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Research paper

Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation

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ABSTRACT

Natural plant growth biostimulants are intensively used nowadays for plant growing in normal and adverse conditions. Severely affected by salt and drought stresses, squash (*Cucurbita pepo* L.) is an important vegetable crop that highly ranked in economic importance worldwide. The current study aimed to evaluate whether leaf extract of Moringa *oleifera* (MLE), as a novel natural biostimulant for plant growth, could play a role in improving drought tolerance in squash plants under saline condition. In summer and fall seasons of 2016, MLE (3%) was foliar sprayed for plants under full (100% of ETc) or deficit irrigation (DI; 80 or 60% of ETc). The effect of MLE on the growth, yield characteristics and water use efficiency (WUE), physio-biochemical attributes, and leaf anatomy of squash plants exposed to DI stress was assessed. MLE-treated plants exposed to DI had higher growth and yield characteristics, harvest index (HI),WUE, chlorophyll fluorescence (F_v/F_m and PI), photosynthetic pigments, soluble sugars and free proline, leaf anatomy, relative water content (RWC%) and membrane stability index (MSI%) and had lower electrolyte leakage (EL%) compared to MLE-untreated plants. Application of 3% MLE was effective in alleviating damages of drought stresses in squash plants by maintaining higher RWC, WUE, and osmoprotectants, and lower EL.

1. Introduction

Squash (*Cucurbita pepo* L.) classified as one of the most important vegetable crops and it is highly ranked in economic importance worldwide. Egypt, Italy and Turkey found to produce 1/3 of squash world production (Paris, 1996).

Due to the global climate changes, agricultural sector began in recent years to overcome the problem of water scarcity in recent years (World Bank, 2006). To successfully manage a limited amount of available water for agriculture, agricultural practices and understandings of water productivity should be improved (Howell, 2001; Jones, 2004) to increase WUE, especially at a field scale. interactive applications of DI and spraying crops with natural biostimulants, including MLE, used as foliar sprays or seed soaking, appeared to be very promising.

According to previous works, irrigating plants under the maximum water requirements for crops became a successful method for saving irrigation water (Pereira et al., 2002; Abd El-Mageed et al., 2016; Abd El-Mageed et al., 2017). To increase WUE by DI strategy, plants are subjected to drought by decreasing the volume of water used or by decreasing the number of irrigation, either throughout a specific stage or during the whole growing season (Fereres and Soriano, 2007; Abd El-Mageed et al., 2017). Effects of drought on performances of crops (field and/or vegetable) have been widely investigated (Kirda, 2002; Abd El-Mageed and Semida 2015a,b; Abd El-Mageed et al., 2016). Where the impacts of drought stress are crop-specific, it is requisite to assess the potential impacts of DI strategy with many years open field experiment prior popularizing a most suitable scheduling of irrigation system to be adapted to a specific site for tested crop (Igbadun et al., 2008; Abd El-Mageed et al., 2016). It has been shown in early investigations that DI is a good strategy to increase water use efficiency for many crops without causing a significant reduction in yield (Geerts and Raes, 2009; Ballester et al., 2014). However, it is undoubted that the activity of reactive oxygen species (ROS) under moisture stress increases in cellular organelles such as peroxisomes, chloroplasts, and mitochondria. These ROS cause defects in different metabolic processes (Batra et al., 2014), adversely affecting performances of crop plants. Large cellular deterioration and death will occur if water stress is prolonged because ROS production will defeat the scavenging

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action of the antioxidant system (Cruz de Carvalho, 2008). When adopting DI strategy, water scarcity applied could have some deleterious effects on growth attributes and productivity of summer squash (El-Dewiny, 2011), and apply the stressed crops with a natural biostimulant such as MLE will help crops to overcome the negative effects of water scarcity stress.

Biostimulants are plant extracts, which contain a wide number of bioactive compounds. These compounds are able to improve various physiological processes that stimulate plant growth and development and increase nutrient use efficiency, reducing chemical fertilizers without adverse effects on yields and their qualities (Bulgari et al., 2015). *Moringa oleifera* (Lam.) is classified as one of the most current biostimulants (leaves of the trees), Moringa is well known vegetable in Arabia, Africa, India, America (Yasmeen et al., 2013). Its leaves and flowers are used as vegetables (Siddhuraju and Becker, 2003). Moringa leaves extract (MLE) contain powerful natural antioxidants, which can be used by crop producers for crop plants to improve growth and yield attributes of various crops, and to overcome environmental stresses (Howladar, 2014; Elzaawely et al., 2017; Njoku and Adikwu, 1997). Its leaves have high nutritional and medicinal values, where they are rich in essential mineral nutrients, fibers, proteins, sugars, free proline, free amino acids and vitamins (El Sohaimy et al., 2015; Rady and Mohamed, 2015). In addition, leaves are also rich in phytohormones, including cytokinin 'zeatin', auxins and gibberellins as well as many antioxidants (Howladar, 2014). Earlier reports have shown MLE potentiality in improving crop resistance to salinity (Rady et al., 2015) and heavy metal (Howladar, 2014) stresses, nonetheless alleviation of drought stress and improving WUE under DI strategy by moringa extracts has rarely been reported. This study hypothesized that exogenous application of MLE may positively affect the squash performance, WUE, chlorophyll fluorescence, osmoprotectants, tissue health and leaf anatomy. Therefore, our experiment was designed to study the response of squash grown under the saline soil (EC = 9.1 dS m^{-1}) to foliar application of MLE under DI stress conditions.

2. Methodology

2.1. Experimental location, and irrigation water applied (IWA)

Two-growing season (summer; SS and fall; FS) experiments were conducted on 2016, at a special Farm located in Fayoum region, Egypt between latitudes 29° 02′ and 29° 35′ N and longitudes 30° 23′ and 31° 05′ E. According to the aridity index, the experimental soil is fallen under arid conditions (Ponce et al., 2000). The experimental soil which the two experiments were conducted had the top soil (0–90 cm depth) and classified as salt-affected soil (ECe = 9.1 dS m^{-1}, SAR = 12.1, and pH = 7.75 Table 3), the texture was loamy sand, with an average bulk density of 1.57 g cm^{-3}. Total available water (%) was averaged 11.98%/60 cm depth (Table 2) and the soil fertility was (organic matter content 1.1% and total N 0.05%). Soil physio- chemical analysis were conducted according to Klute (1986) and Page et al. (1982).

Every 2-days plants were irrigated by different volumes of irrigation water applied (IWA). IWA was estimated as a percentage of the crop evapotranspiration (ETc) representing the following three treatments: I_{100} = 100%, I_{80} = 80% and I_{60} = 60% of ETc. Daily ETo and ETc were estimated according to Allen et al. (1998) equations.

2.2. *Moringa oleifera* leaf extract (MLE) preparation, treatments, and analysis

Fresh leaves of moringa trees were harvested and extracted at the fully matured stage. For extraction, collected leaves were overnight frozen and pressed in a locally fabricated machine and purified twice by filtering through filter paper (Whatman No. 1). After refining process, the extract was centrifuged at 8000 × *g* for 15 min for supernant that diluted to obtain extraction at a 3% concentration to spray the plant foliage (Yasmeen et al., 2012). The MLE was analyzed and its chemical composition is presented in Table 5. The MLE was used as a foliar spray twice; 20 and 35 days after planting (DAS). Squash plants were sprayed with MLE to run-off using 0.1% (v/v) Tween-20 that added to sprays as a surfactant to ensure optimal permeation into leaves of squash.

2.3. Experimental design and plant management

Each of the two experiments was conducted in a randomized complete block design (Split Plot). 3 irrigation treatments were applied (100, 80 and 60% of ETc that were occupied as main plots) and two MLE treatments (i.e., tap water and 3% MLE that were allocated to sub-plots). The 6 treatments were replicated three times, making a total of 18 plots. The area of the experimental plot was 12 m length × 1.10 m row width (13.2 m^2) and the spacing between plants was 0.5 m within rows.

Squash seeds (hybrid Hi Tech®) were planted 0.5 m apart in each bed at a depth of 0.04 m drip irrigation system with one line was used and one dripper with discharge 4.0 L h^{-1}/plant. The EC of irrigation water was 2.1 dS m^{-1} (Table 4). Seeds of squash were sown on 10 May and 20 September and harvested on 4 August and 17 December in the growing seasons of 2016 (SS and FS), respectively. All treatments were separated by 1.5 m of non-irrigated area. Plants were irrigated well in first irrigation.

One week after full germination irrigation treatments application were started. Mineral fertilization was applied at the typical recommended does for squash production in such region; (150–60–70 kg ha^{-1}) N, P_2O_5 and K_2O, respectively. Pest management, disease, and cultural practices were performed as the instructions of local commercial crop production.

2.4. Measurements of growth and yield characteristics, water use efficiency (WUE) and physio-biochemical attributes

From each experimental plot, nine plants at the end of (SS and FS) seasons were randomly chosen and assessed for growth characteristics. Number of leaves/$plant^{-1}$ was counted, using digital planimeter (Planix 7) total leaf area/$plant^{-1}$ was measured and shoot dry weight $plant^{-1}$ was recorded after oven-drying at 70 °C until a constant weight. HI was determined as a ratio of the yield of fruits divided by the total biomass production on a dry mass basis. WUE values were calculated as kg fruits yield m^{-3} of irrigation water applied for each irrigation treatment using of Jensen, (1983 formulla

$$\text{WUE} = \frac{\text{fruityield}\left(\text{Kgha}^{-1}\right)}{\text{waterapplied}\left(\text{m}^3\text{ha}^{-1}\right)}$$

According to Hayat et al. (2007) the following equation was used to calculate RWC%.

$$\text{RWC}(\%) = \left[\frac{(\text{FM} - \text{DM})}{(\text{TM} - \text{DM})}\right] \times 100$$

where: RWC% is relative water content (%), FM: fresh mass (g), TM: turgid mass (g) and DM is the dry mass (g)

MSI% was determined using the method of Premchandra et al. (1990) and the following formula was used.

$$\text{MSI}(\%) = \left[1 - \left(\frac{\text{C1}}{\text{C2}}\right)\right] \times 100$$

where: MSI% is the membrane stability index, C_1: is the EC of the solution at 40 °C and C_2: is the EC of the solution at100 °C