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Variation in polyphenolic composition, antioxidants and physiological characteristics of globe artichoke (*Cynara cardunculus* var. *scolymus* Hayek L.) as affected by drought stress

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ABSTRACT

Different levels of irrigation regimes (20%, 50% and 80% depletion) were applied to evaluate polyphenolic compounds and physiological traits in leaves and heads of artichoke. According to HPLC analysis, chlorogenic acid was the most abundant phenolic acid in leaves (481 mg/Kg DW) and the second most abundant compound (after caffeic acid) in heads (213 mg/Kg DW) in non-stress condition. During water shortage, chlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid and 1,5-dicaffeoylquinic acid increased, while apigenin, and luteolin decreased in both parts. The elevation in the contents of proline, H_2O_2, malondialdehyde and the activities of antioxidant enzymes were also observed under stress conditions. Catalase was the most active antioxidant enzyme in all treatments. Water stress led to increase in carotenoids and decrease in chlorophyll content of the heads. Finally, the irrigation regimes can be selected by considering the yield components as well as favorite compounds for food and pharmaceutical purposes.

1. Introduction

Globe artichoke (*Cynara cardunculus* var. *scolymus* Hayek L.) is perennial, tall thistle-like plant of the Asteraceae family with edible and medical applications from Mediterranean origin (Vamanu et al., 2011). The head of artichoke (capitulum) is a healthy food owing to low fat and high fiber, protein, minerals and phenolic compounds (Fratianni et al., 2007). Artichoke leaves have therapeutic properties with high amount of antioxidant compounds that can be used for the treatment of digestive disorders, improving bile and liver function and reducing blood sugar and cholesterol (Vamanu et al., 2011).

Phenolic acids and flavonoids are widely distributed as secondary products with high antioxidant activity in plants. They enhance stress defense system in plants and give human health benefits to plant-derived foods as well as therapeutic properties to medicinal plants (Ayaz et al., 2000). Cultivation of vegetables and medicinal plants is widely restricted by drought stress as well as crop plants (Siadat-Jamian et al., 2014). However, the application of biotic and abiotic stresses to plants could be one possible method to elevate these compounds. Indeed, an increase in biosynthesis of polyphenols that occurs in plant as a response to stresses might be exploited as a potential source for economical use (Bettaieb et al., 2011).

Water shortage has always been considered one of the main global crises and it is being escalated by climate change into more acute problem, chiefly in arid and semi-arid regions. Drought restricts agricultural economy, terrestrial ecosystem productivity and threatens the world food security with negative effects on plant growth, development and crop productivity more than any other environmental stresses (Mancosu et al., 2015). Photosynthesis and cell growth are the main processes that can be affected by drought condition. Moreover, plants increase production of reactive oxygen species (ROS) under stress conditions that can cause severe damage to photosynthetic system (Chaves et al., 2008).

Plants display vast range of stress tolerance or stress avoidance mechanism to survive under stress condition (Mirzaee et al., 2013). For example, antioxidant system including enzymatic antioxidants such as catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX), as well as low molecular weight non-enzymatic antioxidants (ascorbic acid, glutathione, carotenoids, phenolic acids, flavonoids, etc.), could be changed as a result of drought stress (Amiri et al., 2015). Variation in the activity of CAT, POX and APX in response to water deficit has been reported in *Sesamum indicum* (Kadkhodaie et al., 2014) and *Amaranthus hypochondriacus* (Slabbert and Krüger, 2014). Plants respond widely to water deficit by a range of molecular, physiological and biochemical processes. Relative water content (RWC) and malondialdehyde (MDA) could also be changed in response to water deficit (Mirzaee et al.,

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2013). MDA is considered as a final product of lipid peroxidation and is a sign of membrane cellular damage by accumulation of free radicals (Soleimanzadeh et al., 2010). Chlorophyll and carotenoids have important roles in photosynthesis and protection of photosynthetic apparatus against harmful free radicals (Kadkhodaie et al., 2014).

In artichoke, previous reports revealed that polyphenolic composition can be affected by plant parts (Fratianni et al., 2007), harvest time and genotype of the plant (Lombardo et al., 2010). Shinohara and Leskovar (2015) also assessed the effect of ABA, antitranspirants and heat stress on plant growth and water status of artichoke transplants. Jorenush and Rajabi (2015) evaluated germination and seedling growth of artichoke under drought and salinity tensions. However, there is no study in respect to the effect of water stress on physiological properties and polyphenolic composition of artichoke in both heads and leaves.

The aims of the present study were: (1) to assess phenolic acids and flavonoids compounds as well as their antioxidant enzymes activity (CAT, APX, and POX) in heads and leaves of artichoke in different levels of irrigation; (2) to evaluate physiological parameters including carotenoids content, proline accumulation and lipid peroxidation in drought stress condition and (3) to find out changes in growth parameters and yield components as a result of water shortage.

2. Materials and methods

2.1. Plant material and experimental condition

Artichoke (*C. cardunculus* var. *scolymus* Hayek) seeds were collected from Isfahan Agricultural and Natural Resources Research Center, Isfahan, Iran. The field experiment was conducted in research farm of Fozveh that is located in west side of Isfahan (32°36′N; 51°26′E and 1612 m above the mean sea level), Iran. The experimental field was located in the territory of the alluvial plain and had semi-arid climate with 16 °C average temperature and 140 mm annual average rainfall. The experiment farm had undeveloped young soil profile with clay loam (31% clay; 24% silt; 45% sand), EC of 2.8 ds m^{-1}, pH of 7.6, a bulk density of 1.36 g cm^{-3}, organic carbon of 0.26%, total N of 0.03%, available P and available K of 15 and 250 mg kg^{-1}, respectively. Seeds were planted with 40 cm distance on the rows and 60 cm space between rows at 3–4 cm depth.

2.2. Irrigation treatment

The field experiment was designed as a randomized complete block with six replication and three irrigation regimes (three treatments). To have appropriate establishment, plants were irrigated with no water deficit in the first year (2013), and treatments were applied on two year plants from May to August 2014.

Irrigation treatments were determined based on the method of Allen et al. (2000). Different irrigation regimes including 20%, 50% and 80% depletion of soil available water were used as non-stress (NS), moderate water stress (MWS) and severe water stress (SWS) conditions, respectively. The irrigation time was adjusted by time domain reflectometry (TDR) device (Model Sabta Barbara 6050X) and creating soil moisture curve.

2.3. Sampling procedure and morphological traits evaluation

Samples were collected in marketing stage of the heads, when the immature flowers were firm and tightly closed and proper for use. Sampling was carried out in the early morning and more than six leaves and heads (large terminal buds) samples were collected from each experimental unit and kept at a temperature of −80° C. For capitula dry weight determination, the samples were oven-dried at 70 °C for 72 h and then weighed. Measurement of grain yield and 1000 grain weight performed after harvesting at the full maturity stage.

2.4. Relative water content

Sampling was carried out an hour after solar noon. Six top fully expanded leaves from different plants per replication were sampled by sharp knife and immediately used. The leaves weighted (FW) and then were hydrated in distilled water for 24 h in the room temperature. Turgid samples were quickly weighted (TW) after drying surface water with paper towels. Dry weights (DW) were measured after drying at 75 °C for 48 h. RWC was calculated according to following equation (Ritchi et al., 1990):

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

2.5. Proline content

Approximately 0.5 g of material was homogenized in 10 mL of 30 g L^{-1} sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. Then 2 mL of filtrate was reacted with 2 mL of acid-ninhydrin (1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 mol L^{-1} phosphoric acid) and 2 mL of glacial acetic acid in a test tube at 100 °C for 1 h. The reaction was terminated in an ice bath and then 4 mL of toluene was added and the product of the reaction was extracted by vortex mixing. The absorption of the upper phase was read at 520 nm using toluene as a blank. Proline concentration was calculated on a dry weight (DW) basis using L-proline for the standard curve (Bates et al., 1973).

2.6. Chlorophyll and carotenoids contents

Sample amount of 0.5 g was grinded and extracted in 10 mL of 80% (v/v) acetone using the mortar and pestle until a colorless residue was obtained. The extract was centrifuged at 3000 rpm for 10 min at room temperature and the residue was removed. The absorbance of the solution was determined at three wavelengths including 663, 645 for chlorophyll and 470 nm for carotenoids. Chlorophyll and carotenoids content were estimated by mg g^{-1} dry weight (Arnon 1949):

$$\text{Chlorophyll a (mg g}^{-1}\text{ DW)} = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \text{mL acetone}/1000 \times W$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ DW)} = [(22.9 \times A_{645}) - (4.69 \times A_{663})] \times \text{mL acetone}/1000 \times W$$

$$\text{Carotenoids (mg g}^{-1}\text{ DW)} = [(1000 \times A_{470}) - (1.9 \times \text{Chl a}) - (63.14 \times \text{Chl b})]/214$$

2.7. Determination of malondialdehyde and H_2O_2

Malondialdehyde (MDA) was measured using 2-thiobarbituric acid (TBA). For this purpose, 0.1 g of powdered plant tissue mixed with 5 mL of TBA (0.6%) in 10% trichloroacetic acid (TCA) using a mortar and pestle. Then, the mixture was boiled at 100 °C for 15 min. After cooling the mixture in ice, it was centrifuged at 5000 rpm for 10 min. The absorbance of the supernatant was read at 450, 532, and 600 nm. The MDA content was calculated on a dry weight basis as follows (Zhao et al., 2006):

$$\text{MDA (μmol g}^{-1}\text{ DW)} = 6.45(OD_{532} - OD_{600}) - 0.56\ OD_{450}$$

Hydrogen peroxide (H_2O_2) was calculated after reaction with KI. Sample of 1 g was ground with 10 mL 0.1% trichloroacetic acid (TCA) and centrifuged for 15 min at 12,000 rpm. Then 0.5 mL of the solution was reacted with 0.5 mL of 100 mM K-phosphate buffer and 2 mL reagent [1 mL KI (w/v) double-distilled water] for 1 h in the dark. The absorption was read at 390 nm and the amount of hydrogen peroxide was measured according to standard curve that was prepared with known concentrations of H_2O_2 (Jana and Choudhuri 1981).