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Full Length Research Paper

Application of exogenous glycine betaine on some growth traits of soybean (*Glycine max* L.) cv. DPX in drought stress conditions

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Glycine betaine is an osmoprotectant quaternary ammonium compound that accumulates in response to stress in plants. In order to evaluation stress tolerance, determination of yield amount and vegetative characteristic of *Glycine max* L. cv. DPX, an experiment was performed under potting conditions in factorial randomized complete block design with 4 replications in natural environmental conditions. Treatments include different levels of drought stress (30, 50, 70 and 100% of field capacity) and exogenous glycine betaine in four levels (0, 2.5, 5 and 7.5 kg ha^{-1}). Morphological index including plant height, number of branch, number of seed per plant, seed weight 1000, and yield were measured after harvesting. The results indicated that plant height decreased significantly as stress rate increased, but different concentrations of glycine betaine had no significant effect on the plant height. The number of branch, number of seed per plant and seed weight 1000 increased significantly with application of exogenous glycine betaine in different levels of drought stress in comparison with control. This research indicated the role of exogenous glycine betaine in reduction of harmful effects of drought stress and its useful effects on yield and yield components.

Key words: Drought stress, exogenous glycine betaine, soybean, yield.

INTRODUCTION

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally, drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. 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). Drought stress reduces the yield of the cultivated plants or affects on the quality of the harvested products (Arafa et al., 2009). The accumulation of solutes such as betaines by plants under osmotic stress, improves their ability to maintain physiological functions (Yancey et al., 1982). Of the wide variety of betaines, found in plants, glycine betaine (GB) accumulates in many families such as the Asteraceae, Chenopodiaceae, Poaceae and Solanaceae (Wyn and Storey, 1981) in response to abiotic stresses (Ladyman, 1982). Not all higher plants are capable of accumulating GB (Yang and Lu, 2005). There is some evidences showing that soybean is normally a low-accumulator of GB (Agboma et al., 1997a), with an average content less than 5 μmol/g dry weights (Makela et al., 1996). Foliar application of GB could increase its content in soybean plant up to 60 μmol/g dry weights, leading to an improvement in photosynthesis activity, nitrogen fixation, leaf area development, and seed yield of both well irrigated and drought-stressed soybean plants (Makela et al., 1996; Agboma et al., 1997b). Thus, as an alternative, exogenous application of GB to non-accumulator plants may be a possible approach to tolerate environmental stress. The accumulated GB may maintain cellular osmotic balance (McCue and Hanson, 1992) and stabi-

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Abbreviations: GB; glycine betaine, **Exo-GB;** exogenous glycine betaine, **FC;** field capacity.

lizes quaternary structures of complex proteins (Papageorgiou and Murata, 1995). Reports by Larkum and Wyn Jones (1979) and Smirnof and Stewart (1985) indicated that GB had a positive influence on key factors contributing to economic yield of plants under stress. Uptake of foliar applied GB has been shown to be active (Ladyman et al., 1980). Exogenous GB (Exo-GB) was absorbed by the leaves and remained stable there, indicating a long-term protective capability. Exo-GB also 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). These effects are highly significant when Exo-GB applies at a critical growth stage (Agboma et al., 1997b). Iqbal et al. (2005) reported that foliar application of 100 mm GB at vegetative stage is more beneficial in alleviating the effects of water stress and improving 100-achene weight and thus, increasing the yield of sunflower. There are many reports demonstrating the positive effects of Exo-GB on plant growth and final crop yield under drought stress; examples include those in tobacco, wheat, barley, sorghum, soybean and common beans (*Phaseolus vulgaris*) (Ashraf and Follad, 2007). The aim of this study was to evaluate the effects of exogenously applied GB in vegetative stages of soybean *Glycine max* L. cv. DPX exposing to drought stress to evaluate the potential use of GB to enhance yield and drought tolerance in soybean.

MATERIALS AND METHODS

Plant materials

Seeds of soybean (*Glycine max* L.) cv. DPX were secured from the Agricultural Research Centre, Gorgan city, Golestan province, Iran and GB was supplied by Sigma Chemical Co., USA.

Soil characteristics, experimental design and treatments

An experiment was arranged in potting conditions including clay loam soil with pH 7.9, EC 0.7 and factorial randomized complete block design with 4 replications in natural environment conditions. Treatments include different levels of drought (30, 50, 70 and 100% of field capacity) and application of Exo-GB in four levels (0, 2.5, 5 and 7.5 kg ha^{-1}) that were applied as foliar for six stages and about 1 week before the flowering and leaves.

Morphological and growth parameters and yield attributes

The morphological growth parameters (plant height and number of branches) were measured at the full bloom stage. Yield attributes (the number of seeds per plant, weight of 500 grains and grain yield) were recorded at maturity. Soybean yield estimation: product per unit area of pot was estimated as yield per hectare.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by a Duncan's mean test (±SE). Each data point was a mean of four replicates (n ¼ 6) and comparisons with P values 0.05 were considered significantly different. Statistical analyses of the data were made with SPSS software.

RESULTS

Results showed that different levels of drought stress have reduced the plant height. Highest and lowest height had shown in 70 and 30% FC treatments, respectively. Application of different concentrations of Exo-GB has no significant effect on plant height of soybean (Table 1). Drought stress reduced the number of branches in soybean (Table 1). This reduction increased as the stress intensity increased. Application of Exo-GB in 50 and 70% of FC had no significant effect on the number of branches, but in 30% FC, drought stress increased the number of branches, significantly (Table 1). There was significant different in seed number per plant between the controls and other levels of drought stress (Table 1). Drought stress reduced the number of seeds per plant in soybean. Exogenous application of GB in 50 and 70% of FC had significant effect on the plants (Table 1). Grain weight of 1000 was decreased with increasing the drought stress and increased with Exo-GB application, significantly (Table 1).

According to this research, field experiments conducted in drought stress have indicated that the grain yield of soybean cv. DPX was decreased following drought stress and increased significantly due to foliar applications of Exo-GB, when sprayed during stress (Figure 1). The seed yield increasing after the application of Exo-GB, was associated with the greater number of lateral branch, number of seed per plant and more grain weight of 1000 in DPX cultivar (Table 1).

DISCUSSION

Our results showed that different levels of drought stress has reduced the plant morphological aspects, including the number of branch, number of seed per plant and grain weight of 1000 (except for plant height) and the application of Exo-GB has regeneration and compensation roles. It has also been reported that plants are able to utilize foliar-applied GB and transport it to almost all plant parts, especially developing organs (Makela et al., 1996b). Variations of GB levels among different organs and at different ages were examined and showed that GB content was markedly different among cotyledons, roots, stems, leaves, flowers and seeds (Yu-Mei et al., 2004). The GB contents of these organs were very low during the earlier stages of plant development and increased as the plant development. The roots accumulated a small amount of GB at every stage of plant development. There are many reports demonstrating the positive effects of exogenous application of GB on plant growth and final crop yield in

Table 1. The number of lateral branches per plant, pods per plant, grain per pods and grain weight of 1000 in soybean (*Glycine max* L.) cv. DPX in drought stress after exogenous application of GB. Values represent mean-SE of four replications plants.

Exo-GB (kg/ha)	Drought (FC%)	Plant height (cm)	Number of branches/plant	Number of grain/plant	Weight of 1000 grain
	30	38.81±1.39^{g}	1.32±0.17^{h}	15.06±0.82^{g}	114.68±0.121^{i}
0	50	43.06±3.00^{efg}	3.20±0.20^{g}	18.50±1.44^{fg}	125.26±3.05^{j}
	70	54.06±5.80^{c}	3.02±0.26^{g}	24.37±3.49^{def}	148.42±0.58^{f}
	100	60.00±1.87^{b}	4.95±0.26^{b}	89.31±3.72^{a}	175.40±0.24^{b}
	30	41.68±3.11f^{g}	3.28±0.10^{g}	21.18±3.00^{efg}	127.50±0.58^{i}
2.5	50	47.62±2.02^{d}	3.65±0.22^{efg}	28.68±3.78^{d}	139.92±1.67^{g}
	70	62.68±4.94^{a}	4.22±0.22^{cde}	40.39±5.12^{c}	162.80±0.38^{d}
	30	40.12±0.77^{fg}	3.35±0.22^{fg}	20.56±1.52^{efg}	128.43±0.43^{i}
5	50	46.66±1.12^{de}	3.95±0.14^{def}	37.97±2.14^{c}	154.63±0.65^{e}
	70	57.12±1.71^{bc}	4.82±0.19^{be}	41.81±3.78^{c}	179.05±0.89^{a}
	30	42.18±1.70^{efg}	3.50±0.00^{fg}	21.12±1.79^{efg}	121.65±1.29^{k}
7.5	50	44.43±1.24^{def}	4.50±0.20^{bcd}	26.00±1.75^{de}	137.97±1.18^{h}
	70	57.06±2.26^{bc}	5.62±0.31^{b}	66.00±3.5^{b}	173.99±2.11^{c}

soybean (Ashraf and Follad, 2007). Foliar application of GB in tobacco plants grown under mild drought conditions in the glasshouse significantly increased the fresh weight, dry weight and leaf area. It also decreased the time to leaf maturity. GB was absorbed by the leaves and remained stable there, indicating a long-term protective capability, and its effects were highly significant, when it was applied at a critical growth stage. The results indicate that GB has the potential to improve drought tolerance and reduces the effects of water deficit on leaf growth of tobacco (Agboma et al., 1997a). Our results showed that the drought stress reduced the number of seeds per plant in soybean (Table 1). Application of 3 kg ha^{-1} GB on soybean for 75 and 100% irrigation levels showed increasing the number of seeds (Agboma, 1997b). There are a few reports suggesting a lack of such positive effects or even apparent negative effects of Exo-GB on plants growing under stress conditions, for example, foliar application of GB did not affect on yield components or endogenous levels of GB in cotton plants grown under drought stress (Meek et al., 2003). Agboma et al. (1997b) reported that the application of GB in the 50% irrigation regime on soybean reduced the weight of seeds and in the 75 and 100% irrigation levels increased the weight of total and large seeds. Increasing of the seed yield following the application of 3 kg/ha GB could be associated with the greater number of seeds and more large seeds (Agboma, 1997b). However, Bergmann and Eckert (1984) reported that the application of GB on winter wheat enhanced grain yield that was related to a larger number of grains per plant. Exogenous application of GB either in the form of foliar spray or seed treatment had no effect on hundred achene weight under normally irrigated conditions and when water deficit was imposed at the vegetative stage, exogenous application of GB was much effective when it was applied at the time of initiation of stress than that of seed treatment or foliar application of GB at the reproductive stage (Iqbal et al., 2005). In contrast, when water deficit was imposed at the reproductive stage, foliar spray of GB before the application of water stress (at the vegetative stage) and at the time of the initiation of stress (at the reproductive stage) had almost similar value (4.0 and 4.1 g, respectively) for hundred achene weight which were significantly higher than that of plants raised from GB-treated seed (Iqbal et al, 2005). The effect of pre-sowing GB treatment was not prominent in

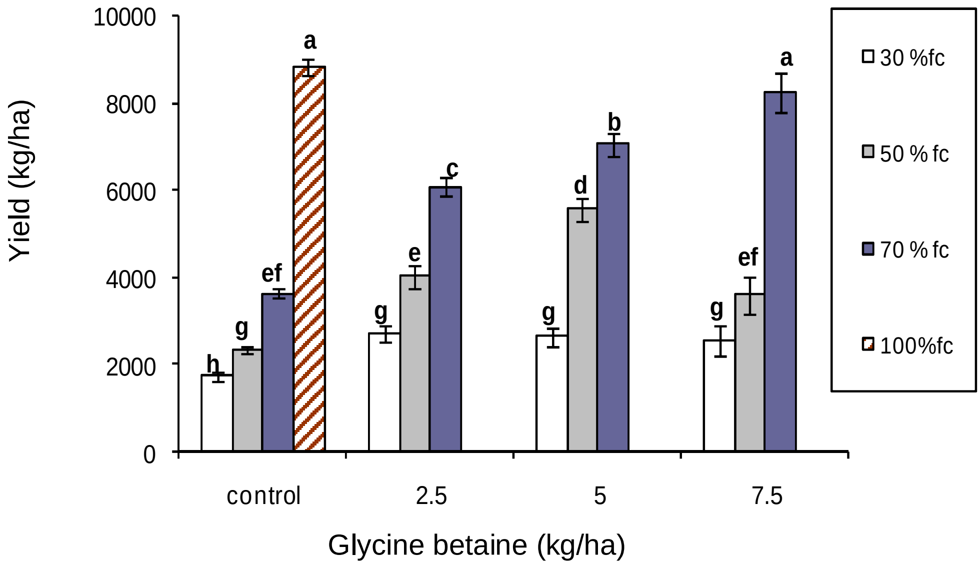

Figure 1. Yield in soybean (*Glycine max* L.) cv. DPX in drought stress after exogenous application of GB. Values represent mean-SD of four replications plants.

terms of 100-seed weight (Tahir et al., 2009). Iqbal et al. (2005) reported that foliar application of 100 mM GB at vegetative stage is more beneficial in alleviating the effects of water stress and improving the 100-achene weight and thus increasing achene yield of sunflower. Therefore, application of Exo-GB on soybean grain weight of 1000 in stress is time-, cultivar- and dose-dependent. There are many reports demonstrating positive effects of exogenous application of GB on plant growth and final crop yield under drought stress; examples include those in tobacco, wheat, barley, sorghum and soybean (Ashraf and Foolad, 2007). When GB applied at pod initiation in the 75% watering regime, 3 kg ha^{-1} GB resulted in a grain yield that was 22% greate than when 1 kg ha^{-1} was applied (Agboma et al., 1997b).

Studies with cereals showed contradictory results. Agboma et al. (1997a) concluded that the application of GB, in field conditions, improved drought tolerance and increased the yield of maize and sorghum, but not for wheat. These researchers found that Exo-GB delayed the canopy senescence of barley, oat and wheat, but these differences were not associated with differences in grain yields (Makela et al., 1996a).

Foliar application of GB did not affect yield components or endogenous levels of GB in cotton plants grown under drought stress (Meek et al., 2003). GB treatment induced increasing of grain yield up to 25% in maize (*Zea mays* L.), and by up to 11% in sorghum (*Sorghum bicolor* (L.) Moench) when plants were suffering from drought.

The explanation to the yield increases of stressed plants after application of GB has been proposed to lie at least partly in the increased net photosynthesis, decreased rate of photorespiration, stomata conductance, induced more efficient gas exchange (Makela et al., 1998b) and thus, better availability of carbon for photosynthetic processes and ability to avoid possibly photo-inhibition (Makela et al., 1999), water use efficiency (Bergmann and Eckert, 1984), chloroplast ultra-structure (Makela et al., 2000), chloroplast volume (Rajasekaran et al., 1997) and increased chlorophyll content in plants (Whapman et al., 1993; Blunden et al., 1997) and GB protects, stabilizes and activates the proteins of photosynthetic reactions (Papageourgiou and Murata, 1995).

Conclusions

Potted experiments have indicated that the crop stability and yield were often increased due to foliar applications of GB, when sprayed during to the stress. Compared to the control plants, the number of branches, number of seeds per plant and grain weight increased with the use of Exo-GB in different levels of drought stress, significantly. Application of Exo-GB on soybean gains weight of 1000 in stress is time-, cultivar- and dose-dependent.

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