IMPACT OF EXOGENOUSLY APPLIED GLYCINE BETAINE ON PHYSIOLOGICAL ATTRIBUTES OF SUNFLOWER UNDER DROUGHT STRESS

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ABSTRACT

Scarcity of water causes physiological, biochemical & oxidative damages in sunflower (*Helianthus annuus* L). Effect of exogenous glycine betaine (GB) application through foliar and Hoagland's nutrient solution in amelioration of water stress of sunflower hybrids was studied. Appropriate time, mode and doze of GB application were tested. Three levels of irrigation were used normal irrigation, no irrigation at vegetative and reproductive stages. Three levels of glycine betaine i.e. 0, 75 mM and 125 mM were applied by means of foliar application at vegetative and flowering stage. Morphological parameters such as root and shoot length, fresh and dry weights were recorded three weeks after the GB application. Physiological and biochemical parameters including leaf water and osmotic potential, turgor potential, relative water content, photosynthetic rate, chlorophyll content, protein, soluble sugars and amino acid content were recorded. Nevertheless exogenous GB improved these attributes. However, the irrigated mode of exogenous GB application was more effective than foliar application, among the doses applied 125mM proved more effective. Role of exogenously applied glycine betaine was more pronounced at flowering stage than vegetative stage.

Keywords: Drought, Glycine betaine, Sunflower, Exogenous.

INTRODUCTION

Water stress is being considered the primary factor in limiting crop production (Ashraf and Harris, 2013) and affect plant growth and productivity (Chaves *et al*., 2009). Glycinebetaine (GB) is an amino acid derivative and many scientists hypothesized that GB in low concentration can improve stress tolerance. GB reduces lipid peroxidation of the cell membranes and prevents deterioration of photosynthetic protein complexes (Holmstrom *et al*., 2000; Iqbal and Ashraf, 2008). Endogenous glycinebetaine concentrations show variation in plants, with some taxa accumulating the compound naturally, whiles not others (Bluden *et al*., 2001).

Sunflower (*Helianthus annuus*) is important oil seed crop and its yield is adversely affected by water stress. Water stress affects sunflower plant growth and biomass production (Tahir and Mehdi, 2001). Water stress at vegetative and reproductive growth phase in sunflower may result in 61% and 40% yield reduction, respectively (Bluden *et al*., 2001; Iqbal, 2004). Osmolytes production in water stress condition is an important physiological adaptation to minimize the detrimental effect of stress and achieved by the accumulation of osmolytes such as proline and glycinebetaine (Holmstrom *et al*., 2000). GB accumulation in plants helps to reduce adverse effect of water stress (Iqbal, 2004; Yang and Lu, 2005). Exogenous GB may have also enhanced the ability of cells to retain water without disturbing the cellular functions. Limited knowledge is available about, effective concentrations of GB, timings and frequency of exogenous GB application. This is prerequisite for GB role in crop stress tolerance. Therefore, the present study is a step towards determining appropriate doze, mode and time of application of GB that could be more beneficial to alleviate the water deficit condition.

MATERIALS AND METHODS

This study was carried out at glass house of department of botany PMAS Arid Agriculture University, Rawalpindi. Seeds of both hybrids (hybrids Hyoleic-41 and Hysun-33) were brought from crop science, NARC Islamabad. Sodium hypochlorite 5% solution was used for surface sterilization. Uniform size seedlings were transplanted to earthen pots containing 10 kg soil and having 40 cm diameter. Soil used: compost, sand, farmyard in a ratio of 2:1:1. Drought stress was imposed by obstructing the water for 9 days at two stages of plant that is vegetative and reproductive stages. Three treatments of glycine betaine were applied with the onset of drought stress at both the stages of sunflower hybrids. Two differnet mode of Glycine betain application were applied wiz irrigation of plant with GB dissolved in Hogland nutrient medium; and through foliar application by hogland solution subsequently.

Morphological Attributes:

Total number of leaves per plant were counted for vegetative growth features after application of GB for three weeks. Root and shoot fresh weight was determined after harvesting. For dry weight of plants the root and shoots were dried at 65 ^{0}C in oven.

Physiological Attributes:

For the determination of relative water content, Unyayar *et al*. (2005) method was adopted. Leaf water potential was calculated by Scholander pressure chamber (Scholander *et al*., 1965). Freezing point osmometer was used for calculation of osmotic potential of flag leaf, turgor pressure was calculated as well (Garnier and Berger, 1985). For each treatment photosynthetic rate was measured using a portable photosynthesis system (Infrared Gas Analyzer. ADC-LCA-4). Leaf chlorophyll content was analyzed by method of Hiscox and Israelstam (1976)

Biochemical Attributes:

A sample extract was used to determine spectrophotometrically by Bradford method for the determination of protein (1976), Ninhydrin method was used for the determination of amino acids in flag leaf extarct (Hamilton and Van Slyke, 1943). Soluble sugars were estimated by Dubois *et al*. (1951) method. Bates *et al*. (1973) method was used to calculate proline content of plants.

Statistical Analysis

Data were statistically analyzed using Statistics 8.1 program by comparing means by LSD at significane level $P \leq 0.05$.

RESULTS

Morphological Parameters

Water stress is one of major abiotic stresses that drastically reduce plant growth and productivity. Water stress decreased shoot fresh and dry weight at both vegetative and reproductive stages. When shoot fresh weight data was subjected to ANOVA ($p \leq 0.05$) significant difference was observed. In stress conditions shoot fresh weight was comparatively higher at reproductive stage 125 mM Gb via foliar application as compared to irrigation GB application (Fig. 1).Among the two varieties v2 (Hyoleic-41) grew better under stress condition. Hyoleic-41 was responeded more efficiently as compared to Hysun-33 under water stress condition Results revealed that maximum value of shoot fresh weight was observe at T8 and minimum value was observed at T3. Shoot fresh weight of plants with foliar 125 mM GB application was highest with increase of 27% as compared to other GB concentrations. Shoot dry weight when subjected to ANOVA ($p \leq 0.05$) showed considerable difference was observed among all treatments. The most effective treatment was 125 mM GB foliar application at reproductive stage (Fig. 2).GB foliar application at reproductive stage show 29% increase in shoot dry weight and 25% increase due to GB application via irrigation at reproductive stage. The highest shoot dry weight was recorded at T8 (foliar 125 mM Gb application @ reproductive stage) Sunflower shoot and root fresh and dry weight considerably decline under water stress condition. For root fresh and dry weight of sunflower plant under water stress condition subjected to ANOVA ($p \leq 0.05$) a considerable difference was recorded among all treatments. Results of root fresh weight depict that highest root fresh weight was observed at 75 mM GB applied through irrigation at reproductive stage (Fig. 3 & 4). The most effective treatment was T11 for root fresh and dry weight followed by T10 (125 mM GB applied via irrigation at vegetative stage). In root fresh and dry weight GB applied via irrigation was more effective as compared to foliar GB application. Root and shoot fresh weight was considerably increased in water stress condition due to GB application. GB act as growth regulator and enhance root length under stress condition. Root length of Hysun-33 and Hyoleic41 was expressively increased at T8 (foliar applied 125 mM GB @ reproductive stage) (Fig. 5). Root length was recorded at T8 and T 12 with an increase of 13% and 12%. ANOVA analysis of number of leaves data showed significant difference among treatments and cultivars. Gb application via foliar or irrigation both methods were effective for number of leaves. Highest number of leaves were observed at T12 with an increase of 46% followed by T8 with an increase of 35% (Fig 6). Shoot growth is severely affected due to water shortage and shoot length was drastically reduced in water stress condition. Statistical analysis of shoot length data was showing significant difference at ($p \leq 0.05$). GB application via foliar spray was seems to more effective in case of shoot length as compared to GB application through irrigation (Fig 7). Hyoleic41 was observed with maximum shoot length as compared to Hysun-33. Shoot length maximum value at T7 & T8 with an increase of 27% and 22%, respectively.

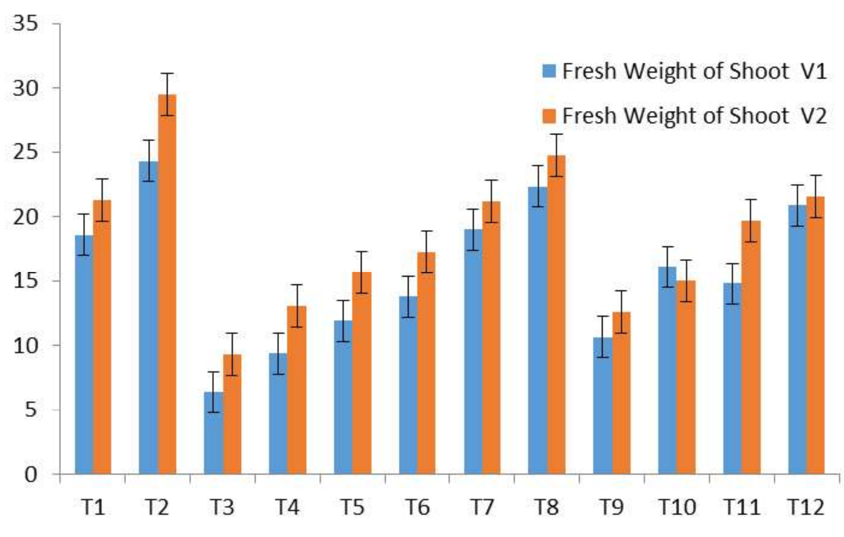

Figure 1: Impact of GB application on shoot fresh weight (gm) of sunflower

T1=Normal irrigation(vegetative), T2= Normal irrigation(reproductive), T3 = stress at vegetative stage, T4= Stress at reproductive stage, T5= Foliar GB application @75mM at vegetative stage, t6= Foliar GB application GB @125mM at vegetative stage, T7= Foliar GB application GB @75mM at reproductive stage, T8= Foliar GB application GB @125mM at reproductive stage, T9= Irrigation of GB @75mM at vegetative stage T10= Irrigation of GB @125mM at vegetative stage, T11= Irrigation of GB @75mM at reproductive stage, T12= Irrigation of GB @125mM at reproductive stage.

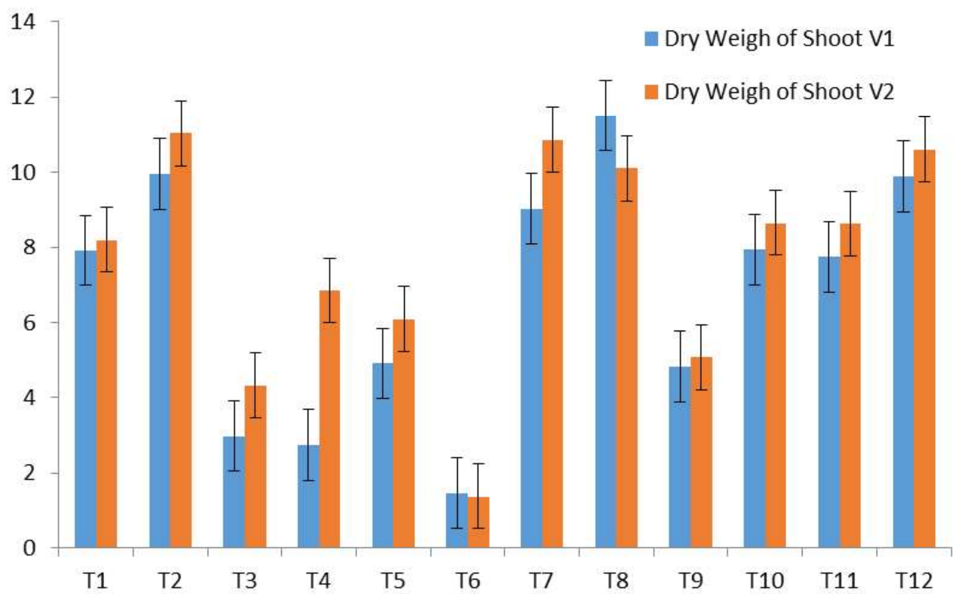

Figure 2: Impact of GB application on shoot dry weight (gm) of sunflower

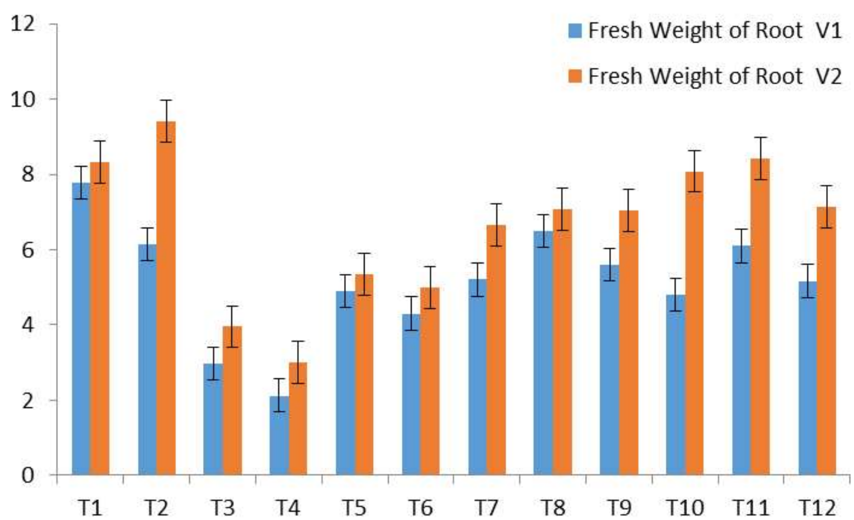

Figure 3: Impact of GB application on root fresh weight (gm) of sunflower

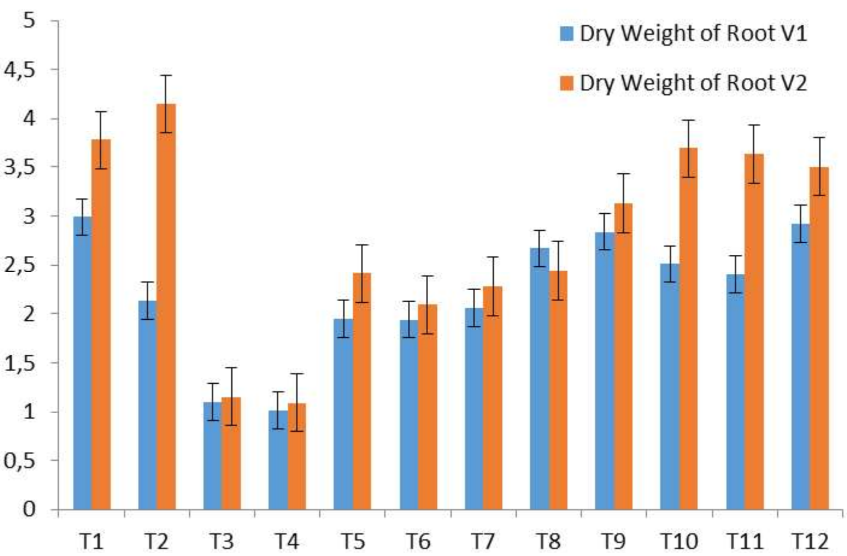

Figure 4: Impact of GB application on root dry weight (gm) of sunflower

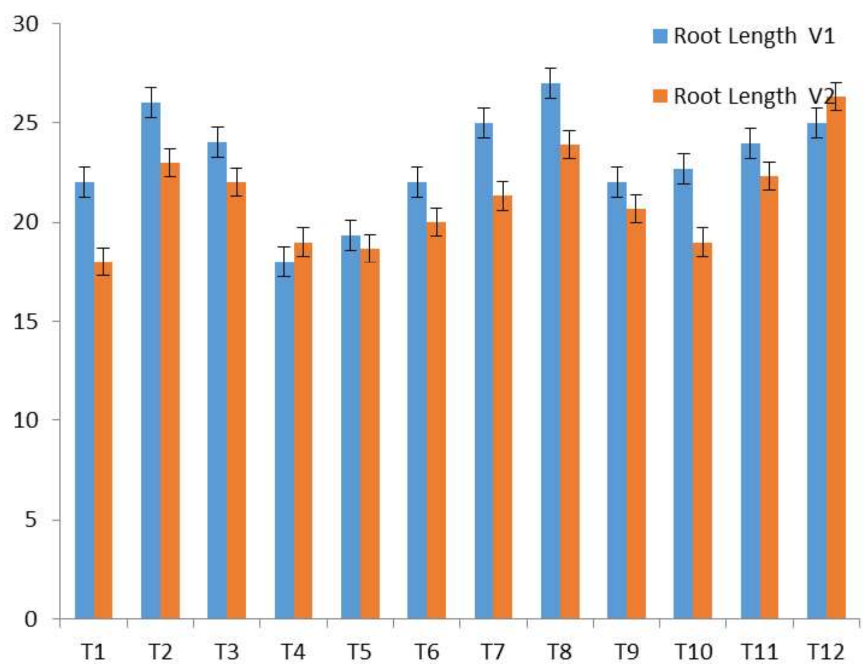

Figure 5: Impact of GB application on root length (cm) of sunflower

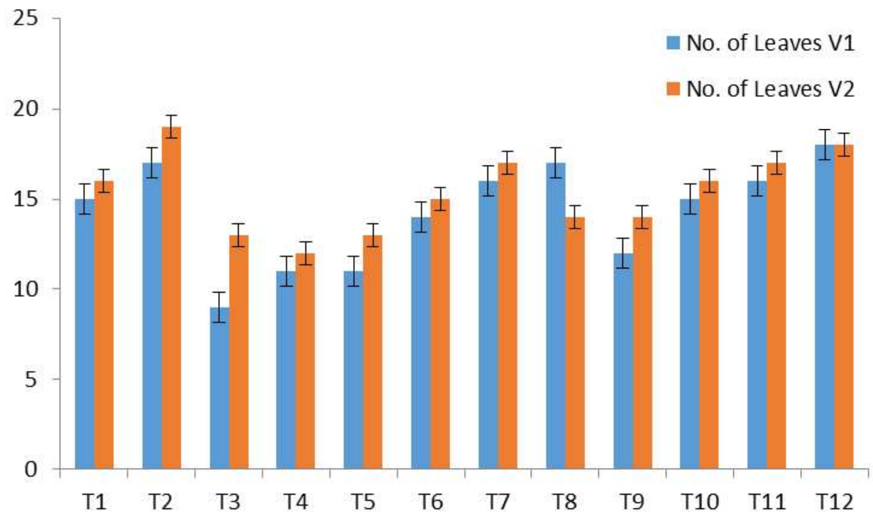

Figure 6: Impact of GB application on number of leaves of sunflower

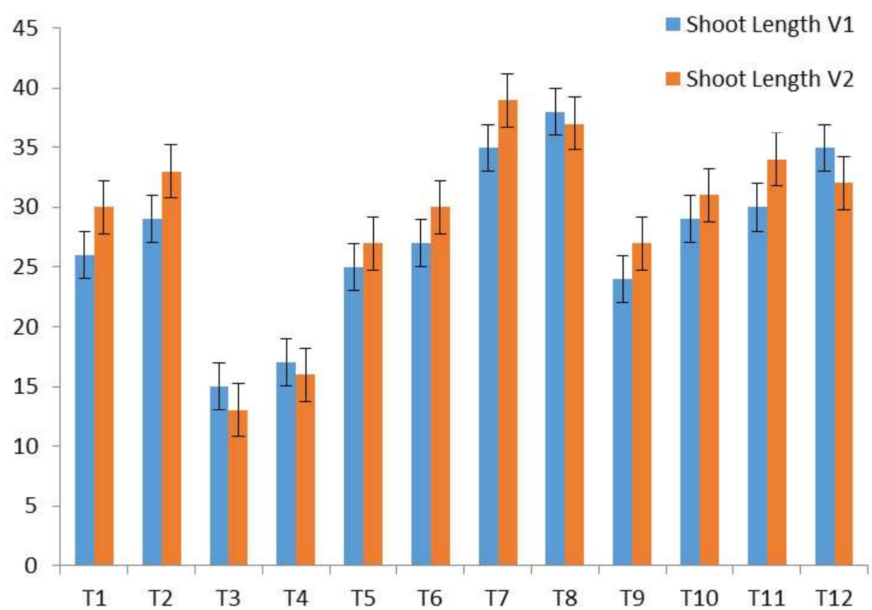

Figure 7: Impact of GB application on shoot length (cm) of sunflower

Physiological Parameters

Drought condition has prominent effect on physiological processes of plant and drastically effect photosynthetic machinery of plant. A considerable decline in total chlorophyll content of plant was observed under water stress condition as compared to control condition. Total chlorophyll content data were subjected to ANOVA ($p \leq 0.05$) and significant difference was detected among all treatments and sunflower cultivars (Fig 8). The most effective treatment for total chlorophyll content in foliar application was T6 (125 mM GB application @vegetative stage) and T8 (125 mM GB application @vegetative stage). Total chlorophyll content showed an increase of 15% and 11% due to GB application via foliar and irrigation as compared to control condition. Chlorophyll a and chlorophyll b show similar trend (Fig 9 & 10). Treatment effects of chlorophyll a (foliar & irrigated application of 125 mM GB) were more significant at reproductive stage with an increase of 10 and 8%, respectively. Chlorophyll b was negatively influenced by water stress and effective treatment for chlorophyll b was T8 with an increase of 15%. Rate of photosynthesis also had a pattern analogous to transpiration rate in normally watered plants. Photosynthetic rate data were subjected to ANOVA ($p \leq 0.05$) and obvious difference was recorded. GB application via foliar spray was effective for photosynthesis rate (Fig 11). Photosynthetic rate were increased 15% because of GB application as compared to respective control. The present study findings confirmed that GB application at vegetative and reproductive stage are effective to enhance photosynthesis. Plant water relation drastically influenced by water stress. Relative water content results were subjected to ANOVA ($p \leq 0.05$) and showed considerable difference. Relative water content of GB treated plants show an increase of 19% as compared to respective control (Fig 12). The most effective treatment was foliar GB application at reproductive stage with an increase of 17%. Results of water potential were subjected to ANOVA ($p \leq 0.05$) and noteworthy difference was observed (Fig 13). In drought condition without GB application 30% reduction in water potential was observed. The most effective treatments for water potential T6 (foliar 125 mM Gb applied @vegitative stage) and T10 (125 mM Gb applied via irrigation @ vegetative stage). Highest values of water potential were observed in plants treated with GB foliar spray. ANOVA analysis of osmotic potential results showed significant difference at ($p \leq 0.05$) (Fig 14). Osmotic potential of plant without GB treatment under water stress with an increase of 17 and 20 % at vegetative and reproductive stages, respectively. Turgor potential play an important role to maintain turgidity of cell. Turgor potential was recorded and a considerable difference was found (Fig 15).

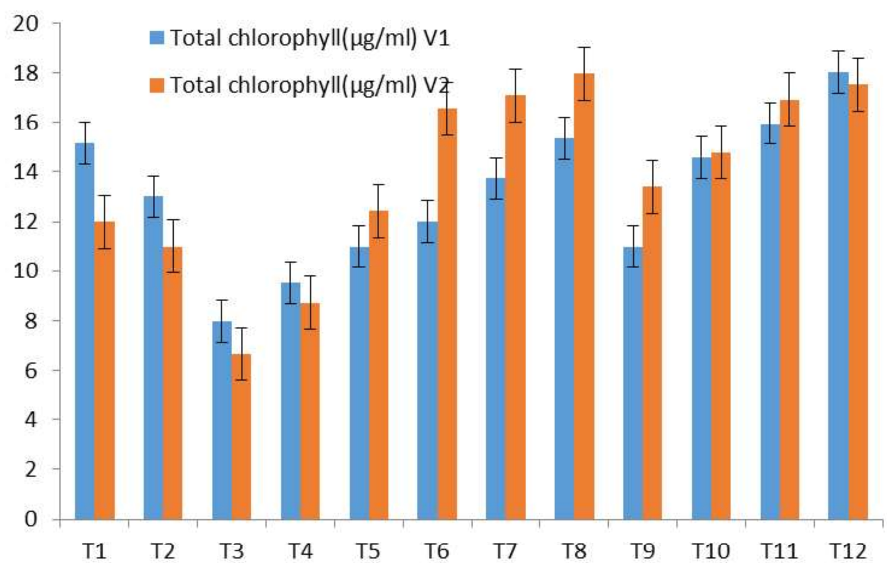

Figure 8: Impact of GB application on total chlorophyll of sunflower

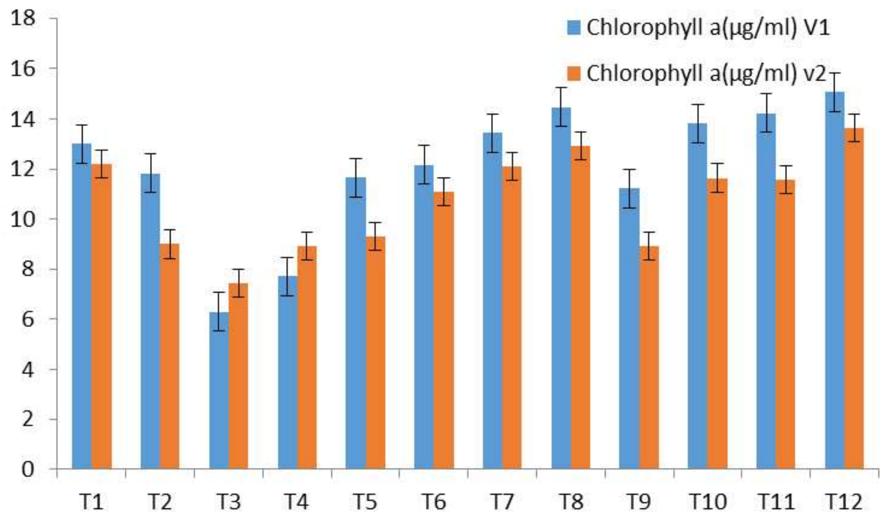

Figure 9: Impact of GB application on chlorophyll a of sunflower

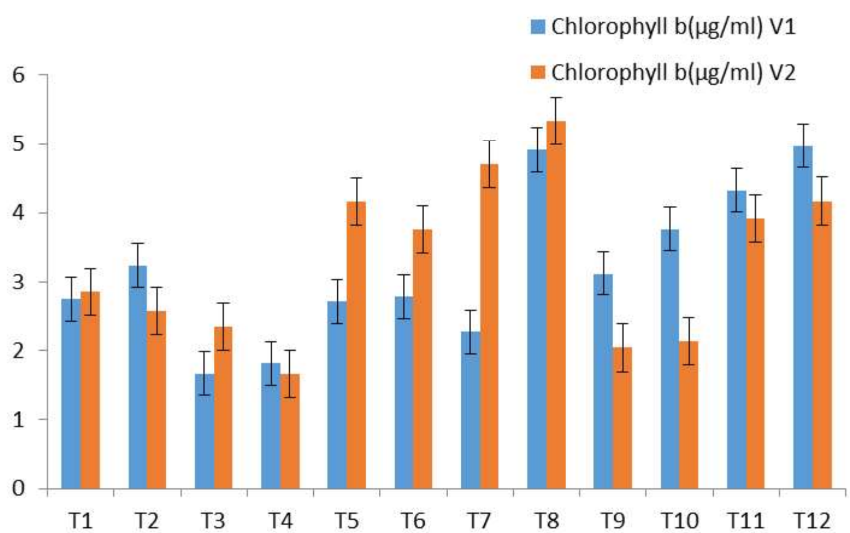

Figure 10: Impact of GB application on chlorophyll b of sunflower

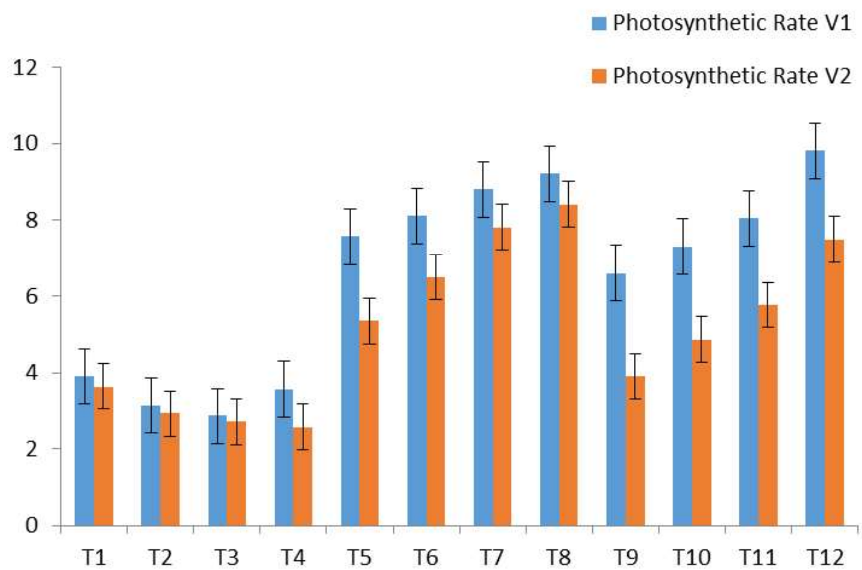

Figure 11: Impact of GB application on Photosynthetic rate of sunflower

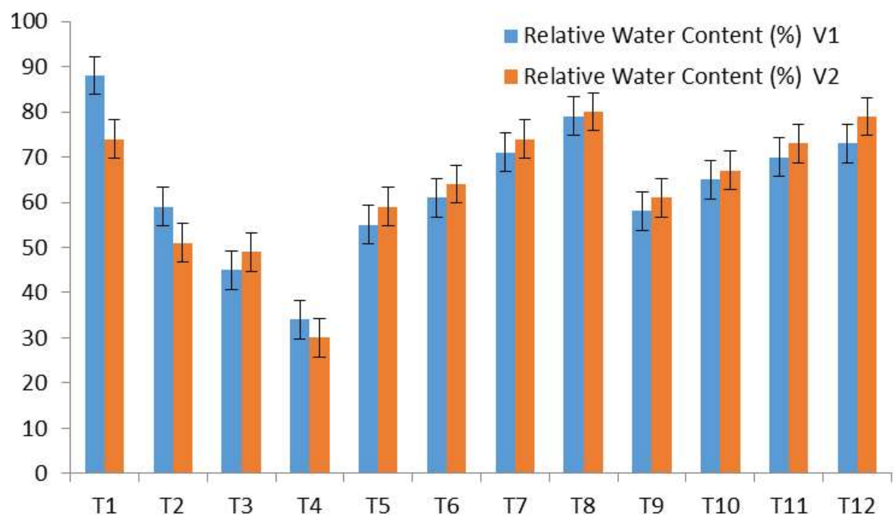

Figure 12: Impact of GB application on relative water content of sunflower

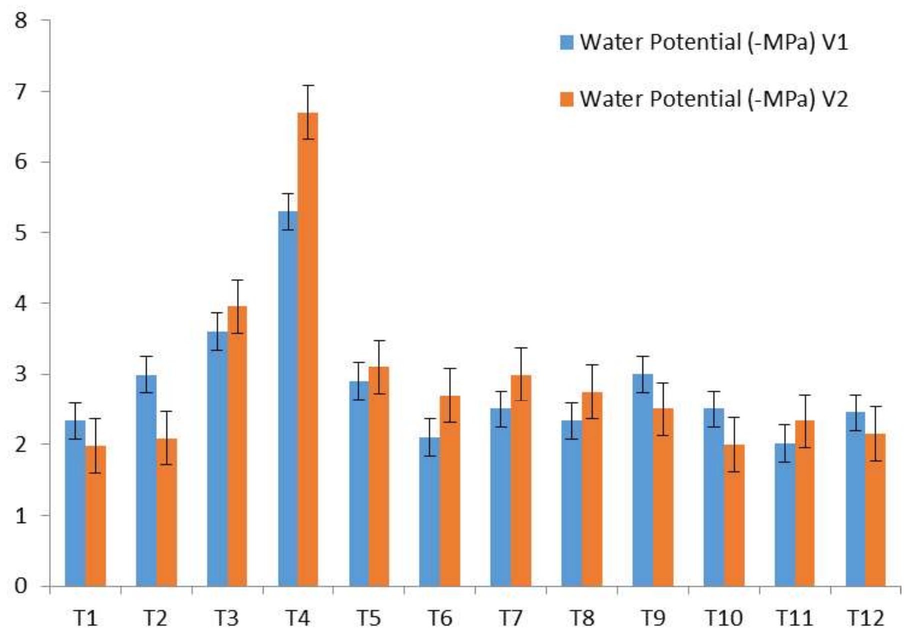

Figure 13: Impact of GB application on Water potential of sunflower

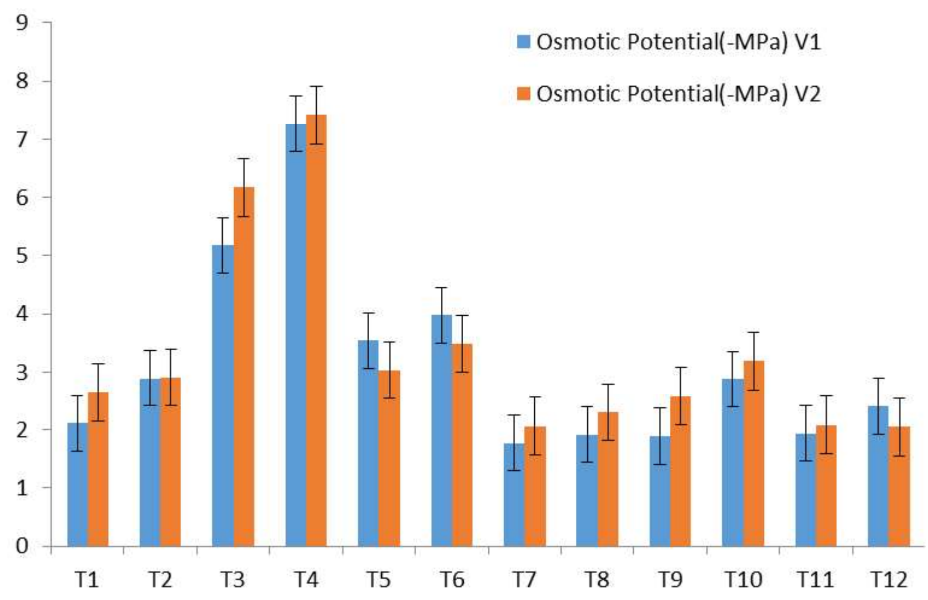

Figure 14: Impact of GB application on osmotic potential of sunflower

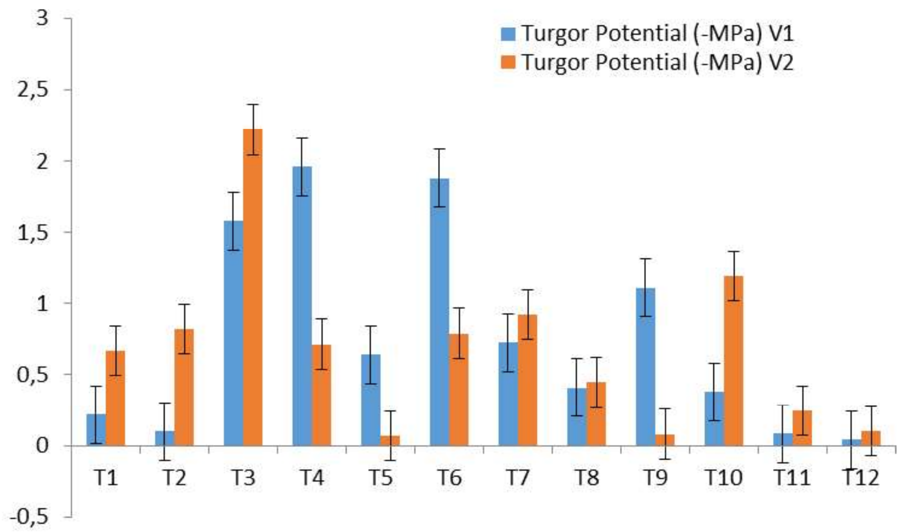

Figure 15: Impact of GB application on Turgor potential of sunflower

Biochemical Parameter

When plants are exposed to abiotic stress condition plant produces different types of osmo protectants that helps plants to tolerate unfavorable condition. Results of protein content were showing significant difference when subjected to ANOVA ($p \leq 0.05$). An increase of 13% of protein content in plants was recorded under water stress (Fig 16). The most effective treatment for GB treated plants were T6 (foliar 125 mM GB application at vegetative stage) and T7 (foliar 125 mM GB application at productive stage). In drought stress condition, it was observed that 21 % increase in free amino acid due to GB treatment via irrigation (Fig 17). The most effective Gb concentration for free amino acid was 125 mM at reproductive stage. Similarly higher content of proline content was observed in water stress condition. Results of proline were subjected to ANOVA and significant difference was observed among treatments (Fig 18). Maximum proline production was observed in GB treated plants with an increase of 23% as compared to respective control. High amount of total soluble sugar was found in water stress (Fig 19). Because in less amount of water, soluble sugar accumulate to combat stress condition. Under water stress without GB treatment an increase of 14% soluble sugar content was observed. Results of soluble sugar content was show significant difference among treatments ($p \leq 0.05$).

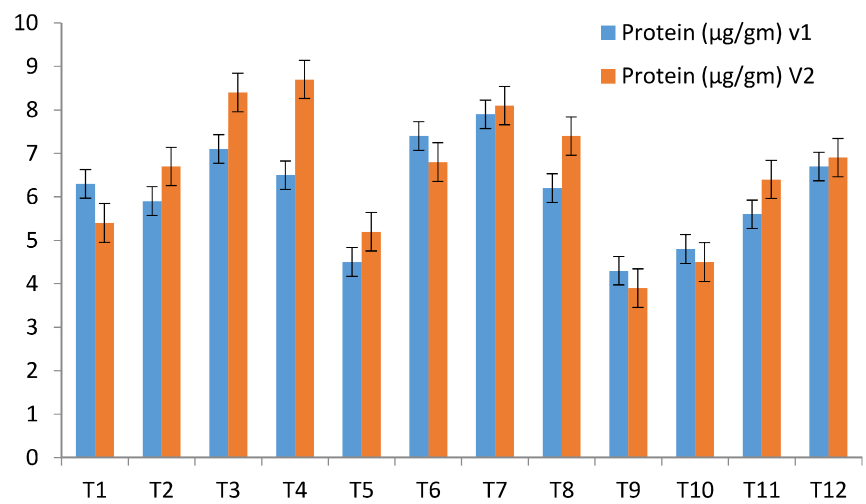

Figure 16: Impact of GB application on protein content of sunflower

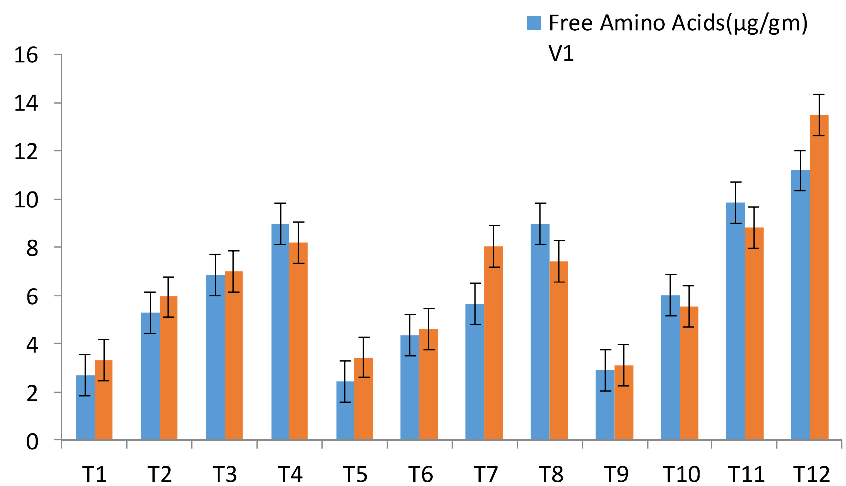

Figure 17: Impact of GB application on free amino acids of sunflower

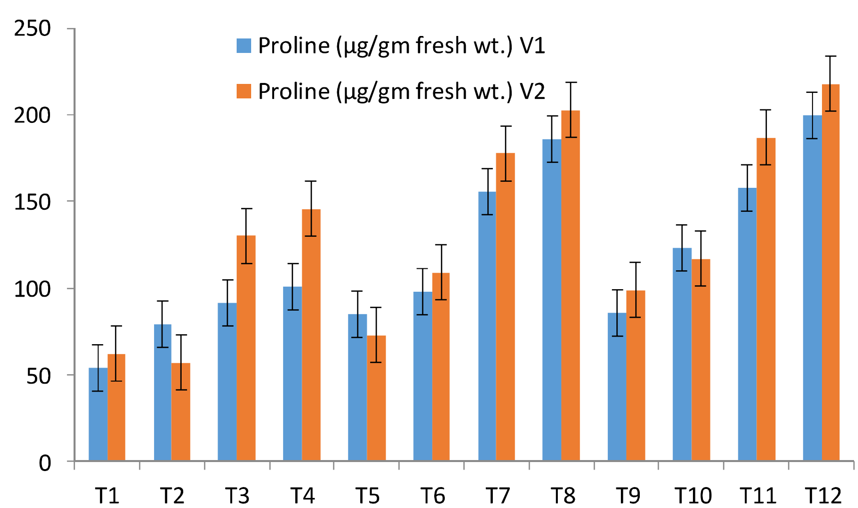

Figure 18: Impact of GB application on proline content of sunflower

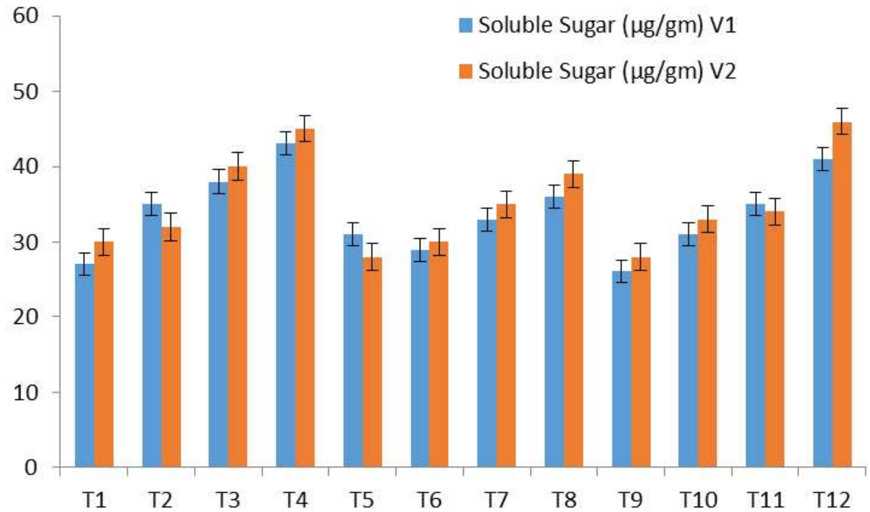

Figure 19: Impact of GB application on soluble sugar of sunflower

DISCUSSION

Drastic decrease in fresh weight of both hybrid plants was recorded under water deficit environment. As water stress considerably reduced the fresh weight of wheat crop (Rane *et al*., 2001), *Abelmoschus esculentum* (Rane *et al*., 2001) and pearl millet (Kusaka *et al*., 2005). The upshot for suppression cell growth and cell expansion due to the lower turgor pressure may results in such drastic decrease. Exogenous application of GB mitigates the stress effect and increased the fresh weight of sunflower hybrids at both the growth stages. Both hybrids have shown better results under irrigated mode of GB application as compared to foliar application.

The effect of irrigation levels was significant for dry weight of both the hybrid varieties under drought condition as water scarcity drastically decreased the dry weight of plants. This decrease in dry weight might be due to reduced photosynthetic activity, leaf senescence and plant growth (Rane *et al*., 2001; Kusaka *et al*., 2005).

Endogenously synthesized glycinebetaine improves the growth of above ground biomass following foliar application of GB. GB stabilize the turgor pressure and enzymes involved in amino acid metabolism, even at leaf concentrations upto 500 milliMole (Sankar *et al*., 2007). Laurie and Stewart (1990) reported that exogenous application of GB improved the growth in sunflower under drought and our results are also in accordance with above mentioned study. Several other reports clearly demonstrated the pronounced impact of exogenous application of GB on plant vegetative and reproductive stages in several crops e.g. maize (Agboma *et al*., 1997a) and soybean (*Glycine max*) (Hussain *et al*., 2008).

Better root system is fundamental adaptive mechanism that improves the ability of a plant to capture water towards drought stress. According to Agboma *et al*. (1997b) the root surface area in *Populus* species decreased in drought stress in the similar way the stem length in *Albizzia* decreased under water shortage (Agboma *et al*., 1997c). Lower turgor pressure reduce the plant height, this reduction in plant height might be associated with decreased cell enlargement and cell growth under stress (Sundaravalli *et al*., 2005).

The economic yield of crops can be estimated by the rate of photosynthesis. Drought stress decreases the photosynthetic activity. Similar results were obtained in present study. However, inhibitory effect of drought stress was ameliorated by foliar application of compatible solute (GB). Exogenous application of compatible solutes increased the net photosynthetic rate and also has been reported in maize (Yang and Lu, 2005) and tomato (Munns and Tester, 2008). Under drought or salt stress, GB not only control the stomatal conductance but also maintain Rubisco activity and chloroplast ultrastructure (Lopez *et al*., 2002).

There was a considerable decline observed for chlorophyll content of both hybrid under drought stress. Chl *a*, Chl *b* and Chl *a* + *b* contents decrease under progressive drought stress in maize (Nawaz and Ashraf, 2007). Exogenous application of GB improved photosynthetic pigments and thus enhanced photosynthetic capacity in various crops and vegetables e.g., tomato (Raza *et al*., 2006) and wheat (Anjum *et al*., 2011).

All water relations were disturbed due to water shortage including, turgor potential leaf water potential, osmotic potential and relative water contents at both growth stages of sunflower (Anjum *et al*., 2011). RWC decline depicts loss of turgor that results in limited water availability resulting in loss of turgidity which stops or decrease the cell expansion process in crop plants. In present study decrease in RWC was observed under drought conditions. Exogenous application of GB reversed the effect of water stress on RWC. This is in accordance with previous finding (Iqbal, 2004; Farooq *et al*., 2008) who reported an increase in RWC in kidney beans by foliar application of GB under abiotic stress. Active lowering of osmotic potential is generally considered as an adaptation under drought to maintain turgor (Iqbal, 2004). A beneficial drought resistance character is osmotic adjustment which is adopted by green plants (Meek *et al*., 2003) at lower leaf water potentials (Iqbal, 2004). To cope up with the stress effect, foliar application of GB is used as an important tool and has strong potential to reverse the stress effects. GB increased the turgor potential of the plant cells by osmotic adjustment (Iqbal *et al*., 2008).

Reduction in protein synthesis and proline accumulation in many crop plants have been widely studied (Iqbal *et al*., 2008) and are of the several biochemical indices of water deficit injury. Reduction in protein contents was observed in our study during exposure of drought stress. In leguminous plants, soluble protein content in both leaves and nodules decreased as drought progressed with more drastic decline in nodule tissues. Drought stress produced drastic effect on the soluble protein contents of leguminous plants in both leaves and nodules but the effect was more severe in nodule tissues. Amino acids accumulation in cell sap is one of the adaptations under water scarcity. Amino acid contents showed an uplift in sorghum plants when exposed to moisture stress conditions (Yaday *et al*., 2005). Similar results were observed in present study when sunflower plants were exposed to water stress. Osmotic adjustment alleviates some of the hazardous water stress effects. Lv *et al*. (2007) reported the accumulation of GB for drought stress tolerance in plants and the accumulation was more effective in transgenic plants containing enhanced activity of GB accumulation as compared to wild plants.

Accumulation of proline is chief indicator of drought stress tolerance in higher plants (Iqbal, 2004). The proline contents of leaf was increased in both sunflower hybrids at both stages. Accumulation of proline in various parts of plants is reported earlier particularly in wheat crop. Elevated level of proline was also observed in vegetable crop *Abelmoschus esculentus* (L.) under drought (Sankar *et al*., 2007). Glycine betaine application under drought condition ameliorated the stress effect at both the stages by accumulating proline in leaves. Exogenous application of GB increased the soluble sugars contents in sunflower at both growth stages (Lin *et al*., 2002; Lv *et al*., 2007) similar results were observed in present study.

CONCLUSION

Exogenous GB application increases chlorophyll content and photosynthetic rate of sunflower under water stress. According to this study, foliar application proved to be more effective among the two modes of application that have been investigated in the present study. The concentration of 125 mM GB were more operative as compared to 75 mM GB . Growth of both sunflower hybrids was effected by drought stress and had shown improvement towards application of GB but the reproductive stage was more sensitive as compared to the vegetative stage. It is concluded that 125mM GB concentration could be applied exogenously at reproductive stage to ameliorate the adverse effects of water shortage.

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