Plant Omics Journal POJ

POJ 5(2):79-86 (2012) ISSN:1836-3644

# Morpho-physiological improving effects of exogenous glycine betaine on tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress conditions

**Mohammad Ali Rezaei[1], Ibrahim Jokar[1], Mahlagha Ghorbanli[1], Behzad Kaviani[2]* and Ardashir Kharabian-Masouleh[3,2]**

**[1]Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran**
**[2]Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran**
**[3]Southern Cross Plant Science, Southern Cross University, NSW 2480, Australia**

***Corresponding author: b.kaviani@yahoo.com**

**Abstract**

Drought stress reduces the yield and production of tomato (*Lycopersicum esculentum* Mill.). Tomato does not naturally accumulate glycinebetaine (GB) in the cells under natural conditions. Effects of exogenous glycinebetaine on some morpho-physiological characteristics of *Lycopersicum esculentum* Mill. cv. PS was evaluated at different levels of drought stress. The experiment was conducted in a factorial design based on completely randomized block design (RCBD) with four replications. Seeds were germinated and arranged in pot conditions. Plants were watered weekly, bi-weekly and tri-weekly once as drought stress and irrigation was based on 100% of field capacity (FC). Exogenous glycinebetaine was applied as foliar at three levels (0, 5 and 10 mM) in three stages with 10 days interval. Root length, leaves number, total leaf fresh weight, total leaf dry weight, leaf area index, relative water content (RWC), and stress tolerance index (STI) were analyzed in the vegetative stage. Moreover, flowering number, fruit number and fruit weight were recorded during reproductive period. Results showed that the vegetative growth parameters [shoot height (maximum; 59.75 cm, minimum; 40.25 cm), root length (highest; 58.25 cm, lowest; 38.25 cm), leaf number (largest; 47.50, lowest; 16.50), leaf area (most; 819.21 $mm^2$, least; 305.63 $mm^2$)] and physiological characteristics [total shoot fresh weight (maximum; 52.68 g, minimum; 48.23 g), total shoot dry weight (maximum; 51.18 g, minimum; 36.67 g), relative water content (highest; 47.08, lowest; 25.95) and stress tolerance index (highest; 1.220, lowest; 0.548)] decreased with the increment of drought stress. In addition, all the above mentioned traits increased (70%, 73%, 187%, 193%, 168%, 9%, 72% and 122%, respectively) by exogenous application of glycinebetaine, significantly. Reproductive growth parameters [the number of flower (largest; 46, least; 23), fruit number (largest; 45, least; 22) and weight of fruit (maximum; 34, minimum; 20)] decreased under drought stress and increased significantly by 86%, 115% and 125%, respectively under exogenous application of glycinebetaine. Based on studied reproductive growth parameters in two levels of drought stress, the 10 mM exogenous glycinebetaine was the best treatment and recommended to alleviate effects of drought condition.

**Keywords**: Drought stress, exogenous glycinebetaine, field capacity, stress tolerance index, vegetative and reproductive parameters, tomato.
**Abbreviations:** GB_Glycinebetaine; FC_Field Capacity; RWC_Relative Water Content; STI_Stress Tolerance Index.

## Introduction

Stresses such as drought reduce the yield of cultivated plants or affect the quality of the harvested products (Arafa et al., 2009). Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within species (Caeruty, et al., 2009). Tomato (Solanaceae) has berry fruit and used as a vegetable (Yilmaz, 2001; Petro-Turza, 1978) and is a warm season perennial (Gould, 1983). Tomato needs enough irrigation based on climatic conditions and soil type, every week about 20 to 70 mm (Kazemi et al., 2009). In Golestan province (Iran), tomato is watered once a week. Tomato was used as a model system for investigating the effects of GB applications on plants supply (Makela, 1998b), as this species is unable to synthesis GB naturally (Wyn Jones and Storey, 1981; Makela, 1998b), but is sensitive to exogenous supply (Makela, 1998b). GB is a quaternary ammonium compound present in bacteria, cyanobacteria, algae, animals, and plants of several families (Rhodes and Hanson, 1993). The compatible solute GB, a small organic metabolite soluble in water and nontoxic at high concentrations, is a compound that can potentially play a crucial role in effective protection against salt, drought, and extreme temperature stress (Ashraf and Harris, 2004; Ashraf and Foolad, 2007; Chen and Murata, 2008). Moreover, there are many other purposes, in which exogenously applied GB can be used to improving drought tolerance (Iqbal and Ashraf, 2006; Ma et al., 2006; Ma et al., 2007; Zhao et al., 2007; Farooq et al., 2008). It accumulates in response to water deficit or salt stress in the leaves of some plants (Hanson, 1980; Weretilnyk et al., 1989; Arakawa et al., 1990; Ishitani et al., 1993) and this accumulated GB in leaves may confer tolerance to the osmotic stress (Styrvold et al., 1986; Grumet and Hanson, 1986; Saneoka et al., 1995). GB biosynthesis and accumulation in halophyte species have been widely investigated (Rhodes and Hanson, 1993; Hanson et al., 1995; Yu-Mei et al., 2004). But some higher plants are not capable to accumulating GB (Yang and Lu, 2005). For example, tomato plants (*Lycopersicon esculentum* Mill.) do not naturally accumulate GB (Wyn Jones and Storey, 1981). Thus, as an alternative, exogenous application of GB to non-accumulator plants may be a possible approach to tolerate environmental stress (Yang and Lu, 2005). There are contradictory reports on foliar application of GB in inducing abiotic stress tolerance in crops. For example, foliar application of GB improved salt and drought tolerance in tomato (Makela et al., 1998b; Heuer, 2003). Uptake of foliar applied GB has been shown to be active (Ladyman et al., 1980).

Exogenous GB was absorbed by the leaves and remained stable which improves the growth, survival, and tolerance of a wide variety of accumulator/non-accumulator plants under various stress conditions (Harinasut et al., 1996; Rajasekaran et al., 1997; Diaz-Zorita et al., 2001). Ashraf and Foolad (2007) suggested that effectiveness of foliar applied GB depends on a number of factors including plant species, plant developmental stage at which GB applied, concentration of GB, and number of applications. It is suggested that GB is not a compatible organic osmoticum for all plants or cause of phytotoxicity, when applied either at higher concentration or by increasing the number of applications. The broadleaf species such as bean, tomato, and grape are more sensitive to high concentrations of GB than grass species/cereals. Therefore, it is important to determine optimal concentration of GB, number of applications, and time of application for each crop species (Muhammad Ibrahim et al., 2006). In our previous studies, exogenous application of GB by spraying techniques, in vegetative and reproductive stages, did not lead us to a comprehensive conclusion on plant morphological characteristics and growth parameters of tomato after imposing the drought stress. Thus, the purpose of this study was to evaluate the improvement of drought tolerance, morphological characteristics, and growth parameters of tomato (*Lycopersicum esculentum* Mill.) cv. PS after exogenous and foliar application of GB.

**Results and Discussion**

Morphological characteristics in vegetative stage including shoot height, root length, leaf number, leaf area (Table 1) and physiological characteristic of total leaves fresh weight (except; total dry weight of leaves) decreased with increment of stress. All morphological and physiological values increased significantly by exogenous application of GB ($p \leq 0.05$) (Table 2). In comparison with the control (50.56 and 36.67 g), total leaves fresh weight decreased (48.92 and 48.23 g) and total leaves dry weight increased (43.42 and 41.23 g) under drought stress. Conversely, these parameters increased by exogenous application of GB. RWC (38.2 in control) decreased in D14 (25.95) and increased in D21 (39.99) under drought stress and GB had increasing effect on RWC in 10 mM of GB at all drought stress levels (Table 2). STI decreased in D14 (0.702) and D21 (0.548) under drought stress and influenced by GB application (Table 2). Morphological characteristics at reproductive stage, including number of flower, fruit number and weight of fruit increased significantly by exogenous application of GB, (Figs. 1, 2 and 3). It can be concluded that drought stress, at vegetative and reproductive stage, effectively influences the morphological characteristics of all studied traits (except for total dry weight of leaves) and exogenous application of GB will significantly alleviate the negative effects of stress condition. In the present study, investigated morphological and physiological characteristics, in vegetative and reproductive stages, were reduced under drought stress and then adversely increased by exogenous application of GB.

***Morphological characteristics at vegetative stage***

Results of analysis (Table 1) showed that drought stress had significant decreasing (27% in D14 and 32% in D21) effect on plant height ($P \leq 0.05$). Reduction in plant height due to drought stress has already been reported for tomato (Brown et al., 1992). When plants were watered bi-weekly, there was significant reduction in the plant height compared to daily watering (Milton et al., 1992). Moisture stress at vegetative growth stage caused a maximum reduction in plant height, when compared to control. Rana and Kalloo (1989) studied the morphological attributes associated with the adaptation under water deficit conditions in tomato. Their data revealed that some, resistant genotype recorded highest plant height compared to some other other individuals, whereas susceptible genotype had recorded least plant height. Application of GB decreased plant height (Mohammed and Tarpley, 2011). This is different from the findings of Farooq et al. (2008 a, b), which state that seed priming of maize (*Zea mays* L.) with GB increased shoot length under optimal conditions. However, Exogenous application of GB significantly increased the maize's plant height with increasing GB concentrations in the range of 2-20 mM and when the concentration of GB was higher than 20 mM, there was a decrease in plant height, compared to the control. This indicates that high GB concentrations might have an adverse effect on growth of maize plants (Xinghong and Congming, 2006). We interpret decrease in root length (32% in D14 and 34% in D21) by drought stress (Table 1) as an inhibition of cell division and root growth that ameliorate by exogenous GB adversely, via water relations in resistant and susceptible tomato genotypes (Table 1). Farooq et al. (2008 a, b) suggested that the adverse effects of stress on plant height and root length may be due to its effects on meristimatic cell division and elongation as well as root penetration. Studies of Farooq et al. (2008 a, b) also showed that seed priming of maize (*Zea mays* L.) with GB increased shoot length, root length and biomass under optimal conditions. Root length of resistant and susceptible tomato genotypes under water deficit condition revealed significant difference and resistant genotypes showed maximum root length, where susceptible genotype showed the minimum root length (Rana and Kalloo, 1989). Khafagy et al. (2009) reported that increasing salinity levels (NaCl) up to 6000 ppm decreases the plant height, root length and shoot fresh as well as dry weights in sweet pepper, indicating that application of GB and ASA increased plant height, root length, fresh and dry weights of shoot. GB at 2000 ppm proved to be more effective than ASA to reduce the harmful effect of salinity.

In the present research, drought stress and exogenous application of GB were two inverse factors affecting the leaf number per plant (Table 1). Leaf number per plant was decreased (21% in D14 and 51% in D21) under drought condition, whereas increased by application of GB (42% in 10 mM of D21) (Table 1). In this case different results have been reports. For example, leaf number per plant was not affected by water stress (Seghatoleslami, 2008). A significant inter-specific differences between two sympatric *Populus* species were found in the total number of leaves under drought stress (Wullschleger et al., 2005). Water stress reduced the leaf number, relative leaf expansion rate and plant height depends on the stage of development, particularly when water stress occurred during the vegetative stage. After re-watering, plants stressed during the vegetative and flowering stages increased leaf numbers. The number of leaves for the stressed plants, at different developmental stages, was significantly lower than the unstressed plants, indicating the failure of plants full recovery. Plants stressed during the pod filling stage had a 0% recovery of leaf number after re-watering compared to control plants and

**Table 1.** Plant height, root length, leaf number and leaf area in tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress after exogenous application of GB. Values represent mean-SE of four replications plants.

| Drought*-GB treatment (day/mM) | Plant height (cm) | Root length (cm) | Leaf number | Leaf area ($mm^2$) |
|---|---|---|---|---|
| D7 (Control) | 59.75±0.845a | 58.25±0.866a | 33.00±5.21b | 682.58±5.11b |
| D14 | 43.50±4.349bc | 39.25±2.428c | 26.00±2.42c | 547.90±2.39e |
| D14 × GB5 | 44.25±3.614bc | 41.50±0.957c | 30.25±3.40b | 632.18±3.39c |
| D14 × GB10 | 58.50±0.289a | 50.00±0.913b | 33.25±1.84b | 683.16±1.84e |
| D21 | 40.25±3.119c | 38.25±2.175c | 16.50±1.19d | 305.63±1.19b |
| D21 × GB5 | 52.50±4.490ab | 51.50±3.349b | 34.00±0.14b | 551.58±2.92c |
| D21 × GB10 | 58.25±1.439a | 56.25±1.364a | 47.50±4.53a | 819.21±4.59a |

*D7; weekly irrigation, D14; bi-weekly irrigation and D21; tri-weekly irrigation.

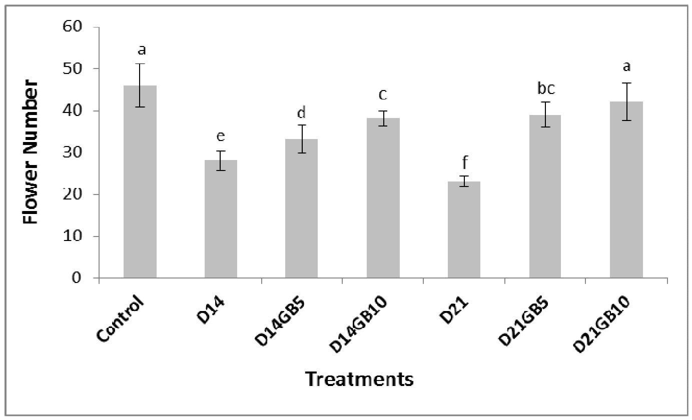


**Fig 1.** Number of flowers in tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress (D7; weekly irrigation, D14; bi-weekly irrigation and D21; tri-weekly irrigation) after exogenous application of GB (0, 5 and 10 mM). Values represent mean-SE of four replications plants.

all the treatments caused leaf abscission at the end of the growing season (Vurayai et al., 2011). Water stress reduced the number of leaves per plant in all stressed bambara groundnut plants. Water deficit stimulates leaf abscission as drought stress induces production of ethylene in a variety of species (Apelbaum and Yang, 1981; Kacperska et al., 1989). Mohammed and Tarpley (2011) reported that there was no significant difference among untreated plants and GB-treated plants with respect to leaf number per plant at the early grain-fill stage. Application of GB to the leaves of grape resulted in reduced growth rate and longer leaf development periods. Application of 50 mM and higher GB resulted in thicker leaves. When 100 or 200 mM GB was applied, growth completely ceased, and the leaves exhibited severe phytotoxic symptoms (Mickelbart et al., 2006). In current study, leaf number per plant was reduced by drought stress and the number of leaves for the GB-treated plants was still significantly higher than that of the stressed and control plants, indicating the possibility of improving and fully recovery by GB in cv. PS. Our results for leaf area (Table 1) supported the study of plant like populus (Wullschleger et al., 2005), soybean (Zhang et al., 2004) and many other species (Farooq et al., 2009), that drought or water deficit stress mostly reduced leaf growth and, in turn, the leaf areas. Drought stress reduces leaf area (7.3% in D14 and 55% in D21), whereas adversely increased by GB application (16.7% in 10 mM of D21), and if the stress occurs before flowering and after flowering reduced the green leaf duration (Banzinger et al., 2000). At any time of crop development, stress reduced crop photosynthesis rate, by which the total assimilates are being available to the crop. Thereby, under stress, cell expansion is inhibited, which expresses itself as reduction of leaf area expansion (Banzinger et al., 2000). Lopez et al. (2002) found that leaf area was increased significantly in unstressed plants, when 10 mM GB was applied. However, at 30 mM GB produced no increase in leaf area. Application of GB to plants grown in a controlled environment slightly reduced growth rates, resulting in a lower shoot leaf area at concentrations of 50, 100, or 200 mM. In contrast, application of GB also increased the average number of days to maximum leaf area, and increased specific leaf weight (Mickelbart et al., 2006). Application of exogenous GB in glasshouse experiments on tobacco (*Nicotiana tobaccum*) at various water stress levels showed positive responses of plant such as increase in the fresh and dry weights of leaves and the leaf area (Agboma et al., 1997b). GB increased the leaf area of *Zea mays* cv. Giza2, exposed to 150 mM NaCl by 22.9% as compared with stressed plants. Similar results were reported for soybean [*Glycine max* (L.) Merr.], where exogenous GB tended to increase the leaf area and seed yield of plant by enhancement of photosynthetic activity and nitrogen fixation (Agboma et al., 1997c). The increase in leaf area following the GB treatment maximized the photosynthetic activity (increase in total carbohydrate) and biomass production which could be attributed to the physiological ability of GB to prevent cellular dehydration, maintain turgid pressure and photosynthetic activity under conditions of low water potentials (Agboma et al., 1997b; Mäkelä et al., 1999).

**Table 2.** Total shoot fresh weight, total shoot dry weight, RWC and STI in tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress after exogenous application of GlyBet. Values represent mean-SE of four replications plants.

| Drought*-GB treatment (day/mM) | Total shoot fresh weight (g) | Total shoot dry weight (g) | RWC | STI |
|---|---|---|---|---|
| D7 (Control) | 50.56±0.99c | 36.67±0.63e | 38.20±0.41e | 1.000±0.000b |
| D14 | 48.92±0.26d | 43.42±0.48c | 25.95±0.57c | 0.702±0.018d |
| D14 × GB5 | 51.62±0.47b | 40.91±0.48d | 30.50±1.09d | 0.814±0.031c |
| D14 × GB10 | 52.56±0.36a | 45.72±0.64b | 43.90±0.58b | 1.141±0.019a |
| D21 | 48.23±0.47d | 41.23±0.47c | 39.99±0.80f | 0.548±0.017e |
| D21 × GB5 | 50.72±0.47c | 45.97±0.47b | 34.00±0.63c | 1.044±0.087b |
| D21 × GB10 | 52.68±0.48a | 51.18±0.48a | 47.08±0.66a | 1.220±0.024a |

*D7; weekly irrigation, D14; bi-weekly irrigation and D21; tri-weekly irrigation.

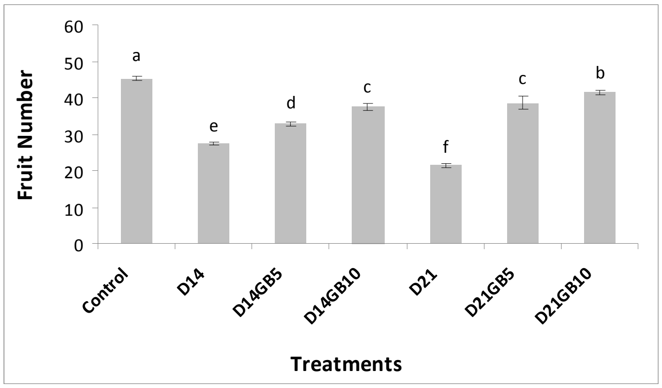


**Fig 2.** Number of fruits in tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress (D7; weekly irrigation, D14; bi-weekly irrigation and D21; tri-weekly irrigation) after exogenous application of GB (0, 5 and 10 mM). Values represent mean-SE of four replications plants.

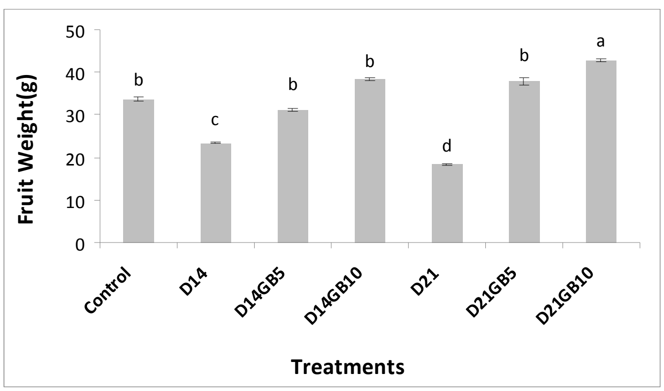


**Fig 3.** Fruit weight in tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress (D7; weekly irrigation, D14; bi-weekly irrigation and D21; tri-weekly irrigation) after exogenous application of GB (0, 5 and 10 mM). Values represent mean-SE of four replications plants.

***Physiological characteristics at vegetative stage***

In our results, the application of exogenous GB showed positive effect and increased the fresh and dry weights of leaves (Table 2). Similarly, application of GB in unstressed conditions on some maize (*Zea mays* L.) characteristics such as shoot fresh and dry weights did not affect by foliar application of GB (Maqsood, et al., 2006). Exogenous application of GB significantly influenced the growth of maize plants, in which fresh and dry weight of shoots and roots increased with increasing GB concentrations in the range of 2-20 mM and when the concentration of GB was higher than 20 mM. There was a decrease in fresh and dry weight of roots and shoots compared to the control, indicating that too high GB concentrations had an adverse effect on growth of maize plants (Xinghong and Congming, 2006). Hewer (2003) reported that fresh weight for both roots and shoots decreased with the application of GB in rice. In the 50% irrigation regime, the application of GB reduced the fresh weight and dry weight in contrast to the 75 and 100% irrigation levels, where its application often resulted in marginal improvements (Agboma et al., 1997b). In contrast, it was also investigated that shoot fresh weight of rice cv. Pokkali and shoot and root dry weight of IR-28 increased by GB application (Damaral and Turkan, 2005). Similar to our results, the application of exogenous GB in tobacco (*Nicotiana tobaccum*) showed positive responses and increased the fresh and dry weights of leaves (Agboma et al., 1997b). GB applied at the vegetative stage was very effective in improving plant dry weight of salt-stressed plants of two maize cultivars (Nawaz and Ashraf, 2007).

In addition, the stimulating effect of GB on plant growth may be attributed to an increase in the viability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and by stabilizing many function units, like oxygen-evolving PSII complex, and ATP synthesis, membrane integrity, and enzyme activity (Tao and Gao, 2003). GB treatment fully overcame the adverse effects on $CO_2$ absorption and chlorophyll fluorescence during water stress (Weibin et al., 1999). In maize growth, $CO_2$ assimilation rate, and stomata conductance increased at low exogenous GB concentrations (2-20 mM), but decreased significantly at high exogenous GB concentrations (30-50 mM) (Xinghong and Congming, 2006). Specifically, under stress conditions, GB can protect photosynthetic activity including photosynthetic enzymes (Incharoensakadi et al., 1986), proteins and lipids of thylakoid membranes (Wiliams et al., 1992 ), and electron flow in the photosystem II complex (Papageorgiou et al., 1991) and its major role in plants exposed to saline soil is probably protecting plant cells from salt stress by osmotic adjustment (Gadallah, 1999), protein stabilization (RuBisCo) (Makela et al., 2000), photosynthetic apparatus protection (Allakhverdiev et al., 2003), and reduction of oxygen radical scavengers (Heuer, 2003). The rate of $CO_2$ uptake by the leaves decreased considerably in response to water stress and was recovered fully by the GB treatment, and influenced same to that, in well-watered control plants. So, GB-treated plants showed better ability to recover from wilting than the untreated plants. In our experiment, exogenous application of GB has positive effect on RWC in drought stress leaves (Table 2), whereas in glasshouse-grown plants of two sugar beet genotypes, RWC of the leaves decreased in response to drought stress (Barbara et al., 2002).

Drought stress prevented N and P absorption and decreased RWC of apple leaves, which caused the decline of photosynthesis and transpiration (Zhang et al., 2010). GB application significantly increased stomata conductance of tomato plants grown in well-watered, water-deficient or saline conditions but not via ABA metabolism or water relations. However, GB did not affect the leaf ABA concentration and RWC (Makela et al., 1998b). Mickelbart et al. (2006) reported that endogenous GB concentrations did neither correlate with RWC and application of exogenous nor result in altered RWC on grapevines. Two tobacco cultivars with different drought tolerance were used to study the effects of GB applied on plant roots. The results showed that GB applied through roots could be absorbed by tobacco roots and then accumulated in leaves and accompanied by negative changes of RWC (Zhi et al., 2006). Changes in RWC due to GB application were almost negligible (Muhammad Ibrahim et al., 2006). On the other hand, accumulation of GB has been linked with plant's ability to better survive by osmotic adjustments under water stress condition (McCue and Hanson, 1990). Exogenously applied GB alleviated the damaging effects of NaCl treatment in two rice (*Oryza sativa* L.) cultivars (salt-tolerant Pokkali and -sensitive IR-28) (Demiral and Turkan, 2004). Similar results for higher GB concentrations were observed which increased the leaf turgor potential in maize (Quan et al., 2004), wheat (Raza et al., 2006), and grapevine (Mickelbart et al., 2006). We did not find any literature about significant relation between exogenous application of GB and calculation of STI in plants. Further work is essential to determine whether the higher GB accumulating ability or exogenous application could be used as a reliable index of stress tolerance in breeding programs as has been shown in barley, wheat, and maize (Naidu et al., 1998). In the present research, RWC and STI of the leaves decreased under drought stress and GB had increasing and positive effect (Table 2). Fernandez et al. (1992) proposed that STI index can discriminates genotypes with high yield and stress tolerance potentials. Talebi et al. (2009) showed that STI is able to discriminate tolerant genotypes under stress conditions. There was a significant correlation between STI and yield under stress. The results indicated that there was a positive and significant correlation among STI and grain yield under normal conditions and grain yield under drought condition. A general linear model regression of grain yield under drought stress on STI has been reported (Talebi et al., 2009). The significant and positive correlation of grain yield under normal conditions and STI showed that this index were more effective in identifying high yield cultivars under different moisture conditions (Talebi et al., 2009) and this conforms with our results in drought stress and exogenous application of GB.

***Morphological characteristics at reproductive stage***

Morphological characteristics of reproductive stage including the number of flower (46-23) (Fig. 1), fruit number (45-22) (Fig. 2) and weight of fruit (34-20 g) (Fig. 3) decreased under drought stress conditions and significantly increased by exogenous application of GB (86%, 115%, 125%, respectively). The total number of flowers in some varieties may be reduced up to 47% under drought conditions affecting the number of pods per plant (Mwanamwenge et al., 1999; Abelardo et al., 2005). The drought stress affected the vegetative growth of both, main stem and branches of the dry bean plants; however branch growth was the most affected, limiting the number of flowers, pods and final yield significantly. This indicated that branch growth may be important trait that could be used by plant breeders to achieve higher yield in determinate dry bean varieties (Abelardo et al., 2005). The effect of drought stress on flowering and pod setting on the different plant axes had influence on yield and yield components. The most affected yield component during the stress period was pods per plant, with a reduction of 63.3%, compared to 28.9% and 22.3% reduction of seeds per pod and weight of seeds, respectively (Abelardo et al., 2005). The decrease in yield of legumes grain grown under drought conditions is largely due to the reduction in the number of pods per plant (Muchow, 1985; Lopez et al., 1996; Pilbeam et al., 1992). On the other hand, irrigation deficit in the first growth period of tomato reduced the number of flowers leading to a decrease in the number of fruits and in the marketable yield (Colla et al., 1999). Drought stress improved fruit quality, organic acid, and vitamin C, increased sugar/acid ratio, but decreased yields. Under simulated drought stress, the number of fruits almost did not change but the weight per fruit was reduced. Dry matter content per fruits increased, and the total dry matter content per section did not change (Mingchi, 2010). GB treatment of tomato plants in a commercial vegetable producer's greenhouse in Southern Finland increased the yield and the number of tomato fruits (Makela et al., 1998b). There have been significant yield increases in tomato crops following application of GB (25, 50 and 100 mM) in normal growing conditions. However, GB at concentrations above 100 mM was toxic to plants, causing leaf burn (Makela et al., 1998a, b). In one study conducted on codA-transgenic tomato plants, an interaction was found between GB accumulation, increased size of flowers and fruits and changes in the expression of genes involved in cell division (Park et al., 2007b). Foliar application of tomato plants in greenhouse with 0.05 M GB during early flowering period clearly increased fruit yields. The increasing yield of tomato with 0.05 M GB was partially due to increased number of fruits, while the application of 0.1 M GB at the early flowering decreased the number of tomato fruits. Foliar application of tomato plants in field condition with 3.36 Kg $ha^{-1}$ GB during mid flowering period increased fruit yield 36 and 39%, as compared to control in salt and heat stress, respectively (Makela et al., 1998b).

In conclusion, GB applied at the vegetative growth stage was more effective in ameliorating the adverse effects of drought stress on tomato cv. PS, due to GB-induced improvement in plant water status. The adverse effects of drought stress on tomato can be alleviated by the exogenous application of GB at different growth stages by modulating water relations. The decrease in yield of tomato fruit number and weight grown under drought conditions is largely due to the reduction in the number of flowering per plant and plant growth.

**Materials and methods**

Seeds of tomato (*Lycopersicum esculentum* Mill.) cv. PS, is cultivated in Golestan province of Iran, provided by the Falatiran Company, were germinated (5 May 2010) in 36 pots containing silt: clay lome: soil (56%: 34%: 10%), EC=1.4, pH 7.4, absorbable K and P of 108, and 16.7 ppm, respectively, saturated percent of 57.9 and % O.C= 1.06. The pots were arranged on plastic trays (30 × 30 cm). Treatments were three different levels of irrigation [weekly (D7), bi-weekly (D14) and tri-weekly (D21) once)] of drought stress [irrigation based on 100% FC]. Exogenous GB (Sigma Chemical Co., USA) was supplied by application in three levels (0, 5 and 10 mM that were applied as foliar in three stages with 10 day interval. Root length, leaves number, plant total leaf fresh weight, plant total leaf dry weight, root length, leaf area index, RWC (Barrs and Weatherly, 1962), stress tolerance index (STI) (Fernandez, 1993), were analyzed in the vegetative stage and flowering number, fruit number and fruit weight were also recorded during reproductive period. Statistical analysis was carried out with SPSS version 19 for all the parameters studied. All data were subjected to Duncan's Multiple Range Test at the 5% level of probability to test differences between treatment means.

**References**


Agboma PC, Sinclair TR, Jokinen K, Peltonen-Sainio P, Pehu E (1997) An evaluation of the effect of exogenous glycine betaine on the growth and yield of soybean: timing of application, watering regimes and cultivars. Field Crop Res 54: 51-64.

Agboma PC, Peltonen-Sainio P, Hinkkanen R, Pehu E (1997) Effect of foliar application of glycine betaine on yield components of drought-stressed tobacco plants. Explementary Agric 33: 345-352.

Allakhverdiev SI, Hayashi H, Nishiyama Y, Ivanov AG, Aliev JA, Klimov VV, Murata N, Carpemtier R (2003) Glycine betaine protects the D1/D2/Cyt*b 559* complex of photosystem II against photo-induced and heat-induced inactivation. J Plant Physiol 160: 41-49.

Arafa AA, Khafagy MA, El-Banna MF (2009) The effect of glycine betaine or ascorbic acid on grain germination and leaf structure of sorghum plants grown under salinity stress. Aust J Crop Sci 3 (5): 294-304.

Arakawa K, Katayama M, Takabe T (1990) Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves, and etiolated leaves and roots of barley. Plant Cell Physiol 31: 797-803.

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59: 206-216.

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166: 3-16.

Asma M, Shahbaz M, Aisha Akram N (2006) Influence of exogenously applied glycine betaine on growth and gas exchange characteristics of maize (*Zea mays* L.). Pak J Agric Sci 43: 1-2.

Barbara S, Thomas TH, Cooke DT (2002) Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress. Plant Growth Regul 37 (1): 77-83.

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 24: 519-570.

Brown RD, Eakes J, Jehe BK, Jiliam CH (1992) Moisture stress, an alternative method of height control to B-nine (daminizide). J Environ Hortic 10: 232-235.

Caeruty AJ, Paramasivam M, Abdul W, Muhammad FH (2009) Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11 (1): 100-105.

Chen THH, Murata N (2008) Glycine betaine: an effective protectant against abiotic stress in plants. Trend Plant Sci 13: 499-505.

Colla G, Casa R, Lo Cascio B, Saccardo F, Leoni C (1999) Fertilization in central Italy. Acta Hort 487: 531-535.

Demiral T, Turkan I (2004) Does exogenous glycine betaine affect antioxidative system of rice seedlings under NaCl treatment? J Plant Physiol 161: 1089-1100.

Demiral T, Turkan I (2006) Exogenous glycine betaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environ Exp Bot 56: 72-79.

Farooq M, Basra SMA, Wahid A, Cheema ZA, Cheema MA, Khaliq A (2008) Physiological role of exogenously applied glycine betaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). J Agron Crop Sci 194: 325-333.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29: 185-212.

Farooq M, Aziz T, Basra SMA, Cheema MA, Rehman H (2008b) Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. J Agron Crop Sci 194: 161-168.

Farooq M, Aziz T, Hussain M, Rehman H, Jabran K, Khan MB (2008a) Glycine betaine improves chilling tolerance in hybrid maize. J Agron Crop Sci 194: 152-160.

Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. In: Proceeding of Symposium Taiwan, 13-16 Aug. Chapter 25, 257-270.

Gadallah MAA (1999) Effect of proline and glycine betaine on *Vicia faba* responses to salt stress. Biologia Plantarum 42: 249-257.

Gould WA (1983) Tomato Production, Processing and Quality Evaluation. AVI Publishing Company, Westport, CO., 445 p.

Grumet R, Hanson AD (1986) Genetic evidence for an osmoregulatory function of glycine betaine accumulation in barley. Aust J Plant Physiol 13: 353-364.

Hanson AD (1980) Interpreting the metabolic responses of plants to water stress. HortSci 15: 623-629.

Hanson AD, Rivoal J, Burnet M, Rathinasabapathi B (1995) Biosynthesis of quaternary ammonium and tertiary sulfonium compounds in response to water deficit. In: Environment and Plant Metabolism Series: Environmental Plant Biology (ed. Smirnoff N). BIOS Scientific Publisher, Oxford, UK, pp 189-198.

Heuer B (2003) Influence of exogenous application of proline and glycine betaine on growth of salt-stressed tomato plants. Plant Sci 165: 693-699.

Hoque MA, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2008) Proline and glycine betaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. J Plant Physiol 165: 831-824.

Incharoensakadi A, Takabe T, Akazawa T (1986) Effect of betaine on enzyme activity and subunit interaction of ribulose-1,5-biphosphate carboxylase:oxygenase from *Aphanothece halophytica*. Plant Physiol 81: 1044-1049.

Iqbal N, Ashraf MY (2006) Does seed treatment with glycine betaine improve germination rate and seedling growth of sunflower (*Helianthus annuus* L.) under osmotic stress. Pak J Bot 38: 1641-1648.

Ishitani M, Arakawa K, Mizuno K, Kishitani S, Takabe T (1993) Betaine and betaine aldehyde dehydrogenase in the Gramineae: levels in leaves of both betaine accumulating and non-accumulating cereal plants. Plant Cell Physiol 34: 493-495.

Kazemi M, Tavakoli H, Sobhaani AR (2009) Echophysiological effects of temperature and moisture on tomato yield's and quality. The First National Congress on Tomato Production and Processing Technology, Mashhad, Iran.

Khafagy MA, Arafa AA, El-Banna MF (2009) Glycine betaine and ascorbic acid can alleviate the harmful effects of NaCl salinity in sweet pepper. Aust J Crop Sci 3 (5): 257-267.

Nawaz K, Ashraf M (2007) Improvement in tolerance of maize by exogenous application of glycine betaine: growth and water relations. Pak J Bot 39 (5): 1647-1653.

Kirda C, Baytorun N, Rafat Derici M, Yildiz Dasgan H (2003) Nitrogen fertilizer recovery and yield response of greenhouse grown and fertigated tomato to root-zone soil water tension. Turk J Agric Forest 27: 323-328.

Liu M, Liu X, Hao J, Gao L (2010) Effect of simulated drought stress on plant growth, yield and fruit properties of tomato. Acta Hort 856: 193-202.

Lopez CML, Takahashi H, Yamazaki S (2002) Plant-water relations of kidney bean plants treated with NaCl and foliarly applied glycine betaine. J Agron Crop Sci 188: 73-80.

Ma QQ, Wang W, Li YH, Li DQ, Zou Q (2006) Alleviation of photo-inhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycine betaine. J Plant Physiol 163: 165-173.

Ma XL, Wang YJ, Xie SL, Li F, Wang W (2006) Glycine betaine applied through roots protects the photosynthetic apparatus of tobacco seedlings under water stress. J Plant Physiol Mol Biol 32 (4): 465-472.

Ma XL, Wang YJ, Xie SL, Wang C, Wang W (2007) Glycine betaine application ameliorates negative effects of drought stress in tobacco. Russian J Plant Physiol 54: 472-479.

Makela P, Munns R, Colmer TD, Condon AG, Peltonen-Sainio P (1998a) Effect of foliar applications of glycine betaine on stomatal conductance, abscisic acid and solute concentration in leaves of salt- or drought-stressed tomato. Aus J Plant Physiol 25: 655-663.

Makela P, Jokinen K, Kontturi M, Peltonen-Sainio P, Pehu E, Somersalo S (1998b) Foliar application of glycine betaine- a novel product from sugar beet as an approach to increase tomato yield. Indus Crops Products 7: 139-148.

Makela P, Karkkainen J, Somersalo S (2000) Effect of glycine betaine on chloroplast ultrastructure, chlorophyll and protein content, and RuBPCO activity in tomato grown under drought or salinity. Biologia Plantarum 43: 471-475.

Makela P, Kontturi M, Pehu E, Somersalo S (1999) Photosynthetic response of drought- and salt- stressed tomato and turnip rape plants to foliar-applied glycine betaine. Physiologia Plantarum 105: 45-50.

McCue KF, Hanson AD (1990) Drought and salt tolerance: towards understanding and application. Trends Biotech 8: 358-362.

Mickelbart MV, Chapman P, Collier-Christian L (2006) Endogenous levels and exogenous application of glycine betaine to grapevines. Scientia Hortic 111: 7-16.

Milton E, Giffern MC, John JR, Musiunas B, Morris GH (1992) Tomato and nightshade (*Solanum nigrum* L. and *S. ptycanthum* Dun.) effects on soil water content. J Am Soc Hortic Sci 117 (5): 730- 735.

Mohammed AR, Tarpley L (2009) Impact of high night time temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. Crop Sci 49: 313-322.

Mohammed AR, Tarpley L (2011) Characterization of rice (*Oryza sativa* L.) physiological responses to α-tocopherol, glycine betaine or salicylic acid application. J Agric Sci 3: 3-13.

Muhammad I, Ambreen A, Nabeela K (2006) Four foliar application of glycine betaine did not alleviate advers effects of salt stress on growth of sunflower. Pak J Bot 38 (5): 1561-1570.

Mwanamwenge J, Loss SP, Siddique KHM, Cocks PS (1999) Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L.). Euro J Agron 11: 1-11.

Naidu BP, Cameron DF, Konduri SV (1998) Improving drought tolerance of cotton by glycine betaine application and selection. In: Proceedings of the 9th Australian Agronomy Conference, Wagga Wagga, Australia.

Nawaz K, Ashraf M (2007) Improvement in salt tolerance of maize by exogenous application of glycine betaine: growth and water relations. Pak J Bot 39: 1647-1653.

Nunez-Barrios A, Hoogenboom G, NeSmith S (2005) Drought stress and the distribution of vegetative and reproductive traits of a bean cultivar. Scientia Agricola 62: 18-22.

Papageorgiou GC, Fujimara Y, Murata YN (1991) Protection of the oxygen-evolving photosystem II complex by glycine betaine. Biochemica et Biophysica Acta (BBA)-Bioenergetics 1057: 361-366.

Park EJ, Jeknic Z, Chen THH (2006) Exogenous application of glycine betaine increases chilling tolerance in tomato plants. Plant Cell Physiol 47: 706-714.

Petro-Turza M (1987) Flavor of tomato and tomato products. Food Reviews International 2 (3): 309-351.

Rajashekar CB, Zhou H, Marcum KB, Prakash O (1999) Glycine betaine accumulation and inhibition of cold tolerance in strawberry (*Fragaria ananassa* Duch.) plants. Plant Sci 148: 175-183.

Rana MK, Kalloo G (1989) Morphological attributes associated with the adaptation under water deficit condition in tomato. Vegetable Sci 16 (1): 32-38.

Reza S, Heidari R, Zare S, Norastehnia A (2006) Antioxidant response of two salt-stressed barley varieties in the presence or absence of exogenous proline. Gen Appl Plant Physiol 32: 233-251.

Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. Annual Rev Plant Physiol Plant Mol Biol 44: 357-384.

Sacks MM, Silk WK, Burman P (1997) Effect of water stress on cortical cell division rates within the apical meristem of primary roots of maize. Plant Physiol 114: 519-527.

Seghatoleslam MJ, Kafi M, Majid E (2008) Effect of deficit irrigation on yield, WUE and some morphological and phenologycal traits of three millet species. Pak J Bot 40 (4): 1555-1560.

Styrvold OB, Falkenberg P, Landfald B, Eshoo MW, Bjornsen T, Strom AR (1986) Selection, mapping, and characterization of osmoregulatory mutants of *Escherichia coli* blocked in the choline-glycine betaine pathway. J Bacteriol 186: 856-863.

Talebi R, Fayaz F, Naji AM (2009) Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* Desf.). Gen Appl Plant Physiol 35: 64-74.

Tao R, Gao M (2003) Technologies for improving tolerance to environmental stress: genetic engineering of salt stress tolerance in Japanese persimmon (*Diospyros kaki*) with the genes involved in biosynthesis of compatible solute. Acta Hortic 625: 307-320.

Vurayai R, Emongor V, Moseki B (2011) Effect of water stress imposed at different growth and development stages on morphological traits and yield of bambara groundnuts (*Vigna subterranea* L. Verdc). Am J Plant Physiol 6: 17-27.

Weibing X, Rajashekar CB (1999) Alleviation of water stress in beans by exogenous glycine betaine. Plant Sci 148:185-195.

Weretilnyk EA, Bednarek S, McCue KF, Rhodes D, Hanson AD (1989) Comparative biochemical and immunological studies of the glycine betaine synthesis pathway in diverse families of dicotyledons. Planta 178: 342-352.

Wiliams WP, Brain APR, Dominy PJ (1992) Induction of non-bilayer lipid phase separation in chloroplast thylakoid membranes by compatible co-solutes and its relation to the thermal stability of photosystem II. Biochemica et Biophysica Acta (BBA)-Bioenergetics 1099: 137-144.

Wullschleger SD, Yin TM, DiFazio SP, Tschaplinski TJ, Gunter LE, Davis MF, Tuskan GA (2005) Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. Can J Forest Res 35 (8): 1779-1789.

Wyn Jones RG, Storey R (1981) Betaines. In the Physiology and Biochemistry of Drought Resistance in Plants. Edited by Paleg LG, Aspinal D. 171-204. Academic Press, New York.

Xinghong Y, Congming L (2006) Effects of exogenous glycine betaine on growth, $CO_2$ assimilation, and photosystem II photochemistry of maize plants. Physiologia Plantarum 127: 593-602.

Yang X, Lu C (2005) Photosynthesis is improved by exogenous glycine betaine in salt-stressed maize plants. Physiologia Plantarum 124: 343-352.

Yilmaz E (2001) The chemistry of fresh tomato flavor. Turk J Agric Forest 25: 149-155.

Yu-Mei W, Yu-Ling M, Naosuke N (2004) Changes in glycine betaine and related enzyme contents in *Amaranthus tricolor* under salt stress. J Plant Physiol Mol Biol 30: 496-502.

Zhang J, Yao Y, Streeter JG, Ferree DC (2010) Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phosphorus absorb in different section of leaves and stem of Fugi/M.9EML, a young apple seedling. Afr J Biotech 9 (33): 5320-5325.

Zhang M, Duan L, Zhai Z, Li J, Tian X, Wang B, He Z, Li Z (2004) Effects of plant growth regulators on water deficit-induced yield loss in soybean. Proceedings of the 4$^{th}$ International Crop Science Congress, Brisbane, Australia.

Zhao XX, Ma QQ, Liang C, Fang Y, Wang YQ, Wang W (2007) Effect of glycine betaine on function of thylakoid membranes in wheat flag leaves under drought stress. Biologia Plantarum 51: 584-588.