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Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confer tolerance to salinity stress

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ABSTRACT

Plants face a lot of abiotic stresses, during their life cycle, including salinity that greatly influences their growth and entire metabolism. Osmoprotectants have to enable plant to beat such stress. Two-season field experiments were conducted consecutively in 2015/16 and 2016/17 to study the effect of glycine betaine (GB) foliar application at three levels (0; a control, 25 and 50 mM) on onion growth, physio-biochemical attributes and antioxidant defense system activity was investigated under 4.80 dS m^{-1} salt stress. Under saline soil conditions, GB treatment significantly increased growth indices (e.g., shoot length, leaves area of $plant^{-1}$, shoot fresh and dry weights), bulb yields and water use efficiency (WUE), leaf chlorophylls contents and their efficiency, stomatal conductance and tissue health measured as relative water content and membrane stability index. Additionally, endogenous osmoprotectants (e.g., GB and choline) contents, non-enzymatic antioxidants (e.g., glutathione and ascorbic acid) contents and enzymatic antioxidants (e.g., catalase, superoxide dismutase, and ascorbate peroxidase) were significantly increased with exogenous GB application under salt stress. In contrast, glutathione reductase activity was reduced, while free proline and soluble sugars contents were not affected. A level of 50 mM GB was more effective to be the better treatment by which this study recommends to use for growing onion plants under moderate salt stress.

1. Introduction

Onion (*Allium cepa* L.) is an ancient valuable vegetable/field crop for people nutrition and medicine worldwide. As a vegetable crop, it ranks second after tomato production. In Egypt, the average annual production of onions in the last five years was 2,113,749 tons (FAO, 2015). Where bulbs yield seriously declines for every ECe unit increase in soil salinity, onions are rated as a salt sensitive crop (Shannon and Grieve, 1999).

Salinity is a considerable environmental problem causing a stress that presents a severe threat to crop plants in many world parts, particularly arid and semiarid (i.e., dry) regions. Salinity has a dual effect; besides malnutrition and accumulation of excess ions to potentially toxic levels in plants, it can cause drought stress. Tolerance to salinity can differ spectacularly among species/varieties/cultivars and stage of growth, reflecting usually in a reduction of biomass production, yield or rates of survival (Munns and Tester, 2008). Salinity causes disruptive influences in all growth stages and metabolic processes in plants beginning from a reduction in seed germination as the first critical and most sensitive process, a decrease of node formation, a retardation of plant development to a reduction in final crop yield (Munns and Tester, 2008).

Morphology and physiology of plants are seriously affected by salt stress through a physiological drought problem-induced osmotic stress causing defects in water relations and ion balance in plants that lead to ionic toxicity of all metabolic processes (Munns et al., 2006; Semida et al., 2016). This problem occurs due to an overproduction of reactive oxygen species (ROS; $O_2^{\cdot -}$, OH^-, H_2O_2 and O_1), which trigger an oxidative stress in plant tissues causing degradation of chlorophyll, peroxidation of membrane lipid in terms of accumulated malondialdehyde (MDA), and damages in lipids, proteins and DNA (Yasar et al., 2006). The accumulation of MDA considers as an oxidative stress indicator, which is a tested tool to assess salt tolerance in crop plants (Yildirim et al., 2008). In addition, elevated Na^+ and Cl– levels in soil

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and/or in irrigation water due to salinity reduces photosynthetic attributes, plant growth and development, and induces antioxidative defense systems activities (Abd El-Mageed et al., 2017; Rady et al., 2013; Sairam and Tyagi, 2004). To ameliorate these adverse effects-induced salt stress, plants adopt and promote several mechanisms to stimulate their tolerance and protect their cellular and sub-cellular systems from cytotoxic effects of overproduced ROS by induced activities both non-enzymatic and enzymatic antioxidative defense systems (Rady et al., 2017; Sairam and Srivastava, 2001; Semida et al., 2014b). Besides, ion homeostasis, osmotic adjustments, control and repair of other stress damages are also regulated (Zhu, 2002). Supporting of the endogenous mechanisms of plants, efforts have been originated to control the effects of salt stress by different means, including foliar applications with some osmoprotectants/antioxidants to plants, to adopt the sustainable agriculture in salt-damaged agricultural lands (Abd El-Mageed et al., 2016; Semida et al., 2017).

In response to stress, osmolytes such as proline, soluble sugars, polyols and amino acids, as well as glycine betaine (GB) are endogenously synthesized in excess amounts (Hoque et al., 2007) to adjust the osmotic stress-induced salinity. This osmotic adjustment maintains sub-cellular structures and reduces the oxidative damages caused by ROS under high salinities (Hare et al., 1998). GB endogenous biosynthesis is reported to be stress-inducible, and the level of biosynthesized and accumulated GB depends mainly on the degree of salt tolerance (Sakamoto and Murata, 2000). In this concern, plants unable to accumulate GB can tolerate salt stress by exogenous supplements of GB (Alasvandyari et al., 2017; Cha-Um and Kirdmanee, 2010; Hayashi et al., 1998; Kaya et al., 2013), because GB maintains osmotic regulation in cells of salt-stressed plants (Gadallah, 1999) and supports different transporters for normal functioning. Therefore, GB considers as a protective mean that discriminates Na^+ against K^+ under a saline condition (Mansour, 1998). GB has been reported to enhance root cell vacuolar efficiency to accumulate more Na^+ in salt-stressed plants (Rahman et al., 2002). It has been also reported that GB application improves growth of plants of many crops under stress conditions (Alasvandyari et al., 2017; Gadallah, 1999) and maintain higher antioxidant enzyme activities, which minimize the adverse effect of oxidative stresses in wheat (Ma et al., 2006). Exogenous application of GB increases enzymatic (e.g., catalase and superoxide dismutase) activities and K^+ content and reduces Na^+ and malondialdehyde (MDA) contents in salt-stressed plants (Alasvandyari et al., 2017; Hu et al., 2012).

The main objective of the study herein was to investigate the protective role of GB in onion plants grown on a saline soil through studying the effect of foliar spray of GB on growth and yield performances, water use efficiency, tissue health, osmoprotectant contents, antioxidant (enzymatic and non-enzymatic) defense system activities.

2. Material and methods

2.1. Experimental layout and growth conditions

In two seasons; 2015/16 (SI) and 2016/17 (SII), trials were conducted at the Experimental Farm of the Faculty of Agriculture, Fayoum University, (29°17′N; 30°53′E), Southeast Fayoum, Egypt. Tow field experiments were completely performed using the most common variety (Giza 20) of onion (*Allium cepa* L.) in Egypt for achieving the purpose of this study. Data of the experimental area climate indicate that total rainfalls are approx. 7.5 mm/year, and 14.5 and 31.0 °C are average annual minimum and maximum temperatures in January and June, respectively. Rates of evaporation are synchronizing with temperatures (i.e., lowest evaporation rate; 1.9 mm day^{-1} was recorded in January, and highest value; 7.3 mm day^{-1} was recorded in June). According to the aridity index (Ponce et al., 2000), the experimental Farm considers as hyper-arid climatic area. The landforms of area characterize by surface slopes of less than 3.5% with an elevation vary from 49 to 26 m below and above the sea level, respectively.

Healthy onion seeds were carefully selected and sown on 28 September of both 2015 and 2016. Transportation of transplants was done for replanting on 8 December and harvested on 8 May of both 2016 and 2017. The experiments were performed in a randomized complete blocks design in three replicates. The total experimental area specified for each experiment was 275 m^2 divided into 9 experimental plots of 16.5 m^2 for each (1.1 m wide × 15 m long). To protect against border effects, two guard rows were used to separate the experimental unites. Each experimental plot was contained 12 planting rows set 10 cm apart, and 15 cm was a distance between plants to achieve the typical practice of onion producers.

According to recommendations of the Ministry of Agriculture and Land Reclamation in Egypt, the tested soil was supplemented with 62 kg P_2O_5 ha^{-1} (400 kg calcium super-phosphate; 15.5% P_2O_5), 150 kg N ha^{-1} (450 kg ammonium nitrate; 33.5% N), and 72 kg K_2O ha^{-1} (150 kg potassium sulphate; 48% K_2O).

Onion plants were irrigated 15 days intervals using the surface irrigation method. According to the FAO-PM equation (Allen et al., 1998), the daily ETo was calculated from weather data in Fayoum region (Semida et al., 2016). Using one plastic pipe (spile) of 50 mm diameter for each plot, the applied amount of irrigation water was controlled and conveyed to cover the whole plot. The following equation of Israelsen and Hansen, (1962) was used to calculate the amount of water transferred through each plastic pipe.

$$Q = CA\sqrt{2gh} \times 10^{-3}$$

Where:

Q = discharge of irrigation water, (l. sec^{-1}),
C = coefficient of discharge,
A = cross section area of irrigation pipe, (cm^2),
g = gravity acceleration, (cm. sec^{-2}),
h = average effective head of water, (cm).

The all other recommended agricultural, disease and pest management practices were followed up with the recommendations of the Ministry of Agriculture and Land Reclamation.

2.2. Analyses of soil and experimental treatments

Soil analyses (i.e., physical and chemical properties) were done (Klute and Dirksen, 1986; Page et al., 1982) and data are shown in Table 1. Based on the soil EC_e value and classification of soil according to its salinity (Dahnke and Whitney, 1988), the tested soil includes under the class of moderately saline.

Glycine betaine (GB) was used to spray plant foliage at 3 levels (0; tap water as a control, 25 and 50 mM) 3 times 20 days intervals beginning from 20 days after transplanting to run-off. Based on a preliminary pot study (Table 2), levels of GB, and number and timing of sprays were accurately selected. As a surfactant, Tween-20 (0.1%, v/v) was compiled with the sprays to ensure optimal penetration into leaf tissues.

2.3. Measurements of growth traits, bulb yields and water use efficiency (WUE)

From each experimental plot, 3 plants of 13-week-old (n = 9) were randomly selected and carefully removed to measure shoot lengths with a meter scale and to count numbers of leaves per plant. By counting squares covered by a leaf, a graph sheet was used to measure leaf area. Each plant shoot was weighed (fresh weight; FW), and was then dried on 70 °C using an electric oven to record dry weight (DW).

At each experiment end, all plants of each plot were taken away to assess 3 size bulb yields; < 5.0 cm, 5–7.5 cm and > 7.5 cm, from which total bulb yield was calculated. As kg yield per m^3 water, applied water use efficiency (WUE) of onion plants was calculated after harvest (Jensen, 1983).