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Effects of nano-iron spraying on the antioxidant activities of canola leaf under drought stress

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Abstract

Drought stress limits plant productivity and performance constraining safe food production worldwide. Reactive oxygen species (ROS) are mostly harmful event taking place in plants under abiotic stresses. This study aimed to evaluate nano-iron foliar application in reducing ROS damage through measuring electrolyte leakage, total phenolic content, antioxidant enzymes (ascorbate peroxidase, catalase, and guaiacol peroxidase), and antioxidant activity in canola under 30% FC of drought stress. Results indicated that the effect of drought stress and nano-iron spraying were significant on studied parameters ($p<0.01$). The plants treated with nano-iron compared to untreated plants showed remarkably a low electrolyte leakage and high total phenolic content, antioxidant enzyme activities, and antioxidant activity under 30% FC (LSD<0.05). In conclusion, it is suggested that nano-iron is effective in reducing ROS damage and improving oxidative defense system in canola.

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Introduction

The growth and performance of plants are limited by various biotic and abiotic stresses around the word. Drought stress as an important environmental stresses affects plant growth as others types of stresses at the entire course of plant growth or a part of their growth (Soltani *et al.*, 2006). This stress is a recurring feature of various climates that threats secure food production for growing population (Zarch *et al.*, 2015). The plants grown under drought conditions are unable to absorb adequate water so they experience water deficiency. Under such conditions, the plant cell division and expansion are impaired, resulting in decreasing the plant growth index, physiological and biochemical alteration (Bandehagh *et al.*, 2011; Cakir, 2004; Gharelo Shokri *et al.*, 2016).

Micronutrients serve many essential roles in the plant. Iron is an essential micronutrients required for plant growth. It acts as a cofactor for many enzymes that catalyze chlorophyll synthesis, thylakoid synthesis, chloroplast development, and many other biochemical reactions (Theil, 1987). Stopping each of these enzymes can drastically reduce the plant growth. Micronutrient deficiency considered as one of the important factors that decline the plant growth (Hu and Schmidhalter, 2005). Under drought stress, micronutrient deficiencies are common for some reasons, such as high pH, changing redox potential of the soil solution, and solubility of micronutrients (Zhu *et al.*, 2004). Studies on application of micronutrient as a spray on different plants have indicated that the treated plants show a high growth rate and more tolerance to abiotic stress (Gharelo, 2016; Nasiri *et al.*, 2010; Said-Al Ahl and Mahmoud, 2010; Zayed *et al.*, 2011).

Nanotechnology is the science of dealing with particles with size of a less than 100 nm. One of the applications of nanotechnology in agriculture is nanofertilizer. Nanofertilizer are needed in a low amount for plants and do not have harmful effects of chemical fertilizer (Scott and Chen, 2013). The more surface area of nanofertilizers may help in an efficient absorption of the fertilizers by plants (El-Feky *et al.*, 2013).

Using nanofertilizers increases the growth and yield of the plants (El-Sherbini *et al.*, 2015; Shokri-Gharelo *et al.*, 2016). Application of Nano fertilizers could be one of the promising ways to overcome such problems because uncontrolled using of chemical fertilizer has caused environmental pollutions and human health issues.

Reactive oxygen species (ROS) are the result of the partial reduction of oxygen molecule, existing basically in four forms: singleton oxygen, superoxide radical, hydrogen peroxide, and hydroxide radical. The chloroplast and mitochondria are responsible for ROS production (Cruz de Carvalho, 2008). Under normal growth conditions, plants are commonly producing ROS in a low rate, but enhanced ROS production is unavoidable under drought stress (Bian and Jiang, 2009). When ROS production reaches a high level in the cell, it becoming deleterious and damaging the cell membrane (Mittler, 2002). Plants to cope with such ROS production in a high level have evolved different strategies at molecular level; (A) scavenging enzymes such as catalase, superoxide dismutase, (B) photorespiration (Bian and Jiang, 2009; Gharelo, 2016).

Many studies have demonstrated that the plants that show high antioxidant activity are more tolerance to adverse effects of abiotic stress (Bandehagh *et al.*, 2011; Kapoor *et al.*, 2015; Shi *et al.*, 2015; Shokri-Gharelo *et al.*, 2016). Therefore, this index is appropriate parameter for assessing the ability of plants to scavenger ROSs under drought stress.

Canola (*Brassica napus* L.) is one of the widely cultivated oil crops for producing vegetable oil. This plant tolerates abiotic stresses, however, its growth, yield, and performance are adversely affected (Purty *et al.*, 2008). This study aimed to evaluate effects of foliar application of nano-iron on antioxidant activities of canola, Sarigol, under drought stress. It could be best to our knowledge to determine the ability of nano-iron in raising antioxidant activities of canola under drought stress. It is expected that nano-iron spraying could ameliorate harmful effects of drought stress by increasing antioxidant systems in canola.

Material and methods

Plant materials and cultivation conditions

Brassica napus seeds– cultivar Sarigol– provided from Seed and Plant Improvement Institute (SPII), Karaj, Iran. Seeds were germinated and cultivated in a field condition in Moghan, Ardabil Province, Iran. Moghan climate is subtropical, located at the northwest of Iran. Seeds were sown on 15 October and plants harvested on 5 May before going to flowering stage. Drought stress exerted on plants by reducing times of irrigation. Regularly, canola is irrigated 5 times during its growth course which we decreased irrigation times to 2 times. Field capacity of the field measured continuously to watch precious exertion of drought stress. Totally, 12 blocks each in the size of 1×1m arranged to carry out the experiment. All the agricultural operation on the field including preparing filed, controlling weeds, fungi, and pests conducted as operations commonly performed on other fields in order to provide real field conditions on plants.

Experimental treatment application

Split plot with a completely randomized block experimental design with three replications was carried out in which drought at two levels (0 and 30% FC) was considered as the main factor while foliar application of nano-iron was applied in two levels (presence or absence of nano-iron).

Synthesis of iron nanoparticles

$FeCl_3.6H_2O$ (1.6 g, 6 mM), sodium acetate (4 g, 48.8 mM), trisodium citrate (1.5g, 5.8mM) were solved in 75 cc of ethylene glycol. After vigorous stirring for 1h, resulted yellow solution was transferred into stainless steel autoclave with 100 cc volume and 200°C for 12h. Autoclave slowly cooled under normal temperature. Magnetic nanoparticles of iron were collected using magnet and separately washed several times by distilled water and ethanol. The products were dried under vacuum conditions and 50°C for 12h. Analysis of particle size distribution showed average diameter of 39nm for the iron nanoparticles.

Electrolyte leakage

Lutts *et al* (Lutts *et al*., 1996) described method was followed to determine electrolyte leakage (EL). Young leaves of samples were washed and placed in close vital containing 10ml of deionized water. The vital was incubated at 25°C for 24h followed with determining electrical conductivity of the solution (C_1); subsequently, samples were autoclaved at 120°C for 20min and the last electrical conductivity (C_2) measured. The electrolyte leakage was defined as follows: electrolyte leakage (%) = $(C_1/C_2) \times 100$.

Total phenolic content

The Singleton method (Singleton and Rossi, 1965) was followed to measure total phenolic content. 100mg of samples were extracted using 10 ml methanol-ethanol and diluted by 1 to 100 ratio. 300µl of the diluted extract mixed with 1.2ml of 7.5% sodium carbonate and 1.5ml of 10% folin ciocalteu, and then samples were located in the darkness for 30min, followed by measuring samples light absorbance at 765nm.

Enzyme activities

0.5g of fresh leaf tissue was homogenized in 5ml of phosphate buffer (50mM) with pH of 7, containing 1% polyvinylpyrrolidon. All stage of extraction was performed under 0-4°C. The homogenate was centrifuged at 5000 rpm for 20min. The supernatant obtained was used for assaying enzyme activates and its protein content. Catalase (CAT) was measured using the method described by Chance and Maehly (Chance and Maehly, 1955), based on the initial rate of disappearance of hydrogen peroxide.

The reaction mixture comprised of 50mM phosphate buffer, pH 7.0, 15mM hydrogen peroxide, and 100µl enzyme extract. The decline in hydrogen peroxide absorption was measured at wave length of 240nm.

The reaction mixture for assaying ascorbate peroxidase (APX) was 50 mM phosphate buffer, pH 7.0, 0.5mM ascorbate, 0.1mM hydrogen peroxide, and 150µl enzyme extract. The decline in absorption was measured at wave length of 290 nm (Nakano and Asada, 1981).

The method of Plewa *et al* (Plewa *et al*., 1991) was followed to assay guaiacol peroxidase (GPX). 3ml of the reaction mixture containing 2.77ml 50mM potassium phosphate buffer, pH 7.0, 100µl hydrogen peroxide 1%, 100µl guaiacol 2%, and 30µl enzyme mixture. The increase in absorption was measured at wave length of 470nm.

Antioxidant activity

The antioxidant activity of leaves extraction was measured according to method described by Moon and Terao with some modifications includes adding 2,2-Diphenyl-1-picryl-hydrazul (DPPH) to inactivate free radicals (Moon and Terao, 1998). 2g of fresh leaf was homogenized in 5mL of buffer phosphate 50mM and centrifuged at 4°C, 13000rpm, and 15min to collect sample extraction. 100µL of sample extraction was mixed with Tris-HCL buffer (100mM, pH=7.4), subsequently, 1mL of DPPH 500mM added into the mixture. After 30 min, the light absorbance was measured at 517nm. This measurement was repeated for mixture without DPPH and this measure subtracted from the measurement of the mixture with DPPH. Finally, antioxidant activities for samples were calculated using following equation:

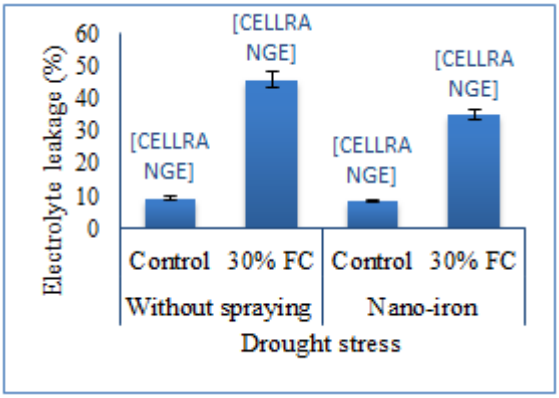
$AA= 1-A_{517}/ A_{517}\times100$

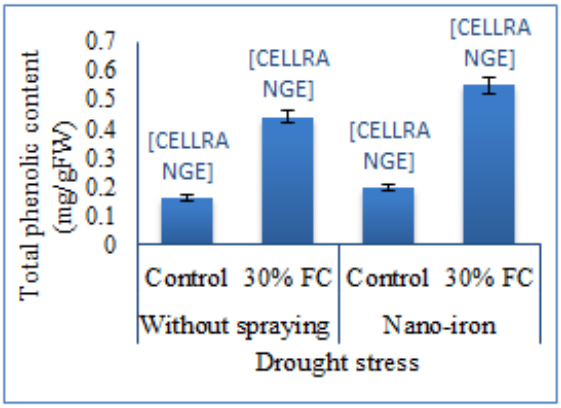
Statistical analysis

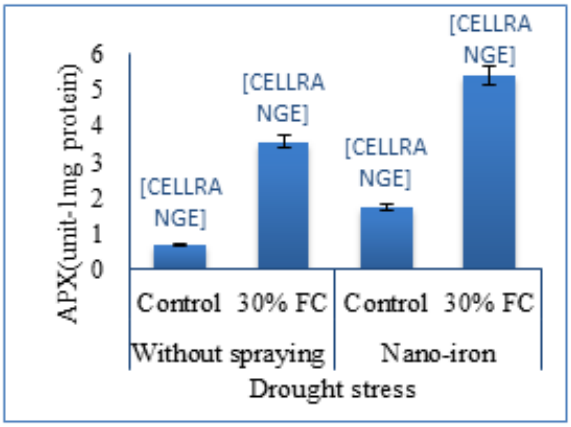
After normalization of data, statistical analysis was performed using JMP 8 (statistical software) and for comparison of means, LSD considered 5% (*p*-value=0.05).

Results

The results of statistical analysis indicated that effects of drought stress and nano-iron foliar application were significant on total phenolic content, antioxidant enzyme activities, and antioxidant activities of the extraction (*p*-value <0.01). Mean comparison showed that plants treated and untreated with nano-iron had not significant difference in electrolyte leakage in the absence of drought stress, while plants treated with nano-iron showed lower electrolyte leakage compared to untreated plants under 30% FC (Fig. 1). Under control and 30% FC, there was statistically significant difference between plants without spraying of nano-iron and plants sprayed with nano-iron (LSD<0.05). Antioxidant enzyme activities (APX, CAT, and GPX) had the highest value under 30% FC and the lowest values observed in plants grown under control conditions (Fig.1). Total phenolic content and antioxidant activities of extraction were high under 30% FC, while there was not significant difference (LSD<0.05) between plants treated and untreated with nano-iron under control conditions. Under control and 30% FC, the activity of antioxidant enzymes was high in plants treated with nano-iron compared to plants without the spraying (Fig.1).







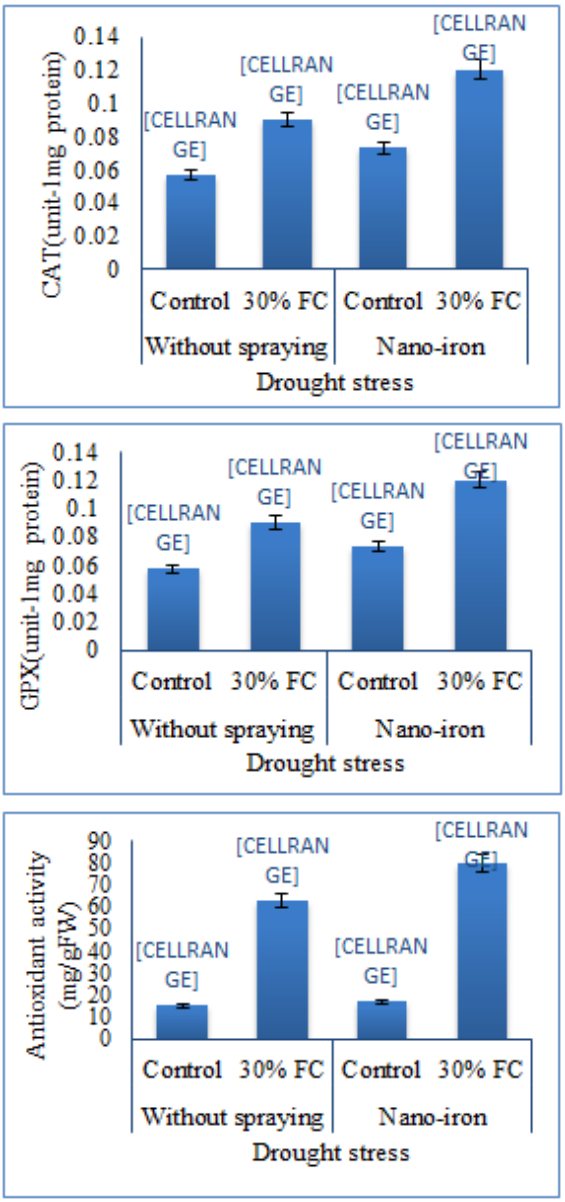


Fig. 1. The effect of drought stress (0 and 30% FC) and spraying of nano-iron on electrolyte leakage, total phenolic content, antioxidant enzyme activities, and antioxidant activities. Data represents the mean comparison with three replicates. Vertical bars indicate ± S.E. APX; Ascorbate peroxidase, CAT; Catalase, GPX; Guaiacol peroxidase.

Discussion

Drought stress is one of the major abiotic stresses that will be crucial constrain in the way of agriculture especially in the future (Farooq *et al.*, 2012). ROS production is a common phenomenon under drought stress in plants that could be result in serious damage to the cell and even the plant death.

Plants, however, have evolved defense mechanisms to cope with ROS production (Uzilday *et al.*, 2012). The ability of plants own mechanisms to encounter with harmful effects of ROS production is sufficient only for surviving in many species and their yield remarkably decrease under such conditions. In other hand, some cultivars of crops are natively sensitive to drought stress, but their cultivation is essential because they have a high quality and quantity products. Therefore, reaching approaches that help plants to tolerate deleterious effects of drought stress could be crucial. Here, we attempted to study effects of nano-iron application on canola cultivar, Sarigol, under drought stress at the field. Conducting the experiment on the field could lead to more practicable results, although other factor effects are likely attributed to observations. The field conditions, however, is where in which methods to ameliorate abiotic stresses must be practicable on plants and give effective results.

Electrolyte leakage shows membrane damage of plants under different kind of stresses (McCollum and McDonald, 1991). The studies have represented that under abiotic stresses such as drought, salinity, and chilling tolerant plants show a low membrane damage, however, sensitive plants show a high membrane leakage (Fan and Sokorai, 2005; Peever and Higgins, 1989). Our results indicated plants treated with nano-iron had lower electrolyte leakage (Fig. 1). This indicates nano-iron application protecting integrity of cell membrane. This could be due to high antioxidant activity of plants treated with nano-iron under drought stress.

CAT, APX, and GPX are three major antioxidant enzymes involved in converting H_2O_2 to water and molecular oxygen (Willekens *et al.*, 1995), using ascorbate to convert H_2O_2 to water (Noctor and Foyer, 1998). All the plants treated with nano-iron showed high activity for these enzymes (Fig. 1). Enhancement of antioxidant enzyme activities is an important sign of tolerance to abiotic stress (Bor *et al.*, 2003; Mittova *et al.*, 2002). Antioxidant enzymes with converting toxic and reactive oxygen species to non-harmful compounds remarkably protect peroxidation of the cell membrane (Gill and Tuteja, 2010).

The high level of these enzyme activities in plants treated with nano-iron could attribute to a low electrolyte leakage as mentioned above (Fig. 1).

Antioxidant activity estimates the free radical scavenging activities of a plant extraction (Re *et al.*, 1999). Our results showed that plants treated with nano-iron had the highest antioxidant activity compared to untreated plants under drought stress (Fig. 1). The highest antioxidant activities have been reported for tolerant plants, suggesting more ability for plants with high antioxidant activity to counter with reactive oxygen species such as free radicals, singlet oxygen, triplet oxygen and peroxides (Gill and Tuteja, 2010; Sairam and Srivastava, 2002; Sairam *et al.*, 2005). High level of reactive oxygen species is a common observation under abiotic stresses (Mittler *et al.*, 2004). The high antioxidant activities of extraction of Sarigol could also attribute to the high level of total phenolic content.

Our results indicated that total phenolic content of plants treated with nano-iron was significantly more than those untreated plants under 30% FC conditions (Fig.1). The phenolic compounds because of their phenol group and free hydroxyl group are able to scavenger reactive oxygen species produced under biotic and abiotic stress, consequently, protecting the cell structure from the damages of free radical species (Al-Amier and Craker, 2007).

Conclusion

Nano-iron foliar application on canola, Sarigol, under drought stress and under the field conditions indicated that nano-iron as one of the important micronutrient in high probability provides co-factor needed for many enzymes including antioxidant enzyme and other enzymes that may be indirectly in association with antioxidant enzymes. By this way, it could decrease electrolyte leakage and increased antioxidant enzyme activities and total phenolic content. It could be suggested that nano-iron application is effective approach to reduce harmful effects of drought stress on canola. However, application of nano-iron in high-scale maybe expensive for farmers and there is need to advance in technologies that provide nano-scale micronutrient in a lower price.

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