

MOLECULAR STUDIES INVOLVED IN SUNFLOWER RESPONSES IN DROUGHT STRESS

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ABSTRACT

Sunflower is a major oil seed crop worldwide that shows adaptations to diverse environmental conditions, such as water and salt stresses. It is adapted to grow in semi-arid conditions. To provide expansion of cultivated area, it is crucial to determine and create drought tolerant sunflower genotypes. There are diverse physiological and biochemical responses against water stress by up- or down-regulating stress tolerance genes and other mechanisms, including hormones and secondary metabolite accumulations. For instance, an increase in the expression of sunflower aquaporin gene *HaTIP7* was observed in guard cells and root phloem under drought conditions. Overexpression of a drought-responsive dehydrin gene, *HaDHN1*, makes the sunflower line tolerant to water-limited conditions. For further studies, outstanding transcriptomic strategies can be applied to examine the genes encoding for transcription factors in sunflower in drought tolerance. Here, we present recent molecular studies associated with drought stress tolerance in sunflower genotypes.

Keywords: sunflower, drought stress, molecular studies, tolerance

INTRODUCTION

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) oil is the fourth most significant vegetable oil after palm, canola and soy in the world. Planted all over the world, sunflower is used for a wide range of purposes including oil production from oil seed sunflower, consumption of seed directly from confectionary sunflower and cosmetics production from ornamental sunflower. Sunflower production is mainly concentrated in Ukraine, Russia, Argentina and India (Gulya 2014). According to Turkish Statistical Institute data, sunflower production is increased 2.6% and became 1.7 million tonnes in Turkey (Tuik, 2015). It is cultivated in over 22 million ha land, and annual production of sunflower is over 9 million tonnes in the world (Fernández-Martínez et. al., 2009). Geographical, archeological, morphological and molecular evidences suggest that sunflower was first domesticated in eastern North America (Rieseberg, 2001). Sunflower breeding programs date back to early 20th century. In 1910-1912, Krasnodar by Vasili Stepanovich Pustovoit started a scientific sunflower breeding from locally developed varieties. In first efforts, breeders tried to cope with parasitic weeds (broomrape, *Orabanche cumana*) and insects (*Homeosome electellum*, sunflower moth) (Fick, 1997) by genetic controlling. Development of varieties including high oil content became a milestone in oil seed sunflower breeding in the world. Another milestone in the development of sunflower was the discovery of cytoplasmic male sterility. This highly reliable method paved the road to the production of commercial hybrid seeds with inherent advantages (Leclercq, 1969). In recent years, sunflower breeding programs have started to concentrate on the development of varieties tolerant to environmental stresses.

Drought stress is the most significant abiotic stress factor that affects the plant production because around one third of the soils are affected by drought worldwide. All plant organs show reactions to drought stress with morphological, physiological, and metabolic alterations. To

minimize yield reduction due to drought stress, there are various mechanisms that are categorized in three groups of (1) dehydration avoidance, (2) drought escape, and (3) dehydration tolerance (Singh, 2000). In the slowly dehydrated attached leaves of drought-stressed plants, dehydration avoidance by osmotic adjustment is improved (Levitt, 1985). The activation of drought escape mechanism in plants is a significant process in early maturation that provides suitable environment for late-season drought stress conditions (Singh, 2000). Water uptake and consumption is minimized by soil drought. In order to withstand drought conditions, plants decrease the transpiration rate by stomatal closure, which in turn leads to overheating in the leaves due to local high temperature. Because of water loss, photosynthesis rate also decreases gradually (Žolkevič, 1968). Increasing the water retention capacity is a protection mechanism in plants to escape from water loss. Reduction in transpiration rate and enhancement of water uptake from soil are together referred to as water or turgor potential under drought conditions. In drought stress, dehydration tolerance increases with leaf number. (Levitt, 1985)

Several morphological and physiological parameters are used to examine the drought tolerance levels in sunflower (Škorić, 2009). They include leaf water potential, yield stability, root xylem diameter, root growth, stomatal conductance, osmotic adjustment, canopy temperature, abscisic acid (ABA) accumulation, seedling recovery after stress, growth under stress, proline accumulation and leaf rolling. These parameters were used to evaluate the differences in drought tolerance levels among sunflower varieties in attempts to develop drought tolerant sunflower genotypes. In an early attempt, Škorić (1992) evaluated over 30 different parameters to study the drought tolerance in sunflowers. In another attempt, Petrović et al. (1992) evaluated the free-proline accumulation rate and nitrate reductase activity under conditions of water stress, and found large amounts of differences between sunflower varieties. Therefore, proline accumulation and nitrate reductase activity levels were proposed to be used as indicators for the estimation of drought stress in sunflower varieties. Unlike other plant species such as *Arabidopsis*, wheat, rice and soybean, water stress tolerance mechanism is still not completely comprehended in molecular basis in sunflower even though most of drought-related genes are cleared in other plant species (Cellier et al., 1998).

MOLECULAR STUDIES OF DROUGHT TOLERANCE IN SUNFLOWER

Morphological, physiological, and metabolic modifications respond to drought stress in whole plants. At the cellular level, water deficit causes cell damage in all plant organs. In adaptive processes, other responses occur (Ingram and Bartels, 1996). In sunflower, there are several management mechanism to cope with drought stress. Water deficit reduces root proliferation, leaf size and stem extension by disturbing water relations. A variety of physiological and biochemical responses are controlled at cellular and whole-organism levels start to manage drought stress (Farood, 2009). CO₂ assimilation in the leaves is reduced by membrane damage, stomatal closure and disturbed activity of various enzymes that function in adenosine triphosphate synthesis and CO₂ fixation. Oxidative load generating reactive oxygen species (ROS) is increased by enhanced metabolite flux through the photorespiratory pathway. ROS cause injury to biological macromolecules under drought stress (Farood, 2009). This is among the major obstacles for growth. Plants display some mechanisms to cope with the drought stress. The major mechanisms that curtail the effects of water loss include the diffusive resistance, enhanced water uptake by deep and prolific root systems, and succulent and smaller leaves to limit the transpirational loss. Moreover, potassium ions provide osmotic adjustment while silicon develops root endodermal silicification and enhances the cell water balance. To sustain cellular functions under drought conditions, low-molecular weight osmolytes including proline, glycine-betaine and other amino acids, polyols and organic acids have significant emerging roles. Plant growth substances such as auxins, gibberellins, salicylic acid, ABA and cytokinins regulate the plant responses toward drought stress. To reduce the adverse effects of drought stress, citrulline polyamines and several enzymes act as antioxidants (Farood, 2009).

In breeding programs, development of tolerant cultivars and selection of drought tolerance is achieved by the help of physiological trait improvements or genetic modifications. More recently, molecular markers were effectively applied to select for enhanced drought tolerance among sunflower varieties (Škorić, 2009). At molecular level, several drought responsive genes and transcription factors have been characterized in sunflower. However, the studies to identify drought responsive genes, such as the genes encoding for late embryogenesis abundant (LEA) proteins, dehydration-responsive element-binding proteins and aquaporins in sunflower have been limited under drought stress conditions. Although a lot of physiological studies have been carried out in sunflower, molecular studies and genetic modifications over drought stress tolerance are limited.

Expressions of many genes are up- and down-regulated by water deficit (Table 1). Liu et al., (2003) analyzed the structural and functional characterization of environmental stress-induced genes under drought and salinity stresses in sunflower. Differential display was used to compare overall differences in gene expressions between drought- or salinity-stressed and unstressed (control) plants of sunflower. Guanylate kinase (signal transduction), *lytB* (antibiotic/drug resistance), selenium-binding protein (heavy metal stress), polyprotein (reverse transcriptase), and AC-like transposable element were identified from sequence analysis of used clones under drought and salinity stress conditions. To regulate water fluxes in plants, aquaporins are one of the major functional transporters in plants. Under water deficit conditions, sunflower aquaporin gene *HaTIP7* accumulated in the roots inducing stomatal closure (Aguado, 2014). In drought and exogenous ABA conditions, sunflower *HaABRC5* of ABI5-Interacting Proteins (AFP family) was up-regulated in roots, seedling shoots and leaves (Liu et al. 2004). This gene is predicted to be an ABA-responsive nuclear protein playing a role in plant stress responses in sunflower. In drought conditions, hydrophilin and LEA proteins are essential as soluble proteins to provide maintenance of cellular integrity. Drought induced transcripts of *HaELIP1*, *HaDHN1*, and *HaDHN2* accumulated in leaves of tolerant sunflower variety under progressive drought (Cellier, 1998). Water stress induced *HaELIP1* gene expression and accumulation of dehydrins in sunflower leaves (Ouvrard et al. 1996). Dehydrins are from D-11 subgroup of LEA proteins that are functional as water stress-induced proteins. Dehydrins accumulate in desiccation tolerant seed embryos during water stress conditions (Close, 1997). Actually, the function of dehydrins in drought stress tolerance mechanism is not clear, but their accumulation in drought stress was shown to be increased in previous studies (Giordani et al., 1999; Hundertmark and Hinch, 2008). Interestingly, the expression of the *HaDHN1A* dehydrin gene was under the control of two possible mechanisms of ABA-dependent or ABA-independent pathways. The expression level of *HaDHN1A* transcript was lower in ABA-deficient sunflower mutant compared to ABA-sufficient non-mutant under water limited conditions, suggesting the involvement of ABA-dependent tolerance mechanism in sunflower responses under drought stress (Giordani, 1999). In ethylene synthesis, ACCO (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE) is a main regulatory enzyme. *HaACCO2* transcript expression increased in sunflower leaves (Liu et al. 1997). Drought and exogenous ABA application induced the expression of this gene as well (Ouvrard et al. 1996).

Gago et.al. (2002) reported that the transcript abundance of a homeodomain-leucine zipper protein, *Hahb-4*, was controlled by water stress conditions in sunflower whole seedlings, roots, stems and leaves. Additionally, ABA was proved to function as a component in signal transduction pathway that regulates *Hahb-4* expression under water stress conditions. ABA is involved in various regulations in plant physiological and developmental stages, and in pathways to cope with drought and salinity stresses (Skriver & Mundy 1990; Leung & Giraudat 1998). The functional analysis of the promoter sequences of genes involved in sunflower drought tolerance identified *ABRE* (ABA responsive elements) consensus sequences indicating the involvement of ABA signaling in drought tolerance in sunflower.

Table 1. Genes involved in sunflower drought tolerance.

Genes	Results	Reference
<i>HaELIP1, HaDHN1, and HaDHN2</i>	drought-induced genes	Ouvrard et al. 1996
<i>HaTIP7</i>	transcript accumulation by water deficits	Sarda et.al. 1997
<i>HaACCO2</i>	induced by drought and exogenous ABA application	Ouvrard et al. 1996
<i>ABI5-Interacting Proteins (AFPs)</i>	involved in ABA response	Garcia et al. 2008
<i>LTP genes</i>	induced by water deficit and ABA application	Colmenero-Flores et al, 1997

Water deficit stress and ABA application induce *LTP* genes encoding for Lipid Transfer Proteins. These proteins are functional as epidermal cell wall proteins that are essential for the secretion of extracellular lipophilic substances (Martin and Brewbaker, 1971). Ouvrard et al. (1996) showed that *HaLTP* gene expression increased in ABA treatment and drought stress conditions in sunflower. Mitotic activity and DNA synthesis activity is constrained by drought stress and ABA treatment (Robertson et al., 1990). Liu et.al (2003) showed that *HaRPS28* expression in different organs of sunflower decreased under drought and salinity stresses. Additionally, they realized that *ABRE* repeats in 3' UTR of *HaRPS28* mRNA are expressed in low levels, suggesting a new comprehension and study about ARE-mediated decay pathway under drought stress conditions in sunflower (Liu, 2003).

Giordani et al. (2011) analyzed 8 genes, namely *NAC1, DREB, ABA-C5, ABP1, DHN, HSP, LTP* and *DES*, which are functional in drought responses in eight sunflower inbred lines with phenotypic characters. Gene expression analyses proved that these genes are putatively essential in drought stress responses. *NAC1* gene belongs to the NAC family of transcription factors functioning in morphogenesis and stress responses (Ooka et al. 2003). Drought-responsive-element-binding (*DREB*) protein encoding genes are transcription factors, which bind DRE cis-elements of drought-responsive genes (Shinozaki and Yamaguchi-Shinozaki 2007). ABA-responsive-C5 (*ABAC5*) encoding gene was involved in ABA-mediated drought response, and there are two copies in the sunflower genome (Liu and Baird 2004). A sunflower specific gene named *AUXIN-BINDING PROTEIN (ABP1)* was shown to be involved in the auxin transport within the cell and was predicted to be the auxin receptor (David et al. 2007). Induction of *ABP1* under drought stress in sunflower suggests the involvement of auxin signaling in drought tolerance mechanism. In addition to these genes, genes encoding for heat shock proteins (*HSP*) and desaturase enzyme are also induced under drought conditions in sunflower. The nucleotide diversity values of four genes (*NAC, ABA-C5, DREB, ABP1*) were shown to be lower than the other four, although they were highly functional genes encoding for proteins involved in the regulation of transcription or signaling cascades under stress conditions (Giordani, 2011).

Drought tolerance of sunflower has not been studied in details although different plant characters have been analyzed and numerous attempts have been carried out to understand the mechanisms involved in drought tolerance in sunflower. Wild sunflower species provide high level of drought tolerance by controlling various sets of genes to create new drought tolerant sunflower lines (Škorić, 2009). Appropriate screening techniques, controlling genetic backgrounds and analyzing physiological mechanisms of drought tolerance can be developed by the help of selection methods and breeding programs (Škorić, 2009).

Molecular Markers in Sunflower Breeding Against Drought

In crop breeding programs, molecular markers play a crucial role in detection of characters. Molecular marker tools can be applied successfully to oil seed crops such as sunflower, soybean and groundnut to control the seed quality or other characters affected by abiotic and biotic stresses (Sujatha, 2009). Ali et al., (2009) analyzed physico-chemical attributes of sunflower seeds under drought stress at different growth stages, i.e. vegetative and reproductive stages. From results of their comprehensive studies, it was concluded that drought affected some constituents of sunflower seed oil in different cultivars. Distinctive parameters such as fatty acid composition, oil yield, iodine value and oil tocopherol content are significant factors that are the most vulnerable to water deficit (Ali et al., 2009). Studies conducted in unfavorable conditions, especially drought showed that it affects the seed composition and seed numbers (Nel, 2001; Anwar et al., 2006). In quantitative trait loci (QTL) identification studies, several physiological traits were associated with genomic locations (Hervé 2001). Hervé et.al. (2001) analyzed traits related to photosynthesis including internal CO₂ concentration, net photosynthesis rate, leaf chlorophyll content, and water status traits including transpiration, stomatal conductance, relative water potential and leaf water potential in recombinant inbred sunflower lines. Analyzed traits showed a correlation between water potential and transpiration, and between transpiration and photosynthesis rates. This study was the first one about the identification of genetic characters involved in water status and photosynthesis in sunflower under drought conditions. Genetic markers associated with these physiological characters were also identified in sunflower inbred lines, and their utilization in future breeding programs was evaluated.

Haddadi et.al. (2011) detected genomic regions associated with leaf related traits and yield components in recombinant inbred sunflower lines under water stress. This study is a suggestive work for the development of future marker-based approaches in sunflower. This study can develop improved understanding of positional cloning of related genes in development, and improvement of near-isogenic lines in sunflower varieties. Kiani et al. (2007) detected QTLs related to water status and osmotic adjustment in sunflower with two water treatments in greenhouse conditions.

In a recent study, Abdi et.al. (2013) compared the relative water content and chlorophyll concentration in 70 recombinant sunflower inbred lines under drought and control conditions. By using 210 simple sequence repeats (SSRs), 11 genes were placed in 17 linkage groups. A total of 10 and 8 QTLs were identified for chlorophyll levels and relative water content, respectively. Utilization of SSR markers to develop an association mapping gives a greater precision in decoding the genetic map. Identification of genomic regions related to drought tolerance phenotypes will develop a future understanding of marker-based approaches in drought stress conditions in sunflower. These molecular studies give new insights to the development of drought tolerant sunflower varieties in molecular ways.

CONCLUSION

Although a large number of drought-induced genes have been characterized in other plant species, molecular basis of sunflower tolerance to water deficit has not been completely understood. There are many reports on molecular mechanism of sunflower drought stress tolerance. Sunflower drought tolerance can be managed by the use of molecular techniques such as marker-assisted selection, QTL identification and associating mapping. At the molecular level, several drought responsive genes and transcription factors have been characterized in sunflower drought stress tolerance. However, the application of different molecular methods such as transcriptomics will help the development of new sunflower cultivars that are more tolerant to drought stress conditions. Applications such as mass-screening and breeding, exogenous application of hormones and osmoprotectants to growing plants are also ongoing studies in sunflower. Additionally, array based

cDNAs will contribute to the understanding of functions of more sets of genes functioning in sunflower drought stress tolerance.

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