Chapter 4
Effect of Drought Stress and Utility of Transcriptomics in Identification of Drought Tolerance Mechanisms in Maize

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Abstract Maize crop encounters a number of abiotic and biotic stresses which reduce the production and the productivity. Abiotic stresses such as drought are unpredicted environmental disturbances during the crop growth which often lead to reduced crop yield or complete crop loss in some cases. Drought occurring at flowering leads to greater yield losses than when it occurs at other developmental stages. Plant responses at various levels such as morphological, physiological, biochemical and molecular changes to cope up with the stress. It is very important to understand the genes involved in drought tolerance as well and their interactions to breed tolerant hybrids in maize. Transcriptome profiling is useful to understand the whole spectrum of genes expressed under drought condition. The assay will be useful to decipher the genes involved in specific pathways and with the help of in silico analyses, interactions of target genes can be studied. Several transcriptome studies have been carried out in maize in different stages and in tissues under drought stress. Genes involved in detoxification, stomatal regulation, photosynthesis, hormone signaling, root architecture and sugar metabolism pathways are considered as important to achieve drought tolerance. The genes identified through gene expression assays could be used as candidate genes in selection programmes to develop drought tolerant hybrids in maize.

Keywords Drought · Candidate genes · Plant responses · Transcriptomes
Tolerant mechanisms

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4.1 Introduction

The changing trends in environmental temperature, precipitation and sea levels are adversely affecting the crops' production worldwide. Though various biotic and abiotic stresses affect agricultural crops; drought, cold, flood and heat have been the most devastating leading to huge yield losses. Drought is an important stress that relentlessly affects the agricultural output worldwide, especially in arid and semi-arid regions (Farooq et al. 2012). Drought is a climatic glitch, represented by deprived moisture as a consequence of sub-normal rainfall, unreliable rainfall circulation, higher water need or an array of all three factors.

Present challenge for researchers is to overcome the problem of water scarcity. It is the biggest threat our agriculture is now tackling, which needs continuous efforts of scientists. It is estimated that more than one third of the arable land of the world is facing the problem of water scarcity. Other types of abiotic stresses often accompany drought stress, thus making it more complex to study (Barnabás et al. 2008; Farooq et al. 2009; Zlatev and Lidon 2012).

Though plants can tolerate drought up to some extent, however, the degree of tolerance varies from species to species (Rampino et al. 2006). They cope up with drought by adopting any of the three strategies; drought escape, drought endurance and drought avoidance to complete their life cycle. Different levels of complex interactions among stress factors and integration of morphological, physiological, biochemical and molecular processes influence plant developmental stages (Farooq et al. 2009; Zlatev and Lidon 2012). To understand the mechanism of drought tolerance, it is important to understand the changes in plants that occur in response to drought stress. The primary responses in plants against drought include reduced leaf water potential and turgor loss, stomatal closure, cessation of cell enlargement and growth, and reduction in water content (Farooq et al. 2009). The changes in gaseous exchange occur as a result in reduction in photosynthetic process and organic solute synthesis. This ultimately affects photosynthesis, respiration, translocation, ion uptake, growth factors, carbohydrate and nutrient metabolism, plant growth and cell elongation. Further, hyped intensity results in photosynthetic arrest, metabolic imbalance and eventually the death of the plant (Farooq et al. 2009; Jaleel et al. 2008). Drought also modifies CO_2 conductance and thus adds to photosynthetic imbalance by histological and leaf anatomical changes. In the following sections, we have reviewed the morphological, physiological and biochemical responses of plant to drought stress followed by responses at molecular and transcriptome levels.

4.2 Morphological Response

Plant responses vary with growth stage, exposure period, stress intensity and level of tolerance (Jaleel et al. 2008). In the subsequent subsections, the morphological changes adapted by plants in response to drought as well as to withstand drought stress conditions have been discussed.

4.2.1 Plant Growth

Growth is defined as an irreversible proliferation in plant mass resulting from both cell division (especially in meristems) and cell extension. As a result of complexity in cell growth and differentiation under drought stress, the morphological transition occurs that leads to poor growth in plants. The reduced growth is considered as an adaptive modification in plants to help them avoid energy loss under unfavorable conditions. Hydrostatic pressure is very essential for cell growth and expansion. This is the reason that cell expansion is very sensitive to water stress, which directly or physically reduces growth as a result of low hydrostatic pressure. Weak photosynthetic activity affects the plant growth, which in turn is controlled by water supply. Therefore, plant suffers a reduction in photosynthesis under poor water supply. Limitation posed in by reduced photosynthesis components results in reduced growth of the plant to conserve the stored energy.

A. **Effect on vegetative growth**: The early phase is one of the most susceptible phases in the life cycle of plants under limited moisture conditions as drought affects both elongation and expansion of cells due to low hydrostatic pressure (Kusaka et al. 2005; Shao et al. 2008). In maize, for example, elongation of stem gets reduced under drought stress during vegetative stage. The water stress condition also affects the rate of tiller appearance that in turn reduces the plant grain yield. Limited supply of moisture reduces leaf expansion rate. Constricted moisture during vegetative growth shrinks the leaf area of the plant considerably and therefore carbon usage gets reduced throughout the growing season. Denmead and Shaw (1960) reported that extended drought during vegetative stage affects the length of the internodes by affecting cell size development and assimilate storage.

B. **Effect on reproductive growth**: Flowering, silking, pollination and grain formation are the important stages of plant development. Among cereal grasses, maize is most sensitive crop to drought stress at flowering stage. The flowering interval in maize is very short and pollen remains viable for a very short time period. It has been reported that per day delay between pollen shed and silk emergence reduces sexual fertilization and increases bareness and yield loss (Sangoi and Salvador 1997). The delicate period lies from one week before silking to two weeks after silking with probable chances of ovules, kernels and ears abortion (Uhart and Andrade 1995). There is a delayed silking under moisture stress so pollen is shed much before the stigmas are formed (Herrero and Johnson 1981). In maize, the anthesis-silking interval (ASI) increases in response to the drought condition. Extended dry conditions reduce ear growth and silk appearance thus escalating ASI. Increased ASI is thought to be a cause of yield loss as it is highly correlated with kernel set (Byrne et al. 1995).

4.2.2 Root

One of the important components of water potential is the matrix potential, which is defined as the energy required by the plant to extract soil water. In low moisture conditions, this force is greatly enhanced and shows a high matrix potential while in dry conditions, it increases further which ultimately results in stress of plant. Another difficulty faced under drought stress is accumulation of solutes in the interior cells of the roots, which leads to reverse cell osmosis. The probable outcome of reverse osmosis is membrane collapse and finally the death of the root cells, which adversely affects the water intake capacity of the plant. Roots being the first to sense the drought conditions are highly influenced by drought than any other aerial part of the plant. Early stages of plant development are highly controlled by a well-developed shallow root system (Johansen et al. 1994). An increase in fresh weight of the roots under drought stress has been reported probably due to better water utilization than shoots. The best symptom for morphological identification of drought tolerant crop is maximum root fresh weight. In many experiments, the reduction of shoot to root ratio as a result of dehydration stress is very well documented. Under water limited conditions, there is a high root to shoot ratio (Wu and Cosgrove 2000) due to better availability of food assimilates to roots. In maize, drought at seedling stage increases the root growth and thus adapting plant to drought stress by making the apical cell walls of the root expansible. Sacks et al. (1997) reported that meristematic cells elongate with reduced cell division per unit length of tissues and cell under drought stress condition.

4.2.3 Leaf Area

Leaf area is a typical trait for plant photosynthesis and transpiration. Photosynthesis along with cell-growth are among the primary processes affected by drought stress (Chaves and Chaves 1991). These processes help plant to attain optimum leaf area for photosynthesis and dry matter establishment. Drought considerably reduces the number of leaves per plant, leaf size and longevity. Restricted photosynthetic area may suppress the leaf expansion due to reduced leaf region (Rucker et al. 1995).

4.2.4 Fresh and Dry Mass

Unpleasant drought conditions may slow the rate of fresh and dry biomass formation (Farooq et al. 2009). Plant yield under drought stress is strongly associated with the processes of dry mass partitioning and biomass distribution (Kage et al. 2004). Process of dry mass accumulation is affected by the water stress at different stages of plant growth. The allocation of dry matter between root and shoot and further partitioning of above ground dry matter into vegetative and reproductive organ are vital for crop yield under stress condition.

4.2.5 Yield

The stage and the duration of stress drastically influences the grain yield. Stress during the early vegetative state has little impact on yield reduction while greatest harm is done when drought stress continues until post vegetative or the reproductive stage of plant growth. Rolling of leaf is most immediate response of plant to drought stress condition at the early vegetative growth. Leaf rolling reduces the rate of photosynthesis hence negatively influencing the yield. If stress progresses to the reproductive stage of the plant, it affects the silk extension and ultimately viability of the pollen grains. If the stress continues further to post-flowering stages, yield is reduced due to reduction in kernel rows and kernel numbers. Another factor affecting the grain yield is evapotranspiration. Evapotranspiration is the loss of water from soil by evaporation as well as loss of water through transpiration. This inadequate availability of water affects the nutrient availability, uptake and transport. In maize, most sensitive stage affecting crop yield is the three-week period of silking, and drought stress at this stage results in kernel abortion, and further continuation of drought stress reduces the seed size.

4.3 Physiological Responses

4.3.1 Photosynthesis

Photosynthesis is one of the main physiological responses of plant negatively affected by drought stress. Drought badly affects photosystem-II than photosystem-I. Photosynthetic rate gets adversely influenced by limited CO_2 supply and metabolic processes under stress. Leaf potential becomes low under water stress and in response to reduced leaf turgor stoma closes. Enzymatic activities slow down under drought stress due to diminished supply of CO_2 to RUBisco that dissipates the energy in photosynthetic apparatus causing down regulation of photosynthesis. Photosynthesis promptly depends on relative water content and leaf potential both of which at low concentration slows the rate of photosynthesis. The major effect of drought is decreased CO_2 availability through limited diffusion through stomata and mesophyll (Flexas et al. 2004, 2007). This decrease in mesophyll conductance is linked to physical interaction or alterations in the structure of the intercellular spaces due to leaf shrinkage (Lawlor and Cornic 2002) or to alterations in the biochemistry (bicarbonate to CO_2 conversion) and/or membrane permeability (aquaporins). This pattern of metabolic changes supports the assertion by (Cornic 2000) that stomatal closing is the principle cause of decrease in photosynthetic rate under mild drought.

4.3.2 Respiration

During respiration process, plants catabolize food for ATP production and other useful metabolites. Enormous research has been conducted with relevance to photosynthesis but very less work has been done to find the effect of stress on respiration. Under drought stress, some studies reported a significant reduction in respiration rate, some showed no changes at all while some reports concluded to have increased respiratory rate under water stress condition. Hence, a unanimous conclusion has not been reached.

4.3.3 Transpiration

Transpiration is a process of evaporation of water from the aerial parts of the plants. It occurs largely when the stomata remains open for gaseous exchange. Thus, the degree of stomatal opening regulates the rate of transpiration. Other factors affecting rate of transpiration are linked to hydration level, humidity, temperature, leaf number and leaf moisture. Roots withdraw water from the soil and draw it up to stomatal openings. As water moves all the way through the system, vital nutrients are transported to different areas of the plant. The stoma releases waste products such as oxygen into the environment and brings in carbon dioxide. In addition, transpiration maintains turgor in plants leading to maintenance of water in cells. Drought often limits the growth of root and shoots, which makes the plant stunted under plant stress. Reduction in growth is followed by complete or partial stomatal closure resulting in reductions in transpiration and CO_2 uptake for photosynthesis. Therefore, stomatal closure under severe drought condition influences the photosynthesis as well as transpiration rate. The water loss by a plant depends on plant dimensions and the quantity of water absorbed in the roots. Transpiration cannot persist if its water uptake efficiency is not in equilibrium with soil water. When roots are unsuccessful in absorbing water to keep up with the rate of transpiration, turgor pressure drops and due to reduction in turgor, stomata close to minimize further water loss. If the loss in hydrostatic pressure stretched through the plant, the plant wilts and dies from lack of nutrients.

4.3.4 Pigments

Photosynthetic pigments are present in chloroplasts and are mainly involved in the process of photosynthesis by trapping sunlight and reducing power production in plants. Soil dryness mainly affects chlorophyll ‘a’ and ‘b’ activity (Farooq et al. 2009) whilst carotenoids still help plants to survive under drought condition. Ratio of chlorophyll ‘a’ and ‘b’ to carotenoids changes in response to drought stress

(Anjum et al. 2003; Farooq et al. 2009). Drought induced photosynthesis limitation has been reported in many studies (Anjum et al. 2003; Lawson et al. 2003) because of stomatal and non-stomatal limitations (Farooq et al. 2009). Carotenoids act as antioxidant defense system that helps to overcome the oxidative damage generated by increased drought stress. β-carotene of all green plants is absolutely bound to the core complexes of PS-I and PS-II. It plays a unique role in protecting photochemical processes and sustaining them (Havaux 1998). Drought has the ability to decrease the concentration of chlorophylls and carotenoids (Havaux 1998; Poormohammad Kiani et al. 2008), mainly with the generation of reactive oxygen species (ROS) in the thylakoids (Ramachandra Reddy et al. 2004).

4.4 Biochemical Responses

4.4.1 ROS and Antioxidative Enzymes

The production of ROS is one of the earliest responses in any type of abiotic stress. Decreased metabolic machinery has been known to trigger the accumulation of free radicals under desiccation. A drop in rate of photosynthesis and limited CO_2 fixation give rise to a number of ROS such as H_2O_2, O_2 and OH^-. These ROS are essential when present in minimal amount but can become deterrent when present in large amounts causing oxidative damage to the plants under water stress (Arora et al. 2002). Many studies on maize have reported increased ROS under drought stress condition. Photorespiration being a wasteful process is the main source of ROS accumulation accounting for approximately 70% of the total hydrogen peroxide production. To minimize the ROS level and fight the oxidative stress caused by them, plants express antioxidative enzymes to strengthen their antioxidative defense system. The antioxidant defense system is comprised of various enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, peroxidase and helps the plant to eradicate excess ROS and minimize the damage caused by them (Li et al. 2013). The equilibrium between ROS production and antioxidative defense system decides the stress responsive pathway of the plant and thus the ability of antioxidative defense system of the plant is directly correlated with the drought resistance of the plant (Anjum et al. 2011). Chugh et al. (2011) reported increased activities of catalase, peroxidase and ascorbate peroxidase in a drought tolerant variety of maize. Polyethylene glycol (PEG)-induced water stress is thought to be relieved by increased ROS, (abscisic acid) ABA accumulation and antioxidative enzymatic activity. In plant cells, different mechanisms are available to prevent the production of toxic molecules but oxidative damage remains an expected problem as it causes perturbations in metabolism (Ramachandra Reddy et al. 2004). In maize, glutathione reductase (GR) and dehydroascorbate reductase (DHAR) were solely located in mesophyll cells whereas most of the superoxide dismutase (SOD) and ascorbate peroxidase (APX) were located in mesophyll and bundle sheath cells. Kingston-Smith and Foyer (2000) suggested that the oxidative damage under stressful conditions in C4 plants remain confined to bundle sheath cells because of inadequate antioxidant protection in this tissue.

4.4.2 Lipid Peroxidation

Lipids are chief components of the membrane system and thus maintain the integrity of the system. Increased ROS production in water stress condition damages the membrane integrity of the cell by the lipid oxidation (Liljenberg 1992). The concentration of malondialdehyde (MDA) content increases which is responsible for damages in the membrane system by altering its fluidity, protein cross-linking, transport etc. (Sharma et al. 2012). Ge et al. (2006) conducted a systematic study to know the effect of drought on antioxidative and lipid peroxidation system of maize plant. He found a significant increase in ROS scavenging enzymes with increase in stress severity along with increased MDA content. Increased MDA, indicator of lipid peroxidation, was also reported by Yin et al. (2012) in two different types of maize plant along with other biochemical changes. The alteration in membrane lipids has become a major biomarker of plant under the stress condition.

4.4.3 Osmolytes Accumulation

Regulating water potential in water stressed condition can be a rescue mechanism for plants facing stress. Presence of water ion/channel proteins and osmolytes has been reported to regulate the osmotic adjustments under drought stress (Ingram and Bartels 1996). Osmolyte accumulations result in reduction of osmotic potential and thus maintain cell turgor pressure and water uptaking capacity to sustain the plant's physiological processes. In support of this, accumulation of sugars such as raffinose family oligosaccharides (RFO), fructose and trehalose have been reported in drought stressed condition (Wanek and Richter 1997). Trehalose, a non-reducing saccharide when present in definite amount acts as a stabilizer of protein and cell membranes (Paul et al. 2008). Proline has been considered as one of the most important osmolytes that accumulates in plants in response to different environmental stresses including water stress. An investigation on importance of osmolytes accumulation under drought stress concluded that osmolytes are beneficial for plant when occur in root tips as they allow deeper root development and increased access to the water deep inside the soil (Serraj and Sinclair 2002).

4.4.4 Carbohydrates Biosynthesis

Association of soluble sugars with drought tolerance is highly reported. Alteration in carbohydrate content is particularly important because of their proximal association with plant's physiological processes. Sugar accumulation upon drought exposure results in osmoregulation as well as induction of sugar-related signaling pathways including mitogen-activated protein kinase (MAPK), $Ca2^+$ and calmodulins in plants (Kaur et al. 2007). Trehalose is the minimally needed simplest sugar which acts as an osmoprotectant while other soluble sugars, chiefly sucrose, have shown to increase in drought stressed condition. Sucrose being a compatible solute acts as an osmolyte and maintains plant's water potential. Sugar accumulation is also important in maintaining other processes. Phosphofructokinase, an important enzyme for glycolytic pathway usually degrades in dehydration condition. *In vitro* studies have shown the involvement of sucrose, maltose and trehalose in enzymatic stabilization under dehydration (Carpenter et al. 1987).

4.5 Molecular Responses

4.5.1 Transcriptional Factors (TFs)

Under abiotic stress, plants often influence the expression of numerous transcriptional regulators (TFs) which in turn up-regulate an array of downstream genes for survival and stress adaptation. Several families of TFs and *cis*-elements have shown to play significant roles in promoter region of stress-related genes and thus control the expression or suppression of these genes. So far, at molecular level, studies focused on identifying plant response to the drought stress condition involving initiation of stress-responsive and stress tolerating genes. ABA stimulation in plant controls stomatal closure to regulate transpiration and stress responsive transcriptional factors under drought conditions (Cutler et al. 2010). Till date, more than 7% of the coding sequences regulating plant responses to environment have been explained (Udvardi et al. 2007). Probably these TFs are thought to regulate the plant late phase under dehydration stress while some may regulate other drought responsive signaling pathways for activating drought responsive genes for tolerance (Kilian et al. 2012).

Nuclear factor Y is a ubiquitous ABA-dependent TF that has been reported to be strongly expressed under drought in maize crop at both transcriptional and post transcriptional level (Nelson et al. 2007; Li et al. 2008a, b). In maize, TF ZmNF-YB2 is shown to have an equal role as AtNF-YB1 in *Arabidopsis* in conferring improved performance under drought conditions (Nelson et al. 2007). A TF belonging to Abscisic acid Stress Ripening protein (ASR) family, ZmASR-1 protein influences branched chain amino-acid biosynthesis and maintains kernel yield in maize under water deficit conditions (Virlouvet et al. 2011). An another group of TF family, bZIP plays a vital role in ABA signaling along with other functions in plant growth and abiotic stresses. *ZmbZIP72*, a bZIP transcription factor gene in maize was found to be over-expressed in various organs by drought, salinity and ABA in seedling stages. Similarly, AP2, ERF, dehydration-responsive element-binding protein (DREB), Cys2His2 Zinc Finger (C_2H_2 ZF) TFs, MYB, bHLH are important plant stress-responsive TFs which have been shown to express or hold an important role in plant stress tolerance mechanism (Ying et al. 2012).

4.5.2 Hormonal Regulation and Signaling

Phytohormones regulate the very aspect of plant growth and development and enable plants to cope with various environmental conditions. They initiate specific signaling pathways to induce responsive gene expressions in stress condition. ABA is the key phytohormone governing plant responses in drought and other abiotic stress conditions. Importance of other phytohormones such as, cytokinins, brassinosteroids, auxins, jasmonate etc., in abiotic stress tolerance is also discovered.

ABA accumulation is very rapid in any stress condition and triggers downstream stress-responsive signaling that helps the plant to survive the stressed condition. Most of the TFs work in an ABA-dependent manner while studies suggested the presence of both ABA-dependent and ABA-independent regulatory systems (Shinozaki and Yamaguchi-Shinozaki 1996). In drought stress condition, ABA accumulation in the shoot induces stomatal closure to reduce water loss from the plant. Equilibrium between ABA biosynthesis and ABA catabolism is critical for plant survival.

Cytokinins, known for their role in cell division, growth and differentiation, decrease under drought stress, which makes shoots more responsive to ABA and ultimately resulting in stomatal closure (Goicoechea et al. 1997). Though little research has been done on the role of auxins in drought condition but a drop in indole-3-acetic acid (IAA) content under drought stress and changes in other genes of IAA biosynthesis pathway and signaling in rice implied its role in drought condition (Du et al. 2013). IAA functions antagonistic to ethylene in ABA regulation and so shut down the ethylene-initiated ABA signaling in plants (Sakamoto et al. 2008). Under drought stress, low level of auxin and increased production of ABA appears to provide drought tolerance in plants.

Salicylic acid is a hormone-like substance, which is important in improving drought tolerance ability in plants. Okuma et al. (2014) investigated salicylic acid accumulating *Arabidopsis* mutant and confirmed that these mutants were more tolerant to drought stress than the wild type by inhibiting light-induced stomatal opening. Jasmonic acid (JA) is also a signaling molecule affecting plants response at molecular level. It imparts drought tolerance by lowering oxidative stress and by enhancing expression of antioxidative enzymes. JA and ABA cross talks in signaling pathways and their interaction helps to regulate the plant signaling cascades in drought conditions.

4.6 Transcriptomes

Nearly, every cell of every organism is composed entirely of the same genome and has same set of genes. Thus, disparity in response of plant in different environmental conditions is entirely because of the differential expression of genes in different stages of cell development. The transcriptome consists of all RNA, including, rRNA, tRNA, mRNA, and non-coding RNAs expressed in one or a population of cells at a given moment. Decoding different transcriptomes associated with different cells at different times gives a more clear view and deeper insights into specific responses of cells. With the comparative analysis of transcripts of an organism in a particular condition, researchers can determine when genes express or switch off.

4.6.1 Role of Transcriptome in Maize for Drought Stress Tolerance

Accessibility of transcriptome and whole-genome sequences in public databases and with the upgradation of bioinformatics tools, detection of genetic variation in genotypes and within genotypes has become easier and more cost-effective. Maize (*Zea mays* spp. *mays* L.) is very sensitive to water constraints, particularly during flowering, pollination and embryo development. Therefore, it is important to locate candidate genes and unravel molecular mechanisms in response to drought in maize to accelerate its genetic improvement through marker-assisted selection. A general idea of identification and exploitation of gene for crop improvement has been explained in Fig. 4.1.

The progress in transcriptome analysis techniques, sequencing and bioinformatics, the genetic basis of drought tolerance in maize has been further improved. Gene expression studies in maize in response to water stress have been investigated in roots (Poroyko et al. 2007), seedlings (Zheng et al. 2004), and developing ear and tassel (Zhuang et al. 2007). Different types of transcriptomic techniques are now available such as array-based, whole-genome-based and candidate-based to understand the gene expression.

4.6.1.1 Array-Based Transcriptome

In mid 1970s, the base for the development of the novel techniques of microarray was formulated when it became possible to monitor the level of expression of nucleic acid by fluorescent labeling. Microarray technology exploits the basic fundamental characteristic of nucleic acid to anneal with its complementary nucleic acid sequence by hydrogen bonds formation. In this technique, spotted samples (cDNA, DNA and oligosaccharides) with known identities are arrested on a solid

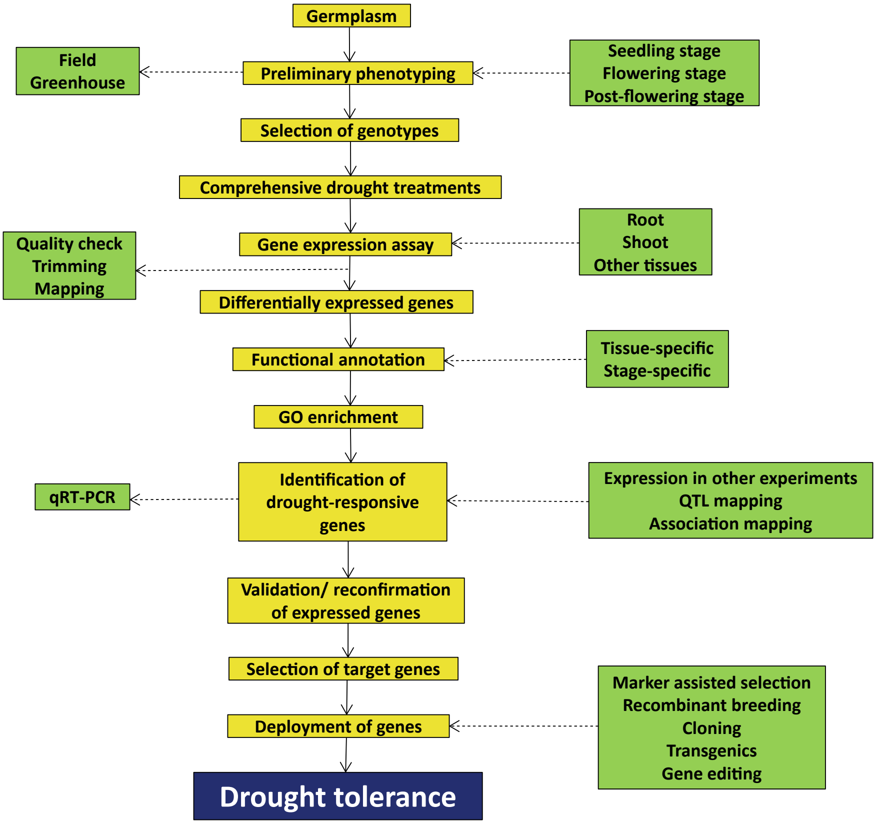

Fig. 4.1 Identification of drought tolerant genes through transcriptome approach

support like glass, silicon, and/or nylon membranes. Each spot represents a single gene, and thus a parallel gene expression for thousands of genes becomes possible at the same time.

Microarray has been successfully employed to maize crop under a range of abiotic stresses for locating potential candidate genes. A cost effective oligonucleotide microarray was developed for the maize community for gene expression analysis in maize. It consists of a total of 5,745,270 mer oligonucleotides representing 25,969 ESTs assemblies, 20,206 singleton ESTs (detected only in a single cDNA library), 9,707 assembled maize sequences, 804 non-redundant repeat elements, 467 organelle sequences, 288 maize community favorites and 11 transgenes. Replicated baseline expression profiles have been generated for 18 tissues and deposited in a database (www.maizearray.org). Advanced and commercial alternative to the public 70-mer array was developed by affymetrix known as the GeneChip Maize Genome Array. This array contains 17,555 probe sets, spanning 14,850 maize transcripts representing 13,339 maize genes. These arrays have 25-mer probes.

A recent advance includes whole genome transcript profiling with a 100 K Maize Affymetrix Gene Chip Array, which contains 100,000 probe sets to detect transcripts from *Zea mays* (Xu et al. 2009). Using microarray chip experiments, gene expression profile under drought stress have been studied in different maize parts including roots, leaves and kernels (Zheng et al. 2004; Hayano-Kanashiro et al. 2009; Marino et al. 2009; Luo et al. 2010; Humbert et al. 2013).

4.6.1.2 Whole Genome Transcriptome

Though microarray studies are relatively inexpensive and the data can be easily generated and analyzed but the detection is limited only to the sequences and homologues on the array. Next generation sequencing (NGS) of RNA (known as RNA-seq) has revolutionized transcriptomic studies by providing scope of multi-dimensional examination of whole cellular transcriptome much more efficiently, allowing identification of novel transcripts (Wang et al. 2009).

High-throughput RNA sequencing (RNA-seq) identifies the abundance of RNA and promises a comprehensive picture of the transcriptome, allowing for the full annotation and quantification of all genes and their isoforms across samples. This technology is extensively applied to identify novel transcripts, study gene expression differences, gene fusion events, alternative splicing and RNA editing.

Several studies have exploited RNA-seq to study transcriptome of many plant species including sorghum (Johnson et al. 2014), tea plant (Liu et al. 2016), maize (Song et al. 2017), lentil (Singh et al. 2017), *Arabidopsis* (Filichkin et al. 2010) and rice (Lu et al. 2010; Zhang et al. 2010). Recently, RNA-seq has become popular to study maize transcriptome and thus so a detailed transcriptome of leaf, root, reproductive leaf meristem and inflorescence has been developed in maize using RNA-seq (Li et al. 2010; Eveland et al. 2010; Opitz et al. 2016; Song et al. 2017). Many comparative studies have been made to test the effectiveness of microarray and RNA-seq in providing the genome-wide expressions in maize (Sekhon et al. 2013). RNA-seq provided extended coverage of the genome along with clarity in expression patterns among paralogs. In yet another study by Hansey et al. (2012), whole seedlings of 21 maize inbred lines were sequenced from diverse North American and exotic germplasm. Kakumanu et al. (2012) used RNA-seq to analyze drought-stressed and well-watered fertilized ovary and basal leaf meristem tissue of maize. The study showed more number of drought responsive genes in ovary (1500) than leaf meristem.

4.6.1.3 Candidate Gene-Based Transcriptome Analysis

Candidate gene is a gene governing a particular trait in an organism at any said environment or condition. Candidate gene approach is based on three successive steps. First is to identify a potential candidate gene based on the physiological, biological and functional importance of the gene in question to that condition or environment or based on linkage data of the locus under study. This is limited to existing knowledge of genes. In the second step, a molecular polymorphism or genetic variant is revealed to calculate statistical co-relation between candidate gene polymorphism and phenotypic variation or the candidate gene can be co-localized on a genetic linkage map to look for the linkage between candidate gene and loci being characterized. Detecting polymorphism in laboratory often involves sequencing of the case and control ones. The third step tests the validity of association and segregation from correlative experiments (Kwon and Goate 2000).

There exist a number of ways to detect candidate genes such as prior knowledge of the biological pathways, linkage studies, expression studies, and quantitative trait locus (QTL) analysis and genome wide association studies (GWAS). Genome wide association mapping and QTL mapping are the genomic tools, which identify a region that may be on or near to a potential candidate gene. As the identified suspected potential candidate gene is believed to have a role in the said biological pathway of the desired trait, finding an association by GWAS studies confirms its role in that pathway (Korte and Farlow 2013).

4.6.2 *Important Gene Families Identified Using Transcriptomes and Their Role in Stress Tolerance*

Harb et al. (2010) made comparisons between moderate and progressive microarray data that showed specific association of cell wall expansion genes under moderate stress while same genes were shown to be down-regulated in the progressive drought condition. The quantification of expansin genes i.e., *EXPA3*, *EXPA4*, *EXPA8*, *EXPA10*, and *EXPANSIN-LIKE B1* was done where most of the genes were found to be expressed in moderate drought stress.

DREB TFs belongs to AP2/ERF superfamily and has have been identified to be one of the main transcription factors to be involved in improving drought tolerance. DREB binds to dehydration responsive element (DRE) in the promoter region of many drought and/or cold stress-inducible genes (Liu et al. 1998). Over-expression of isoforms of DREB, (DREB2A-CA) protein in transgenic plant imparts significant drought and heat tolerance (Sakuma et al. 2006). Liu et al. (2013) cloned 18 *ZmDREB* genes of maize B73 genome and analyzed phylogenetic relationships and synteny with rice, maize and sorghum. They explored a significant link between genetic variation between *ZmDREB2.7* and drought tolerance at seedling stage. Further analysis revealed that the DNA polymorphisms in the promoter region of *ZmDREB2.7* was associated with different levels of drought tolerance among maize varieties.

Humbert et al. (2013) reported molecular responses in maize to drought and nitrogen stresses individually as well as in combination by customized Affymetrix maize microarray. Their study concluded effects of mild and severe drought stress on plant's photosynthetic machinery, Calvin cycle, sucrose and starch metabolism.

Table 4.1 List of important genes identified to provide drought tolerance in maize

Drought related function	Gene ID	Chr	Gene start	Gene end	Function	References
Detoxification	GRMZM2G025992	7	171775019	171778224	Oxygen radical detoxification	McKersie et al. (1996), Castillejo et al. (2008)
	GRMZM2G054559	3	12195404	12200349	Phospholipid hydrolysis	Zhu 2002
	GRMZM2G059991	6	136070517	136074741	Oxygen radical detoxification	McKersie et al. (1996), Castillejo et al. (2008)
	GRMZM2G066120	1	37470728	37476121	Reactive oxygen species homeostasis	Zhu (2002)
	GRMZM2G071021	3	221771183	221775333	Reactive oxygen species homeostasis	Miao et al. (2006), Chen et al. (2012)
	GRMZM2G125268	4	165996358	165999622	Reactive oxygen species homeostasis	Miao et al. (2006), Chen et al. (2012)
	GRMZM2G140667	2	219258176	219261097	Reactive oxygen species homeostasis	Badawi et al. (2004)
	GRMZM2G172322	1	12985602	12991971	H_2O_2 metabolism	Galle et al. (2013)
	GRMZM2G305066	8	152510200	152511639	Reactive oxygen species homeostasis	Zhu (2002)
	GRMZM5G884600	10	138607002	138608876	ROS homeostasis	Miao et al. (2006)
Stomatal closure	GRMZM2G068330	4	11278503	11281332	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G069365	4	160153804	160155930	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G071112	7	112658777	112661470	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G089619	2	50140925	50142374	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G111136	5	88828896	88831814	ABA-dependent pathway	Thirunavukkarasu et al. (2014)
	GRMZM2G122479	6	139464390	139470075	Ion homeostasis-dependent pathway	Laporte et al. (2002)
	GRMZM2G172586	10	2639262	2640147	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G328438	8	73654879	73656447	ABA-dependent pathway	Davletova et al. (2005)

(continued)

Table 4.1 (continued)

Drought related function	Gene ID	Chr	Gene start	Gene end	Function	References
	GRMZM2G330848	7	175907236	175908949	ABA-dependent pathway	Iuchi et al. (2001), Thompson et al. (2007)
	GRMZM2G389379	2	188271896	188273136	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G407181	1	174550907	174553815	ABA-dependent pathway	Iuchi et al. (2001), Thompson et al. (2007)
	GRMZM2G408158	2	235244890	235246909	ABA-dependent pathway	Iuchi et al. (2001), Thompson et al. (2007)
	GRMZM2G417229	5	201282108	201284278	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM2G417954	7	5981141	5983425	ABA-dependent pathway	Iuchi et al. (2001), Thompson et al. (2007)
	GRMZM2G470974	10	2632775	2633842	ABA-dependent pathway	Davletova et al. (2005)
	GRMZM5G858784	3	87358369	87360132	ABA-dependent pathway	Iuchi et al. (2001), Thompson et al. (2007)
	AC197099.3_FGT005	1	96504420	96506132	ABA-dependent pathway	Abe et al. (1997)
	AF466202.2_FGP001	10	138428306	138432705	Ion homeostasis	Laporte et al. (2002)
	GRMZM2G570020	1	166137697	166137798	ABA-dependent pathway	Abe et al. (1997), Seo et al. (2011)
	GRMZM2G008250	1	174845979	174849344	ABA-dependent pathway	Li et al. (2008a, b)
	GRMZM2G009275	4	119500675	119501178	ABA-dependent pathway	Abe et al. (1997), Seo et al. (2011)
	GRMZM5G822829	10	138462252	138463015	ABA-dependent pathway	Abe et al. (1997), Seo et al. (2011)
Photosynthesis	GRMZM2G012397	7	5134217	5135120	Photosystem I reaction center	Zhang et al. (2012)
	GRMZM2G024150	5	3530018	3531150	Oxidation reduction process	Kimata and Hase (1989)
	GRMZM2G033885	7	157314547	157315990	Chlorophyll a/b-binding protein, photosystem II	Ashraf (1994)
	GRMZM2G077755	5	207185179	207187500	Chlorophyll a/b-binding protein, photosystem II	Ashraf (1994)

(continued)

Table 4.1 (continued)

Drought related function	Gene ID	Chr	Gene start	Gene end	Function	References
	GRMZM2G078409	2	24166809	24167435	Electron transfer	Efeoglu et al. (2009)
	GRMZM2G122337	6	1340417	1341388	Oxidation reduction process	Kimata and Hase (1989)
	GRMZM2G178693	2	28493204	28495467	Water transport	Jang et al. (2004), Alexandersson et al. (2005)
	GRMZM2G092125	2	169186912	169190423	Water transport	Jang et al. (2004), Alexandersson et al. (2005)
	GRMZM2G081192	4	143891075	143894123	Water transport	Jang et al. (2004), Alexandersson et al. (2005)
	GRMZM2G154628	5	195239679	195242694	Water transport	Jang et al. (2004), Alexandersson et al. (2005)
	GRMZM2G014914	7	41435006	41438812	Water transport	Jang et al. (2004), Alexandersson et al. (2005)
Hormone signalling	GRMZM2G056120	3	196638145	196644110	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)
	GRMZM2G057935	1	277059620	277064623	Signalling network	Sheehan et al. (2004)
	GRMZM2G066867	5	18469442	18472522	ABA signaling network	Schafleitner et al. (2007), Mao et al. (2010)
	GRMZM2G073750	3	123881899	123888098	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)
	GRMZM2G110908	10	148736929	148738463	ABA signaling network	Schafleitner et al. (2007), Mao et al. (2010)
	GRMZM2G112240	4	170944444	170947965	ABA signaling network	Zheng et al. (2010)
	GRMZM2G116557	8	159641546	159646951	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)

(continued)

Table 4.1 (continued)

Drought related function	Gene ID	Chr	Gene start	Gene end	Function	References
	GRMZM2G137413	4	118717709	118723692	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)
	GRMZM2G142768	4	171402585	171405007	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)
	GRMZM2G165433	9	11782450	11785572	ABA signaling network	Schafleitner et al. (2007), Mao et al. (2010)
	GRMZM2G179121	1	158940439	158942519	ABA-inducible TFs triggering stomatal closure	Furihata et al. (2006), Kim et al. (2012)
	GRMZM2G015605	10	87283919	87284844	Auxin transport	Hund et al. (2009)
Root development	GRMZM2G028648	6	115935425	115937455	Auxin transport	Hund et al. (2009)
	GRMZM2G090576	5	20815035	20817354	Auxin transport	Hund et al. (2009)
	GRMZM2G091819	10	16522572	16525775	Auxin biosynthesis	Overvoorde et al. (2010)
	GRMZM2G104400	8	102056994	102060989	Auxin transport	Hund et al. (2009)
	GRMZM2G371345	10	46790874	46793155	Auxin transport	Overvoorde et al. (2010)
	GRMZM2G014844	10	34733949	34737952	Carbohydrate metabolism	Krasensky and Jonak (2012)
Sucrose metabolism	GRMZM2G016890	10	34240659	34246077	Starch biosynthesis	Hurkman et al. (2003)
	GRMZM2G058310	7	155396510	155399710	Starch degradation	Rizhsky et al. (2004)
	GRMZM2G073054	2	59216710	59226937	Starch degradation	Rizhsky et al. (2004)
	GRMZM2G130043	4	172635729	172706662	Hydrolysis of sucrose	Ruan et al. (2010)
	GRMZM2G152908	9	122479052	122485725	Sucrose metabolism	Gonzalez et al. (1995)
	GRMZM2G175218	3	1535920	1540195	Sucrose metabolism	Gonzalez et al. (1995)
	GRMZM2G175423	1	197301249	197304338	Cellulose hydrolysis	Mei et al. (2009)
	GRMZM2G318780	1	56787028	56794227	Starch biosynthesis	Ober et al. (1991)
	GRMZM2G394450	2	23504839	23509410	Cellulose hydrolysis	Mei et al. (2009)

The genes involved in photosynthesis and Calvin cycle were severely down-regulated while that of later two (sucrose and starch metabolism) were found to be up-regulated. Genes involved in amino acid biosynthesis mainly for asparagines and proline were also over-expressed in this study.

NGS provides more lucid view into the DNA variation, polymorphism detection, marker development and gene expression analysis (Barabaschi et al. 2011; Mastrangelo et al. 2012). Xu et al. (2014) studied transcriptome of maize reference genome B73 by RNA-seq and compared gene expression in fertilized ovaries and basal leaf meristem tissues collected under drought-treated and well-watered conditions. The study identified 6,385,011 SNPs from 15 maize inbreds and B73 reference genome. Several genes such as ADP-glucose pyrophosphorylase (GRMZM2G163437), glucosyltransferase (GRMZM2G179063), putative calmodulin-binding protein (GRMZM2G466563), leucine-rich repeat receptor-like protein kinase family protein (GRMZM2G428554) were identified to involve in drought tolerance (Table 4.1) (Xu et al. 2014).

4.7 Conclusions

Drought stress is one of the major abiotic stresses that affects the crop growth of maize and leads to low yield. Drought affects all developmental stages and plants respond at different levels; morphological, physiological, biochemical and molecular. At morphological level, drought stress responses include reduced plant growth, high root to shoot ratio, reduced number of leaves per plant, reduced leaf size and longevity, low grain yield etc. Physiological responses include decrease in respiratory rate, photosynthetic rate as well as transpiration rate due to stomatal closure. At biochemical level, ROS production, osmolyte accumulation and biosynthesis of carbohydrates are the major responses. At molecular stage, transcription factors and phytohormones play major role in regulation of drought tolerance. The progress in transcriptomic approaches for understanding the gene expression identified various drought-related transcription factor gene families from both ABA-dependent and ABA-independent pathways. These genes and pathways would be helpful for the development of drought tolerant maize hybrids.

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