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The interaction effect of drought and exogenous application of glycine betaine on corn (*Zea mays* L.)

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ABSTRACT

In order to study the effect of glycine betaine on corn under drought condition an experiment was conducted as split-split plot in the base of randomized complete blocks design. The first factor were irrigation interval cycle (4 and 8 days) and second factor including different concentrations of glycine betaine (50 ppm, 100 ppm,150 ppm) and third was spraying in two times (stem elongation stage and before flowering). Analysis of variance showed that effects of three factors on all of parameters except for carotenoids were significant. However, foliar application of glycine betaine reduced effect of stress conditions on the plant so that extent of chlorophyll (a and b), plant height, yield and 1000 seed weight increased significantly. Spraying by glycine betaine caused improving plant performance in stress conditions, so that in the concentration of 150 ppm produced the highest quality in all of characterizations that preference was more evident before flowering. By considering these results it could be said that spraying with 150 ppm of glycine betaine before flowering in the condition of stress and without drought stress could improve the extent of chlorophyll (a and b), plant height, yield and yield components. Eventually it could be concluded that external glycine betaine with 150 ppm concentration while spraying before flowering had great positive effects and usage of that material is affected by time of application concentration and more stress severity.

Keywords: compatible solutes, osmotic adjustment, stress tolerance, irrigation

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INTRODUCTION

Water scarcity and drought are the major factors limiting crop production in arid and semi-arid zones of the world. Irrigation is today the primary consumer of fresh water on earth [42], and thus agriculture has the greatest potential for solving the problem of global water scarcity. Improvements in management of agricultural water and improvement of crop performance in deficit water conditions continue to be called for to conserve water, energy and soil while satisfying society's increasing demand for crops for food and fiber [22]. Generally, drought stress reduces growth [28] and yield of various crops [12] by decreasing chlorophyll pigments, photosynthetic rate, stomatal conductance and transpiration rates [10, 11].

It had been showed that biochemical, physiological and morphological changes occur in plants in response to water deficit [37]. One of the adverse effects of drought on plants are production of some reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^*OH$) and singlet oxygen (^1O).

Compatible solutes with low molecular weight are non toxic and protect plants from different stresses through osmotic adjustment, and membrane stability and protecting protein and enzymes [4,16]. One of the prevalent groups of compatible solutes are betaines which the most abundant of them in plants is glycine betaine. Glycine betaine generally produced in many plants species in response to environmental stresses especially osmotic stresses and play as osmoregulator [32]. The production of glycine betaine was reported in many crop plants such as barley, wheat, sorghum and corn [43].

As plants may produce insufficient endogenous glycine betaine for alleviating stress damage, exogenous application of glycine betaine had been suggested as an approach for this purpose. Exogenous application of compatible solutes like proline and glycine betaine before or during environmental stress can protect plants against stress damage through increasing inside plant solutes [26]. Positive effects of exogenous application of glycine betaine on growth and yield of crop under stress condition [25]. Harinasut *at al.* [31] showed that exogenous glycine betaine application in rice prevent PSII from salt stress damages. According to Makela *et al.* [32] glycine betaine penetrate rapidly penetrate to plants leaves and immediately move to plant roots, meristems and leaves protect plant parts against stress damages. Smirnoff and Stewart [15] showed that exogenous application of glycine betaine cause increase in growth and reproduction of bean after recover from stress condition. Murata *et al.* [30] reported that glycine betaine protect the PSII and ATP synthase complex from inhibitory effects of NaCl. However, in cotton under drought stress foliage application of glycine betaine had no significant effects on yield components, physiological processes and endogenous level of glycine betaine [8].

Corn is one of the important cereal crop grown in the Iran and sometimes its yield limits by drought conditions. Keeping in the view the role of glycine betaine in plants under water deficit conditions, we hypothesize whether or not exogenously applied glycine betaine as foliar spray alter morpho-physiological and yield responses of corn under water deficit conditions.

MATERIALS AND METHODS

The experiment was conducted at 2011 in Fars province, Iran (latitude of 28° 52', longitude 52° 36' and altitude of 1600 m above sea level). This region was semi-arid and the soil was clay-loam with pH= 7.8, EC= 1.45 ds/m and 0.78% of organic matter.

The experimental design was split split plot in a completely randomized blocks design with four replications. The main factor was tow irrigation treatment (4 day irrigation and 8 day irrigation intervals), the sub factor was tow glycine betaine spraying time (early in stem elongation and pre anthesis) and sub sub factor was four spraying concentration (0, 50, 100 and 150 ppm). The soil was under wheat cultivation in previous year and plowed before corn cultivation. Corn (cv SC 704) was planted in plot with size of 5.4m x 3m. Each plot consisted of 8 planting rows with 3m length and 0.7 m row spacing. Corn was hand seeded on 1 July 2011. Before planting P and K fertilizers were incorporated with soil at 100 kg ha^{-1}. Nitrogen fertilizer (150 kg ha^{-1}) was applied at two stages (early after planting and during stem elongation).

At flowering stage Chlorophyll and proline was measured at last fully expanded leaf. Proline was evaluated using methods described by Bates *et al.* [26]. The chlorophyll a evaluated according to Arnon [26] procedure. At physiological maturity the area of 4 m^2 of each plot was harvested and the traits grain yield, biological yield, number of seed per ear (by counting 20 randomly selected ears in each plot), thousand grain yield (by counting 1000 randomly selected grain) and plant height (in 30 randomly selected plants) were measured. Data was subjected t analysis of variance using SAS software and mean was compared using Duncan multiple range test at 5% level.

RESULTS AND DISCUSSION

Chlorophyll

The results of analysis of variance showed that the main effects of irrigation intervals, time of spraying, glycine betaine (GB) concentration and the interaction effects of GB × time of spraying, irrigation × time of spraying, concentration× irrigation and irrigation × concentration ×time of concentration was significant chlorophyll a (Table 1). By increasing irrigation interval from 4 to 8 days the chlorophyll a concentration decreased by 18.5% (Table 3). This reduction may be due change in nitrogen metabolism in relation of synthesis nitrogen metabolites such as proline. In stress condition the chlorophyll precursor, glutamate, are less locates in chlorophyll biosynthesis pathway.

With increasing concentrations of glycine betaine application from 0 to 150 ppm chlorophyll content increased from0.574 to 0.994 mg/gFW (Table 3). Exogenous application of glycine betaine concentrations appear to increase internal precursor choline in leaves and prevent of chlorophyll degradation and inhibit activity of chlorophylase enzyme therefore chlorophyll concentration increased in leaf. Harinasut *et al. [31]* showd that exogenous application of glycine betaine in rice protected PSII from salt stress damage and increased chlorophyll concentration. The application of GB at flowering had the more effect than stem elongation on chlorophyll content (Table 3).

The triple interaction of treatment showed that in normal irrigation conditions (4 days irrigation interval) spraying pre flowering by 1500 ppm GB produced the highest chlorophyll a concentration and control treatment (without spraying) the showed lowest chlorophyll concentration (Fig 1). In all treatment 8 day irrigation intervals caused a decrease in chlorophyll but, spraying GB in both growth stages reduced the adverse effects of drought on chlorophyll.

Drought stress results in increasing ROS production in chloroplast that destructs chlorophyll and chloroplast membrane [24]. In this exogenous application of GB prevent chlorophyll from degradation. 8 day irrigation interval significantly reduced chlorophyll a in both spraying times (Fig 1). This may be due to carbohydrate consumption in some metabolites biosynthesis such as proline [21].

Proline

The main effects of irrigation, time of spraying and interaction of concentration × time and concentration× time× irrigation were significant for proline at 1% level also, the effects of irrigation × time of spraying and concentration× irrigation were significant at 5% level (Table 1). By increasing irrigation interval from 4 to 8 day proline content significantly increased (by 15.4%) (Table 3). In drought condition proline content increases in plant that serve as nitrogen storage or osmoregulator solute and help plant to tolerate stress condition [26].

Comparison glycine betaine (GB) concentration showed that 150 ppm GB produced the highest proline content which statistically similar to 100 ppm and the lowest proline content observed in the control treatment (Table 3).

The interaction effect of irrigation interval × GB concentration was shown in Fig 2. In drought (8 day interval) condition proline content increased by increasing GB concentration. Proline accumulation can reduce lipid proxidation and prevent membrane degradation in stress by reducing ROS molecules [33].

Plant height

The results of analysis of variance showed that main effects of GB concentration and interaction effect of GB concentration× time were significant at 1% and effects of irrigation interval, time, irrigation × time, and concentration× irrigation× time were significant at 5% level (Table 2).

Plant height was higher in 4 day irrigation than 8 day irrigation interval (Table 4). Increasing irrigation interval reduced height by 8.39%. This may be due reduction in water and nutrients uptake and shortening stem internodes. Spraying GB pre flowering increased corn height by 4.5% in comparison with spraying at stem elongation (Table 4). By increasing GB concentration plant height increased significantly, as the lowest and highest height observed in control and 150 ppm respectively (Table 4). Plant height in 50, 100 and 150 ppm GB increased by 4.04, 5.56 and 9.13% in comparison with control respectively. Glycine betaine causes increase in plant height through increasing stomatal conductance, cell torgur maintenance and increasing cell elongation [44].

Interaction effects of concentration× time of spraying and irrigation intervals showed that in both spraying time the highest corn height belonged to 4 day irrigation interval (Fig 3). The highest corn height in pre flowering spraying was observed in 150 ppm concentration and 4 day irrigation interval and the lowest corn height was observed in stem elongation spraying and 8 day irrigation. Drought stress (8 day irrigation interval) significantly reduced corn height and spraying GB at stem elongation had no effect on this reduction (Fig 3). According to Arakawa and Timasheff [40] the positive effects of GB in drought is relating to its interaction with a protein which play role in cell growth and by this increasing plant height. Gibon *et al*. [45] showed that application of exogenous GB increased internal GB concentration and by osmotic adjustment improved cell growth.

Seed number per ear

All of main effects and interaction were significant for number of seed per ear (Table 2). 4 day irrigation interval produced higher seed number than 8 day irrigation interval. By increasing irrigation interval seed number decreased by 22.98% (Table 4). Drought condition causes reduction in nutrients availability and translocation of carbohydrates in plant and therefore number of seed decreases in this condition [28].

Spraying with GB at pre flowering stage produced more seeds number (10.3% more seed) than stem elongation (Table 4). Although seed number determines in vegetative stages, in reproductive stages high water requirement exist for seed development and GB application by improving water relations reduced seed abortion and number of seed per ear increased. Increasing GB concentration increased seed number as, control treatment produced the lowest seed number and 150 ppm GB produced the highest seed number. There was no significant deference between 50 and 100 ppm concentration (Table 4).

Evaluation interaction effects of spraying time, GB concentration and irrigation interval showed that spraying at stem elongation stage there was no significant deference between concentrations of GB at 8 day irrigation interval (Fig 4). In fact drought effects of 8 day irrigation intervals were so severe that any concentration of GB could compensate these effects. But at pre flowering stage spraying by 150 ppm GB improved seed number in 8 day irrigation interval while concentrations of 50 and 100 ppm don not shown this improvement. Application of GB pre flowering caused the damage of drought on sensitive reproduction processes diminished and lower number of seeds abort due to drought. Iqbal and Shahabuddinn [27] reported the positive effects of application GB on cell turgescence and water use efficiency in cotton.

Grain weight

Effects of irrigation× time of spraying and the interaction effect of concentration× irrigation interval× time of spraying were significant at 5% level and the effects of time of spraying, concentration of GB and the interaction effects of concentration× time of spraying were significant at 1% level (Table 2). By increasing irrigation interval from 4 to 8 day seed weight decreased by 7.49%. (Table 4). This reduction was due to reduction in water and nutrient uptake and transport in plant which affects rate of translocation of photosynthates to seed filling. Serraj and Sinclair [34] showed that the effects of drought on seed weight were highest during seed filling period. Under drought condition plants shorten its growth cycle which reduces grain filling period and lower deed weight.

Spraying pre flowering increased seed weight by 5.92 in comparison with sparying at stem elongation (Table 4). Increasing GB concentration significantly increased seed weight so the highest seed weight observed in 150 ppm concentration of GB.

Interaction effects of concentration× time of spraying× irrigation interval showed that application of GB increased seed weight at both 4 and 8 day irrigation interval and in both stages of spraying (Fig 5). The highest seed weight obtained at 150 ppm concentration in 4 day irrigation interval.

Grain yield

Corn grain yield significantly affected by irrigation, GB concentration and time of spraying and all main and interaction effects were significant (Table 2). 4 day irrigation interval produced 19.5% more grain yield than 8 day irrigation (Table 4). 8 day irrigation interval as a drought stress reduced grain yield. Cell growth is the most sensitive plant process to drought due to turgescence requiring for cell expansion [28]. Drought stress also, affects cell division due to reduction in construction of DNA, RNA and cell wall materials [7].

Spraying GB before flowering produced 11.88% more grain yield than spraying at stem elongation stage (Table 4). This may be due to increasing internal GB derived from exogenous application and producing essential amino acids at this stage of growth. Concentration of 150 ppm GB produced the highest grain yield and the lowest obtained in control treatment. Application of 50, 100 and 150 ppm GB increased grain yield by 8.82, 17.31 and 25.62% respectively in comparison with control treatment (Table 4).

The interaction effects of experimental treatments showed that the highest grain yield obtained in 4 day irrigation interval and application of 150 ppm GB before flowering (Fig 5). The results showed that detrimental effects of drought on grain yield was more severe in vegetative stage than reproductive stage therefore spraying with GB at vegetative stage had less effects on yield improvement while, at reproductive stage (spraying pre flowering) application of GB by improving water relation and translocation of assimilate increased grain yield.

Agboma *et al.* [25] observed that at drought stress condition the internal GB content of sorghum at vegetative and reproductive stage reach to 31 and 68%, respectively and application of exogenous GB can protect plant from detrimental effects of drought. Drought stress by decreasing leaf water content, increasing ABA and reducing photosynthesis reduces assimilate availability for growing grains and therefore reduces grain yield while, application of exogenous GB improves this condition for plants [44]. Similarly Naidu *et al.* [6] observed that application of GB increased sunflower grain yield.

Table 1- Mean square of traits

Source of variation	df	Chlorophyll a	Proline
Replication	2	0.001 ns	32.62 ns
Irrigation (A)	1	0.2309**	1053.77**
Ea	2	0.0004	4.69
Time of spraying (B)	1	0.0653**	515.70**
Irrigation*Spraying	1	0.0003**	105.24*
Eb	4	0.0005	19.44
Glycine betaine (C)	3	0.3059**	7.58 ns
Glycine betaine*Irrigation	3	0.0412*	15.41*
Glycine betaine*Sparying	3	0.0073**	51.47**
Glycine bataine*Irrigation* Spraying	3	0.0210**	49.35**
Ec	24	0.0020	4.28
CV (%)	df	6.11	5.41

** and ** significant al % and 1% level respectively and ns not significant*

Table 2- Mean square of traits

Source of variation	df	Height	Seed /ear	1000 seed weight	Grain yield
Replication	2	17.8 ns	359.8 ns	5.1 ns	517969.9 ns
Irrigation (A)	1	4144.1*	149596.4**	3622.7*	32526278.1*
Ea	2	81.8	1351.0	124.0	747726.1
Time of spraying (B)	1	1140.7*	24735.3*	2227.7**	10799602.4**
Irrigation*Spraying	1	1408.3*	10990.7*	1036.0*	6637477.3*
Eb	4	136.4	1381.0	83.0	362334.5
Glycine betaine (C)	3	2093.0**	37115.9**	2908.1**	10242362.7**
Glycine betaine*Irrigation	3	141.6 ns	4075.1*	85.5 ns	1150655.2*
Glycine betaine*Sparying	3	523.6**	8943.9**	933.2**	5279954.8**
Glycine bataine*Irrigation* Spraying	3	298.9*	3519.4*	265.1*	1038869.3*
Ec	24	79.5	1168.3	86.4	343455.1
CV (%)		4.20	7.95	4.16	7.81

** and ** significant al % and 1% level respectively and ns not significant*

Table 3- Effect of irrigation interval and glycine betaine concentration and time of application on chlorophyll, carotenoid and praline content

	Chlorophyll a (mg/gFW)	Proline (mg/gFW)
Irrigation interval		
4 days	0.8107 a	1.858 b
8 days	0.6720 b	2.374 a
Spraying time		
Stem elongation	0.705 b	2.128 a

Before flowering	0.778 a	2.104 a
Glycine betaine(ppm)		
0	0.574 d	1.904 c
50	0.705 c	2.022 b
100	0.840 b	2.235 a
150	0.994 a	2.053 ab

Table 4- Effect of irrigation interval and glycine betaine concentration and time of application on corn height, yield and yield components

	Plant height (cm)	Seed/ ear	1000 seed weight (g)	Grain yield (kg/ha)
Irrigation interval				
4 days	221.6 a	485.7 a	232.2 a	8309.9 a
8 days	203.1 b	374.1 b	214.8 b	6689.4 b
Spraying time				
Stem elongation	207.5 b	407.2 b	216.7 b	7025.05 b
Before flowering	217.2 a	452.6 a	230.3 a	7973.7 a
Glycine betaine(ppm)				
0	197.6 d	370.3 c	206.9 c	6459.3 d
50	205.9 c	416.9 b	216.6 b	7084.3 c
100	217.3 b	427.8 b	226.5 b	7812.2 b
150	228.6 a	504.6 a	243.9 a	864.7 a

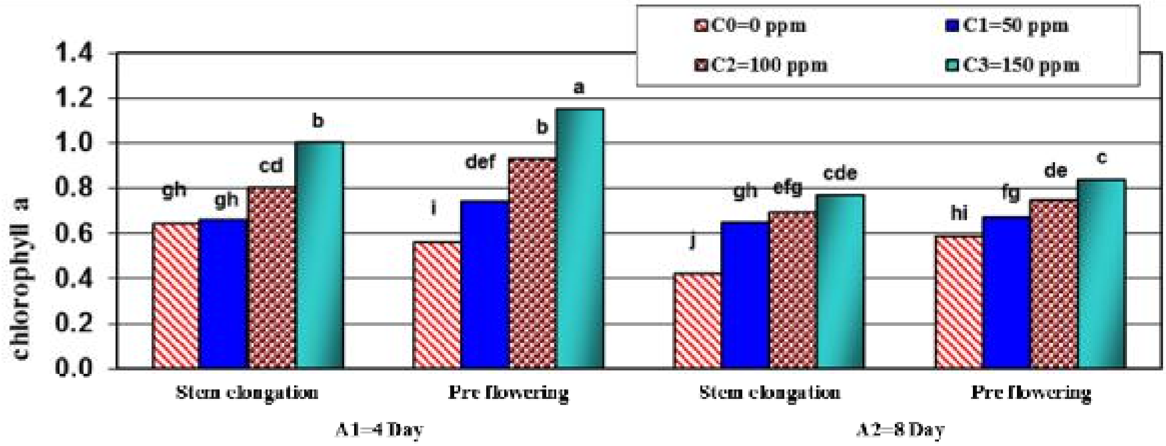

Fig. 1- Interaction effect of irrigation, glycine batine spraying time and concentration on chlorophyll a

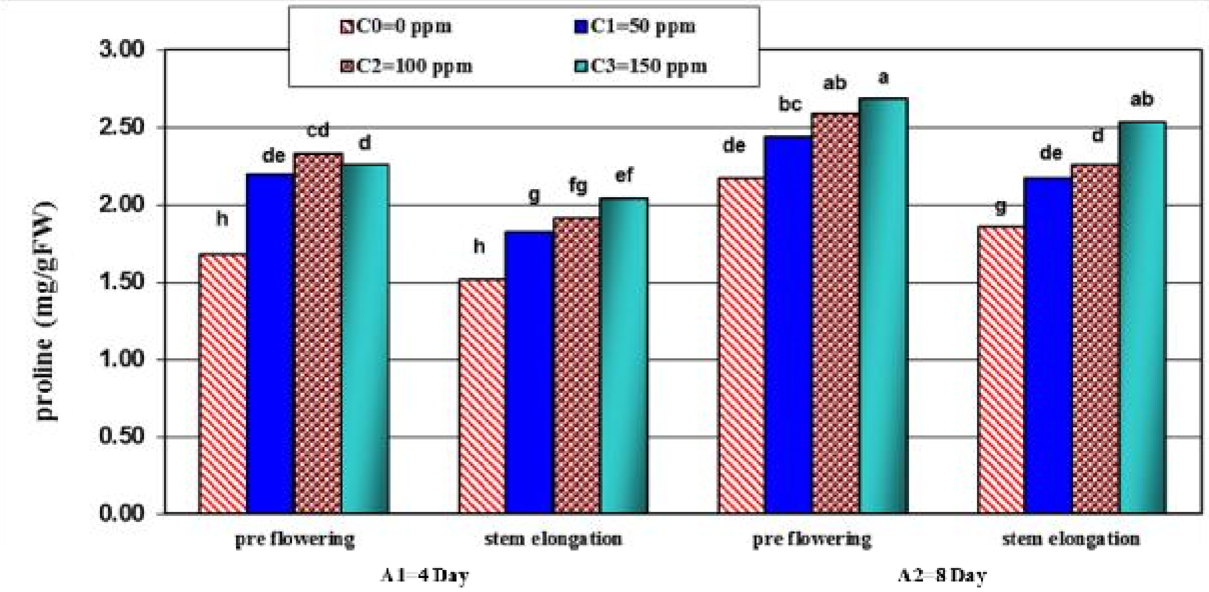

Fig. 2- Interaction effect of irrigation, glycine batine spraying time and concentration on proline content

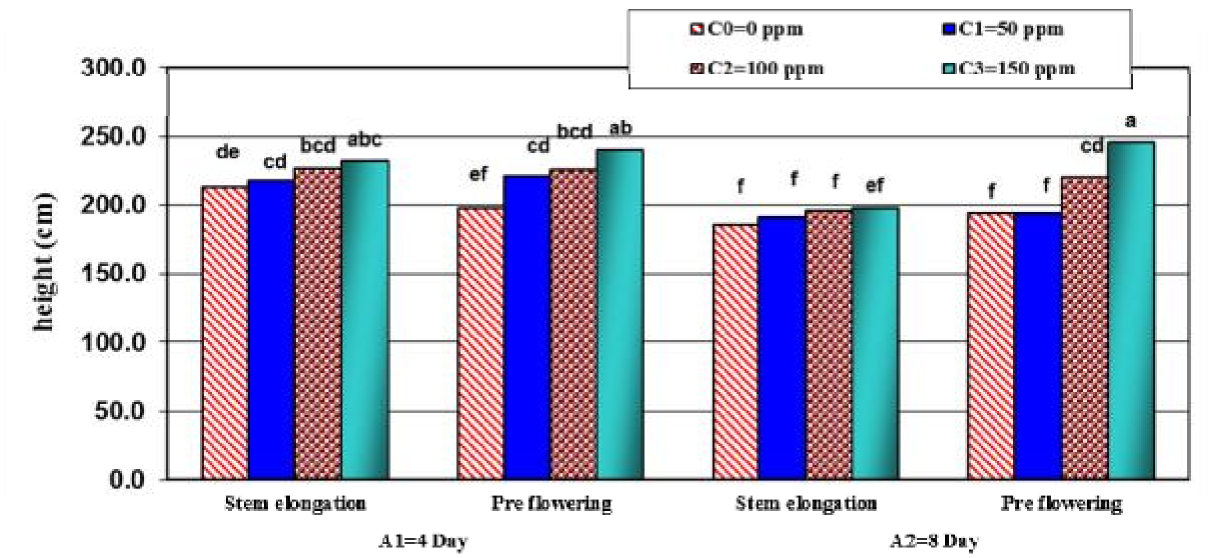

Fig. 3- Interaction effect of irrigation, glycine batine spraying time and concentration on corn height

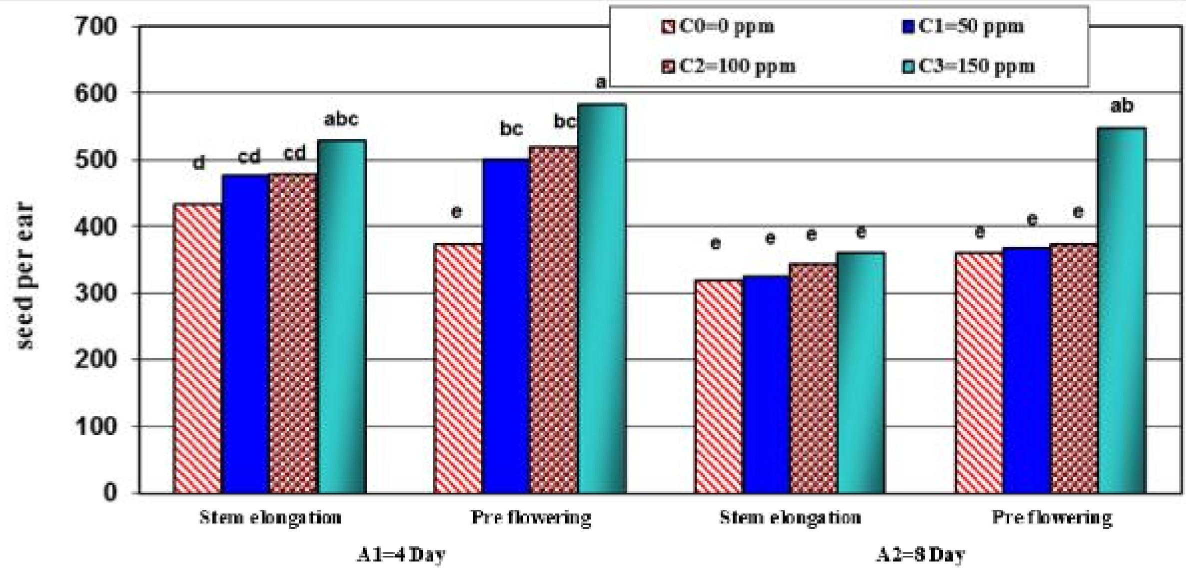

Fig. 4- Interaction effect of irrigation, glycine batine spraying time and concentration on seed per ear

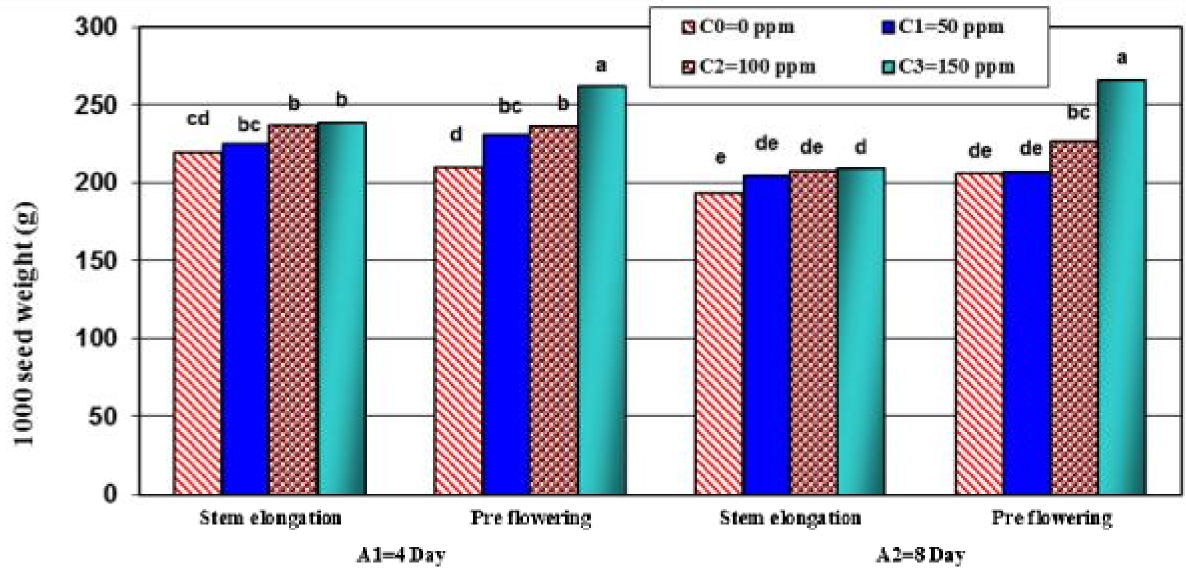

Fig. 5- Interaction effect of irrigation, glycine batine spraying time and concentration on 1000 seed weight

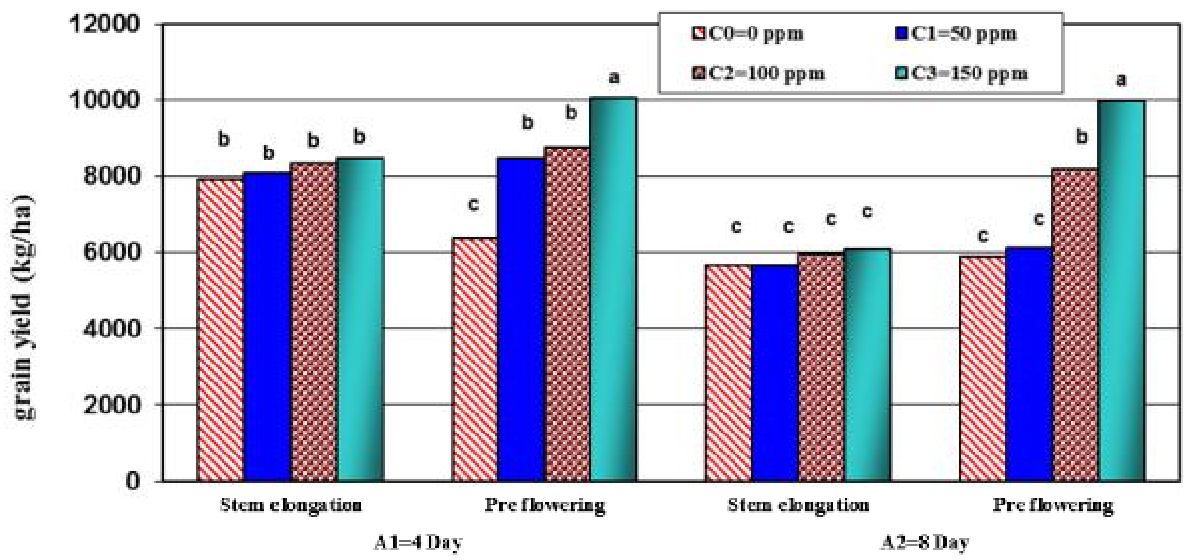

Fig. 6- Interaction effect of irrigation, glycine batine spraying time and concentration on corn yield

CONCLUSION

The results of this experiment showed that drought (by increasing irrigation interval) significantly decreased chlorophyll a, plant height, seed number per ear, seed weight and grain yield while proline content increased. Spraying with glycine betaine at both stem, elongation and pre flowering stages diminished detrimental effects of drought. Concentration of 150 ppm GB was superior to other concentration and pre flowering spraying was better than stem elongation. Therefore it could be suggest that in drought condition application of 150 ppm GB protects corn from detrimental effects of drought in similar agro-climate condition.

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