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Role of exogenous nitrogen supply in alleviating the deficit irrigation stress in wheat plants

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ABSTRACT

Efficient nitrogen (N) nutrition has the capability to assuage water stress in crops by via sustaining the metabolic activities even at a low tissue water potential. The study aimed to evaluate the role of N-supply in improving the drought tolerance in wheat plants under a deficit irrigation (DI) condition. Two-pot experiments were conducted during the two successive seasons of 2015 and 2016; N-fertilizer (0.3 and 0.6 g N/kg soil) was added for plants under full crop water requirement (100% of ETc) or deficit irrigation (60% of ETc). The effect of N - supply on the growth, yield characteristics and water use efficiency (WUE), stem anatomy, physio-biochemical attributes, and antioxidant enzymes activities (SOD and CAT) of wheat plants exposed to DI stress was assessed. Results approved that the nitrogen-treated plants exposed to DI had higher growth and yield characteristics compared to the untreated plants. The grain yield, WUE, photosynthetic pigments, soluble carbohydrates, soluble proteins, total soluble phenols and free proline, relative water content (RWC%), and antioxidant enzymes activities as well as a positive changes in the stem anatomy and had lower relative membrane permeability (RMP) compared to nitrogen-untreated plants were significantly improved as the result of N-supplying. Application of $N_{0.6} + I_{60}$ treatment was more effective in alleviating the damages of drought stress in wheat plants by maintaining higher RWC, WUE, and osmoprotectants, antioxidant system and lower RMP.

1. Introduction

Cereal crops are a major staple food worldwide, which contribute more than 50% of total human calorie input directly. Among them, wheat (*Triticum aestivum* L.) occupies the prominent position as source of dietary protein and calories forever burgeoning population of the world (FAO, 2009).

Due to the global climate changes, the agricultural sector began in recent years to overcome the problem of water deficiency in recent years (World Bank, 2006). To manage the limited amount of available water for agriculture successfully, the agricultural practices and understanding of water productivity should be improved to increase WUE (Jones, 2004) to increase WUE. A combined practice of deficit irrigation (DI) and supplying the crops with N-fertilizer.

According to previous works, irrigating plants under the maximum water requirements for crops became a successful method for saving water (Pereira et al., 2002; Abd El-Mageed et al., 2017). To increase WUE by DI strategy plants are subjected to drought by decreasing the volume of the used water or by decreasing the number of irrigation, either throughout a specific stage specific stage or during the whole growing season (Fereres and Soriano, 2007; Abd El-Mageed et al., 2017). Effects of DI on growth and productivity of many crops have been intensively investigated (Fereres and Soriano, 2007; Agami, 2013a,b and Agami et al., 2017). It has been shown that DI is a good strategy to increase water use efficiency for many crops without causing a severe reduction in yield (Geerts and Raes, 2009; Ballester et al., 2014). However, it is well known that water deficit stress increases the production of reactive oxygen species (ROS) in cellular organelles such as chloroplasts, peroxisomes and mitochondria. These ROS cause negative effects in the metabolic processes and growth (Batra et al., 2014). Huge cellular deterioration and death will occur if water deficit stress is prolonged because ROS production will defeat the scavenging action of

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the antioxidant system (Cruz de Carvalho, 2008). Drought stress severely influenced plant water status by reducing the water potential and the relative water content (RWC) in wheat (Siddique et al., 2001). Drought stress can increase organic compounds required for the osmotic adjustments, such as soluble sugars and proline (Garg et al., 2001; Molinari et al., 2007). When adopting deficit irrigation strategies, water deficit stress could have some negative effects on growth and productivity as reported by Cui et al. (2015) however, supplying the stressed plants with nitrogen fertilizers will help the crops to tolerate water deficit stress.

Nitrogen is a vital structural component of proteins, rubisco, nucleic acids, chlorophylls as well as some hormones and N fertilization is an essential agronomic management practice to enhance the crop productivity (Ata-Ul-Karim et al., 2016). Functional activity of the photosynthetic apparatus of leaf depends on the availability of N in plants (Brennan, 1992). In addition, efficient N-nutrition has been reported to have the potential to alleviate the drought stress damages by maintaining the metabolic activities even at low tissue water potential (Zhang et al., 2007). An appropriate N - supply could stimulate plant growth, improve water-use efficiency (WUE), and alleviate the effects of drought stress (Wu et al., 2008; Brueck et al., 2010). Under drought conditions adequate N - supply could enhance plant drought tolerance (Chapin, 1991; Arora et al., 2001). This study hypothesized that exogenous application of N - fertilizer may positively affect on the wheat performance, WUE, chlorophyll concentration, osmoprotectants, antioxidative enzymes activities, and tissue health as well as stem anatomy. Therefore, the experiments were designed to study the response of wheat plants grown under water deficit stress conditions to the exogenous supply of N - fertilizer.

2. Methodology

2.1. Plant culturing and growth conditions

Two-pot experiments were conducted during the two successive seasons of 2015 and 2016, at a special Farm located in El-Fayoum region, Egypt between latitudes 29° 02′and 29° 35′ N and longitudes 30° 23′and 31° 0′5E. Two-irrigation treatments were carried out: well-watering (100% of crop evapotranspiration) and deficit irrigation (60% of crop evapotranspiration), with three N-application rates (0.0, 0.3 and 0.6 g N/kg soil, designated as $N_{0.0}$, $N_{0.3}$ and $N_{0.6}$, respectively). Thus, six treatments, i.e. $N_{0.0} + I_{100}$, $N_{0.3} + I_{100}$, $N_{0.6} + I_{100}$, $N_{0.0} + I_{60}$, $N_{0.3} + I_{60}$ and $N_{0.6} + I_{60}$ were included in this study. Before sowing, the wheat seeds (cv. Giza-168) were surface-sterilized with 0.5% hypochlorite solution for 2 min. Twenty surface-sterilized uniform seeds were planted in plastic pots having 25-cm and 35- cm height and diameter, respectively. The pots were filled with 12 kg air-dried and sieved (0.5 mm) clay soil having 14% soil moisture, taken from the cultivated field. The soil had the following physicochemical characteristics: sand, 2.7%; silt, 28.8%; clay, 68.5%; pH, 7.23 (1:2, w/v, soil and water solution); ECe, 3.39 dS m^{-1}(1:2, w/v, soil and water solution); $CaCO_3$ 10.61% and organic matter 2.59%; total nitrogen, 37.6 (mg/kg dry soil); available phosphorus, 31.8 (mg/kg dry soil); extractable potassium, 8.33 (mg/kg dry soil); iron, 3.20 (mg/kg dry soil); manganese, 9.8 (mg/kg dry soil); zinc, 0.80 (mg/kg dry soil); copper, 0.60 (mg/kg dry soil)). Soil physiochemical analysis was conducted according to Klute (1986) and Page et al. (1982). At the time of soil filling, 0.8 g P_2O_5 and 1.8 g K_2O were applied per pot for each treatment. The first experiment was conducted on 13 November 2015 and the second one was conducted on 17 November 2016 in an open greenhouse. Table 1 shows the meteorological data of El-Fayoum during the period of the experiment. Pots were arranged in the greenhouse in a complete randomized block design with four replications for each treatment. Thinning was carried out at the third leaf stage, and seven uniform seedlings per pot were selected for the subsequent studies. To ensure the regular emergence and growth of wheat, there was no water deficit during the seedling stage until tillering stage, after which the deficit treatment was implemented. At the stage of tillering, the plants were either well-watered to maintain 100% crop evapotranspiration (ETc) or drought-stressed by reducing the amount of water added to the plants to reach 60% of the crop evapotranspiration

2.2. Drought stress and nitrogen treatments

2.2.1. Application and management

The experiment consisted of two irrigation treatments, namely, well-watering i.e. 100% of evapotranspiration (ETc) and deficit irrigation (DI), in which the irrigation amounts were 60% of evapotranspiration, and with three N-application rates (0.0, 0.3 and 0.6 g N/kg soil, designated as $N_{0.0}$, $N_{0.3}$ and $N_{0.6}$ respectively). For N-treatments 50% N of each level was distributed at the time of sowing, 30% at jointing and 20% at booting stages, respectively to each pot. Drought stress treatments to each N level were applied on separate pots starting from the stage of tillering to the watery ripe stage.

2.3. Irrigation water applied (IWA)

Crop water requirements (ETc) were estimatedusing the crop coefficient according to Allen et al. (1998), as follows:

$$ETc = ETo \times Kc$$

Where ETc is the crop water requirement (mm day^{-1}), ETo is the reference evapotranspiration (mm day^{-1}), and Kc is the crop coefficient.

The daily ETo was computed using the pan equation as follows:

$$ET_o = E_{pan} \times K_{pan}$$

Where:

ETo = Reference evapotranspiration (mm day^{-1}),

E_{pan} = Evaporation from the Class A pan (mm day^{-1}), and

K_{pan} = Pan coefficient (FAO pp. No. 24).

Wheat plants were irrigated 2 days intervals by different amounts of irrigation water applied. The amount of irrigation water applied (IWA) for each treatment during the irrigation regime was determined by using the following eqution:

$$IWA = \frac{A \times ETc \times Ii}{Ea \times 1000}$$

Where IWA is the irrigation water applied (cm^3), A is the pot area (cm^2), ETc is the crop water requirements (mm day^{-1}), Ii is the irrigation intervals (day), and Ea is the application efficiency (%). Measuring cylinders were used for measuring the irrigation water volumes manually

2.4. Measurements of growth, yield characteristics and water use efficiency (WUE)

Samples of wheat plants (36 per plants per treatment) were collected after 90 days from sowing to assess morphological characteristics. Length of shoots and spikes (cm) was measured using a meter scale. Numbers of fertile tillers per plant and numbers of spikelets per spike were counted. Flag leaf area (cm^2) was measured using a digital leaf meter (LI-3000 Portable Area meter Produced by LI-COR Lincoln, NE, USA). Samples of flag leaves were collected to estimate the concentration of total chlorophylls and total carotenoids, free proline, total soluble protein, total soluble carbohydrates and total soluble phenols, relative membrane permeability, relative water content and activities of antioxidant enzymes. The experiment was terminated when the plants were 130-days-old, then plants were collected, and the various measurements and analysis were carried out terminated after 130 days after sowing. Wheat plants were removed from the pots and moved smoothly to remove the adhering soil particles by dipped them in a bucket filled