



# Combined effect of foliar-applied salicylic acid and deficit irrigation on physiological–anatomical responses, and yield of squash plants under saline soil



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## ABSTRACT

Salicylic acid (SA) controls plant growth and induces water deficit tolerance in plants. Summer and fall season experiments were conducted in 2013 to study the effect of 1 mM SA on growth, anatomy, yield, chlorophyll fluorescence, osmoprotectants and water use efficiency (WUE) of squash plants under three levels of irrigation ( $I_{100} = 100\%$ ,  $I_{80} = 80\%$  and  $I_{60} = 60\%$  of crop evapotranspiration). Growth, chlorophyll fluorescence, leaf anatomy, leaf photosynthetic pigments, total soluble sugars (TSS), proline, harvest index (HI), yield and WUE were significantly affected by both deficit irrigation (DI) and the combined DI + exogenously applied SA treatment. The combined DI + SA treatment modified the adverse effects of DI and enhanced all aforesaid parameters, while decreased proline concentrations. The combined treatment of  $I_{60}$  or  $I_{80}$  + SA produced plants having yields and WUE as produced with the plants generated under full irrigation conditions without SA treatment. From these results, we conclude that with exogenously applied 1 mM SA, the  $I_{60}$  or  $I_{80}$  strategy studied here could be successfully applied during summer and fall seasons for the production of commercial squash allowing water savings of 20%–40% without any detrimental effect on plant growth or yield.

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## 1. Introduction

As one of the most important vegetable crops, squash (*Cucurbita pepo* L.) is grown in many temperate and sub-tropical regions, and is highly ranked in economic importance worldwide. One-third of the world's production of squash is from Turkey, Italy and Egypt (Paris, 1996). In recent years, the agricultural sector began to face a problem of water shortage across the globe. The rapid population growth and greater incidence of drought caused by climate change and different human activities have caused this problem (World Bank, 2006). Better agricultural practices and enhanced understandings of water productivity are some of the means that could lead to the successful management of a limited amount of water available for agricultural uses (Howell, 2001; Jones, 2004). Among the water management practices for increasing water use efficiency (WUE), particularly at field scale, combined practices of deficit irrigation (DI) and providing crop plants with some antioxidants, including the use of salicylic acid for seed soaking and/or as foliar sprays, appears to be very promising.

Irrigation with water below the optimum crop requirements is a strategy for water-saving by which crops are subjected to a certain

level of water stress either during a particular period or throughout the whole growing season (Pereira et al., 2002). The increase in the WUE by reducing the amount of water applied and/or by reducing the number of irrigation events is the main objective of applying the DI strategy (Kirda, 2002). Effects of DI on growth and productivity of many vegetables and field crops have been intensively investigated (Feres and Soriano, 2007; Abd El-Mageed and Semida 2015b). Where the effects of DI are crop-specific, it is necessary to evaluate the impact of DI strategies with multi-year open field experiments before generalizing the most appropriate irrigation scheduling method to be adapted in a specific location for a given crop (Scholberg et al., 2000; Igbadun et al., 2008; Abd El-Mageed and Semida 2015a). It has been shown that DI is successful in increasing water productivity for different crops without causing severe yield reduction (Geerts and Raes, 2009).

Undoubtedly, water deficit (WD) is one of the most common and self-evident observations. It is well known that WD stress increases the production of reactive oxygen species (ROS; superoxide,  $H_2O_2$ , singlet oxygen and hydroxyl radical) in cellular organelles such as chloroplasts, peroxisomes and mitochondria, negatively affecting various processes such as transpiration, photosynthesis, stomatal conductance and growth (Alscher et al., 1997; Batra et al., 2014). Extensive cellular damage and death will occur if WD stress is prolonged because ROS production will defeat the scavenging action of the antioxidant system

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(Cruz de Carvalho, 2008). One of the physiological processes that occur when plant is subjected to WD stress is the stomatal closure in order to avoid water losses through transpiration (Ache et al., 2010). When adopting deficit irrigation strategies, WD stress applied could have some negative impacts on growth and productivity as reported by El-Dewiny (2011) on summer squash, and applying the stressed plants with some antioxidants such as salicylic acid will help crop plants to tolerate WD stress.

Salicylic acid (SA) is classified as a phenolic growth regulator, known as an antioxidant compound that can regulate plant growth, prohibiting the activity of ROS (Amanullah et al., 2010; Hayat et al., 2010). It is also recognized as a signal molecule, and has been intensively investigated for its role in plant adaptation to the adverse effects that occur in growing environments. It is found to improve plant tolerance to various environmental stresses (Manaa et al., 2014; Semida et al., 2015), including water stress (Singh and Usha, 2003; Hesami et al., 2013). It is well known that SA ameliorates the impairments that arise from WD in plants (Hussain et al., 2009). It is involved in different processes in plants such as stomatal conductance, membrane permeability, plant water relations and nutrient uptake and transport (Gunes et al., 2005; Hayat and Ahmad, 2007; Hayat et al., 2010). The effect of SA on plant physiological processes varies depending on species, developmental stage, SA concentration and environmental conditions (Shraiy and Hegazi, 2009). Water use efficiency, rate of transpiration and internal CO<sub>2</sub> increased after supplementation with SA (Kumar et al., 2000). Different studies showed that plant growth, productivity and biochemical attributes in various crops have been improved by SA application under water deficit stress (Khan et al., 2003; Shirani Bidabadi et al., 2012; Hesami et al., 2013).

The current study aims to assess the efficacy of foliar-applied SA under deficit irrigation strategies on water use efficiency, growth and productivity of squash as a model plant in different growing seasons.

## 2. Materials and methods

### 2.1. Experimental site

Two experiments were conducted in both growing summer season (SS) and growing fall season (FS) of 2013, at farmer's field located in El-Fayoum province, which occupies a depression west of the Nile at 90 km southwest of Cairo, Egypt between latitudes 29°02' and 29°35'N and longitudes 30°23' and 31°05'E. Supplementary Table 1 shows the climatic data of El-Fayoum during the months of the study. According to the aridity index (Ponce et al., 2000), the area is located under a hyper-arid climate. Some initial physical and chemical properties of the irrigation water and the experimental site soil were analyzed according to Klute (1986) and Page et al. (1982) and are given in Supplementary Tables 2–4. According to Ayers and Wesctcot (1985), the scale used for the irrigation water lies within the second categories for salinity and sodicity levels ( $C_2S_1$ ,  $EC_{iw} = 0.75\text{--}3.00\text{ dS m}^{-1}$  and  $SAR < 6.0$ ).

### 2.2. Irrigation water applied (IWA)

The squash plants were irrigated at 2-day intervals with different amounts of irrigation water. IWA was specified as a percentage of the crop evapotranspiration (ETc) representing one of the following three treatments:  $I_{100} = 100\%$ ,  $I_{80} = 80\%$  and  $I_{60} = 60\%$  of ETc. The daily ETo was computed according to Eq. (1) (Allen et al., 1998) as follows:

$$ETo = \frac{0.408 \Delta (R_n - G) + \gamma \frac{900}{T_{\text{mean}} + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34 u_2)} \quad (1)$$

where ETo is the reference evapotranspiration ( $\text{mm day}^{-1}$ ),  $\Delta$  is the slope of the saturation vapor pressure curve at air temperature

( $\text{kPa } ^\circ\text{C}^{-1}$ ),  $R_n$  is the net radiation at the crop surface ( $\text{MJ m}^{-2} \text{d}^{-1}$ ),  $G$  is soil heat flux density ( $\text{MJ m}^{-2} \text{d}^{-1}$ ),  $\gamma$  is the psychrometric constant ( $= (0.665 \times 10^{-3} \times P) \text{ kPa } ^\circ\text{C}^{-1}$  (Allen et al., 1998),  $P$  is the atmospheric pressure (kPa),  $u_2$  is the wind speed at 2 m height ( $\text{m s}^{-1}$ ),  $e_s$  is the saturation vapor pressure (kPa),  $e_a$  is the actual vapor pressure (kPa),  $(e_s - e_a)$  is the saturation vapor pressure deficit (kPa), and  $T_{\text{mean}}$  is the mean daily air temperature at 2 m height ( $^\circ\text{C}$ ).

The average of daily ETo in El-Fayoum was 10.16, 10.74, 10.66, 9.90, 8.64, 6.61, 4.63 and 3.49  $\text{mm day}^{-1}$  in May, June, July, August, September, October, November and December, respectively. The crop water requirements (ETc) were estimated using the crop coefficient according to Eq. (2):

$$ETc = ETo \times Kc \quad (2)$$

where ETc is the crop water requirement ( $\text{mm day}^{-1}$ ) and Kc is the crop coefficient. The duration of the different crop growth stages were 25, 35, 25 and 15 days for initial, crop development, mid-season and late season stages, respectively, and the crop coefficients (Kc) of initial, mid and end stages were 0.60, 1.00 and 0.75, respectively, according to Allen et al. (1998).

The amount of IWA to each treatment during the irrigation regime was determined by using Eq. (3) as follows:

$$IWA = \frac{A \times ETc \times li \times Kr}{Ea \times 1000 \times (1 - LR)} \quad (3)$$

where IWA is the irrigation water applied ( $\text{m}^3$ ),  $A$  is the plot area ( $\text{m}^2$ ), ETc is the crop water requirements ( $\text{mm day}^{-1}$ ),  $li$  is the irrigation intervals (day),  $Ea$  is the application efficiency (%) ( $E_a = 85$ ),  $Kr$  is the covering factor, and to calculate ( $Kr$ ), the Decroix and Cecroix method was used (Eq. (4); Vermeiren and Jobling, 1980):

$$Kr = (0.10 + G_c) \leq 1 \quad (4)$$

where  $G_c$  is the ground cover. In addition,  $LR$  is the leaching requirements, which was estimated according to Eq. (5):

$$LR = \frac{EC_w}{2MaxEC_e} \quad (5)$$

where  $EC_w$  is the electrical conductivity of the irrigation water ( $\text{dS.m}^{-1}$ ) and  $MaxEC_e$  is the maximum electrical conductivity of the soil saturation extract for a given crop (see the table shown according to Doorenbos and Pruitt (1984) and Keller and Bliesner (1990).

### 2.3. Salicylic acid (SA) treatments

From a preliminary pot study (data not shown), 1 mM SA and three time applications (i.e., at 21 and 30 days after sowing) were selected. These concentration and application times generated the best growth of squash plants grown under 20% and 40% water depletion. For the main study, there were 6 treatments including SA that was applied in 3 treatments. These treatments were as follows:  $I_{100}$  (irrigation with 100% of ETc),  $I_{80}$  (irrigation with 80% of ETc),  $I_{60}$  (irrigation with 60% of ETc),  $I_{100} + 1\text{ mM SA}$ ,  $I_{80} + 1\text{ mM SA}$  and  $I_{60} + 1\text{ mM SA}$ .

### 2.4. Experimental design

The experiments were conducted in a complete randomized block design. In either summer or fall season, the 6 tested treatments were replicated three times for each, making a total of 18 plots. The experimental plot area was 13.2  $\text{m}^2$ ; 12 m length  $\times$  1.10 m row width, and 0.5 m spacing between plants within rows.

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