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Effect of glycine betaine and salinity on photosynthetic pigments and ion concentration of safflower

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Abstract

The influence of exogenous application of glycine betaine (GlyBet) was examined on photosynthetic pigments and ions concentration of safflower under salinity stress conditions. The experiment was performed in a randomized complete block design arranged as a factorial with three replications. Salinity treatments (0, 50, 100, and 150 mM) were applied using sodium chloride (NaCl). GlyBet (0, 10, 30, and 60 mM) sprayed onto leaves of safflower. The results showed that an increase in salinity levels led to an increase in Na^+ content and Na^+/K^+ ratio and a reduce in shoot and root fresh weight, chlorophyll, carotenoid, photosystem II (PS II) activity , K^+, and Ca^{2+} contents. In all stress levels, foliar application of 30 and 60 mM GlyBet led to an increase in chlorophyll a, b and total chlorophyll, carotenoids and PS II activity than control. Also, in all salinity levels, foliar application of GlyBet reduced Na^+ content and Na^+/K^+ ratio of the plants and increased their K^+ and Ca^{2+} contents. These results suggest that the application of 30 and 60 mM GlyBet under salt stress conditions had the largest effect on photosynthetic pigments, PS II activity, and K^+ and Ca^{2+} contents of safflower.

***Keywords*:** Glycinebetaine; Chlorophyll; Safflower; Salinity stress

1. Introduction

Salinity stress is one of the major stresses, especially in arid and semi-arid areas, which limits plant growth and productivity (Koca *et al*., 2007). There is a competition among urban, industrial, and agricultural sections in different areas for freshwater which decreases the share of freshwater usage for agriculture (Tilman *et al*., 2002). Salinity affects plant performance through various mechanisms. Osmotic stress and ion toxicity are two major consequences of salt stress with different effects on plant growth, which influence plant metabolism depending on salt concentration (Munns and Tester, 2008). Osmotic stress occurs in low salt concentration, while ion toxicity effect, which disturbs plant growth, occurs in high salinity levels (Hasegawa *et al*., 2000). Threshold salt concentration for these two effects is strongly affected by the amount of salt uptake, accumulation, and sensitivity of plant species (Parida and Das, 2005).

Photosynthesis is the most important process which is influenced by salinity. Reduced photosynthesis under salinity stress not only results in the closing of stomata and reduction in intracellular CO_2 concentration, but also affects non-stomata factors. Based on evidences, salinity influences photosynthetic enzymes, chlorophyll, and carotenoid as well (Stepien and Klobus, 2006).

GlyBet is an amphoteric quaternary ammonium compound which plays an important role as compatible solutes in plants under various types of environmental stresses such as high salinity level and low temperature. Plant species have different capacities for GlyBet synthesis, while some plants like spinach and barley accumulate relatively high levels of GlyBet in their chloroplasts, other plants such as arabidopsis and tobacco are not able to synthesize this compound (Sakamoto and Murata, 2002). It has been reported that plants

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which are capable of accumulating GlyBet naturally, grow well under drought and saline environment (Chen and Murata, 2008). Exogenous application of GlyBet may contribute to salinity stress tolerance in plants through its role in Na^+/K^+ ion homeostasis (Hamdia and Shaddad, 2010). Foliar application of GlyBet is a suggested method for inducing tolerance to stress conditions in crops which accumulate compatible solutes in low amounts or are unable to accumulate them (Ashraf and Foolad, 2007). Exogenous application of GlyBet was used for alleviating salinity stress in plants whether accumulating or not accumulating GlyBet, e.g. corn (Yang and Lu, 2005). There is a positive correlation between the level of GlyBet and the degree of salt tolerance in plants (Meek and Oosterhuis, 2000). The uptake rate and GlyBet concentration in plant tissues seem to be not only dependent on plant organs and their age but also on crop species and environmental factors. When GlyBet is used, it is quickly absorbed by the leaves and is transported to other organs, which help improve salinity stress tolerance (Makela *et al*., 2000).

Safflower (*Carthamus tinctorius* L.) is an oil seed crop that grows in arid and semi-arid areas. It has been reported that safflower is more sensitive to salinity in germination stage than other growth stages (Weiss, 2000). Seedling growth reduction has been reported in different cultivars of safflower under salt stress in germination stage (Kaya *et al*., 2003; Hussain *et al.,* 2016) and in maturity (Elias and Kaffka, 2002).

The aim of this study is to investigate the effect of foliar application of GlyBet on reducing the effects of salinity stress in safflower. This study evaluates the effect of GlyBet on photosynthetic pigments and Na^+, K^+, and Ca^{2+} contents of safflower under salinity stress conditions.

2. Materials and Methods

2.1. Growth conditions and treatments

A pot experiment was conducted at the research greenhouse of the Department of Agriculture, Vali-e-Asr University of Rafsanjan-Iran, on April, 2015 to assess the influence of GlyBet as foliar application on the shoot and root fresh weight photosynthetic pigments and ions contents of safflower (*Carthamus tinctorius* L. var. Soffeh) under non-stress (control) and salinity stress conditions. The experiment was performed in a randomized complete block design arranged as a factorial with three replications. The Factors were salinity in four levels (0, 50, 100, and 150 mM) and GlyBet in four levels (0, 10, 30, and 60 mM).

The pots (diameter: 20cm, height: 30cm and volume of 8 liters) were filled with perlite and coco peat (1:1) and planted 10 seeds of safflower in each pot. Nine days after seedling emergence, the pots were irrigated with a nutrient solution with half strength Hoagland's solution. Irrigation at intervals of 2 days was applied to the plant. Then the plants were thinned to keep five in each pot. After the fourth true leaves appeared (20 days after planting), salinity stress in the pot was created by adding 10, 30, and 60 mM NaCl, to half strength Hoagland's solution. Control plants (Non-stress treatment) were only irrigated with half strength Hoagland's solution. When plants were at approximately the six-leaves stage, (28 days after planting), Glybet spray was applied with 0, 10, 30, and 60 mM (in 0.1% Tween-20 solution) over three stages within a 7-day interval. Plant growth was carried out in a glasshouse with 14-h d^{-1} photoperiod, irradiance of 250 µmol m^{-2} s^{-1}, 32/20°C day/night temperature, 50–55% air humidity. After 50 days of sowing, samples of leaves were collected for evaluation of photosynthetic pigments and ions concentration.

2.2. Measuring the fresh weight of root and shoot

The plants were removed carefully with the root system and washed thoroughly. The aerial parts were separated from the roots. Then, the fresh weight of roots and shoots was measured in grams using an electronic balance, and the average was calculated.

2.3. Photosynthetic pigments

Chlorophyll a, b and carotenoeid contents were determined according to the method of Arnon (1949). For chlorophyll determination, the fully expanded young leaves were detached from the plants and were cleaned with deionized water to remove any surface contamination. Fresh leaf samples (0.2 g) were grounded in 80% acetone using a pestle and mortar. The absorbance was measured at 645 nm and 663 nm for chlorophyll and 470 nm for carotenoeid.

2.4. Measurement of PS II activity

The activity of PS II, expressed as F_v/F_m, was measured with a chlorophyll fluorimeter (model Pocket PEA, Hansatech, England).

Measurements were made on intact leaves with the fluorimeter after the plants were adapted to darkness for 30 min.

2.5. Determination of Na^+, K^+ and Ca^{2+} contents

The leaves were dried in 60 ^oC for 48-h. Then 1 gr of samples was powdered and burned in 550^oC for 6-h d^{-1} to obtain ash then ashes were digested in 10 mL of 1N HCl. The concentration of Na^+ and K^+ in the digested samples was determined using a flame photometer (Jenway, Model PFP7, England) and Ca^{2+} by atomic absorption (Atomic Absorption Spectrophotometer- GBC Avanta Ver. 1.33, Australia)

2.6. Statistical analysis

All statistical analysis was performed using the SAS 9.1.3 (SAS Institute, 2004) software. Mean comparison was performed using LSD test at the 5% level of significance ($P < 0.05$).

3. Results and Discussion

Root and shoot fresh weight was affected by the salinity and GlyBet (Table 1). The highest and lowest root fresh weight was observed in the control treatment and salinity of 150 mM, respectively. Application of GlyBet increased root fresh weight compared to the control (0 mM GlyBet) (Table 2). The highest and lowest shoot fresh weight obtained the non-stress conditions and 150 mM stress, respectively. The application of 60 mM GlyBet increased of fresh weight of shoot (Table 2). It has been reported that salinity stress decreased Root and shoot fresh weight in canola (Tuncturk *et al*., 2011) and sunflower (Akram *et al*., 2007). So, salinity stress may disturb plant metabolism and result in reduced growth.

Table1. Analysis of variance for root and shoot fresh weight in safflower under salinity and GlyBet treatments

S.O.V	d.f	Root fresh weight	Shoot fresh weight
Block	2	0.03^{ns}	2.31^{ns}
NaCl	3	5.24^{**}	72.97^{**}
GlyBet	3	1.78^{**}	26.40^{**}
NaCl * GlyBet	9	0.20^{ns}	1.57^{ns}
Error	30	0.29	3.05
C.V (%)		15.57	18.94

$^{ns, *}$ and ** Not significant, significant at 5% and 1% probability levels, respectively. d.f degree of freedom; CV, coefficient of variation, GlyBet, glycinebetaine

Table 2. Effects of GlyBet and salinity stress on root and shoot fresh weight in safflower

Treatments	Levels	Root fresh weight (g)	Shoot fresh weight (g)
GlyBet (mM)	0	2.991± 0.123b	7.71± 0.585c
	10	3.483±0.221a	8.41± 0.912bc
	30	3.641±0.236a	9.65± 0.874ab
	60	3.908±0.283a	11.09±0742a
NaCl (mM)	0	4.416±0.234a	12.30±·0.672a
	50	3.475± 0.132b	9.92±0.627b
	100	3.283± 0.202bc	8.04±0.503c
	150	2.850± 0.116c	6.60± 0.578c

Values are means ± SE of three replicates. Means followed by the samer letter are not significantly different (P<0.05), using LSD test

The effect of salinity, GlyBet and the interaction effect of them were significant on photosynthetic pigments and Fv/Fm (Table 3). In non-stress conditions, foliar application of 30 and 60 mM GlyBet to plants increased chlorophyll a content than control. In salinity levels of 50 and 100 mM, 30 and 60 mM GlyBet application increased chlorophyll a content compared to that in the control plants. Application of all GlyBet levels had no effect on chlorophyll a content in 150 mM salinity level (Table 4). The highest chlorophyll b content was related to non-stress level and 60 mM GlyBet treatment, while the lowest content was related to salinity level of 150 mM without the application of GlyBet (Table 4). In all salinity levels, application of GlyBet increased chlorophyll b content compared to the control plant.

In non-stress level and in salinity stress levels up to 100 mM, application of all GlyBet concentrations caused an increase in total chlorophyll compared to control plants. In stress level of 150 mM, foliar application of 30 and 60 mM GlyBet increased total chlorophyll content compared to the control plants (Table 4). In levels without salinity stress and 50 mM salinity, although there was no significant difference in carotenoid content among all GlyBet concentrations, they increased its

amount compared to non-stress plants. In 100 and 150 mM salinity, levels of 30 and 60 mM GlyBet increased carotenoid content compared to control (Table 4).

Salinity greatly influences the photosynthesis due to reduced chlorophyll and adverse effects on membrane stability (Parida *et al*., 2002). Reduced chlorophyll content in maize (Kaya *et al*., 2013) and safflower (Aymen *et al*., 2014; Siddiqi *et al*., 2011) during salinity stress has been reported. In salt-affected plants, reduction in chlorophyll content is probably due to the changes in protein-lipid ratio of protein-pigment complex or increased activity of chlorophyllase enzyme (Iyengar and Reddy, 1996). In salinity and drought stress, lack of balance between light absorption in photosynthesis and using NADPH in carbon fixation may induce excess energy and cause damage to the photosynthetic apparatus (Hasegawa *et al*., 2000). Higher leaf chlorophyll content is another factor (non-stomatal limitation) which may contribute to the higher photosynthetic capacity of plant in salinity conditions (Raza *et al*., 2007). Also, other researchers have reported that using GlyBet increased chlorophyll b and total chlorophyll contents in wheat (Raza *et al*., 2007), chlorophyll a and b contents in wheat (Kaya *et al*., 2013), and total chlorophyll content in perennial Ryegrass (Hu *et al*., 2012) under salinity conditions. Makela *et al*. (2000) have also reported that foliar application of GlyBet improved chlorophyll content in tomatoes under salinity stress. It seems that high accumulation of GlyBet in safflower plants has considerably helped protect the photosynthetic capacity and integrity of cell membranes under salinity stress.

Table 3. Analysis of variance for photosynthetic pigments and Fv/Fm in safflower under salinity and GlyBet treatments

S.O.V	d.f	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotonoied	F_v/F_m
Block	2	0.01 ^{ns}	0.0002 ^{ns}	0.016 ^{ns}	0.0005 ^{ns}	0.0006 ^{ns}
NaCl	3	0.79 **	0.0747 **	1.343 **	0.0783 **	0.0714 **
GlyBet	3	0.22 **	0.1340 **	0.687 **	0.0845 **	0.1357 **
NaCl * GlyBet	9	0.02 *	0.0025 *	0.030 *	0.0059 *	0.0025 *
Error	30	0.01	0.0011	0.010	0.0024	0.0011
C.V (%)		9.16	9.7500	7.280	8.1200	6.2100

$^{ns, *}$ and ** Not significant, significant at 5% and 1% probability levels, respectively. d.f degree of freedom; CV, coefficient of variation, GlyBet, glycinebetaine

Table 4. Different concentrations effects of GlyBet on photosynthetic pigments and F_v/F_m in safflower under salinity stress conditions

NaCl (mM)	GlyBet (mM)	Chlorophyll a (mg.g $^{-1}$FW)	Chlorophyll b (mg.g $^{-1}$FW)	Total Chlorophyll (mg.g $^{-1}$FW)	Carotonoied (mg.g $^{-1}$FW)	F_v/F_m
0	0	1.11± 0.005cd	0.313±0.063efgh	1.42±0.067de	0.601±0.004cde	0.513±0.065efg
	10	1.26±0.049bc	0.403±0.003cd	1.67±0.047c	0.676±0.024abc	0.606±0.007cd
	30	1.38±0.050b	0.430±0.015c	1.81±0.059bc	0.694±0.053ab	0.626±0.013c
	60	1.57±0.041a	0.590±0.021a	2.16±0.053a	0.729±0.013a	0.796±0.019a
50	0	1.03±0.014d	0.276±0.008 h	1.31±0.010e	0.559±0.010ef	0.480±0.011gh
	10	1.15±0.015cd	0.340±0.00ef	1.49±0.015d	0.666±0.004abc	0.54±0.006ef
	30	1.35±0.009b	0.360±0.00de	1.71±0.009c	0.667±0.010abc	0.56±0.00de
	60	1.42±0.060ab	0.516±0.019b	1.93±0.050b	0.727±0.031a	0.716±0.019b
100	0	0.81±0.065e	0.180±0.00i	1±0.066fg	0.513±0.032f	0.383±0.008ij
	10	1.02±0.054d	0.280±0.00gh	1.31±0.055e	0.571±0.016def	0.480±0.00gh
	30	1.14±0.050cd	0.323±0.022efgh	1.46±0.046de	0.613±0.011bcde	0.526±0.019efg
	60	1.18±0.01c	0.486±0.003b	1.67±0.019c	0.712±0.039a	0.686±0.009b
150	0	0.736±0.006e	0.143±0.003j	0.87±0.006g	0.354±0.062g	0.340±0.005j
	10	0.746±0.023e	0.220±0.00i	0.96±0.023fg	0.403±0.003g	0.436±0.003hi
	30	0.753±0.116e	0.300±0.00fgh	1.06±0.114f	0.572±0.012def	0.503±0.003fg
	60	0.796±0.098e	0.333±0.003efg	1.10±0.144f	0.649±0.024abcd	0.540±0.006ef

Values are means ± SE of three replicates. Means followed by the samer letter are not significantly different (P<0.05), using LSD test

As salinity increased, F_v/F_m reduced. In all salt stress levels, using GlyBet increaseded increased F_v/F_m significantly (Table 4). It seems when plants are exposed to drought or salinity stress, Fv/Fm plays a critical role in photo inhibition (Maxwell and Johnson, 2000). Results show that by increasing salinity, F_v/F_m was reduced and in all salinity levels, foliar application of 30 and 60 mM GlyBet increased F_v/F_m content compared to that of the control plants (Table 4). Reduced F_v/F_m has been reported in some safflower cultivars in salinity conditions by Erdal and Cakirlar (2014). Our results confirm the effect of GlyBet on maintaining activity and integrity of cell membrane of safflower leaf in photosystem II.

The effect of salinity, GlyBet and the interaction effect of them were significant on

Na^+ content, K^+ content, Na^+/K^+ ratio and Ca^{2+} content (Table 4). In 50 mM salinity, all concentrations of GlyBet reduced Na^+ content in comparison to the control plants and 100 and 150 mM levels of stress, also the application of 30 and 60 mM GlyBet reduced Na^+ content in comparison to the control plants (Table 6). Salinity stress decreased K^+ content and GlyBet was able to mitigate this reduction. In non-stress level application of 60 mM GlyBet increased K^+ content compared to the control plants. In 50 mM salinity, all concentrations of GlyBet increased K^+ content. In 100 mM level of stress, application of 30 and 60 mM increased K^+ content. In 150 mM level of salinity stress, there was no significant difference between different concentrations of GlyBet and the control plants in K^+ content (Table 6). Increasing the salinity level increased the ratio of Na^+/K^+. In all salinity levels, the application of 30 and 60 mM GlyBet created a significant difference in comparison to the control plants and reduced Na^+/K^+ ratio (Table 6).

In absence of salinity stress and 50 mM salinity, all levels of GlyBet increased Ca^{2+} content compared to that of the control plants. In 100 mM salinity stress level, foliar application of 60 mM GlyBet increased Ca^{2+} content compared to that of the control plants. In 150 mM salinity, levels of 30 and 60 mM GlyBet increased Ca^{2+} content (Table 6).

Under salinity stress, antagonistic interaction between Na^+ and K^+, reduced K^+ concentration in stems and roots (Karmoker *et al*., 2008). It has also been demonstrated that salinity stress has reduced K^+ content in safflower (Siddiqi *et al*., 2011) and maize (Kaya *et al*., 2013) and reduced Ca^{2+} content in various plant species including safflower (Siddiqi *et al*., 2011), tomato (Navarro *et al*., 2000), and strawberry (Kaya *et al*., 2002). High levels of Na^+ or Na^+/K^+ ratio can disturb different enzymatic processes in cytoplasm (Blaha *et al.*, 2000). It has been reported that GlyBet may play a role in protecting cytosolic K^+ and reducing Na^+ uptake by an apoplastic flow (Sobahan *et al*., 2009). Foliar application of GlyBet reduced Na^+ content and accumulation, and maintained K^+ in rice stem in salinity conditions (Lutts *et al*., 1999). Kaya *et al*., (2013) also showed that under salinity stress, the application of GlyBet reduced Na^+ content or Na^+/K^+ ratio and increased K^+ and Ca^{2+} contents in maize. Also, the application of GlyBet led to an increase in K^+ concentration in tomato (Heuer, 2003), rice (Rahman *et al*., 2002), and barely (Cuin and Shabala, 2007) and increased Ca^{2+} content in wheat (Badran *et al*., 2015) under salinity stress conditions**.** Maintaining high concentrations of cytosolic K^+ and Na^+ and K^+ balance is considered to be one of the most fundamental mechanisms of tolerance to salinity in plants. Our results confirm the effect of GlyBet on maintain K^+ content and reduce Na^+ content.

Table 5. Analysis of variance for ion concentrations in safflower under salinity and GlyBet treatments

S.O.V	d.f	Na^+	K^+	Na^+/K^+	Ca^{2+}
Block	2	000005^{ns}	0.0004^{ns}	0.0005^{ns}	0.001^{ns}
NaCl	3	0.00166^{**}	0.0082^{**}	0.0396^{**}	0.132^{**}
GlyBet	3	0.000896**	0.0026**	0.0200**	0.271**
NaCl * GlyBet	9	0.000029^{**}	0.0008^{**}	0.0014^{**}	0.004^{*}
Error	30	0.000008	0.0001	0.0003	0.001
C.V (%)		5.83	4.86	10.23	7.83

$^{ns, *}$ and ** Not significant, significant at 5% and 1% probability levels, respectively. d.f degree of freedom; CV, coefficient of variation, GlyBet, glycinebetaine

Table 6. Different concentrations effect of GlyBet on ion concentrations in safflower under salinity stress conditions.

NaCl (mM)	GlyBet (mM)	Na^+ (mmol/g)	K^+ (mmol/g)	Na^+/K^+ Ratio	Ca^{2+} (%)
0	0	0.041±0.001fg	0.30±0.003bc	0.13±0.0033gh	0.466±0.033de
	10	0.040±0.00gh	0.30±0.133c	0.13±0.0066gh	0.600±0.00c
	30	0.033±0.001i	0.30±0.003bc	0.11±0.0033h	0.600±0.00c
	60	0.026±0.002j	0.33±0.0152a	0.07±0.0120i	0.866±0.033a
50	0	0.058±0.003cd	0.24±0.015g	0.23±0.030cd	0.400±0.00e
	10	0.047±0.002e	0.30±0.003c	0.15±0.008fg	0.533±0.033cd
	30	0.044±0.001efg	0.30±0.003c	0.14±0.006fg	0.566±0.033c
	60	0.035±0.001hi	0.32±0.006ab	0.11±0.0100h	0.800±0.00a
100	0	0.062±0.0005bc	0.25±0.013fg	0.24 ±0.013bc	0.400±0.00e
	10	0.059±0.0011bcd	0.26±0.008def	0.22 ±0.008cd	0.400±0.00e
	30	0.054±0.0003d	0.27±0.006de	0.20±0.005de	0.466±0.033de
	60	0.045±0.0030ef	0.28±0.0d	0.17 ±0.012ef	0.700±0.00b
150	0	0.071±0.0006a	0.25±0.00fg	0.29 ±0.005a	0.266±0.033f
	10	0.069±0.0013a	0.25±0.00fg	0.27 ±0.0ab	0.300±0.00f
	30	0.063±0.0013b	0.25±0.0066gf	0.25 ±0.008bc	0.433±0.033e
	60	0.045±0.0003e	0.25±0.0033efg	0.15 ±0.006fg	0.566±0.033c

Values are means ± SE of three replicates. Means followed by the samer letter are not significantly different (P<0.05), using LSD test

4. Conclusion

The results of the present study show that GlyBet had a modifying role in the performance of photosynthetic and nutrition system under salinity stress conditions. Foliar application 30 and 60 mM GlyBet had more chlorophyll a, b, total chlorophyll, and carotenoid contents compared to that of the control plants under salinity stress conditions. When plants were exposed to salinity stress, foliar application of GlyBet increased K^+ and Ca^{2+} uptake by inhibiting Na^+ uptake. Therefore, GlyBet can be taken into consideration as a promising substance for removing the effects of salt stress and its positive aspects is employed in agriculture of saline areas. Of course, field experiments are clearly required to completely ensure the effectiveness of exogenous application of this substance.

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