

Analysis of Sugar Biosynthesis-Related Proteins of Sorghum Bicolor and Investigation of their Role in Drought-Stress Tolerance

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Abstract

The world's major production constraint consists of drought alone or in combination with the other stresses. Drought alone or in conjunction with stresses, sorghum, one of the major cereal crops, has been affected; however, sorghum has evolved adaptive responses to combined stresses. Drying stress causes remarkable changes in physiology and growth impediments that decrease the plant biomass and crop yields significantly. Some plant species, however, are resilient and keep their yields almost normal under severe water deficits. Sorghum's two-dimensional difference gel electrophoresis (2D-DIGE) was tested for its adaptive response against drought stress and landraces from Egypt. Their relative control values following the stress of drought and recovery were compared to the physiological measurements and proteomic alterations in accession number 11434, drought resistant and accession number 11431, drought sensitive. In addition, a significant number of genes of sorghum for drought tolerance were investigated. However, there is a little understanding of the molecular mechanism underlying the drought response. The variation in amino acids, polysaccharides and their derivatives is a part of the metabolic changes. A total of 188 compounds were found in the two sorghum varieties, including 142 recognized metabolites and 46 unknown small molecules. This study provides the candidates genes of Sorghum bicolor responsible for the drought-stress tolerance..

Key words: Drought-stress tolerance, Metabolites, Proteomic alterations, Sorghum bicolor, Two-dimensional difference gel electrophoresis (2D-DIGE).

Introduction

The abiotic factors such as drought, salinity, floods, extreme temperatures, and insufficient farming practices have a negative effect on crop yield [1]. Such stressors will reduce the yield by up to 80% separately or combined. The availability of water for plant growth is considered to be a significant limit. Drought, as any other abiotic stress, is a common and destructive phenomenon that has a greater impact on agriculture and human life [2]. The 5th largest major cereal in terms of production, Sorghum (*Sorghum bicolor* (L.) Monche), a C4 grass and highly genetically variable, among others are increasingly affected by the drought [3]. Drought is one of the key constraints on food production particularly in African and Asian developing countries. There is an increasing need for agriculture, in order to maximize farming, which can withstand harsh environmental conditions such as drought. The roots, shoots and leaves of green plants at morphological, physiological and biochemical level are characterized by the adverse effects of water deficiency.

The drought activates the phytohormone abscisic acid ABA, which is a key hormone in stomatal closure to trigger the signal transduction in order to decrease the transpiration. Drought also suppresses the cell growth and efficiency of photosynthesis, increases respiration and induces several other genes that respond to the abiotic stress. Many molecular and genomic analyses have been documented in the *Arabidopsis*, rice, and other plants for drought-inductive genes involved in a broad range of functions. The abundant mRNA may be rapidly degraded or inefficiently translated resulting in a non-proportional abundance of mRNA and protein. In addition, only a fraction of a given mRNA pool is recruited further for translation into the polyribosomal assembly. In addition, several transcripts generate more than one protein by alternative splicing or post-transcriptional amendments. Several proteins also experience the changes that deeply affect their activities after translation. The genes that are inducible to drought are divided into two classes. The first group includes proteins that work in tolerances of the abiotic stresses, such as chaperones, late embryogenesis abundant (LEA) proteins, osmotin, mRNA-binding proteins, key osmolyte biosynthesizing enzymes, water channel proteins, transporters of metabolites, detoxifying enzymes, and various other proteases. The intracellular osmotic potential is decreased by the osmotic adjustment, including accumulation of sugar alcohols, amino acids, organic acids and glycine betaine. The second group consists of the regulatory proteins that contribute to further regulating signal transduction and stress-responsive gene expression, such as protein kinases, protein phosphatases, phospholipid enzymes, and other signaling molecules, including calmodulin-binding protein [4].

Various agricultural sectors are affected by the drought and salinity, drought and extreme temperatures and salinity and heat. This combination of stress exists in many areas around the world and causes the major losses of farmers by many folds rather than the damage caused by the individual stresses alone [5]. Drought and stress with all the co-diversity factors impacts not only the plant life and production but also leads to the possibility of global food security which is being more challenging.

Drought exposure contributes to complex organic responses in the plant and involves a number of suitable solutes, as well as up-regulation of common dry-induced proteins such as heat shock proteins (HSPs) and dehydrins (DHNs). Compatible solutes are osmolytes that support plants and preserve the cytosolic osmotic balances in extreme plant conditions against osmotic stresses. The accumulation of compatible solutes in various herbaceous plants was probably due to drought. The accumulation of compatible solutions has been defined as a symptom of stress or as a mechanism for defense that can mitigate the effects of stress. On the one hand, they are seen as a plant signalling mechanism which can potentially mitigate the production of reactive oxygen species (ROS), while on the other hand, they are considered to help in maintaining a cytosolic cell energy balance and their accumulation which is improved by up-regulation of abscisic acid (ABA) [6]. The accumulation of compatible solutions has been defined as a symptom of stress or as a mechanism for defense that can mitigate the effects of stress. For instance, the compatible solutes which have been identified by the drought-sensitive including nitrogen-containing compounds, such as glycine

betaine, proline and sugars (trehalose, glucose and sucrose) [7]. The osmolytes are metabolically costly and are an essential source of nitrogen in the case of amino acids. While cellular homeostasis is thought to continue to be the compatible solutes, there is still no consensus about the role and mechanism of plant regulation *in vivo*, especially during the drought. The exogenous application of the above-mentioned amino acids and their derivatives increases the stress resistance. Nevertheless, it has been considered that the accumulation of solutes may lead to stress instead of an adaptive response.

Proteome and cellular metabolism reprogram transcriptomic changes which are driving the drought-induced signaling. The significance of most of the proteins is still not understood properly. Although, others have a role in signal transduction and activation of additional genetic expression, while others clearly support the cellular homeostasis and drought-stress survival adaptive response. Some of the scientists have reviewed the different classes of proteins which are utilized during the plant adaptation to drought [8]. These include water movement aquaporins through membranes and biosynthetic enzymes, which are essential for the osmotic rebalancing, for osmolytic sugars, proline, and glycine-betaine. Cell detoxifying enzymes such as ascorbate peroxidase, glutathione-S-transferase, catalase and superoxide dismutase prevent the oxidative damage, while chaperones, mRNA-binding proteins, late-embryogenesis abundant proteins, and the similar protein that protect the membranes and the macromolecules. Increased protein turnover, aided by the enzymes and proteins, such as ubiquitin, Clp protease, and thiol proteases, facilitates the efficient re-programming of transcriptome and proteome. Transgenic plants that over-express some of these genes are resistant to drought, which means that the gene products actually function under stress [9].

In the genomic period, the dynamic signal transduction mechanism was analyzed with developments throughout the order to identify the cross-references between various signaling pathways. Advances to sequence technologies from next generation have provided new conditions for comprehensively analyzing medium- and large-scale data on drought-related stress tolerance that enables the use of deep *de novo* and reference-based genome sequences. This efficient and powerful high-performance technology is commonly used in various studies including signaling network discovery and regulatory processes that underlying the complex drought-related responses. Molecular analysis of signaling pathways allows for the identification of proteins that are essential for the molecular events that lead to stress response from plants [10]. Plant hormone like abscisic acid (ABA) plays a typical role in monitoring signals for individual and combined stresses. An integrative function for regulatory hormones may allow the modeling of specific signaling mechanisms and cross-communication between regulatory pathways. According to a study, it has been demonstrated that Most of these signaling proteins such as MAPK and phytohormones like ABA have been proven to be the fundamental targets in metabolic engineering to boost crops such as sorghum in order to react to the combined stresses. While the previous studies have elucidated that the over-expression of the single gene sets are different from the over-expressed gene set in plants under individual stress which may be essential in response to plant stress. Drought-stress response strategies were recently examined using integrated

approaches in sorghum to identify and evaluate the genes for tolerance to multiple stresses across various species [11]. While these research studies have so far increased our understanding of stress tolerance mechanisms mainly at the physio-biochemical level, the interaction between the multiple individual stresses and stress combinations, the results should be explored in order to promote the knowledge of molecular mechanism which supports the stress tolerance associated with the natural conditions. Figure 1 shows plant molecular approaches and strategies under drought.

Most of the experimental research work has been carried out on the application of drought-sensitive model species like *Arabidopsis thaliana* to molecular responses to drought. The normal, drought-tolerant, highly-genetic cereal Sorghum (*Sorghum bi-color* L. Moench) is the good model to research on the drought-adaptive reactions, particularly to identify the new genes for use in drought-tolerant crops [12]. Various studies have reported the sequence of the genome of Sorghum and the analysis of its transcriptomic and proteomic function of leaf responses.

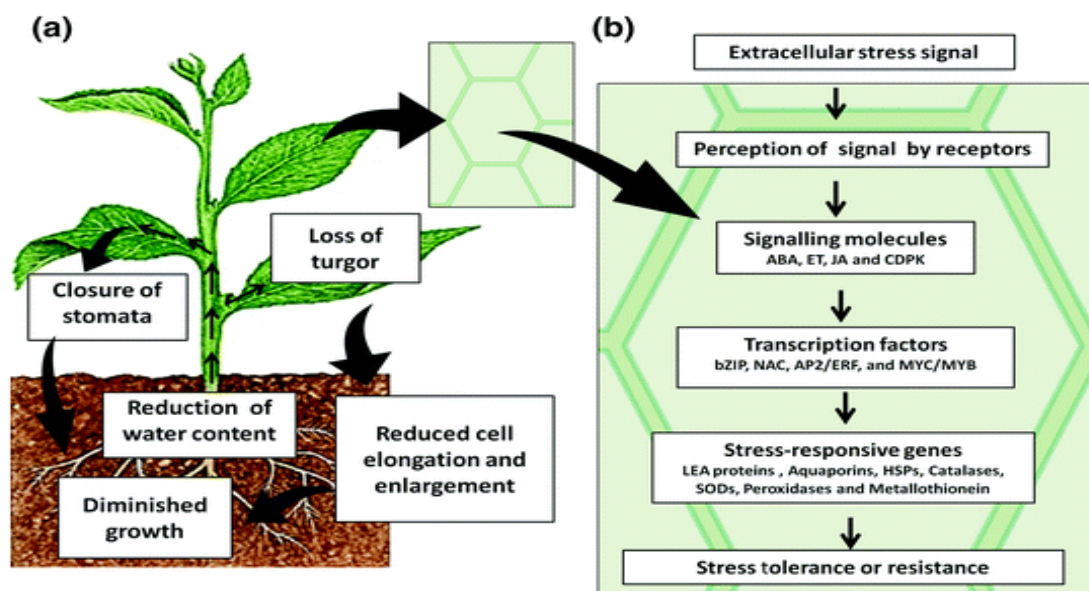


Fig.1: Plant molecular approaches and strategies under drought.

C4 grass *Sorghum bicolor* is one of the drought tolerant species of the most widely cultivated crops. The C4 biochemistry enables photosynthesis to be maintained at low CO₂ levels, for instance, if stomata are substantially locked. Nevertheless, its close relative maize is closer to C4, but does not have much lower drought resistance. In two types of sorghum, Samsorg 17 or Samsorg 40, have previously shown the contrasting responses with different levels of dry tolerance, particularly with regards to high constituency of sugar and protein loss. The observation was done for their morphological and physiological drought responses. In pots containing Samsorg 17, soil has been dried down slower than in pots containing Samsorg 40 but both species have lost specific areas in response to drought. Samsorg 40 had higher overground biomass and, unlike Samsorg 17, lacked chlorophyll in each region of leaf

[13]. Samsorg 17 have the ability to maintain the efficiency of relative water content, open stomata, net assimilation and photosystem II longer than the Samsorg 40.

1. Identification of Sorghum cell suspension culture ECM proteins:

An experimental study has demonstrated the isolation of the fractions which are supplemented with the soluble phase of the extracellular matrix (ECM) of Sorghum. The proteins were identified and analyzed in response to their osmotic stress. The Sorghum cell suspension was utilized as a source of the easily extractable soluble proteins of ECM from the culture growth medium. Based on the preliminary information from the growth curve, an exponential phase 8-day crops culture for the stress treatment was analyzed. Cells were collected every 24 hours up to 72 hours for RNA extraction which were treated with 400mM Sorbitol³¹. The Sorghum homologues of Arabidopsis have been identified, which include ERD1 and DREB2A, which are considered as “ERD1-1 (SORBI_3004G162400), ERD1-2 (SORBI_3006G065100), DREB2A-1 (SORBI_3009G101400), and DREB2A-2 (SORBI_3003G058200)”. Apart from DREB2A-2, sorbitol treatment triggered all the genes, with a median expression of 48 hours (figure 2). Hence, the time of 48 hours was chosen for applying the sorbitol to cell crops for the extraction of proteins in subsequent experiments. Four biological replicates for the treatment and regulation of sorbitol ensured the detection of proteins with highly reproductive responses.

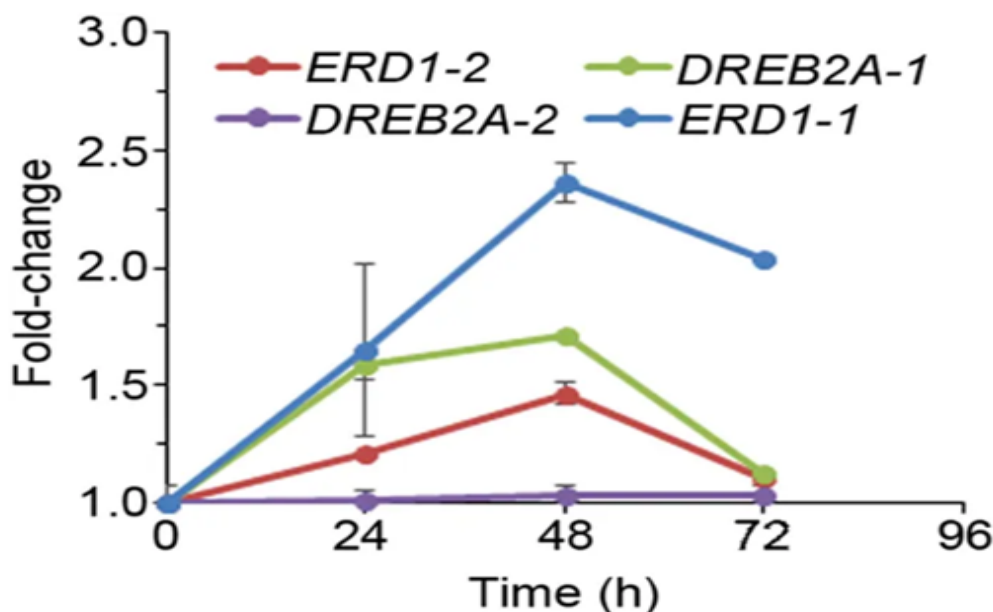


Fig.2: Activation of Sorghum ERD1 and DREB2A expression in response to sorbitol. Sorghum cell suspension cultures were treated with sorbitol and cells harvested at the indicated time-points.

Conclusion

The drought genotype proteome analysis has shown that the combined activities of various protein groups can enable plants to survive drought stress and to recover efficiently after the

stress conditions are removed. Drought tolerances are the difficult factors governing the physio-biochemical and molecular processes. To clarify that, a system biology approach has been developed that provides a comprehensive and integrated analysis of drought-related molecular pathways and candidate genes. The most characteristic features obtained from this study include an efficient mechanism for protein stability, metabolites allocation in newly developed structures and effective protein synthesis. On the other hand, the most obvious characteristic elements of drought-sensitive genotype were the elements of cell death combined with the production of proteases. Drought-tolerance was shown to be significantly correlated with the key functional modules in the interaction network and other substantially related groups. Notable evidence showed that the drought-specific sub-network extraction was involved in the primary pathway signals associated with the drought tolerance, both in the original genes DSRhub genes and additional candidates.

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