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TREHALOSE-INDUCED IMPROVEMENT IN GROWTH, PHOTOSYNTHETIC CHARACTERISTICS AND LEVELS OF SOME KEY OSMOPROTECTANTS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER DROUGHT STRESS

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Abstract

Accumulation of trehalose, a sugar rarely found in plants, is believed to be involved in improving drought stress tolerance in different plant species. To assess the influence of trehalose on regulation of different gas exchange attributes, chlorophyll contents and osmoprotectants of sunflower (*Helianthus annuus* L.) plants, an experiment was conducted under drought stress conditions. Fifteen day-old seedlings of two sunflower cultivars i.e. Hysun 33 and FH 598 were exposed to non-stress (100% field capacity) and water limited stress (60% field capacity). After 30 days of drought stress treatments, four levels of trehalose (0, 10, 20 and 30 mM) were applied to the plant leaves of both sunflower cultivars. Water deficit conditions suppressed shoot and root fresh and dry weights, chlorophyll *a* and *b* contents, photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), and C_i/C_a ratio of both sunflower cultivars while it significantly enhanced the WUE as well as accumulation of proline and glycinebetaine (GB)contents in both sunflower cultivars. Foliar-applied different levels of trehalose significantly improved shoot fresh and dry weights, root dry weight, chlorophyll *a* and *b*, *A*, *E*, g_s, C_i, C_i/C_a, *WUE*, proline and GB contents under water stress conditions. Of both sunflower cultivars, cv. Hysun 33 was superior in shoot and root fresh and dry weights, *A*, *E*, g_s, *Ci*, C_i/C_a under water limited conditions, whereas cv. FH 598 in WUE. Of all trehalose levels used, 20mM and 30mMwere more effective for improving shoot fresh weight, chlorophyll *b*, g_s, C_i, and C_i/C_a in both sunflower cultivars. Overall, exogenous application of trehalose was effective in enhancing drought stress tolerance in sunflower plants by improving osmoprotection and gas exchange characteristics.

Key words: Chlorophyll pigments, Gas exchange characteristics, Glycinebetaine, Proline, Trehalose.

Introduction

Various environmental stresses including drought caused a significant decline in crop productivity every year (Sadiq *et al.,* 2017). Drought stress is one of the main abiotic stresses that influence adversely crop development and yield (Sadiq *et al.,* 2016). Water limited regimes cause a prominent decrease in plant photosynthetic processes, mainly due to impaired stomatal conductance, which reduces CO_2 entry into leaf tissues or due to impairment of the activity of Rubisco, a key enzyme of photosynthetic metabolism (Ashraf & Harris, 2013). Water deficit conditions also lower down considerably plant water contents and perturb different metabolic processes including protein synthesis, rate of photosynthesis and respiration, synthesis of primary and secondary metabolites, generation of reactive oxygen species (ROS), hormonal homeostasis and levels of essential nutrients in plants (Ahmad *et al*., 2010). To cope with such adverse conditions caused by abiotic stresses, plants adopt different mechanisms including accumulation of compatible organic osmolytes through their metabolic pathways (Ashraf, 2009). Plants can regulate cellular osmotic adjustment, a key physiological protective process occurring in plants under water scarce conditions. Water-stressed plants possess the ability to accumulate a variety of organic osmolytes but the extent of accumulation varies from species to species and from variety to variety. However, the intrinsic levels of the osmolytes have been reported to be linked to plant drought tolerance (Khan *et al*., 2010). On the basis of pattern of accumulation of organic osmolytes, plants have been categorized as high, low and non-accumulators (Slama *et al*., 2015). For raising the levels of osmolytes particularly in low accumulators or non-accumulator's efforts are underway to genetically engineer such plants to enable them to produce/accumulate desired levels of the key osmolytes. However, considering this approach a very cost-intensive and time-consuming, alternatively a shot-gun approach wherein osmolytes are supplied to plants through exogenous means so as to raise the intrinsic concentrations of osmolytes to a desired level.

A variety of organic osmolytes are being supplied exogenously to plants for achieving enhanced drought tolerance. They include soluble sugars, amino acids including proline, quaternary ammonium compounds including glycinebetaine, synthetic plant growth regulators, etc. (Rao *et al*., 2012). Of different soluble sugars known for exogenous supply to plants, trehalose has gained ground as an effective osmolyte for enhancing drought tolerance in most plants (Govind *et al*., 2016). Trehalose, a non-reducing disaccharide, is believed to protect biological membranes and molecules in living organisms during water deficiency (Akram *et al*., 2016). Although, it is present in small quantity, it can play a vital role in metabolic reactions affected by abiotic stresses (Luo *et al*., 2010). Furthermore, it is now widely reported that trehalose can be readily taken up when applied exogenously and plays a role in plant protection against drought stress (Akram *et al*., 2016). For example, exogenous application of trehalose at the range of 10-30 mM was found to be very effective in improving plant biomass, antioxidative potential, nutrients status and chlorophyll pigments etc. in different plants e.g. maize (Ali & Ashraf, 2011) and radish (Shafiq *et al.*, 2015; Akram *et al*., 2016) etc.

Sunflower (*Helianthus annuus* L.) being a high yielding oil-seed crop, is ranked as the world's fourth largest oil-seed crop (Andaleeb *et al*., 2008; Akram *et al.*, 2012). Furthermore, it has a short life span (90-120 days) and can be grown twice in a year without disturbing any cash crop cultivation (Hussain *et al*., 2013). Drought stress is reported to significantly reduce sunflower head diameter, number of achenes along with their oil contents and finally seed oil yield (Oad *et al*., 2002). Earlier, Sayed & Gadallah (2002) reported that application of 5 and 10 mg L^{-1} thiamin either through root growing mediumor as foliar spray on salt stressed sunflower plants enhanced dry mass, chlorophyll contents, soluble sugar levels, and absorption of K^+. Similarly, Hussain *et al*., (2008) externally applied 0.724 mM salicylic acid (SA) and 100 mMglycinebetaine which neutralized the adverse effects of water deficiency on achene yield of sunflower whereas the quality of oil contents remained unaltered. According to another report, foliar spray of 20 and 80 mg L^{-1} of 5-aminolevulinic acid proved to be effective in improving growth in sunflower both in saline stress and non-stress conditions (Akram & Ashraf, 2011). The principal objective of the present study was to examine if foliar application of trehalose could mitigate the adverse effects of drought on plant biomass, different gas exchange attributes, pattern of accumulation of some key non-enzymatic metabolites such as proline and glycinebetaine in two commercially grown sunflower cultivars.

Materials and Methods

Experimental site: This study on the role of exogenous supply of trehalose in mitigating the effect of drought stress on sunflower (*Helianthus annuus* L.) plants was conducted in the Botanical Garden of the GCUF, Pakistan from January to March in the spring season, 2015. The seed (achenes) of two sunflower cultivars Hysun 33 and FH 598 were collected from the Ayub Agricultural Research Institute Faisalabad, Pakistan.

Plant growth and external applications: The crop was planted in plastic pots (height 24.5 cm and diameter 21 cm). Pot soil (7 kg) was filled in each plastic pot. The seeds were dipped in distilled water for two hours before sowing. Ten seeds of each sunflower cultivar were sown in each pot. Thinning was done after 15 days of planting so as to maintain five plants in each pot.

Drought stress treatment: After three-week growth, water stress treatments (100% and 60% of field capacity) were applied. The moisture contents of drought hit pots were monitored and maintained regularly considering the weight of each pot equal to that calculated for 100 or 60% field capacities through addition of normal irrigation, if needed. At the vegetative stage, after 4 weeks of drought stress treatment, four levels (0, 10, 20 and 30 mM) of trehalose [formula, $C_{12}H_{22}O_{11}$, molecular weight, 342.29 g/mol, density 1.58g/cm^3, Company MP Bio USA and 100% pure] were applied as a foliar spray. The stock solution of trehalose was prepared by dissolving 11.34 g/Ltrehalosein distilled water. Tween 20 (0.1%) was added to varying concentrations of trehalose prior to foliar spray. Before foliar spray, all pots were covered witha paper to avoid addition of solution to the root growing medium. All plants were allowed to grow for a further two weeks and then the data for the following attributes were determined.

Plant biomass: -Plant biomass (dry and fresh weights of roots and shoots) of the sunflower plants was determined individually after two weeks of spray. After recording the data of fresh weights, the plants were placed in an oven at 60°C for 3 days and then dry weights measured.

Gas exchange characteristics: Infra Red Gas Analyzer *(IRGA)* Ci-301 PS (CID, INC, USA), a portable photosynthesis apparatus was used to measure gas exchange parameters. Data recording time was 10.00 am to 11.00 am, when sunflower leaves experienced irradiance just close to 1200 micromol/m^2/s. Leaf temperature was 25 + 5°C.

Chlorophyll contents: Different steps for chlorophyll *a* and *b* determination were followed as described by Arnon (1949).

Leaf proline estimation: Proline estimation was determined according to the protocol of Bates *et al*., (1973).

Glycinebetaine content (GB): The method described by Grieve& Grattan (1983) was followed for the measurement of glycinebetaine contents.

Statistical analysis: To test the significance level of each treatment, analysis of variance (ANOVA) wasemployed on the recorded data.

Results

Imposition of water-deficit stress (60% field capacity) significantly ($p \leq 0.001$) suppressed the plant biomass, i.e., shoot and root fresh and dry weights of both Hysun 33 and FH 598 sunflower cultivars (Fig. 1). Foliar-applied varying levels (10, 20 and 30 mM) of trehalose significantly improved the shoot fresh weight ($p \leq 0.001$), shoot dry weight ($p \leq 0.01$) and root dry weight ($p \leq 0.01$) of both sunflower cultivars under stress and non-stress conditions of both sunflower cultivars, cv. Hysun 33 was relatively superior to cv. FH 598 in fresh and dry weights of shoots and roots under water deficit conditions (Fig. 1).

A significant ($p \leq 0.05$) reduction in chlorophyll *a*pigment and a non-significant effect on chlorophyll *b*were observed due to water limited supply in both sunflower cultivars. Exogenously applied trehalose was proved effective in improving chlorophyll *a* and *b* pigments in both sunflower cultivars. Of all levels of trehalose used 20 and 30 mM were more effective in improving chlorophyll pigments particularly chlorophyll *b* contents in both sunflower cultivars. The response of both sunflower cultivars was similar in accumulation of chlorophyll *a* and *b* pigments under stress and non-stress conditions (Fig. 1).

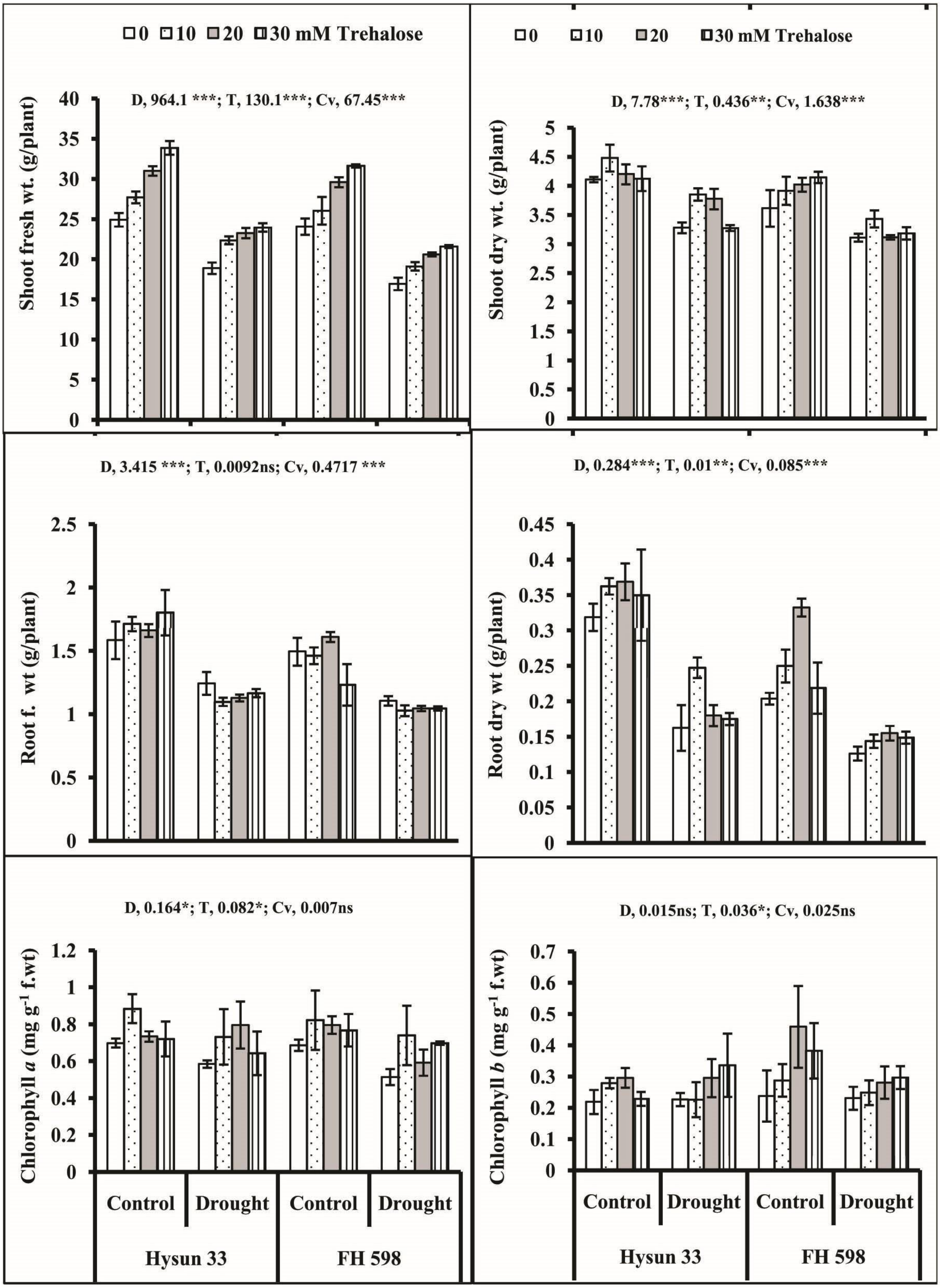

Fig. 1. Shoot and root fresh and dry weights, chlorophyll *a* and *b* contents of water-stressed and non-stressed plants of two cultivars of sunflower (*Helianthus annuus*) subjected to foliar-applied varying levels of trehalose (mean ± S.E.) D, Drought; T, Trehalose; Cv, Cultivars; ns, Non-significant, *, ** and ***, significant at 0.05, 0.01 and 0.001 levels, respective.

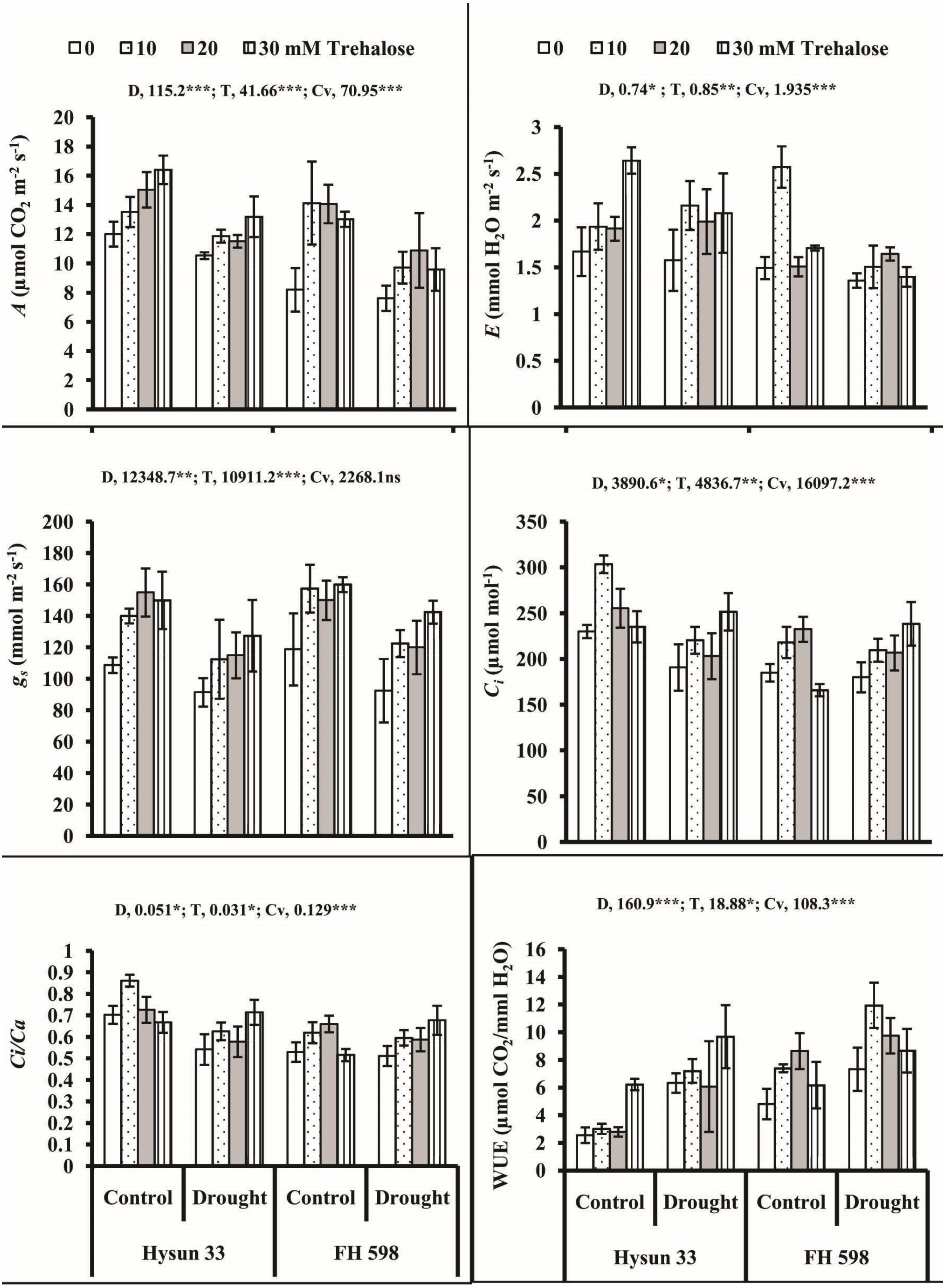

Fig. 2. Photosynthetic rate (*A*), transporation rate (*E*), stomatal conductance (g_s) sub-stomatal CO_2 concentration (C_i), water use efficiency (*A/E*), C_i/C_a of two cultivars of sunflower (*Helianthus annuus*) subjected to foliar-applied varying levels of trehalose (mean ± S.E.) D, Drought; T, Trehalose; Cv, Cultivars; ns, Non-significant, *, ** and ***, significant at 0.05, 0.01 and 0.001 levels, respective.

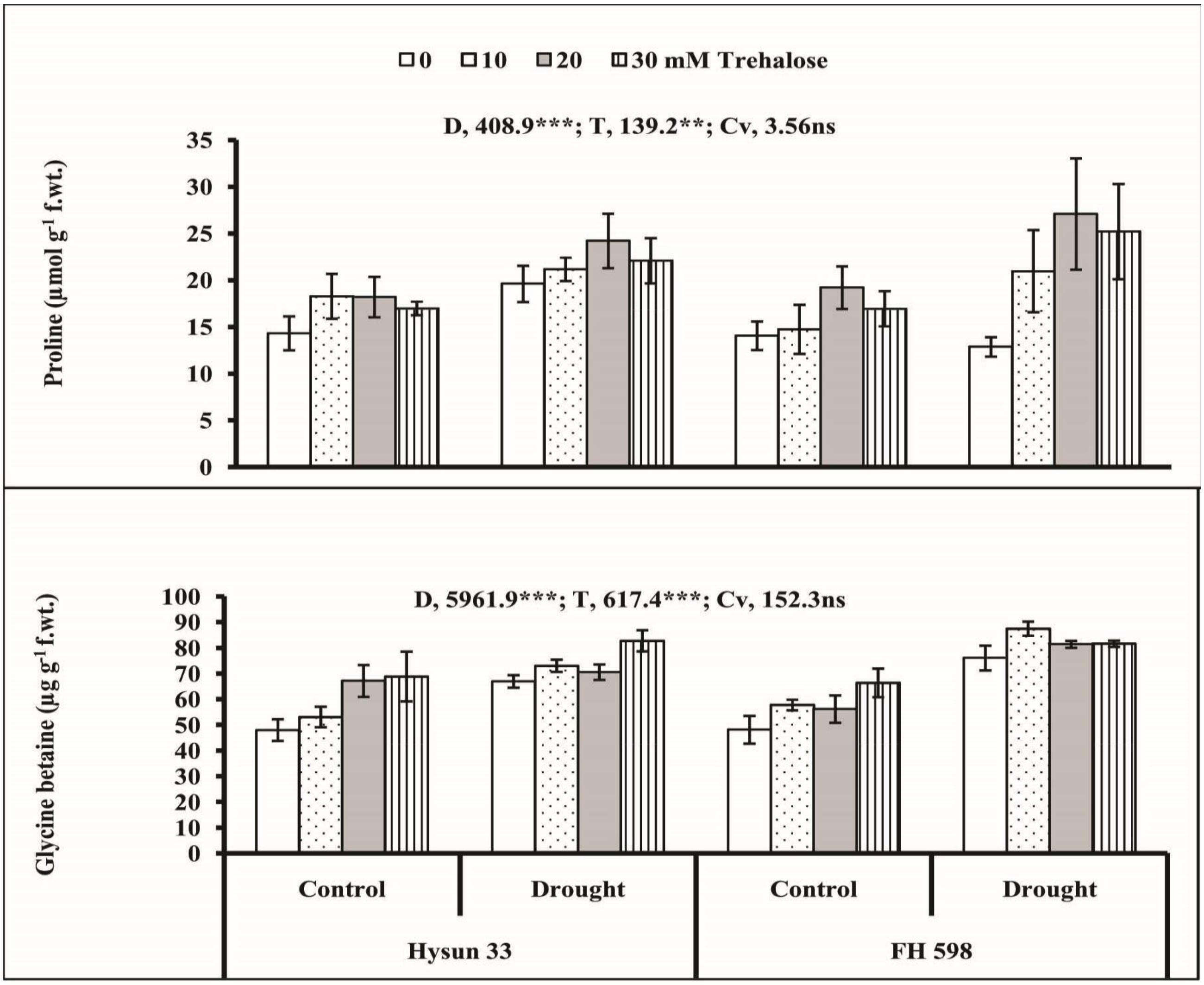

Fig. 3. Leaf proline and glycinebetaine contents of water-stressed and non-stressed plants of two cultivars of sunflower (*Helianthus annuus*) subjected to foliar-applied varying levels of trehalose (mean ± S.E.) D, Drought; T, Trehalose; Cv, Cultivars; ns, Non-significant, *, ** and ***, significant at 0.05, 0.01 and 0.001 levels, respective.

Data for different gas exchange characteristics showed that drought stress (60% field capacity) significantly suppressed the photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i) and C_i/C_a ratio, but increased the water-use-efficiency (WUE) of both sunflower cultivars. Exogenous application of trehalose was effective in improving all the earlier-mentioned gas exchange characteristics in both sunflower cultivars under stress and non-stress conditions. Of both sunflower cultivars, cv. Hysun 33 was superior to the other cultivar in A, E, C_i, C_i/C_a, while the reverse was true in WUE. The response of both sunflower cultivars to g_s was similar under control and moisture deficit conditions (Fig. 2).

Under water deficit conditions, leaf proline and glycinebetaine (GB) accumulations increased considerably ($p \leq 0.001$) in both sunflower cultivars (Fig. 3). Trehalose-treated plantsof both sunflower cultivars were significantly ($p \leq 0.01$; 0.001) higher in proline and GB accumulation, particularly under water stress conditions. Both sunflower cultivars were similar in response to proline and GB accumulation under water stressed conditions (Fig. 3).

Discussion

During different phases of life, plants are naïve to be exposed to various stresses like water shortage, soil salinity, extremes of temperature etc. To survive under such adverse cues, plants employ different defensive adaptive mechanisms. However, the most promising is osmoregulation (Holmstrom *et al*., 2000). During this activity, certain low molecular weight carbohydrates, proteins and amino acids play an important role (Hasegawa *et al*., 2000; Akram *et al*., 2016). If the levels of these low molecular weight substances are low within the plant body, the plant is generally not able to withstand harsh environmental cues. Thus alternatively as a shot-gun approach, such substances can be applied exogenous to bring their intrinsic concentrations to the optimum levels. Foliar spray of such organic compounds has been advocated as a viable strategy to impart vitality to plants so that they can thrive well under unfavorable stress conditions. Of various sugars involved in stress tolerance, trehalose is known to stabilize the desiccated enzymes, proteins and lipids effectively as well as it protects biological membranes from damage during dehydration

(Garg *et al*., 2002). In the present investigation, water deficit conditions significantly impaired growth parameters (shoot and root fresh and dry weight) of both sunflower cultivars (Hysun 33 and FH 598). However, foliar application (10, 20 and 30 mM) of trehalose significantly improved these growth parameters (Fig. 1). These results can be related to earlier studies which also used exogenous application of trehalose on different plants under stressful conditions as in maize (Zeid, 2009), *Catharanthus roseus* (Chang *et al*., 2014), *Brassica* species (Alam *et al*., 2014), wheat (Ibrahim & Abdellatif, 2016), and radish (Akram *et al*., 2016), etc. The trehalose-induced plant growth improvement was suggested to be associated with upregulation of oxidative defense system, maintenance of turgor potential and nutritional balance as well as suppression in the levels of reactive oxidants under stress conditions (Alam *et al*., 2014; Akram *et al*., 2016).

Upon exposure to abiotic stresses, reactive oxygen species (ROS) are generated in plants which rapidly deteriorate the chlorophyll contents (Baker *et al.*, 2007). Similar results were observed in the present study. The moisture deficient stress significantly reduced the chlorophyll contents, particularly chlorophyll *a* contents. The trehalose treatment significantly improved both photosynthetic pigments in both sunflower cultivars under stress and non-stress conditions (Fig. 1). Similar findings were reported under water stress in *Vicia faba* (Gadallah, 1999), wheat (Kamran *et al.*, 2009), and maize (Ashraf *et al.*, 2007). Likewise, Akram *et al*., (2016) reported that in radish plant, trehalose pretreatment caused a significant increase in chlorophyll *a* contents. In Fenugreek plant, Sadak (2016) reported enhanced levels of photosynthetic pigments in trehalose-treated plants as compared to those in the non-treated plants.

Stomatal closure is contemplated as the first line of defense against the onset of soil desiccated conditions. This modification in stomatal regulation affects photosynthetic activity due to denaturation of proteins related to photosystems. Data recorded for different gas exchange components in this experiment depicted that limited soil moisture conditions negatively affected the photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), sub-stomatal CO$_2$ concentration (C_i) and C_i/C_a ratio, but they increased the water-use-efficiency (WUE) of both sunflower cultivars. The foliar spray of trehalose played a beneficial role in all the above-mentioned gas exchange attributes in both sunflower cultivars but an opposite trend was noted for water use efficiency. In this respect, Ali& Ashraf (2011) found that treatment of trehalose significantly improved the *A, E, Ci, gs, A/E,* and *Ci/Ca* in maize cultivars under drought stress and non-stress conditions. Similarly, Akram *et al*., (2016) reported that priming radish seed with trehalose significantly raised plant water use efficiency. This discussion affirms that trehalose spray on plants induces positive adjustment in sunflower plants between carbon utilization and transpiration.

The compatible osmoprotectants such as glycinebetaine and proline play a significant role in plants to endure the prevailing abiotic stress conditions. These solutes upregulate plant key physiological processes under stress adversaries and make them acclimatized (Nounjan *et al.*, 2012). In the present study, leaf proline and glycinebetaine (GB) accumulation increased considerably in both sunflower cultivarsunder water deficit stress. The trehalose-treated plants of both sunflower cultivars were significantly higher in proline and GB accumulation, particularly under water stress conditions (Fig. 3). Our results are analogous to those of Alam *et al.,* (2014) who observed enhanced accumulation of proline and GB in *Brassica campestris* seedlings during a combined treatment of trehaloseand drought stress. Not only foliar spray, but also pre-sowing seed treatment with trehalose induced higher levels of proline in water stressed radish plants (Akram *et al.,* 2016) but in contrast, Nounjan *et al*., (2012) reported that in salt stressed rice seedlings, externally applied (rooting medium) trehalose suppressed endogenous proline levels. In the case of GB accumulation in drought stressed sunflower plants, our results are in agreement with those of some earlier research reports e.g., sunflower (Akram *et al.,* 2012), and radish (Shafiq *et al.,* 2015) under water shortage conditions. They suggested that trehalose induced increase in GB accumulation could play an important role in drought stress tolerance.

In conclusion, exogenous application of trehalose significantly improved the plant growth in terms of shoot fresh and dry weights, and root dry weight of both sunflower cultivars under water deficit stress. In addition, trehalose-induced significant improvement was also observed in chlorophyll *a* and *b*, *A*, *E*, g_s, *Ci*, C_i/C_a, *WUE*, proline and GB contents predominantly under water stress conditions. Of all levels, 20 and 30 mM of trehalose were more effective for improving growth, gas exchange characteristics and osmoprotection components in both sunflower cultivars.So, it can be suggested that foliar applied trehaloseis an effective mode for achieving enhanced drought stress tolerance in sunflower plants by improving osmoprotection and gas exchange characteristics.

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