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Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress

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ABSTRACT

Plant species and cultivars have different physiological mechanisms in response to drought stress. A biennial field experiment was conducted to evaluate the effect of drought severities on enzymatic and non-enzymatic antioxidants, photosynthetic pigments, chlorophyll florescence and water relations in sunflower cultivars. Eight cultivars of sunflower (Azargol, Iroflor, Armavirovski, Lakumka, Alstar, Master, Sirna and Pumar) were subjected to well-watered, moderate and severe drought stress. Drought treatments were started at the beginning of stem elongation. The results showed that catalase activity, carotenoid content, proline content and electrolyte leakage were increased with drought severity, but chlorophyll-a, chlorophyll-b, maximum photochemical efficiency of photosystem-II, photosynthetic performance index, relative water content, and stomatal conductance were decreased. Peroxidase activity was not affected by drought stress. There was a positive correlation between enzymatic and non-enzymatic antioxidants. Catalase activity was correlated positively with proline content but negatively with chlorophyll content and chlorophyll florescence. Carotenoid had a negative correlation with chlorophyll-a as well as chlorophyll-b, but had a positive correlation with proline content.

1. Introduction

Plants are subjected to several rough environmental stresses. Several biotic and abiotic factors affect the growth of higher plants in the field (Lichtenthaler, 1996). Among these, drought is a major abiotic factor that limits agricultural crop production. Plants experience drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two mentioned conditions often coincide under arid and semi-arid regions (Reddy et al., 2004). Decreasing water supplies either temporarily or permanently affects morphological and physiological processes in plants adversely (Iqbal et al., 2008). Understanding the physiological mechanisms providing drought stress tolerance is very important in terms of developing selection and breeding strategies. However, despite an extensive literature on plants' responses to drought, there are few documented examples where a physiological understanding of drought has identified traits that limit yield under drought and where these have been used in successful crop improvement programs to enhance crop yields (Richards, 2006).

Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oil crops in the world (Flagella et al., 2002). There are two types of sunflowers: oil types containing about 40% oil and non-oil types with about 30% oil. Oil types represent 80–95% of sunflower seed production. The oil is mainly used for cooking and frying. Industrial uses include lighting, paints, cosmetics, resins, lubricants and biofuel. Non-oil types are mostly used in confectionery products such as roasted and/or dehulled seeds (FAO, 2010; Grompone, 2005). Sunflower oil is extracted from the seeds. Each environmental factors that decreases the growth of sunflower plant, finally leads to decreasing of seed production, oil yield and industrial products. Sunflower is deep-rooted crop that has been shown to deplete available soil water. This makes sunflower more tolerant to short periods of water stress (Karam et al., 2007).

Plants under drought stress are affected by secondary damages caused by oxidative stress. One of the mechanisms aiming drought avoidance is stomatal closure (Ozkur et al., 2009). Decreasing of CO_2 uptake due to stomatal closure leads to over-excitation of the

Abbreviations: ROS, reaction oxygen species; CAT, catalase; POD, peroxidase; PS-II, photosystem II; F_v/F_m, maximum photochemical efficiency of PS-II; PI, photosynthetic performance index; Chl-a, chlorophyll a; Chl-b, chlorophyll b; CAR, carotenoid; RWC, relative water content; EL, electrolyte leakage; CMS, cell membrane stability.

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reaction centers of photosystem II (Ahmed et al., 2009) and formation of reaction oxygen species (ROS) (Fu and Huang, 2001). Chloroplast, mitochondria and peroxisome are the common cellular organelles of ROS production (Ishikawa et al., 2010). ROS include superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^-) and singlet oxygen ($^1O_2$) (Reddy et al., 2004). The excess production of ROS is harmful to lipid, proteins and nucleic acids (Halliwell and Gutteridge, 1989), whose oxidation may lead to detrimental effects such as enzyme inhibition, chlorophyll degradation, disruption of membrane integrity, loss of organelle function and reduction in metabolic efficiency and carbon fixation (de Campos et al., 2011). Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic and non-enzymatic antioxidants (Reddy et al., 2004). Among the enzymatic antioxidants, superoxide dismutase (SOD) has an important role in the antioxidant defense system as it scavenges O_2^- free radicals converting them into H_2O_2. The H_2O_2 is then further scavenged by catalase (CAT) and peroxidase (POD) into H_2O and O_2 (Wang et al., 2009). The commonly non-enzymatic antioxidants are carotenoids (Munne-Bosch and Penuelas, 2003), glutathione (Gómez et al., 2004), and ascorbate (Huseynova, 2012). These responses depend on the species, the development and the metabolic state of the plant, as well as the duration and severity of the stress (Reddy et al., 2004).

The photosynthetic apparatus consists of a series of thylakoid-localized multi-subunit protein complex. Photosystem-I (PS-I) and photosystem-II (PS-II) are each composed of a core reaction center surrounded by specific light harvesting complexes called LHC-I and LHC-II, respectively (Green and Durnford, 1996). The LHC family is the most plentiful pigment protein complex in the thylakoid membrane and binds roughly half of the total chlorophyll (chl a and b) in chloroplasts (Peter and Thornber, 1991). Chl-a is a component of both the photosynthetic reaction centers and LHC, whereas chl-b is restricted to the LHC (Oster et al., 2000). Light energy is harvested by the LHC and drives electron transfer in the reaction centers (Kang et al., 2012).

Chlorophyll a florescence measurement, a non-destructive technique, is widely used to study the plant's response to environmental stresses (Oukarroum et al., 2007, 2009). Florescence yield can be quantified by exposing dark-adapted leaf to light of a defined wavelength and measuring the amount of light re-emitted at longer wavelengths (Maxwell and Johnson, 2000). The illumination of this dark-adapted leaf results in characteristic changes in the intensity of chlorophyll a florescence, known as the Kautsky effect (Boureima et al., 2012). In a florescence induction curve, at the minimal florescence (F_o) all the reaction centers are open and at the maximal florescence (F_m) all the reaction centers are closed.

Osmotic adjustment is possible with accumulating compatible solutes, such as amino acid proline. Besides proline's role in osmotic adjustment, it helps in the conservation of tissue water and to protect proteins and cellular membranes against osmotic and oxidative stresses (Ashraf and Foolad, 2007). Proline has an important role as singlet oxygen quencher (Alia et al., 2001) and scavenger of hydroxyl radicals (Smirnoff and Cumbes, 1989), so proline can stabilize DNA, proteins and membranes (Alia et al., 2001). Proline has another role in storage of carbon, nitrogen and energy (Hare and Cress, 1997). It has been reported that proline improved drought resistance in sunflower (Hussain et al., 2008), peper (*Capsicum annuum* L.) (Anjum et al., 2012), maize (*Zea mays* L.) (Anjum et al., 2011), rice (*Oryza sativa* L.) (Iyer and Caplan, 1998) and barley (*Hordeum vulgare* L.) (Villadsen et al., 2005).

Determination of water relation components at the whole plant or cellular level is important for determination of resistance of species or cultivars to drought stress (Turner, 1986). Among several methods used to characterize internal plant water status under drought conditions, relative water content (RWC) was used successfully to identify drought resistance in barely (Matin et al., 1989), maize (Efeoğlu et al., 2009), common bean (*Phaseolus vulgaris* L.) (Rosales-Serna et al., 2004) and sunflower (Angadi and Entz, 2002). Sinclair and Ludlow (1985) proposed that RWC was a better indicator of water status than was water potential.

The cell membrane is one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water deficit conditions is a major component of drought tolerance in plants (Bajji et al., 2002). Selection for slow leaf electrolyte leakage under heat stress has been proposed as a method for increasing heat tolerance and heat resistance of several grain crops by enhancing membrane thermostability (Blum et al., 2001; Thiaw and Hall, 2004). The degree of cell membrane injury induced by drought stress or heat stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji et al., 2002; ur Rahman et al., 2004).

Mediterranean climate regions are characterized by cool wet winters and hot dry summers (Leport et al., 1998). In many areas of this region, irrigation resources, such as rivers and wells, are decreasing during summer. Hence, summer crops such as sunflower are exposed to drought stress. Thus, the objective of this work was to study the responses of some physiological traits including enzymatic and non-enzymatic antioxidants, chlorophyll a and b concentration, chlorophyll florescence, proline concentration, cell membrane stability and water relations in sunflower cultivars against well-watered, moderate and severe drought conditions.

2. Materials and methods

2.1. Study location

A biennial research was conducted at experimental field of Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran, in 2010 and 2011. This place is located in latitude 34°21′ North and longitude 47°9′ East with elevation 1319 m above sea level and belongs to semi-arid zone. Weather characteristics of the study area during the growing seasons were presented in Table 1. Experiment was conducted on a clay soil with pH 7.6, N 0.112%, P_2O_5 9.8 mg kg^{-1}, K_2O 360 mg kg^{-1}, Mn 6.0 mg kg^{-1}, Fe 5.5 mg kg^{-1}, Zn 1.10 mg kg^{-1}, and Cu 2.2 mg kg^{-1}.

2.2. Treatments and agronomic operations

Each experiment was conducted as split plot based on randomized complete block design with three replications. Irrigation regimes were considered as main-plots and cultivars as sub-plots. Main-plots were control (irrigation after 10 days = IR_{10}), moderate drought stress (irrigation after 15 days = IR_{15}) and severe drought stress (irrigation after 20 days = IR_{20}). Sub-plots were eight sunflower cultivars including Azargol, Iroflor, Armavirovski, Lakumka, Alstar, Master, Sirna and Pumar. Sunflower seeds were hand sown as furrow method at 25 June in both years. Each plot consisted of 5 rows, 4.5 m length, 55 cm distance between rows, and 25 cm distance between plants. All plants were well-watered and received irrigation uniformly until the imposition of the treatments. Drought treatments were started at the beginning of stem elongation. Weeds were hand controlled continuously during sunflower vegetative growth. Based on the results of soil testing, 250 kg ha^{-1} urea and 150 kg ha^{-1} superphosphate triple fertilizers were consumed. After pollination, flower heads covered with paper to protect against birds attack.