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## Breeding and agronomical approaches for the management of drought stress in sunflower

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### Abstract

In a changing climate scenario, drought is the most severe crop production limitation, and its intensity is severe in the future. Sunflower is a prominent oilseed crop, contributing for 8 percent of world oil production. Despite its drought resistance, severe drought affects seed and oil yield. As a consequence, knowing the physiological, biochemical, genetic, and agronomic characteristics of dehydration is required for long-term regulation of sunflower achene and oil production. Even though the impacts of drought on various aspects of sunflower have been reported, no single work has examined the physiological, biochemical, and genetic basis of dryness at the molecular and crop levels in sunflower. At the cell, plant, and crop levels, the influence of drought on sunflower achene output and oil quality has been thoroughly investigated. The consequences of drought stress on physiological and biochemical characteristics (such as photosynthesis, relative water content, mineral nutrition, and oxidative damage), morphological and growth parameters, achene output, and oil content have all been widely investigated in the literature. Many management techniques, such as breeding for stress tolerance (traditional or biotechnological), exogenous injection of hormones and osmoprotectants, seed treatment, and soil nutrient treatment, have been evaluated and evaluated in light of the discussion on the impact of drought stress. Following the discussion, it was determined that sunflowers respond to water stress by making osmotic adjustments, maintaining turgor, maintaining carbon absorption, and regulating hormones. Extensive review on the integration of multiple management methods, such as agronomic management, traditional breeding, and biotechnological breakthroughs, is desired for lengthy improved performance of sunflower achene yield and oil content under drought conditions. This could also be important in a climate change situation.

**Keywords:** Drought resistance, relative water content, achene output, oxidative damage, osmoprotectants

### 1. Introduction

Sunflower has 40–50% oil & 17–20% protein, and hence has the ability to bridge the worldwide gap between the manufacturing and utilization of edible oil and animal feed. It is, in fact, a tropical and subtropical crop with a semi-arid to arid environment that is usually farmed in dry fields or with additional irrigation. As a result, the crop is influenced by environmental factors such as heat and drought. Any element that reduces yield, whether present or absent, can be classified as stress (Tollenaar and Wu, 1999) [8]. Drought can also be imposed when a plant's evapo-transpirational demands are not met. It can also be defined as "the lack of adequate water availability (including precipitation and soil moisture storage capacity) in quantity and distribution throughout a crop's life cycle, limiting the expression of the crop's full genetic yield potential (Sinha, 1996) [9]."

Droughts can take many forms; for example, a meteorological drought occurs when precipitation falls significantly below expectations for the time of year and region. When water from all sources is insufficient to create significant crop yield deficiencies, an agricultural drought is declared. A physiological drought occurs when water is present in the soil but the plant is unable to absorb it due to salts lowering the soil's osmotic potential. Owing to a reduced rate of water absorption by roots compared to the rate of water loss in the form of transpiration due to strong winds or high temperatures, a plant's leaves may temporarily wilt. Furthermore, a microbe obstructing a plant's pores might cause persistent wilting.

Drought is a multi-faceted stress that affects plants at many levels of their organisation (Yordanov *et al.*, 2000) [10]. Drought conditions are characterised by large changes in precipitation, amount, and distribution within and across seasons (Swindale and Bidinger, 1981) [11]. Stress usually manifests itself as a reduction in photosynthesis and growth (Yordanov *et al.*, 2000) [10].

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## 2. Management Strategies

Darwin's theory of evolution describes "survival of the fittest," which means that fit individuals survived while others declined. Only crops that can withstand stress are successful in harsh environments. Crop scientists must develop strategies to adapt crop plants to harsh environmental conditions in the face of climate change. To improve sunflower performance under drought stress, different scientists in different agro-climatic regions have chosen various management strategies. The following section critically examines these strategies.

### 2.1 Breeding approaches

#### 2.1.1 Screening of drought tolerant germplasm and development of tolerant variety through breeding

Exploration of genetic variation is the first step toward improving crop drought resistance. Such variation exists in wild types and modified genotypes that have evolved through natural selection and are thought to be the best source of resistance traits. Appraising these resources in the field using an integrated phenotyping and genotyping method, as well as identifying traits that are directly related to yield, is critical for improving drought resistance.

Significant variations exist among sunflower cultivars to achieve better yield under drought stress, so genetic variability must be expanded for developing successful drought tolerant lines (Rauf, 2008) [18]. A high level of genetic variability in water status, osmotic adjustments, root characteristics, gas exchange parameters, seedling establishment, and drought susceptibility index was found in a large number of sunflower genotypes from various origins. As a result, improvements in these traits through selection from available germplasm could potentially improve sunflower drought tolerance. When selecting genotypes under drought stress, leaf area index with net assimilation rate may be preferred over leaf area (Hemmati and Soleymani, 2014) [19]. Most cultivated hybrids or open pollinated varieties evolved under near optimum agronomic conditions and often have some common parentage and history of origin. Therefore, breeding for drought tolerance must expand genetic variability. This depends on the incorporation of diverse germplasm so that potential sources of drought tolerance may be identified and subsequently incorporated to ensure yield when drought occurs.

The first approach for the development of a drought tolerant line is to screen high yielding germplasm accompanied by superior yield contributing traits. It is likely that this germplasm may also contain extensive variation for stress tolerant traits (Vasal *et al.*, 1997) [14]. Over the years, experience of handling plant material for drought tolerance at different research institutes (CIMMYT and ICARDA) indicates that improvement in drought tolerance can be made by screening both local and introduced germplasm belonging to various origins (Beck *et al.*, 1990; Edmeades and Bänziger, 1997) [15, 16]. However, the usefulness of introduced material depends on the performance under local conditions. Therefore, it is important to make selection of introduced material under local conditions, and a decision should be made accordingly: either it should be used as the source populations per se or to cross them with locally adapted materials or both (Beck *et al.*, 1990) [15].

Results showed significant variation existed between the sunflower genotypes for yield under drought stress. Fereres *et al.* (1986) [17] conducted field experiments at Cordoba, Spain to evaluate the yield responses to drought of 53 sunflower

genotypes and found substantial variability among genotypes both in dryland yield and under frequent irrigation

#### 2.1.2 Introgression from wild relatives

There are 51 species and 19 subspecies in the genus *Helianthus*, with 14 annual and 37 perennial species (Seiler, 2007) [20]. Wild sunflower species may contain water stress tolerance genes that can be exploited through specific hybridization. Induction of drought-tolerant genes from wild relatives in locally planted sunflower varieties/hybrids may thus aid in the development of drought-tolerant sunflower varieties/hybrids (Mohan and Seetharam, 2005) [21].

Sunflower was ranked fifth out of thirteen most important crops surveyed between 1980 and 2005, and it could be improved through introgression from wild relatives (Seiler *et al.*, 2017) [22]. It has been reported that all *Helianthus* species, with the exception of *Helianthus agrestis* P., can be used to introgress traits using the traditional back cross method. Sunflower breeders have extensively used *Helianthus argophyllus* and *Helianthus anomalus* (wild species) to introgress drought tolerant traits such as higher water use efficiency, improved drought sensitivity index, and a high harvest index under water stress to cultivated genotypes (Baldini and Vannozzi, 1998; Baldini *et al.*, 1999; Griveau *et al.*, 1998; Seiler, 2007) [24, 20]. Introgression of wild species genes improves fatty acid composition, protein quality, and tolerance to nutrient stress (Korrell *et al.*, 1996; Brouillette and Donovan, 2011) [25]. Seiler *et al.* (2010) [27] described two sunflower cultivars (HA 429 and HA 430) that had salt and water stress tolerance traits introgressed from wild relatives. However, in some cases, the introduction of drought-tolerant genes from many wild species has been linked to a decrease in oil yield in cultivated sunflower (Seiler, 2007) [20], which must be corrected through successive backcross breeding. It is concluded that selection and traditional breeding methods are viable tools for developing drought-tolerant sunflower cultivars. However, far too few studies have been conducted to fully exploit the potential of these approaches. To accelerate the process of genotype selection and development, traditional approaches must be combined with molecular techniques. Additionally, wild relatives may be used more extensively to enhance the genetic variability of available germplasm for drought stress tolerance.

#### 2.1.3 Molecular Breeding for Drought Tolerance in Sunflower

The emergence of developing and novel plant cell and molecular biology technologies supplied us with a potent tool to augment and enhance existing plant improvement strategies. As a result, most of the recent research has focused on isolating and assessing the expression of drought-tolerant genes. Following subtractive hybridization of cDNA generated from RNA obtained from drought stressed and unstressed plants, new stress responsive genes were discovered. Furthermore, they only showed expression in drought tolerant types when compared to drought sensitive genotypes (Roche *et al.*, 2007) [1]. Expression of these genes was not confined to a single organ. However, gene *sdis* and *HaRPS28* showed highest expression in fully expanded leaves and they were linked to the production of certain ACC oxidase antioxidant or dehydrins while *HAS1* and *HAS1.1* showed more expression in root when compared with leaves. A few genes were shown to effect the expression of other genes to induce drought tolerance.

Plants overexpressing Hahb-4 were less sensitive to exogenous ethylene and entered the senescence pathway later, according to Manavella *et al.* (2006)<sup>[2]</sup>. This transcriptional factor suppresses the expression of genes involved in ethylene production, such as ACO and SAM, as well as genes involved in ethylene signalling, such as ERF2 and ERF5. Abscisic acid synthesis is triggered by drought stress. Aside from having a differential effect on biomass partitioning (Rauf and Sadaqat, 2007b), it also regulates the expression of drought-tolerant genes such as HaDhn2, Sdis (sdi1, sdi5, sdi9, sdi6, sdi8), Ha-RPS28, and Hahb-4 (Ouvrard *et al.*, 1996; Cellier, 1998)<sup>[3, 4]</sup>. Genes related to drought stress such as dehydrins (Dhn1) have also been used to study the phylogenetic and genetic variability between cultivated and wild sunflower for this gene. The expressed protein showed diversification of biochemical properties between annual and perennial *Helianthus* species. However, cultivated sunflower contained lower genetic variability for dehydrin genes than wild sunflower (Giordani *et al.*, 2003; Natali *et al.*, 2003)<sup>[5]</sup>. It was also concluded that the stress responsive gene (Dhn1) can also be used to study the phylogeny of *Helianthus*.

The concept of DNA-based markers has revolutionised our ability to directly access any part of the plant genome, opening up new possibilities such as screening a large number of segregating populations for QTLs related to drought tolerance without actually exposing them to drought and regardless of plant growth stage. The bulk of physiological features in nature are quantitative trait loci (QTL). Quantitative traits have continuous phenotypic distributions that are influenced by a large number of genes and exhibit strong environmental interaction (Tanksley, 1993)<sup>[6]</sup>. However, they concluded that these QTLs were identified in greenhouse conditions and the usefulness of these QTLs for marker-assisted selection should therefore be evaluated under field conditions and validated in other genetic backgrounds. Similarly, Jamaux *et al.* (1997)<sup>[7]</sup> identified RFLP and STS molecular markers of relative water loss and osmotic adjustment by RAPD bulked analysis.

### 3. Agronomic approaches

#### 3.1. Mitigating Drought Stress through Exogenous Application of $\beta$ -Aminobutyric Acid

Drought stress is a serious danger to sustainable crop production around the world, particularly in arid and semi-arid countries, and is one of the most significant restrictions to sunflower (*Helianthus annuus* L.) production. Different drought mitigation measures, such as foliar spraying of osmolytes such as  $\beta$ -aminobutyric acid (BABA), aid the crop in combating drought.

Drought-relieving treatments included foliar spray of BABA at three concentrations: 0 mM (control), 25 mM, 50 mM, and 75 mM. Under drought conditions, foliar treatment of BABA had a significant impact on physiological parameters, yield, and yield-related variables. The administration of a 75-mM BABA solution to the leaves enhanced the SPAD-chlorophyll value and the membrane stability index while maintaining larger relative water levels. It also increased 1000-achene weight and achene yield, producing 41% and 44% greater achene production in 2018 and 2019, respectively, when compared to other treatments. Under varied water stress circumstances, significant and positive relationships were also detected between sunflower yield and yield-contributing characteristics.

#### 3.2 Use of mineral nutrients and organic manures

Cechin *et al.* (2015) found that applying 10 M sodium nitroprusside (SNP), a NO donor, increased the concentration of ROS scavenging enzymes in sunflower, which improved drought tolerance. The use of triazole compounds such as Hexaconazole, Tebuconazole, and Propiconazole, which have been shown to strengthen the antioxidant defence system of sunflower by increasing the activity of antioxidant enzymes such as SOD, APX, and CAT, has helped to mitigate the negative effects of drought stress in sunflower (Rabert *et al.*, 2014, 2016). Exogenous application of osmoprotectants, phytohormones, micro and macro nutrients, and other compounds has been shown to improve sunflower growth, yield, and oil quality under drought stress. As a result, crop nutrition is an agronomic tool that can be used to improve crop yield drought resistance of sunflower. The application of triazole compounds such as Hexaconazole, Tebuconazole, and Propiconazole, which have been reported to strengthen the antioxidant defence system of sunflower through increased activities of antioxidant enzymes such as SOD, APX, and CAT, has partially alleviated the adverse effects of drought stress in sunflower (Rabert *et al.*, 2014, 2016). Exogenous application of osmoprotectants, phytohormones, micro and macro nutrients, and a variety of other compounds has been shown to improve sunflower growth, yield, and oil quality under drought stress. As a result, proper crop nutrition is an agronomic tool that can be used to improve sunflower drought resistance. Drought tolerance by improving protein synthesis, stomatal regulation, homeostasis, and osmoregulation by quenching ROS (Cakmak, 2005)<sup>[31]</sup>. The role of N in improving growth and plant water relations under drought stress is well established (Saneoka *et al.*, 2004)<sup>[32]</sup>. Similarly, increased K nutrition improves water uptake, maintains plant turgor, and regulates stomatal aperture (Bukhsh *et al.*, 2012)<sup>[33]</sup>. Hussain *et al.* (2016)<sup>[34]</sup> recently reported that combining N and K improves drought resistance of sunflower by improving turgor maintenance, increasing osmoprotectant accumulation, increasing stomatal conductance and net photosynthesis, and decreasing ROS production, resulting in higher yield. Different potassium (K) levels in soil play an important role in sunflower growth regulation and water uptake ability. It is well known that a lack of K reduces plant resistance to drought stress via stomatal closure (Marschner, 1995), lowering the transpiration rates (Hsiao and Lauchli, 1986). However, some authors have reported that moderate K deficiency can cause abnormal stomatal behaviour and increase transpiration in crops such as wheat and sunflower (Brag, 1972; Lindhauer, 1985)<sup>[36, 37]</sup>. According to Fournier *et al.* (2005)<sup>[38]</sup>, moderate K deficiency promotes water uptake and reduces WUE in sunflower plants when compared to well-watered plants. In K+-deficient plants, ABA regulation may prevent stomatal closure, increasing water uptake and transpiration. Furthermore, the increased uptake in K+-deficient plants could be attributed to an increase in transpiration rate, which forced the plant to extract more water from the soil in order to maintain a water balance. Micronutrient application also improves sunflower drought stress performance (Zafar *et al.*, 2014; Babaeian *et al.*, 2011; Rahimizadeh *et al.*, 2007; Shehzad *et al.*, 2016)<sup>[39, 40, 41]</sup> by improving antioxidant defence, stay green, achenes weight, achene yield, biological yield, and oil yield. The application of Fe + Zn + Cu + Mn to sunflower resulted in a 48–89 percent increase in the production of antioxidant enzymes (SOD, CAT, and GPX), indicating the importance of



micronutrient application for improving drought resistance (Rahimizadeh *et al.*, 2007; Babaeian *et al.*, 2011) <sup>[40]</sup>. Significant improvements in drought-stressed sunflower physiological traits have also been reported following Zn and CaCl<sub>2</sub> application (Zafar *et al.*, 2014) <sup>[39]</sup>. Ibrahim *et al.* (2016) <sup>[42]</sup> recently reported positive effects of CaCl<sub>2</sub> foliar application on leaf relative water contents, leaf pigments (such as chlorophyll a, chlorophyll b, carotenoids, anthocyanins, carotenoids), leaf minerals (N, P, K, and Ca), organic osmolytes (proline and soluble sugars), and phenolic related enzymes such as phenylalanine ammonia lyase (PAL) and peroxidase (POD) in sunflower under drought stress. Silicon application is also beneficial in reducing drought-induced yield losses in oilseed crops such as sunflower. In one study, silicon application under drought stress improved K, sulphur, magnesium, iron, copper, and manganese uptake while leaving zinc unchanged (Gunes *et al.*, 2008b). Gunes *et al.* (2008b) found that exogenous silicon application reduced leaf MDA while increasing relative water content and the activities of enzymatic (e.g. catalase) and non-enzymatic antioxidant defense systems in sunflower (Gunes *et al.*, 2008a). Exogenous silicon application increased root/shoot dry weight, net assimilation rate, relative water contents, CO<sub>2</sub> absorbance, root amino acid contents, root water uptake, root proliferation, and the activities of superoxide dismutase and peroxidase in a canola crop study (Habibi, 2014) <sup>[45]</sup>. Organic manures are another viable option that, when used alone or in conjunction with synthetic fertilisers, improve drought tolerance (Esmaeilian *et al.*, 2012) <sup>[46]</sup>. These manures are a good source of major nutrients and influence the temporal dynamics of nutrient availability by improving soil physicochemical properties (Paul and Beauchamp, 1993) <sup>[47]</sup>. Drought tolerance was significantly improved when organic manure was applied to drought stressed sunflower alone or in combination with synthetic fertilisers (Aowad and Mohamed, 2009) <sup>[48]</sup>. Similarly, organic manures, both alone and in combination, improved sunflower nutrient uptake under drought stress, resulting in higher yield (Esmaeilian *et al.*, 2012) <sup>[46]</sup>.

### 3.3 Arbuscular mycorrhizal fungi and polymers

Polymers may improve drought tolerance of sunflower by maintaining nutrient balances.

### 3.4 Seed priming

Seed priming is a technique that involves partially hydrating seeds to the point where germination-related metabolic processes begin but the radicle does not emerge. Hydropriming, salt priming, on-farm priming, and chemical priming are some of the seed priming techniques that have been widely used to improve germination and crop establishment. Seed priming, particularly osmopriming with KNO<sub>3</sub> and hydropriming, improved sunflower crop germination and stand establishment under stress (salt and drought) and normal conditions (Kaya *et al.*, 2006; Hussain *et al.*, 2006). Under drought stress, Moghanibashi *et al.* (2012) <sup>[53]</sup> found that hydropriming sunflower seeds improved germination percentage, germination index, root/shoot length, and root/shoot dry weight. Hydropriming sunflower seeds improved the germination percentage, mean emergence time, and seedling dry weight of two sunflower genotypes, Azargol and Hysun-36, in another study (Sheidaie *et al.*, 2013) <sup>[54]</sup>. Seed priming also improved seed vigour, according to Kausar *et al.* (2009). Biological priming of seeds is a new technique

that gives plants tolerance to a variety of biotic and abiotic stresses (Singh *et al.*, 2015) <sup>[55]</sup>. Significant improvements in drought tolerance of sunflower have been reported using biologically primed seeds with *Azotobacter chroococcum*. The symbiotic relationship between plant roots and arbuscular mycorrhizal fungi is also an important strategy for improving mineral nutrition in plants under abiotic stress (Brachmann and Parniske, 2006). Arbuscular mycorrhizal fungi, in fact, provide surface area for plant roots to absorb water and nutrients (e.g., P, N, Zn, Cu) (Smith and Read, 2008). In one study, the use of two species of mycorrhiza (*Glomus mossea* and *Glomus setanicatum*) improved seed yield and seed nutrient content in sunflower, with *Glomus setanicatum* being more beneficial for improving seed yield and nutrient content of sunflower (Heidari and Karami, 2014) <sup>[50]</sup>. However, more research is needed to investigate the role of arbuscular mycorrhizal fungi in improving sunflower drought stress performance under a variety of environmental conditions. The use of super absorbent in arid and semi-arid climates is another way to improve the water use efficiency and achene yield of sunflower under drought stress. According to Boman and Evans (1991), super absorbent polymers can hold 400–1500 g of water per dry gramme of hydro gel, increasing water absorption and retention under drought stress. A study found that applying super absorbent polymers at 2.25–3 g/kg of soil improved water use efficiency under drought stress (Nazarli *et al.*, 2010). Finally, drought stress disrupts mineral nutrition, whereas the use of macro- and micronutrients, organic manures, arbuscular mycorrhizal fungi, and *Bacillus polymyxa*, either alone or in combination (Singh *et al.*, 2015) <sup>[55]</sup>. Both growth-promoting bacteria increased the activity of antioxidant enzymes, reducing the negative effects of drought stress in sunflower. Gholamhoseini *et al.* (2013) discovered that inoculating sunflower plants with two mycorrhizal fungi species, *Glomus mosseae* and *Glomus hoi*, increased biomass, achene, and seed yield compared to non-inoculated plants. It is concluded that seed priming, either chemical or biological, may aid in the improvement of drought-stressed sunflower performance. As a result, seed priming can be used to significantly reduce the negative effects of drought stress on sunflower germination, growth, and yield.

### 4. Conclusion and future directions

Drought stress has a negative impact on sunflower crop seed germination, seedling growth, plant water relations, mineral nutrition, stay green, photosynthesis, transpiration, and grain partitioning, affecting seed yield and oil quality. However, various management practises such as conventional or biotechnological drought tolerance breeding, exogenous application of hormones and osmoprotectants, seed treatment, and soil nutrient management may be beneficial in improving drought tolerance in sunflower. The RNA-mediated silencing and DNA methylation processes of specific genes are recent options investigated in the developing era of functional genomics for improving abiotic stress tolerance in plants, which can be successfully used to improve drought tolerance in sunflower. Several physiological parameters, including improved stomatal conductance, shoot and root dry weight, harvest index, root system, leaf hydraulics, and stay green, must be considered when screening sunflower genotypes for drought stress tolerance breeding programmes. The use of leaf carbon isotope discrimination technique, identification of QTLs responsible for efficient water use under drought stress,

and application of super absorbent may be quite beneficial for improving the water use efficiency of sunflower under drought stress. For the long-term improvement of sunflower achene yield and oil quality under drought stress, comprehensive research on the integration of various management options, including agronomic approaches, conventional breeding, and modern biotechnological advances, is required.

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