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Effects of Exegenous Glycine Betaine Treatments on Growth and Some Physiological Characteristics of Tomato under Salt Stress Condition

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ABSTRACT: The effects of the exogenous foliar application of glycine betaine (GB) with different doses (0, 5, 10 and 20 mM) on plant growth, chlorophyll content, stomatal conductance, phenolic and proline contents in tomato grown under salt stress were evaluated. Tomato plants (*Lycopersicon esculentum* Mill.) were imposed to salinity stress for 2 months with 75 mM NaCl. Salt stress reduced the growth, chlorophyll reading values and stomatal conductance in tomato. GB application decreased the inhibation of salt stress on tomato plant growth, and increased stomatal conductivity and chlorophyll content. The effective dose of GB was 10 mM to increase tolerance against salt stress. Plant height significantly increased in 5, 10 and 20 mM GB treatments by 2.2, 13.1 and 18.9%, respectively compared to the control. 5 and 10 mM GB treatments significantly increased chlorophyll reading values (51.18 and 53.44, respectively) compared with control (48.92). 10 mM GB exhibited a rapid increase in stomatal conductivity by 11.3% compared with control. Moreover, 10 mM GB showed an increase in proline content by 53.4% compared with control. According to results of our study, it can be suggested that exogenous GB treatments could mitigate the deleterious effects of salt stress in tomato.

Keywords: Glycine betaine, Plant tolerance, Salt stress, Tomato

Glisin Betain Uygulamalarının Tuz Stresi Altında Domatesin Bazı Büyüme ve Fizyolojik Özellikleri Üzerine Etkisi

ÖZ: Çalışmada, farklı dozlarda (0, 5, 10 ve 20 mM) yapraktan glisin betainin (GB) uygulamasının tuz stresi altında yetiştirilen domateslerde bitki büyümesi, klorofil içeriği, stoma iletkenliği, fenolik ve prolin içeriği üzerindeki etkileri değerlendirilmiştir. Bu amaçla, domates bitkileri (*Lycopersicon esculentum* Mill.), 2 ay boyunca 75 mM NaCl ile tuzluluk stresine maruz bırakılmıştır. Tuz stresinin, büyüme parametrelerinde, klorofil içeriğinde ve stoma iletkenliğinde belirgin bir düşüşe yol açtığı tespit edilmiştir. GB uygulaması, domates bitkisinin büyümesi üzerine tuz stresinin engelleyici etkisini azaltarak, stoma iletkenliğinin ve klorofil içeriğinin artmasına neden olmuştur. Tuz stresine karşı toleransı artırmada en etkili GB dozunun 10 mM olduğu belirlenmiştir. Bitki boyu 5, 10 ve 20 mM GB uygulamalarında kontrol grubuna göre sırasıyla % 2.2, % 13.1 ve % 18.9 oranında artmıştır. 5 ve 10 mM GB uygulamaları, klorofil değerlerini (sırasıyla 51.18 ve 53.44) kontrole kıyasla önemli derecede artırmıştır (48.92). 10 mM GB, stoma iletkenliğinde kontrol ile karşılaştırıldığında % 11.3 oranında bir artış göstermiştir. Ayrıca, 10 mM GB, prolin içeriğini kontrole kıyasla % 53.4 artırdığı saptanmıştır. Çalışmamızın sonuçlarına göre, GB uygulamasının, tuzluluk koşullarında büyümeyi arttırmak için domates üretiminde faydalı bir uygulama olabileceği sonucuna varılmıştır.

Anahtar kelimeler: Glisin betain, Bitki toleransı, Tuz stresi, Domates

INTRODUCTION

Plants are influenced by environmental conditions such as drought, low and high temperature and salinity. Salinity exhibits many constraints in vegetable growing that limits yield and quality. About 830 million hectares area is salt-affected worlwide (Martinez-Beltran and Manzur 2005). The problem of soil salinity is increasing due to the poor drainage, excessive fertilization and growing near seashore lands (Chinnusamy et al. 2005). Tomato, an important vegetable crop, is cultivated worldwide which is classified as moderately salt tolerant (Maas 1986).

Salt load in plant body affects growth rate and plant morphology (Munns 1993; Moya et al. 1999; Massai et al. 2004). Growth reduction due to the presence of NaCl at certain concentrations has been reported for various crops such as pepper (Chartzoulakis and Klapaki 2000; Yildirim and Guvenc, 2006), eggplant (Chartzoulakis and Loupassaki 1997), lettuce (Yildirim et al., 2011; Ekinci et al., 2012; Yildirim et al., 2015). Growing of tomato plants under salinity causes many deleterious effects including inhibition of root and shoot development (Cruz and Cuartero 1990), decline in fruit quality and yield (Mitchell et al. 1991; Van Ieperen 1996). Furthermore, salinity stress in tomato plant has been shown to reduce plant water status, nutrient uptake, photosynthesis, metabolism and increase in membrane permeability (Mitchell et al. 1991; Santa-Cruz et al. 2002).

One strategy to increase tomato plant tolerance against salinity is utilization of compatible solutes such as glycine betaine. Exogenous application of these compounds helps plants to adjust osmotic balance by stabilizing the structure of proteins such as Rubisco related with photosynthesis (Mäkelä et al. 2000). Therefore, some beneficial phytochemicals can be applied to plants as an alternative approach to improve fruit yield and quality under stress conditions. More recently, glycine betaine (GB) has been used to alleviate many abiotic stress factors such as cold (Aras and Eşitken 2013), drought (Farooq et al. 2008) and high temperature (Mäkelä et al. 1998). In addition, many studies showed exogenous GB could alleviate the salinity damage in maize (Yang and Lu 2005), lettuce (Yıldırım et al., 2015), bean (Gadallah 1999). GB is an amino acid derivative that accumulates in plant cells (Chen and Murata 2002). The exogenous application of GB to leaves or roots can be used to improve plant abiotic stress resistance (Kanechi et al. 2013).

This research was designed to investigate the role of GB in alleviating salt stress in tomato. We examined the effect of GB on some plant growth and physiological responses against salinity.

MATERIAL AND METHOD

The study was conducted in the greenhouse of Department of Horticulture at Bozok University in Turkey. The mean photosynthetically active-radiation (PAR) measured varied from 956 to 1264 μmol m^{-2} s^{-1} with PAR sensor-JYP 1000. Tomato (*Lycopersicon esculentum* Mill. cv Joker) seedlings were cultivated under natural light conditions, the mean day/night temperatures of 32/19°C, and 42% relative humidity during the study. Tomato seeds were sown into 45 celled trays filled with peat. Thirty days after planting, seedlings were transplanted to 2 L pots filled with mixture of soil:sand (1:1, v:v). Pots were put randomly on the tables in the greenhouse. There were 120 pots in the experiment with three replication, 5 plants for each replication.

Three days after transplanting, four different GB doses (0, 5, 10 and 20 mM) were treated three times with once a week interval to plant leaves. GB (C5H11NO2, molecular weight = 117.146; Sigma-Aldrich, Japan) was initially dissolved in methanol, and concentrations of 0, 5, 10, and 20 mM were made up with distilled water containing 0.02% Tween 20 (polyoxyethylenesorbitan monolaurate; Sigma Chemicals, UK) as a surfactant. Plants were treated in distilled water amended with Tween 20 served as the control. Four days after transplanting all plants were irrigated with tap water or with 75 mM NaCl solution. Two months after the salinity set up, experiment was finished. Plant diameter and plant height were determined.

Chlorophyll content (SPAD measurement): Chlorophyll content was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd, Osaka, Japan). For this determination, the midrib on all fully expanded leaves on both sides were taken at three locations and then averaged (Khan et al. 2003).

Stomatal conductance: Stomatal conductivity (mmol $(H_2O)m^{-2}$ s^{-1}) was measured with a leaf porometer (Decagon Devices SC-1).

Proline content: The proline was determined according to Bates et al. (1973).

Total phenolic content: For the quantification of total phenols, methanol was used for extraction. Total phenolic content was assayed by A765 with Folin-Ciocalteau reagent (Singleton and Rossi 1965). The results were expressed as μg of p-hydroxycinnamic acid (g fresh weight). Phenolic content was expressed as gallic acid equivalents (GAE).

Statistical analysis

Statistical analyses were performed with the statistical software package SPSS, version 20.0. The means were compared by the Duncan’s multiple range test (DMRT) test at 5%.

RESULTS

The tomato plants were exposed to moderate salinity at 75 mM for 2 months and some morphological and physiological responses were evaluated. GB treatments provided better plant growth. Plant height significantly increased in 5, 10 and 20 mM GB treatments by 2.2, 13.1 and 18.9%, respectively compared to the control. Stem diameter increased with 10 mM GB by 8.3% compared with control (Figure 1). GB treatments influenced the chlorophyll content of tomato plants exposed salinity. 10 mM GB treatments significantly increased chlorophyll reading values (53.44) compared with control (48.92).

10 mM GB exhibited a rapid increase in stomatal conductivity by 35 % compared with control. 5 and 20 mM GB foliar treatments also significantly increased stomatal conductivity compared with control. Higher phenolic acid content was obtained from all GB treatments than control.. The lowest content of phenolic acids was obtained from the control (12.45 mg/g). 10 mM GB showed an increase in proline content by 53.4% compared with control, while 5 and 20 mM GB leaded reductions in proline content compared with control (Table 1).

Table 1. Effect of GB on Stomal Conductance, Total Phenolic Acid and Proline Content of Tomato under Salt Stress

	Stomatal conductance (mmol(H_2O)m^{-2} s^{-1})	**Phenolic Acid (mg/g)**	**Proline (mM/g)**
Control (Salt)	68,07d	12,45 c	0,103 b
Salt + 5 mM GB	94,03b	14,46 b	0,098 b
Salt + 10 mM GB	104,70a	17,11 a	0,158 a
Salt + 20 mM GB	78,53c	14,81 b	0,075 c

Data followed by a different letter in same column were significantly different (P≤ 0.5) according to the DMRT.

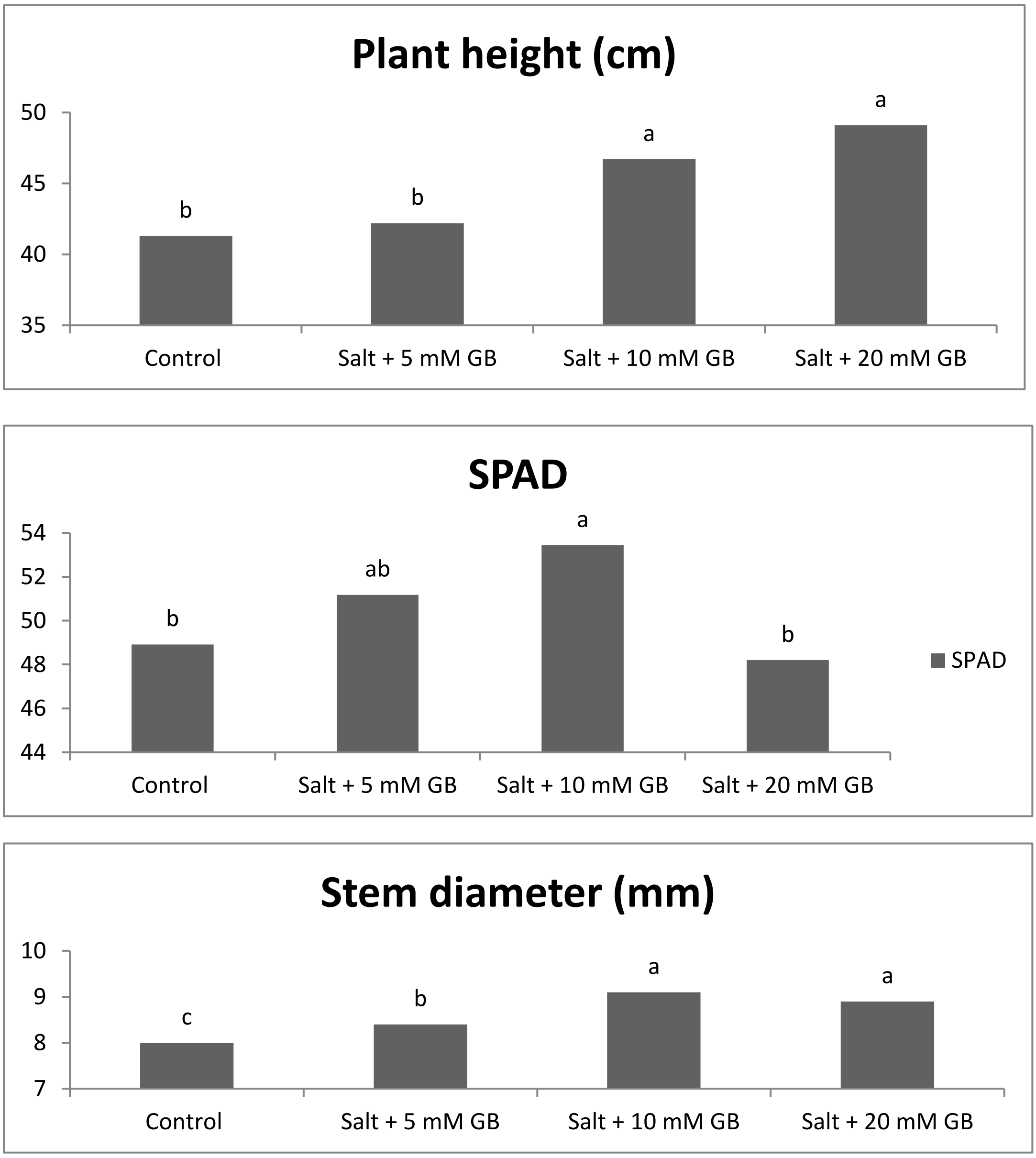

Figure 1. Effect of GB on Plant Height, Stem Diameter and SPAD of Tomato under Salt Stress. Data followed by a different letter on bars were significantly different (P≤ 0.5) according to the DMRT.

DISCUSSION

Salinity is an important environmental stress factor that may cause malignant effects on tomato plant growth and physiological traits (Cuartero and Fernández-Muñoz 1998). Poor drainage, excessive fertilization and inadequate irrigation cause salt stress in plants (Ghassemi et al. 1995). In our experiment, we applied 75 mM NaCl solution to tomato plants as moderate salinity stress. Furthermore, we applied the GB with different three doses in order to deal with the malignant effects of salinity. In the current study, all plants survived with slight leaf burn under 75 mM NaCl irrigation for two months.

Exogenous application of compatible solutes triggers tolerance mechanism of plants against stress factors. Supplemental irrigation with GB was effective in promoting plant growth of tomato. Improvements in plant growth by GB treatment under salt stress condition were previously reported in many plants (Hu et al. 2012; Korkmaz et al. 2012). Kanechi et al. (2013) reported that applying 1 mM GB to tomato plant increased plant growth, the number of harvested fruits and fruit weight. In our experiment, plants pre-treated with GB had considerably higher plant height and stem diameter compared to non-treated plants. These results correlate well with the findings of Abbas et al. (2010) who reported that foliar application of 50 mM GB resulted in higher shoot height. Decline in plant growth is a malignant effect of salt stress (Nazar et al. 2011). In our study, the GB applications decelerated the depression of the tomato plants height and stem diameter compared to control. The protective effect of GB on the plant growth under salinity was also reported by Hu et al. (2012) and Yıldırım et al. (2015).

GB treatments markedly increased SPAD value. 10 mM GB increased stomatal conductance that may lead to increase in yield. Kanechi et al. (2013) have reported an increase in the chlorophyll content of tomato leaves under salinity when treated with GB. Rajasekaran et al. (1998) have alleged that GB treatment has alleviating effect on chloroplast maintenance under salt stress. Stomatal conductance recorded on plants treated with 10 mM GB was 11.3% higher than those recorded in control plants. Decrease in stomatal conductance has frequently been reported as an effect of salt stress (Flexas et al. 2004). 10 mM GB clearly increased stomatal conductance. The results are in accordance with the findings of Abbas et al. (2010) showing that exogenous GB increased stomatal conductance under salinity condition. Increase in stomatal conductance and chlorophyll content by 10 mM GB treatment may be a reason of plant growth improvement under salinity condition.

Phenolics are important compounds in plants on account of acting as antioxidant (Rice-Evans et al. 1997). Increased accumulation of phenolics in stressed plants may help the plant tolerate stress (Parida et al. 2004). Similarly, our results exhibited that GB treatment elevated the phenolic content when compared with salt treatment without GB. Plants may accumulate compatible solutes such as proline under salt stress in order to provide plant tolerance (Khadri et al. 2006). In the current study, endogenous leaf proline content was increased in 10 mM GB treated-tomato plants. Proline accumulation due to the presence of salt stress and GB application has been reported for various cultures such as eggplant (Abbas et al. 2010), pepper (Korkmaz et al. 2012) and rice (Demiral and Türkan 2006).

CONCLUSION

In summary, the current study indicated that exogenous GB at 10 mM concentration was effective in ameliorating the deleterious effects of salinity on tomato plant. The benefical effect of applied GB to plants under salt stress may be attributed to its protective effect on chlorophyll and stomatal conductance. According to these findings, it is concluded that GB application can be useful as a supplement in tomato production to improve growth under salinity condition.

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