a cascade of diverse signals. The physiological responses of plant to drought include leaf wilting, leaf abscission, a reduction in leaf area and stimulation of root growth through directing nutrients to the underground plant parts (Alexandre Bosco). Plants also survive severe drought stress through a plant stress hormone called abscisic hormone (ABA) which induces closure of leaf stomata thus reducing water loss through transpiration. All these improve water use efficiency (WUE) of plants (Joung kang, 2002).

Plant response to drought is also accompanied by activation of genes which are involved in both perceptions of drought stress and transmission of the stress signals. Examples of these genes are those that govern the accumulation of compatibles solutes, stabilization of cell structures from desiccation by reactive oxygen species (ROS).Other genes regulate transduction of stress signal and modulate gene expression (M manuela, 2003). All this gives an idea of potential improvement of DR of crops in field trials though the biochemical together with a molecular basis of drought perception, stress adaptation remains the major challenges for the genetic improvement of DR.

The Genetics Of Drought Resistance

Genetic control of leaf Traits related to drought resistance

Various leaf traits have been associated with DR mechanisms and these traits are highly used to evaluate DR of crops. This includes both morphological and physiological traits which are usually used as drought avoidance (DA) by plants. Plants also have drought tolerance (DT) mechanisms for osmotic adjustment and sugar content. All these together with other traits are among the factors considered by many breeders and researches who elucidate the genetic bases of leaf related DR traits by quantitative trait locus (QTL) mapping approaches. Little work has been done on improving crops for drought resistance through the integration of conventional breeding; marker assisted breeding (MAB) and genetic engineering (M, 2009).

Complex functional traits have been achieved by many researchers through QTL mapping approach due to the development of gene based markers and availability of functional information for various plants which ease functional annotation with QTL studies. Use of high throughput genotyping maps together with functional annotation have narrowed down the position of loci of the targeted traits thus identifying positional candidate genes for these QTL (David Sanchez, 2014).

Detection of QTL involved in photosynthesis and WUE in the context of drought resistance is a mile towards understanding the genetic basis regulating the expression of these traits. Photosynthesis ability is mainly determined by stomata density and stomata aperture but genetic variation of these factors are unknown making it harder for researchers. Plants when exposed to water stress, drought or other harsh conditions have to cope with the stress or else die. The sensitivity and response time to drought differ from different species (Sober Anu, 2010).Repeated drought encounter increases the sensitivity to environmental changes that induces stomata closure, while the sensitivity to changes that induce stomata opening is reduced (Sober Anu, 2010).

Leaf rolling is another drought avoidance trait to overcome harsh environmental conditions. Leaf rolling reduces water loss in addition to reducing the leaf area exposed to heat and light radiation. Various plants differ to roll leaves under similar water deficit and there is evidence that enhanced ability to roll leaves confers a yield advantage under drought conditions (Boopathi N Manikanda)

Genetic control of physiological traits related to Drought Resistance

DT in crops has been associated with physiological traits like osmotic adjustment and cell membrane stability. Osmotic adjustment (OA) is defined as the lowering of osmotic potential due to the accumulation of solutes in response to water deficit. This accumulation of solutes has various functions related to OA which results to increased water retention and turgor maintenance. In some plants like peas (*Pisum sativum L.),* OA has been associated with increased yields under drought stress conditions (Eusebio F, 1998).The overall effect of OA on plant production under stress conditions involves the whole plant development and this brings about deep root growth or higher root length density in the soil (Jingxian Zhang, 1999).QTL mapping analysis for OA under stress conditions has been done in various crops but care must be taken in mapping QTLs for OA and other physiological traits. Evaluation of genotypic difference in OA is supposed to be performed at the same level of stress in all genotypes because solute accumulation in a given genotype is a function of leaf desiccation (Zhang e. a., 1998).

Numerous field studies have been done in crops like wheat and Barley to examine the association of yields and water use efficiency (WUE) under drought stress conditions. Results indicated that plant utilizes different mechanism for moderating water use like reducing plant size and leaf area under stress conditions. An indirect indicator of WUE was difference in carbon isotope discrimination which assisted plant breeders in drawing a conclusion (Condon, 2002)

Plants also use ABA under stress condition as a DA thus playing a role in pre-invasive defense. Other than ABA's role in drought and low temperature stress tolerance (Maud, 2013),ABA has also been reported to mediates plant defense responses (Venkategowda, 2015).Despite the importance of ABA in DA ,its genetic basis of drought –induced ABA accumulation in relation to morphological traits like leaf size and root growth remains unknown.

Genetic control of root traits related to Drought Resistance

Plant roots play a vital role in the growth of a plant during stress conditions. Root hairs which are associated with maintaining plant productivity during drought conditions includes root qualities like diameters, length and density especially at depths in soil with available water (Louise, 2013).In dry areas, small xylem diameters in deep roots help in improving root acquisition of water when ample water at depth is available. Genetic control of root traits through breeding has been a bottleneck because of the complexity of the root system architecture (RSA).

Soil exploration increment by the root systems is a positive feature for improving drought tolerance and thus large genetic variability of root system characteristics is a source of genetic progress (De Dorlodot S, 2007).In

fields with deep soil and water table, roots grow deeper in the soil layers thus improving both water status and access to water because of hydraulic properties (Javaux M, 2008).

Multiple genes usually control various root traits where each governs small effects with a degree of interaction effects that can change with environmental conditions (Draye X, 2007).The QTL that contribute to root traits can therefore be considered as either constitutive or adaptive and this makes it useful in selecting traits which are useful in the target environment (Collins N C, 2008).Both constitutive and adaptive methods are difficult to phenotype and this is one of the reasons why most genetic researchers focus on plants part above the soil as compared to the one below (Herder G D, 2010).

Root QTLs have shown a greater potential for marker assisted selection (MAS) mostly when chosen roots traits contribute to drought tolerance in the target environment. The selected function for use in MAS can vary depending on the environment at hand and the objective of the researcher (Blum, 2011). QTLs that have been identified in green houses conditions are supposed to be validated under field conditions and should relate to improvements in productivity before use in MAS program..

Improvement of productivity under drought conditions through utilization of molecular markers is a challenge in crop improvement simply due to root variation being difficult to phenotype. Marker assisted selection in a breeding population of about hundreds of genotypes assist breeders with different options to select combinations of favorable traits above and below grounds. Molecular marker with strong linkage disequilibrium with the QTL of the desired root traits together with the gene are mostly identified and assist in plant breeding program for the success of MAS. Accurate phenotyping becomes the challenge of using MAS and this can result in greater QTL accuracy locations in higher density molecular maps (Francia E, 2005).

Functional Genomics Of Drought Resistance At Various Levels

Due to complex genetic basis and diverse mechanism of DR where thousands of genes are up or down regulated under drought stress conditions in specific organs, functional genomics seems to be a way out to study molecular basis of DR at the various levels which include proteomic, transcriptomic, metabolomic and epigenetic levels.

Transcriptomic analysis has mostly been used to identify drought responsive genes in various plant species (Deyholos, 2010).Transcriptome sequencing (RNA-seq) has improved the throughput of gene expression profiling in drought stressed plants. Microarrays have facilitated the identification of various drought resistant genes and reveal that the transcription level in response to drought is influenced by time of day and genotype (Raj S, 2010).Common drought-responsive genes in different tissues and genotypes have specific functions in regulating stem development under drought. Using Affymetrix Gene Chip Popular Genome Arrays (wilkins O, 2009) identified divergent responses in gene expression profiles in response to drought stress between two populous hybrids and conclude that it's not possible to describe a common genus-specific drought-driven Transcriptome based on few genotypes.

Proteome-wide profiling approach is another way used in characterizing plant phenotypes where the method targets different proteins and understand their role in stress. This makes it important to understand the complex biological mechanism which is involved in response to a biotic stress tolerance. Post-translational changes are also important where a single gene can translate into different proteins and few genes can lead to a diverse proteome. This explains the limitation to genomics and transcriptomic approaches more so when translational changes govern phenotype (Rupesh Deshmukh, 2014).

Epigenetic regulation is another way beside genetic control in which drought response can be regulated. This is through epigenetic mechanisms like DNA methylation and Histone modification. DNA methylation, plays a role in regulating genome wide gene expression and maintaining genomic stability in response to drought stress (Saze H Tsugane, 2012).Genome Wide methylation in Arabidopsis were recently shown to increase in the progeny of plants exposed to extreme temperature, UV-B, flooding and salt stress but decease in the progeny of drought stressed plants (Boyko A, 2008).

In Histone modification, post translation modification of specific residues on the core histone (H2A,H2B,H3 and H4) play a role in regulating genome functions by recruiting of transcriptional factors or other proteins that affect chromatic structure and state (Hake S B, 2007). Upon water deficiency, nucleosome pattern and histone modification specifically on the H3N-tail of four Arabidopsis drought responsive genes were altered to activate their coding regions (Kim W T, 2008).

Genetic Engineering Of Drought-Resistance Genes In Crops

Through the studies of molecular, biochemical and genetics, drought stress signaling have been revealed to be regulated by transcriptional activation of drought induced genes, protein kinase, nitrogen –activated protein

kinase cascades and other post translational processes. All these genetic regulations can be divided into transcriptional, post-transcriptional and post-translational aspects.

Transcriptional reprogramming plays a vital role in plant adaptation to drought and some of the transcriptional factors act as a central hub to modulate the expression of multiple-drought regulated genes (Thapa G dey, 2011).Transcriptional Factors (TFs) are example of Denovo synthesized regulatory proteins and in *Arabidopsis Thaliana*, more than 1700 genes encoding TFs in above 50 families have been identified (Guo A Y, 2008) where a good percentage of them have been associated with plant response to drought. Many transcription factors like basic leucine zipper (bZIP), ABA responsive element binding protein. NAM (no apical meristem), CUC2 (cup-shaped cotyledon), (NAC) like stress-responsive NAC and zinc-finger like C2H2 zinc finger protein (ZFP)) families have been characterized according to their roles in drought stress response regulation (Shinozaki K, 2003) and expression of these TFs resulted in improved crop tolerance to drought (Xiao B Z, 2009).

In a post –transcriptional regulation, accumulation of small RNAs under drought has a role in stress related responses. In Arabidopsis, levels of 117 miRNAs have been analyzed using miRNA chips and 17 stress –inducible miRNAs have been identified (Kinoshita e. a., 2012).Genome wide identification of drought responsive miRNAs have been carried in different plants like soybean, sugarcane with a number of conserved and non-conserved miRNAs related to drought response (Kulcheski, 2011)

Post-translational modification plays a bigger role in signaling cascades within highly interconnected networks in response to biotic and abiotic stimuli. Protein Farnesylation is a post translational protein modification where farnesyl group to a target protein where it regulates many different growths and development processes (Galichet A, 2003). Farnesyltransferase consist of α- (FTA) and a β- (FTB) subunit where loss of function of FTB in Arabidopsis resulted in mutants that have an enhanced response to ABA.

Protein phosphorylation plays a vital role where some protein kinases need to be phosphorylated to become active. Various protein kinases have been associated with response to osmotic stress based on their transcriptional responses to different environmental stimuli (Chae L, 2009).Some of the protein kinases are Mitogen Activated Protein Kinases(MAPKs),High Osmolarity glycerol (HOG1) etc. Some protein kinases involved in stress signaling are activated by calcium and they include CIPKs. This is through interaction with calcium binding proteins during the ABA response (Hirayama T, 2007).

In plant cell, accumulation of reactive oxygen species (ROS) is mainly caused by abiotic stresses which results in damage to genetic material thus interfering with cellular energy homeostasis through activation of Poly(ADP-ribose) polymerase which catalyzes long branching of poly(ADP-ribose) polymers covalently attached to proteins by consuming NAD+ pools (Bakondi E, 2002).

Breeding In Drought Resistance Crops

Conventional breeding and marker assisted selection (MAS) are examples of breeding techniques carried out in drought resistance crops. Conventional breeding for DR involves backcross strategy to develop new varieties with improved yield potential and DR. This breeding method requires the identification of genetic variability to drought among crop varieties where genetic variability traits are introduced into cultivars with the required characteristics. This method has been applied in breeding programs of various crops like rice, wheat, maize and one of its short backs is being a slow process which is limited by the availability of suitable genes for breeding and temporal variation in the field environment.

Marker assisted selection is a technique deployed to improve crop DR though traits like disease resistance. In this method, major QTLs for drought resistant donor genotypes are introduced into high yielding but fewer drought-resistant parents and thus the developed superior cultivars have only the major QTLs. Molecular markers like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and isozyme facilitate development of drought resistant genotypes more effectively because their expressions are independent of environmental effects. This application of marker assisted selection in evolving drought resistant genotypes is still in progress and more experiments are being carried out.

CONCLUSIONS

Various approaches for engineering and breeding of drought resistant crops have been made for the past years and various approaches have been integrated to study genetic and molecular basis of drought resistance and identify QTL or gene for engineering or breeding. Through this and other methods, various QTLs have been identified but few have been successful due to various challenges. In field conditions, crops are subjected to variable levels of multiple stresses.Therefore; plant response to multiple stresses cannot be inferred from the

response of a single stress thus the need for breeding programme for drought resistance aiming at pyramiding a number of relevant traits in a crop.

Marker assisted breeding approach is another better method for developing drought resistance crops not only because of its less time consuming but also labor and cost effective. Molecular mapping and analysis of QTL have been carried out in various traits related to drought and have resulted in greater magnitude of better understanding drought traits. However, there is a bigger challenge of using this knowledge to manipulate genes in an effective way to improve specific traits in a crop. These challenges include precise QTL identification, genetic and environmental interaction, various numbers of genes regulating yields and invalid use of mapping of QTL. In addition to this, QTL from a specific background do not show significant effects in different backgrounds even under analogous growth conditions (Collins, 2008).

Genetic engineering seems to offer a substantial improvement in a desired trait within a short period of time. Engineering genes encoding different traits like osmolytes, antioxidants, plant growth regulators and transcription factors have resulted in better crop performance under controlled stress conditions. However, with few exceptions, performance of these transgenic crops has been rarely field tested and thus the performance under field stress conditions remains unknown because of different environmental stress under natural field conditions. This makes a conclusion that, even though a number of prominent genes effective for drought resistance have been fully characterized, the function of most of them in a mechanism of environmental stress is yet to be investigated.

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