*Pak. J. Agri. Sci., Vol. 59(3), 435-448 ; 2022*
*ISSN (Print) 0552-9034, ISSN (Online) 2076-0906*
*DOI:10.21162/PAKJAS/22.52*
http://www.pakjas.com.pk

# Influence of Exogenously Applied Trehalose on Morphological and Physiological Traits of Flax (*Linum usitatissimum*) Plant Under Water Shortage Conditions

Abida Abid and Muhammad Shahbaz*

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
*Corresponding author's e-mail: shahbazmuaf@yahoo.com

Water scarcity is the biggest issue which interrupts the physiological, morphological and biochemical process of the plants. Different osmolytes (trehalose, proline, glycine betaine etc) are applied to plants through rooting medium, pre-sowing method and foliar application which are helpful to detoxify the negative effects of drought stress and enhance the yield. Trehalose, an osmolyte which decreases the effects of water deficit conditions by protecting the structure of protein and lipid and function of enzymes. An experiment was performed to study the significant effect of trehalose on flax (*Linum usitatissimum* L.) under water deficit situation. Two levels of drought (normal watering and 50% FC (field capacity)) and four levels of trehalose (non-soaked, water soaked, 10 and 20 mM) were used. Total 64 pots were used in which 50% drought was applied on 32 pots (in which 16 pots (in which four levels of trehalose were applied with 4 replicates of each treatment) as drought of Roshini and 16 pots as drought of Chandini) while other 32 pots (16 pots as control of Roshini and 16 pots as control of Chandini) used as control treatment. Consequences showed that water deficit conditions (50% FC) reduced the growth attributes, yield parameters, carotenoids content, POD (peroxidase) activity, nutrient use efficiency and uptake in both shoot and root while improved the total phenolics, MDA (malondialdehyde), $H_2O_2$ (hydrogen peroxide) and total free proline as compared to control conditions. Foliarly applied trehalose enhanced the shoot fresh weight, MDA, TSP (total soluble protein) and GB (glycine betaine), shoot length, leaf ascorbic acid and $H_2O_2$ in both flax varieties and reduced the *a*/*b* ratio and MDA in Roshini. Trehalose increased the carotenoids contents in Chandini, POD, GB, total free proline, number of seeds/plant and number of capsules/plant in Roshini, and SOD (superoxide dismutase), CAT (catalase) activities in both flax varieties under 50% FC as compared to control conditions. Overall, performance of Chandini was better when 10 mM trehalose application was applied. Drought tolerance of several cereal and medicinal crops elevated by using different osmoprotectants, antioxidants and plant growth regulators. Trehalose application applied to ameliorate the effects of water stress and recovers the flax seed production, but limited information is available in literature on the role of trehalose on flax. How trehalose regulates the biochemical and physiological processes under both control and stress conditions?
**Keywords:** Flax, drought stress, trehalose application, photosynthetic pigments, antioxidants.

## INTRODUCTION

Pakistan's population increased (2.4% yearly), different strategies are used to decrease the effect of abiotic stresses and increased the yield of various crops to fulfil the food demands (GoP, 2018-19; Sivakumar *et al*., 2020). Water deficit conditions decreased the crop yield up to 50-60% (Zulfiqar *et al*., 2021). Crops production is adversely affected by biotic and abiotic factor world-over (Fathi and Tari, 2016). Dehydration in plants reduces the yield in plants under water deficit conditions. Drought stress damagingly influence the growth, stomatal conductance, protein synthesis, physiology, relative water content and photosynthetic rate and increased the ROS production (Hasanuzzaman *et al*., 2014). Water deficit conditions reduces the stomatal conductance as a result movement of $CO_2$ inside the leaves decreased which reduces the photosynthetic activity and transpiration rate (Aldesuquy *et al*., 2018). Plants regulate photosynthesis, membrane system, water relations and gas exchange to survive under water stress conditions (Krasensky and Jonak, 2012). Plants also generate antioxidant to ameliorate the influence of oxidative stress caused by ROS (Tani *et al*., 2019). Under insufficient supply of water, plants consume water efficiently to improve the yield (Mostafavi, 2011). At grain filling phase, water deficit condition is destructive which lowered the number, size, quality and quantity of seed (Sehgal *et al*.,

Abid, A. and M. Shahbaz. 2022. Influence of Exogenously Applied Trehalose on Morphological and Physiological Traits of Flax (*Linum usitatissimum*) Plant Under Water Shortage Conditions. Pakistan Journal of Agricultural Science.59: 435-448.

[Received 11Mar 2022; Accepted 20 May 2022; Published 27 Jun 2022]

2018). Water shortage conditions also diminished the fertilization, germination rate, quantity of flowers and pods (Pushpavalli *et al*., 2014). Water deficit conditions reduced the yield production in barley 49-57% (Samarah, 2005), rice 60% (Basnayake *et al*., 2006), Chickpea 45-69% (Nayyar *et al*., 2006), potato 13% (Kawakami *et al*., 2006) and soybean 46-71% (Samarah *et al*., 2006).

Plants accumulate various osmolytes to protect themselves from the effect of water shortage situations. Osmolytes are low molecular weight solutes, which have potential to diminish the over production of ROS and retain the turgor pressure (Sadak *et al*., 2019). Trehalose acts as a signaling metabolite in plants under various abiotic stresses (Kosar *et al*., 2018). Trehalose defends the plant from desiccation and membranes destruction under drought stress (Pampurova and Dijck, 2014). High trehalose is not synthesized by plants required to reduce the negative effect of the environmental stress that why trehalose applied exogenously to improve the endogenous level of trehalose (Sadak, 2019). During dehydration, trehalose protects the lipid, membranes, proteins, nucleic acid and biomolecules from destruction and scavenge the free radicals (Jin *et al*., 2016). Trehalose defends the plant from environmental stresses and promotes the plants performance (Yang *et al*., 2014). Under drought stress conditions, trehalose improves the development, antioxidant enzymes, photosynthetic pigments and production in *Brassica* species, cowpea and sunflower (Alam *et al*., 2014; Khater *et al*., 2018; Kosar *et al*., 2018 respectively).

Flax plant is majorly cultivated for oil and fiber (Qamar *et al*., 2019). Flax seed has significant quantity of carbohydrates, oil, protein and minerals. Its fiber has resistance against the environmental fluctuations (Dash *et al*., 2017). Its seed contains protein, omega 3 and 6 which are beneficial to stave off the cardiovascular diseases and improves the immunity (Qamar *et al*., 2019). Remaining seed part, after oil extraction is utilized to graze the animals because it has a major portion of nutrients (Coskuner and Karababa, 2007). Drought stress diminishes the percentage (12%) and germination rate (42%), plumule length (66%), radical length (87%) growth and production of the flax plant (Kadkhodaie and Bagheri, 2011). Trehalose application, very helpful to enhance the production of crops but limited knowledge is available on flax plant under water deficit conditions. Needs to know how trehalose application effect the physiological and chemical processes of flax plant to enhance the production under water stress. The main objective of this experiment was to elaborate the impact of trehalose on flax attributes under water shortage situations and to explore the best trehalose level effective to enhance the production and yield of flax under drought and control conditions. Study the effect of trehalose on different attributes of flax under both control and stressed conditions. Find out the level of trehalose which is more helpful to diminish the effect of drought stress. It is assumed that foliar application of trehalose has ability to ameliorate the effect of drought stress on flax.

## MATERIALS AND METHODS

An experiment was executed to inspect the impact of trehalose on flax plant under drought stress. Experiment was organized in Old Botanical Garden, University of Agriculture, Faisalabad-Pakistan (31°25’45’’ N, 73°04’18’’ E, altitude 1644 m) with completely randomized design. Total 64 pots were used in which 50% drought was applied on 32 pots (in which 16 pots (in which four levels of trehalose were applied with 4 replicates of each treatment) as drought of Roshini and 16 pots as drought of Chandini) while other 32 pots (16 pots as control of Roshini and 16 pots as control of Chandini) used as control treatment. During experiment, two drought levels (Control and 50% FC), two flax varieties (Roshini and Chandini) and four trehalose (UNI-CHEM) levels (Control, Distal water, 10 and 20 mM) were used. These levels of trehalose were used because these were helpful to improve the rice yield (Shahbaz *et al*., 2017). Flax seeds were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Two varieties were used to find the best variety, which gives better production under drought stress as well as control conditions. The climatic conditions during the experiment were temperature 17.38 ℃, relative humidity 72.62%, rain fall 29.53 mm and day length 6.8 h. Each pot was containing 5.5 kg of soil. During experiment sandy clay (2 parts of soil and 1 part of humus) were used (clay 60%, sand 25%, silt 15% and pH 6.3). The crop was planted on 2$^{nd}$ November and harvested on 21$^{st}$ April. At the start of November, fifteen seeds were sown in each pot by using the surface sowing method. After 60 days of sowing, water stress was maintained and trehalose application was applied foliarly. After 15 days of the trehalose treatment, morphological data were recorded, and harvesting was done at the maturity.

***Determination of field capacity*:** Field capacity was determined by Nachabe, 1998. At the time of pot filling, soil samples (3 x 200 g) were taken and dried in oven at 100 ˚C for seven days. After that weight of the samples were determined to find the soil moisture than oven dried soil (100 g) taken in a plastic beaker to form saturated paste by adding distal water.

Field capacity = Saturation percentage/2

***Growth attributes*:** To determine the shoot fresh weight, shoot dry weight and shoot length of flax, two plants were uprooted from each pot and measured the shoot fresh weight with the help of balance and shoot length by measuring tape. After that plant samples were placed in oven for a week at 65 ˚C then find out the shoot dry weight (Shahbaz *et al*., 2017).

***Photosynthetic pigments*:** Fresh younger leaves (0.1 g) were chopped and dipped in 5 ml 80% acetone than placed in dark at 4 ˚C for overnight. Next day the extract was used to read the absorbance with the help of spectrophotometer (IRMECO U2020, Lütjensee, Germany) at the wavelength of 480, 645 and 663 nm (Arnon, 1949). Chlorophyll *a*, *b*, *a*/*b*, total chlorophyll and carotenoids was determined by using given formulas:

Chl *a* (mg/g f.wt.) = [12.7(OD 663)-2.69(OD 645) x V/ 1000 x W]

Chl *b* (mg/g f.wt.) = [22. 9(OD 645)-4.68(OD 663) x V/ 1000 x W]

Carotenoids (mg/g f.wt.) = $A_{car}$/Em x100

V = volume of the aliquot and W= weight of tissue

$A_{car}$= OD 480 + 0.114(OD 663)-0.638(OD645) and $E_m$ = 2500

***Enzymatic antioxidants*:** At 4 ˚C, fresh leaf (0.25 g) was ground in phosphate buffer (5 ml) having pH 7.8. The extract was centrifuged at 10,000 rpm for 15 min and used to determine the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). To determine the SOD activity, distilled water (0.4 ml), phosphate buffer (0.25 ml), triton-X (0.1 ml), L-methionine (0.1 ml), riboflavin (0.05 ml), 0.05 ml NBT (nitroblue tetrazolium) and extract (0.05 ml) were poured in a set of cuvettes. Then cuvettes were placed under light for 15 min. After that absorbance was determined by using spectrophotometer at 560 nm (Giannopolitis and Ries, 1977). Chance and Maehly (1955) procedure was used to determine the POD and CAT activities. To find out the POD, 40 mM hydrogen peroxide (0.1 ml), guaiacol (0.1 ml), phosphate buffer (0.75 ml) and extract (0.05 ml) were poured in cuvette and determined the absorbance with the help of spectrophotometer at 470 nm for 120 s with interval of 20 s. For CAT activity, phosphate buffer (1.9 ml), 5.9 mM hydrogen peroxide (1 ml) and extract (0.1 ml) were poured in cuvette and read the absorbance with spectrophotometer at 240 nm for 120 s with interval of 30 s.

***Total phenolics*:** Fresh flax leaf (0.25 g) was ground in 80% acetone (5 ml) and centrifuged at 10,000 rpm for 15 min. Supernatant (100 µl) was taken in test tube and made the volume 1 ml with distilled water. After that Folin Ciocalteau's phenol reagent (500 µl), 20% sodium carbonate (2.5 ml) were added and made the 5 ml with distilled water. The mixture was used to read the absorbance at 750 nm with the help of spectrophotometer (Julkenen-Titto, 1985).

***Leaf ascorbic acid*:** Fresh leaf (0.25 g) was ground in 6% trichloroacetic acid (5 ml) and centrifuged. Supernatant (2 ml) and dinitrophenyl hydrazine (1 ml) poured in test tubes then added one drop of thiourea (prepared in 70% ethanol). Test tubes were placed in water bath for 15 min at 100 ˚C. After that cooled the mixture at room temperature, 5 ml sulfuric acid (80%) was added in each test tube and read the absorbance at 530 nm (Mukherjee and Choudhuri, 1983).

***Malondialdehyde (MDA)*:** Fresh flax leaf (0.25 g) was crushed in 5 ml trichloroacetic acid (1% w/v) at 4 ˚C and centrifuged for 15 min at 10,000 rpm. Supernatant (0.5 ml) and thiobarbituric acid (0.5 ml) was poured in test tubes and placed these test tubes in water bath for 30 min at 100 ˚C. Mixture was cooled at room temperature and used to read the absorbance at 532 and 600 nm by using spectrophotometer (Carmak and Horst, 1991).

***Hydrogen peroxide ($H_2O_2$)*:** Fresh leaf (0.25 g) was homogenized in 5 ml trichloroacetic acid (0.1 % w/v) and centrifuge for 15 min at 10,000 rpm at 4 ˚C. Aliquot (0.5 ml), phosphate buffer (0.5 ml) and 1 ml potassium iodide (1M) were taken in test tubes and mixed gently. Read the absorbance at 390 nm by using spectrophotometer (Velikova *et al*., 2000).

***Glycine betaine (GB)*:** Fresh leaf (0.25 g) was crushed in distilled water (5 ml) at 4 ˚C and centrifuged the mixture at 10,000 rpm for 15 min. Extract (1 ml) and 2 N $H_2SO_4$ (1 ml) were taken in test tube and mixed it well. After that 500 µl from the mixture was taken in another set of test tube and placed in ice for 1.5 h. Then potassium tri-iodide (200 µl), 1,2 dichloroethane (6 ml) and distilled water (2.8 ml) were added in chilled mixture. After vortexing, two layers of the mixture were appeared and lower layer was used to measure the absorbance at 365 nm by spectrophotometer (Grieve and Grattan, 1983).

***Total free proline*:** Fresh leaf (0.25 g) was ground in 3% sulfosalicylic acid (5 ml) and centrifuged the extract. Supernatant (1 ml), acid ninhydrin (1 ml) and glacial acetic acid (1 ml) were taken in test tubes and placed in water bath for 20 min at 100 ˚C. The mixture was cooled down and poured 2 ml toluene in test tubes. Two layers of the mixture were established after vortexing. Upper layer was used to measure the absorbance at 520 nm by spectrophotometer (Bates *et al*., 1973).

***Total soluble proteins (TSP)*:** Homogenized the fresh leaf (0.25 g) in phosphate buffer (5 ml) and centrifuged (10,000 rpm; 15 min) at 4 ˚C. Bradford reagent (5 ml) and 0.1 ml supernatant were mixed in test tubes. The absorbance was measured at 595 nm with the help of spectrophotometer (Bradford, 1976).

***Mineral nutrients*:** Dry shoot and root material (0.1 g) were chopped in digestion flask than added 2 ml $H_2SO_4$ and incubated over night at room temperature. The mixture was heated at 250 ˚C, then cooled and added $H_2O_2$ (2-3 drops). This process was repeated until the mixture became colorless. After that made the volume 50 ml with distilled water and filtered it. This aliquot was used to determine the $Na^+$, $K^+$ and $Ca^{2+}$ ions by using flame photometer (Sherwood 410, Cambridge, UK) (Wolf, 1982).

***Phosphorus determination*:** Filtrate of digestion (1 ml) and Barton reagent (1 ml) were taken in test tubes, then 23 ml of distilled water was added. The absorbance was noted at 460 nm by spectrophotometer (Jackson, 1962).

***Nutrient use efficiency and nutrient uptake*:** Nutrient use efficiency and uptake of mineral ions in shoot and root of the flax plant were analyzed by using the given formula.

*Nutrient use efficiency* ($g^2\ mg^{-1}$) *= 1/ Nutrient conc.* ($mg\ g^{-1}$) *x Shoot or root dry weight (g)*

*Nutrient uptake (mg) = Nutrient conc. (mg g$^{-1}$) x Shoot or root dry weight (g)*

***Yield attributes*:** Number of capsules/plant, total seed weight, 1000 seed weight and number of seed/plant were measured at maturity stage.

***Statistical analysis*:** An experiment was arranged with completely randomized design. Three-way ANOVA was used to evaluate the analysis of variance of data by means of co-stat software (Steel and Torrie, 1986).

## RESULTS

***Growth attributes*:** Fresh weight, dry weight and length of flax shoot considerably ($P \leq 0.001$) 50-57% reduced in the flax plant due to water shortage (50% FC). Trehalose (20 mM) application 16% enhanced ($P \leq 0.05$) the shoot fresh weight in Chandini but decreased 2% in Roshini. Shoot length considerably ($P \leq 0.001$) 94% increased by 10 mM trehalose application while 80% decreased by 20 mM trehalose in flax plant. Foliarly applied trehalose caused non-significant effect on growth attributes of both flax varieties (Roshini and Chandini) under water shortage situations (Fig. 1).

***Photosynthetic pigments*:** Water scarcity caused non-significant effects on chlorophyll *a*, *b*, *a*/*b* ratio and total chlorophyll of flax plant. Trehalose application caused non-significant effect on chlorophyll *a*, *b*, and total chlorophyll under both control and stress conditions. Trehalose application considerably ($P \leq 0.05$) reduced the *a*/*b* ratio in Roshini and in case of Chandini *a*/*b* ratio increased by 10 mM trehalose but reduced by 20 mM trehalose. Under water shortage conditions, trehalose showed non-significant value with respect to *a*/*b* ratio. Photosynthetic pigments were better ($P \leq 0.001$) in Chandini as compared to Roshini. In flax plant, carotenoids contents were reduced ($P \leq 0.001$) under water deficit conditions. Trehalose treatment (20 mM) lowered ($P \leq 0.001$) the carotenoids content in Chandini but enhanced in Roshini. Interactive effect of trehalose and water shortage enhanced ($P \leq 0.001$) the carotenoids in Chandini but in Roshini only 20 mM trehalose enhanced the carotenoids (Fig. 1 and 2).

***Enzymatic antioxidants*:** Catalase (CAT) activity reduced ($P \leq 0.001$) in Chandini while increased in Roshini under water deficit conditions. Drought stress considerably decreased ($P \leq 0.01$) the POD activity in flax plant. Trehalose treatment caused non-significant effect on CAT and POD activities in flax plant. Interactive effect of water shortage and trehalose treatment enhanced ($P \leq 0.001$) the CAT activity in flax. Combine effect of water stress and trehalose treatment enhanced ($P \leq 0.01$) the POD activity in Roshini while in Chandini 10 mM trehalose increased ($P \leq 0.01$) the POD activity and 20 mM trehalose decreased the POD activity. Due to water deficit conditions, SOD activity improved ($P \leq 0.001$) in Chandini and reduced in Roshini. Trehalose treatment as foliar application decreased ($P \leq 0.05$) the SOD activity in Chandini and in case of Roshini SOD activity improved by 10 mM trehalose while reduced by 20 mM trehalose. Interactive effect of water scarcity and trehalose application considerably ($P \leq 0.001$) enhanced the SOD activity in both flax varieties. Foliarly applied trehalose greatly ($P \leq 0.05$) improved the SOD activity in Roshini as compared to Chandini under normal and water shortage situations (Fig. 2).

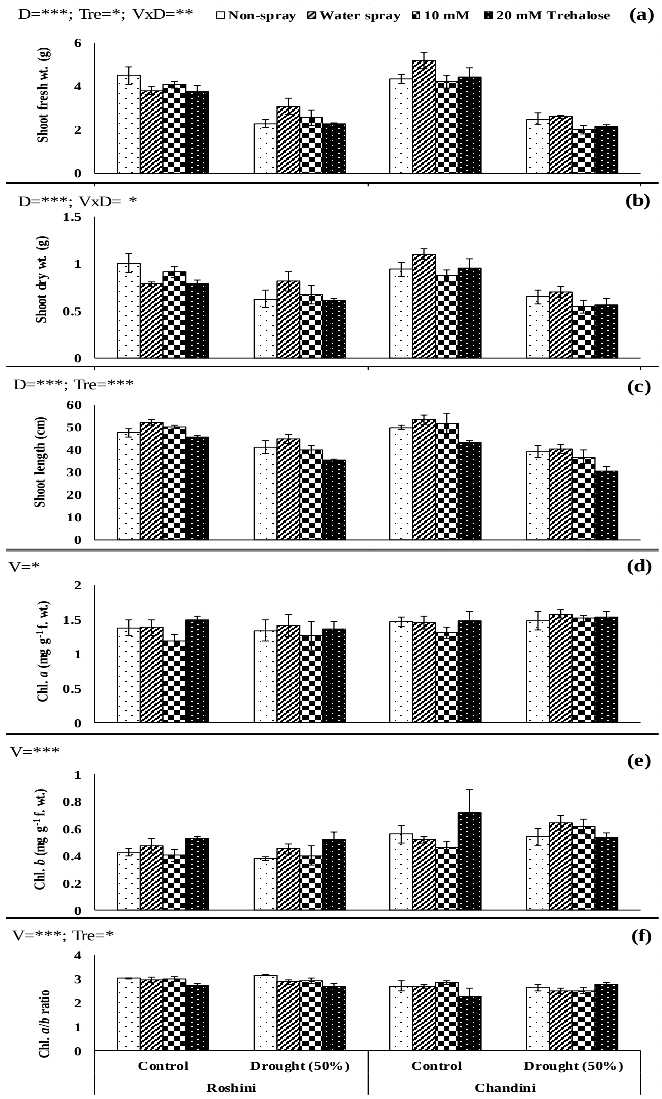


**Figure 1. Effect of application of trehalose on shoot fresh wt. (a), shoot dry wt. (b), shoot length (c), chlorophyll *a* (d), *b* (e) and *alb* (f) ratio in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for shoot fresh wt. (a), shoot dry wt. (b), shoot length (c), chlorophyll *a* (d), *b* (e) and *alb* (f) in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** =**

**Significant at 0.05, 0.01 and 0.001 levels respectively.**

***Non-enzymatic antioxidants and reactive oxygen species*:** Leaf ascorbic acid increased ($P \leq 0.05$) in Roshini but decreased in Chandini due to water stress conditions. In both flax varieties, leaf ascorbic acid enhanced ($P \leq 0.05$) by the foliar application of trehalose while under drought stress trehalose showed non-significant effect on leaf ascorbic acid. Water shortage enhanced ($P \leq 0.001$) the total phenolics, MDA content and $H_2O_2$ (hydrogen peroxide) in flax plant. Trehalose application caused non-significant effect on total phenolics and $H_2O_2$ under water shortage conditions. Foliar application of trehalose decreased ($P \leq 0.001$) the MDA content in Roshini but increased in Chandini. In Chandini, 10 mM level of trehalose increased ($P \leq 0.001$) the MDA content but in case of Roshini, 20 mM trehalose improved the MDA content under water stress. Trehalose treatment considerably ($P \leq 0.05$) improved the $H_2O_2$ production in flax plant (Fig. 2 and 3).

***Glycine betaine (GB)*:** Glycine betaine decreased ($P \leq 0.05$) in Chandini and increased in Roshini under water deficit conditions. Trehalose application as foliar spray enhanced ($P \leq 0.001$) the GB content in Roshini and reduced in Chandini. Interactive effect of water stress and trehalose increased ($P \leq 0.05$) the GB content in Roshini but in Chandini, 10 mM trehalose decreased the GB content and increased by 20 mM trehalose (Fig. 2 and 3).

***Total free proline*:** Under water deficit conditions, total free proline improved ($P \leq 0.001$) in both flax varieties while trehalose caused non-significant effect on total free proline. Combined effect of water stress and trehalose noticeably ($P \leq 0.001$) reduced the total free proline in Chandini but enhanced the total free proline in Roshini. Under both control and stress conditions, performance of Roshini was better ($P \leq 0.05$) than Chandini with respect to total free proline (Fig. 3).

***Total soluble proteins (TSP)*:** Drought stress caused non-significant effect on TSP of both flax varieties. Trehalose application significantly ($P \leq 0.01$) enhanced the TSP in Chandini but in case of Roshini, TSP decreased by 10 mM trehalose and enhanced by 20 mM trehalose. Under water shortage conditions, trehalose reduced ($P \leq 0.01$) the TSP in flax plant (Fig. 3).

***Mineral nutrients*:** Shoot $Na^+$ in flax showed non-significant consequences under water scarcity. Under both control and stress conditions, trehalose treatment caused non-significant effect on shoot $Na^+$. Due to water deficit conditions, root $Na^+$ considerably ($P \leq 0.001$) reduced in Roshini while improved in Chandini. Exogenously applied trehalose lessened ($P \leq 0.05$) the $Na^+$ in flax root. Interactive effect of water scarcity and trehalose improved ($P \leq 0.05$) the root $Na^+$ in Roshini and decreased in Chandini. Shoot $K^+$ in flax plant considerably ($P \leq 0.001$) enhanced under drought stress. All levels of trehalose improved ($P \leq 0.05$) the shoot $K^+$ in Roshini but in case of Chandini only 20 mM trehalose improved the shoot $K^+$. Under water deficit conditions, trehalose showed significant ($P \leq 0.05$) value with respect to shoot $K^+$. Drought stress caused non-significant effects on root $K^+$ of flax. Foliar application of trehalose showed non-significant value with respect to root $K^+$ under normal and stress situations (Fig. 4). Water stress revealed non-significant effect on shoot and root $Ca^{2+}$ of flax. Application of trehalose reduced ($P \leq 0.01$) the shoot $Ca^{2+}$ in both flax varieties. Collective effect of trehalose and water shortage condition considerably ($P \leq 0.001$) improved the shoot $Ca^{2+}$ in Roshini and reduced in Chandini.

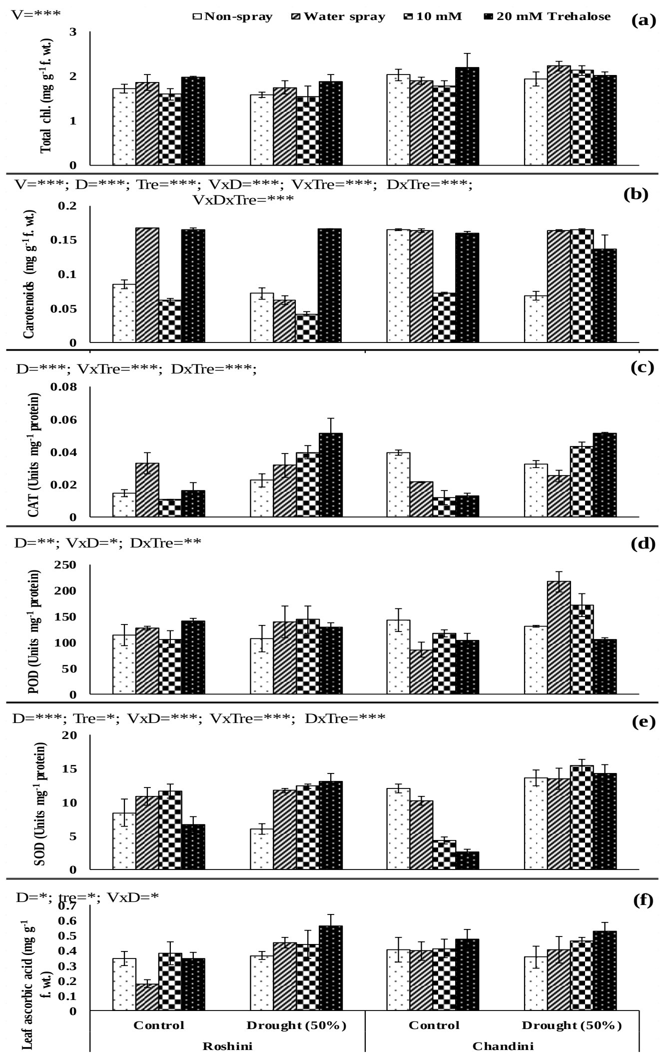


**Figure 2. Effect of application of trehalose on total chlorophyll (a), carotenoids (b), CAT (c), SOD (d), POD (e) and leaf ascorbic acid (f) in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for total chlorophyll (a), carotenoids (b), CAT (c), SOD (d), POD (e) and leaf ascorbic acid (f) in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** =**

**Significant at 0.05, 0.01 and 0.001 levels respectively**

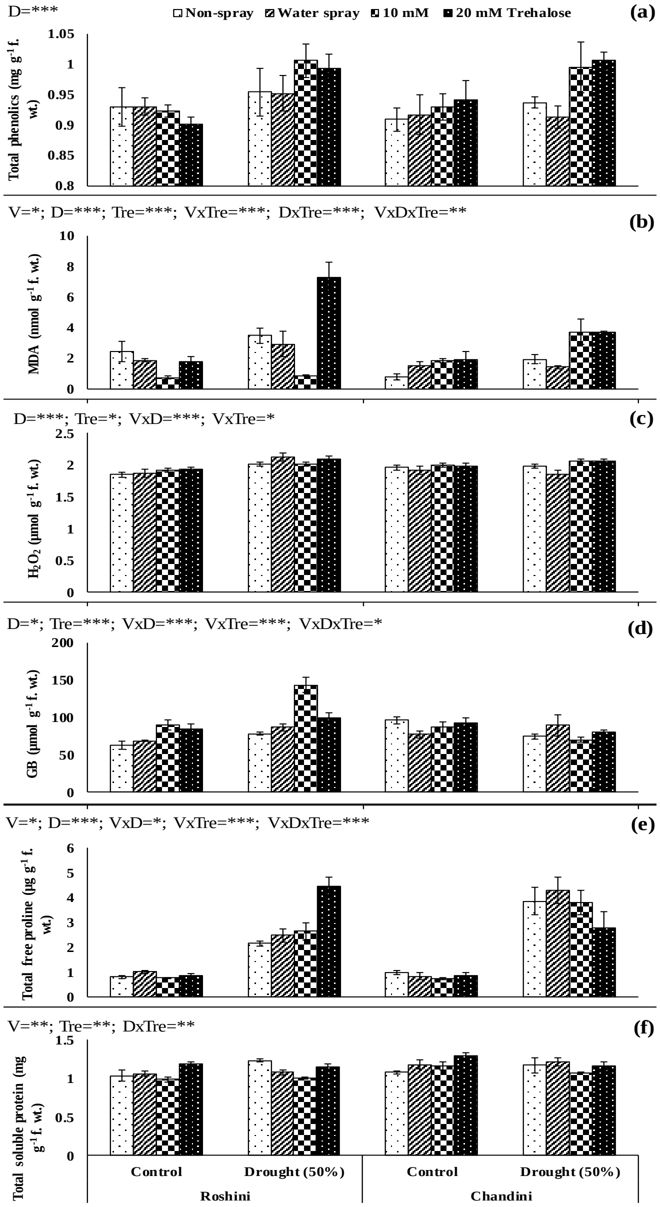


**Figure 3. Effect of application of trehalose on total phenolics (a), malondialdehyde (b), hydrogen peroxide (c), glycine betaine (d), total free proline (e) and total soluble protein (f) in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for total phenolics (a), malondialdehyde (b), hydrogen peroxide (c), glycine betaine (d), total free proline (e) and total soluble protein (f) in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** = Significant at 0.05, 0.01 and 0.001 levels respectively**

Effect of trehalose treatment was non-significant on root $Ca^{2+}$ of flax plant under both normal and water stress situation. Water shortage did not alter the shoot P concentration in flax. Under both normal and water stress situation, application of trehalose showed non-significant effect on shoot P of both flax varieties. Due to water shortage, P concentration enhanced ($P \leq 0.001$) in flax root. Trehalose treatment as foliar spray reduced ($P \leq 0.01$) the root P in Roshini and improved in Chandini. Under water scarcity, trehalose application (10 mM) significantly ($P \leq 0.05$) enhanced the root P but 20 mM trehalose treatment decreased the P concentration in flax root (Fig. 4 and 5).

***Nutrient use efficiency:*** Due to water scarcity, sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{2+}$) and phosphorus (P) use efficiency decreased ($P \leq 0.001$) in shoot and root of the flax plant. Trehalose application did not affect the shoot and root $Na^+$ use efficiency significantly in flax plant under both normal and stress conditions. Exogenously applied trehalose considerably diminished $K^+$ use efficiency in the shoot ($P \leq 0.01$) and root ($P \leq 0.05$) of flax. Integrated effect of foliarly applied trehalose and water shortage conditions decreased ($P \leq 0.05$) the shoot $K^+$ use efficiency in Chandini, on the other hand 10 mM trehalose level improved while 20 mM trehalose decreased the shoot $K^+$ use efficiency in Roshini. Collective effect of water stress and trehalose showed non-significant value for $K^+$ use efficiency of the flax root. Shoot $Ca^{2+}$ use efficiency of flax did not affect by trehalose treatment. Under drought stress, 10 mM trehalose level improved ($P \leq 0.05$) and 20 mM trehalose level lessened the shoot $Ca^{2+}$ use efficiency in Roshini, while in Chandini 10 mM trehalose significantly ($P \leq 0.05$) decreased and 20 mM trehalose enhanced the shoot $Ca^{2+}$ use efficiency. Trehalose application lowered ($P \leq 0.001$) the root $Ca^{2+}$ use efficiency in Roshini but in Chandini 10 mM trehalose enhanced and 20 mM trehalose reduced the $Ca^{2+}$ use efficiency of the root. Under drought stress conditions, trehalose ($P \leq 0.01$) reduced the $Ca^{2+}$ use efficiency of root in Chandini and improved in Roshini. In flax plant, shoot P use efficiency decreased ($P \leq 0.01$) by trehalose spray. Integrated effect of water stress and exogenously applied trehalose (10 mM) improved ($P \leq 0.05$) the shoot P use efficiency in Roshini and in Chandini all levels of trehalose reduced the P use efficiency of shoot. In both flax varieties, trehalose did not affect the root P use efficiency significantly. Under water scarcity, 20 mM trehalose considerably ($P \leq 0.01$) increased the P use efficiency of flax root (Fig. 5 and 6).

***Nutrient uptake*:** Under water deficit conditions shoot $Na^+$ uptake reduced ($P \leq 0.001$) in both flax varieties. Trehalose treatment considerably ($P \leq 0.05$) lessened the shoot $Na^+$ uptake in flax. Combined effect of trehalose and drought stress did not affect the shoot $Na^+$ uptake significantly.

Drought stress as well as trehalose treatment reduced ($P \leq 0.01$) the root $Na^+$ uptake in flax plant. Under water scarcity, trehalose application improved ($P \leq 0.05$) the root $Na^+$ uptake in Roshini while diminished in Chandini. Water stress considerably ($P \leq 0.001$) declined the $K^+$ uptake in shoot and root of the flax plant. Under control as well as stress condition, application of trehalose caused non-significant effects on $K^+$ uptake of shoot and root in both flax varieties (Fig. 6 and 7).

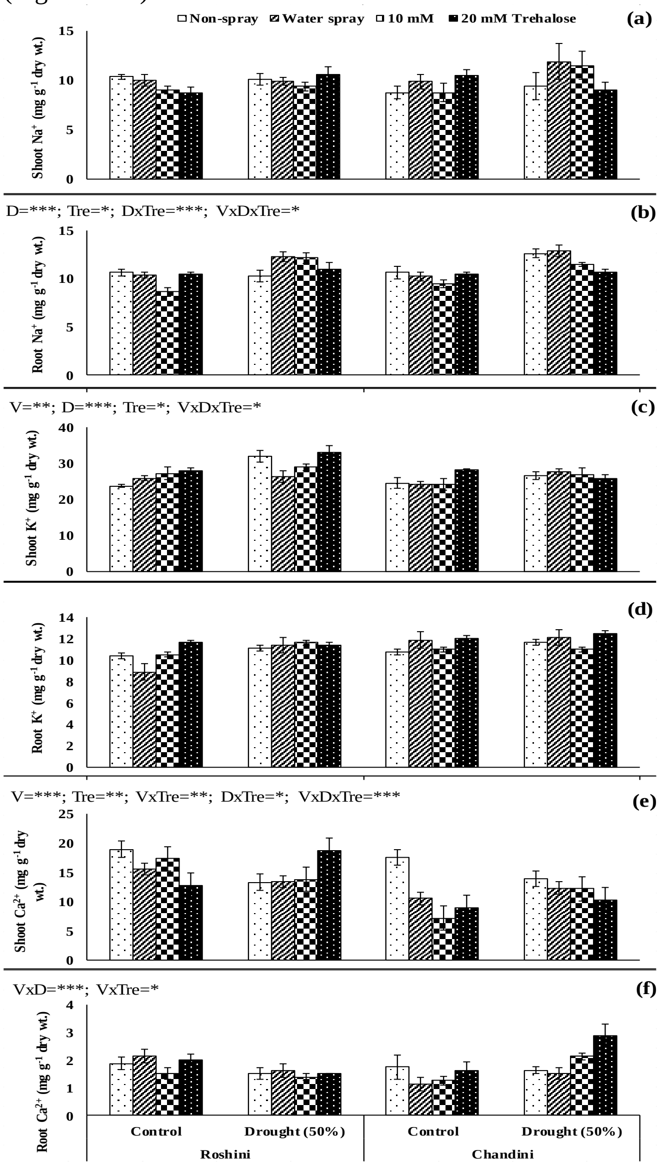


**Figure 4. Effect of application of trehalose on shoot and root $Na^+$ (a, b), $K^+$ (c, d) and $Ca^{2+}$ (e, f) in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for shoot and root $Na^+$ (a, b), $K^+$ (c, d) and $Ca^{2+}$ (e, f) in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** = Significant at 0.05, 0.01 and 0.001 levels respectively**

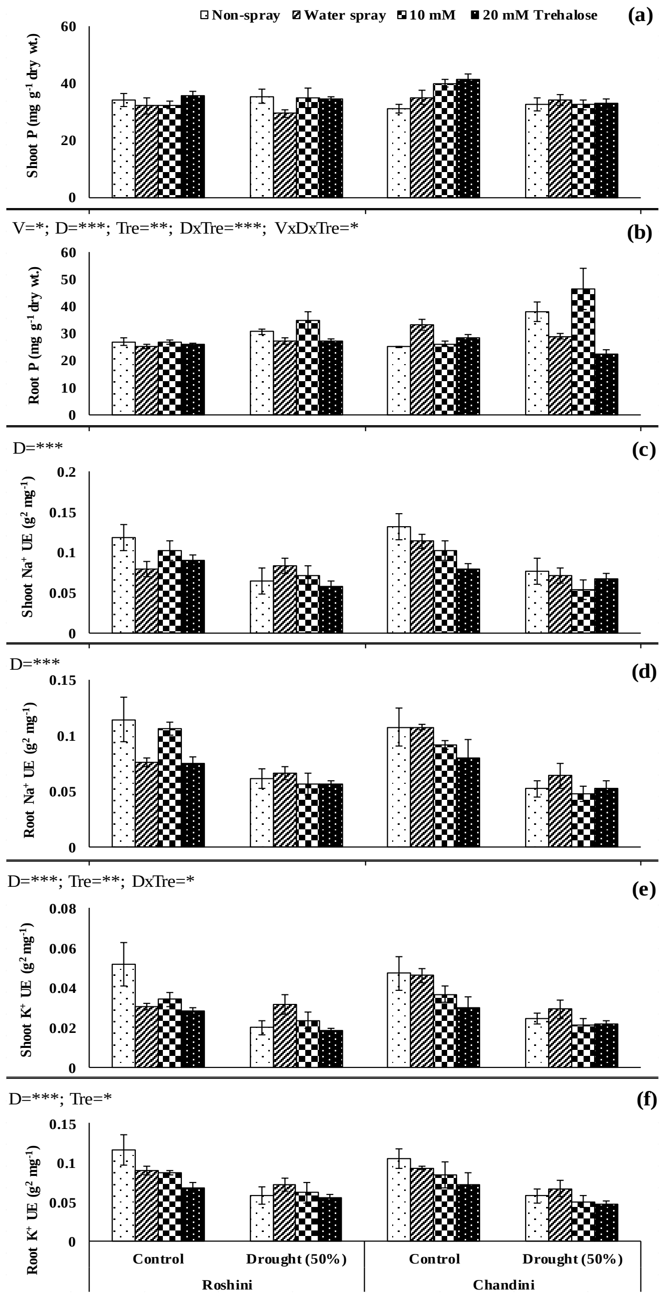


**Figure 5. Effect of application of trehalose on shoot and root P (a, b), shoot and root $Na^+$ (c, d) and $K^+$ (e, f) use efficiency in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for shoot and root P (a, b), shoot and root $Na^+$ (c, d) and $K^+$ (e, f) use efficiency in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** =**

**Significant at 0.05, 0.01 and 0.001 levels respectively**

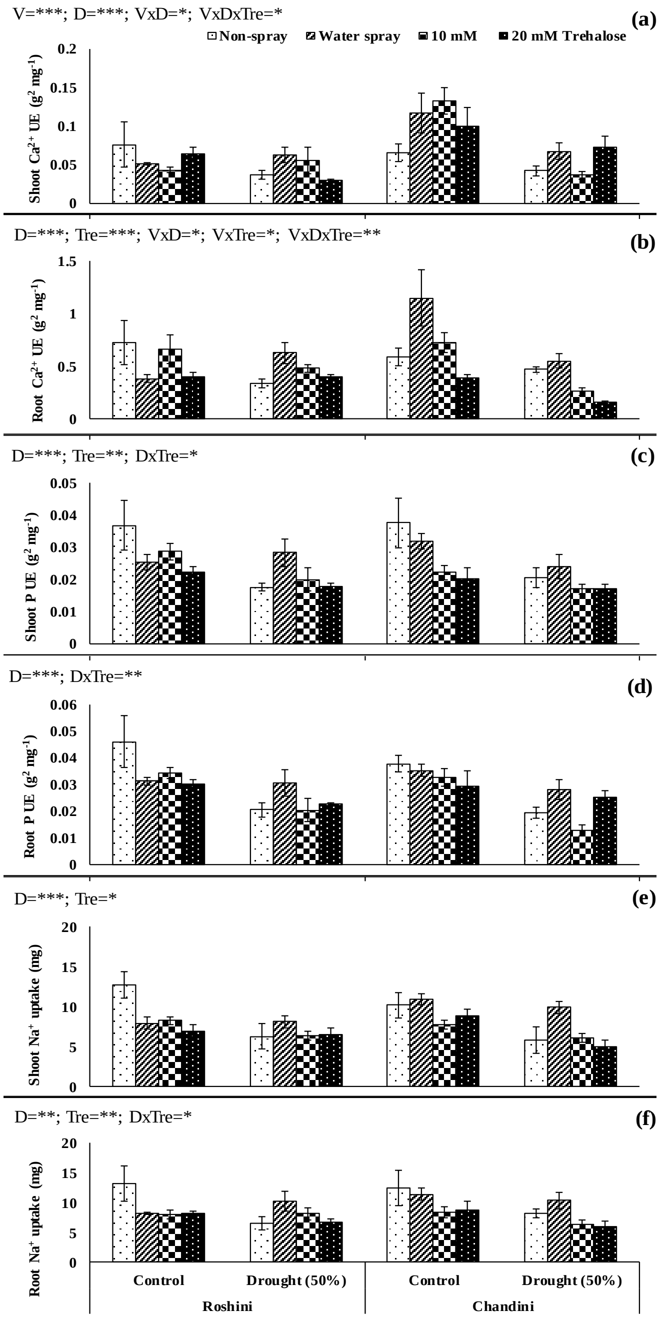


**Figure 6. Effect of application of trehalose on shoot and root $Ca^{2+}$ (a, b) and P (c, d) use efficiency and shoot and root $Na^{+}$ (e, f) uptake in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for shoot and root $Ca^{2+}$ (a, b) and P (c, d) use efficiency and shoot and root $Na^{+}$ (e, f) uptake in two flax varieties subjected to trehalose under control and drought stress**

**conditions. *, **, *** = Significant at 0.05, 0.01 and 0.001 levels respectively**

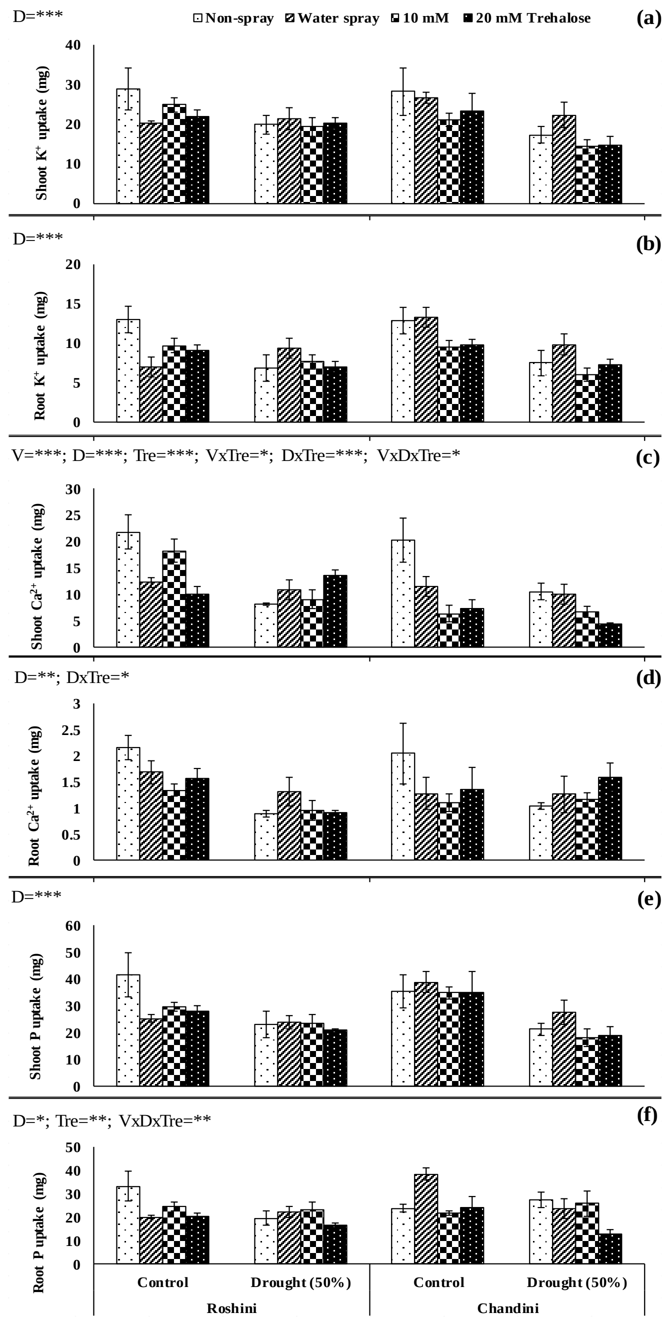


**Figure 7. Effect of application of trehalose on shoot and root $K^{+}$ (a, b), $Ca^{2+}$ (c, d) and P (e, f) uptake in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for shoot and root $K^{+}$ (a, b), $Ca^{2+}$ (c, d) and P (e, f) uptake in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, ***

**= Significant at 0.05, 0.01 and 0.001 levels respectively**

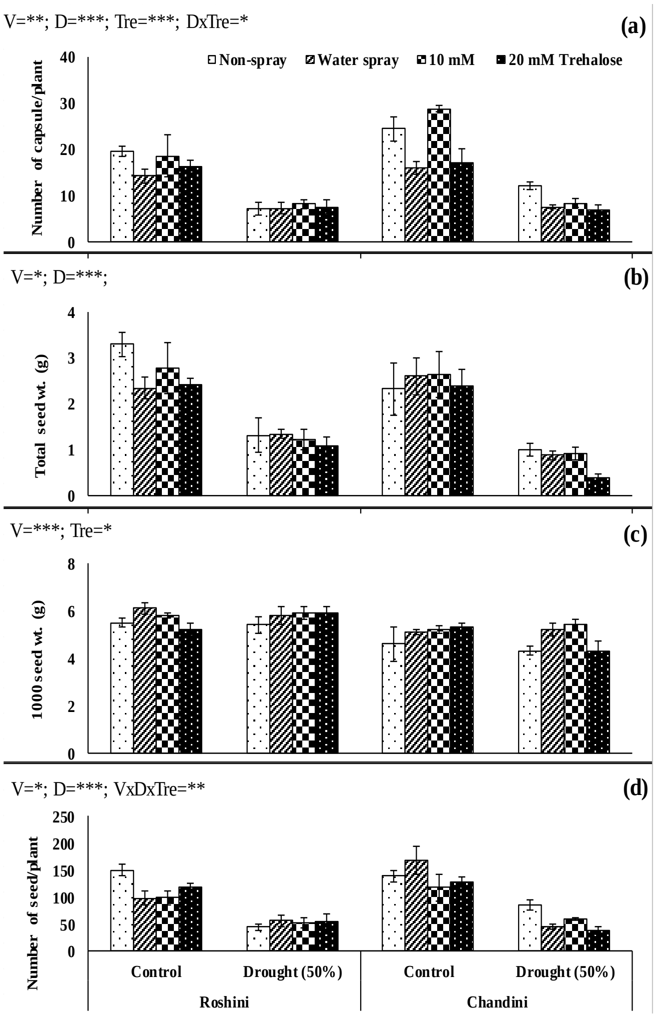


**Figure 8. Effect of application of trehalose on number of capsule/plant (a), total seed wt. (b), 1000 seed wt. (c) and number of seed/plant (d) in two flax varieties under control and drought stress conditions (mean ± S.E; n = 4). Mean squares from ANOVA of data for number of capsule/plant (a), total seed wt. (b), 1000 seed wt. (c) and number of seed/plant (d) in two flax varieties subjected to trehalose under control and drought stress conditions. *, **, *** = Significant at 0.05, 0.01 and 0.001 levels respectively**

Water deficit conditions significantly decreased the $Ca^{2+}$ uptake in shoot ($P \leq 0.001$) and root ($P \leq 0.01$) of flax. Exogenously applied trehalose lessened ($P \leq 0.001$) the shoot $Ca^{2+}$ uptake in flax. Integrated effect of water shortage and trehalose application enhanced ($P \leq 0.001$) the shoot $Ca^{2+}$ uptake in Roshini but decreased in Chandini. More shoot $Ca^{2+}$ uptake ($P \leq 0.001$) observed by Roshini. Trehalose application had non-significant effect on root $Ca^{2+}$ uptake of the flax. In drought affected plants, trehalose treatment improved ($P \leq 0.05$) the $Ca^{2+}$ uptake by root in flax. Water deficit condition markedly ($P \leq 0.001$) reduced the shoot P uptake in flax. Trehalose application caused non-significant effect on shoot P uptake under control as well as stress conditions. Water shortage condition enhanced ($P \leq 0.05$) the root P uptake in Chandini while decreased in Roshini. Foliarly applied trehalose reduced ($P \leq 0.01$) the root P uptake in Roshini but in case of Chandini, 20 mM level of trehalose slightly enhanced the root P uptake. Under water scarcity, 10 mM trehalose application lessened ($P \leq 0.01$) the root P uptake in Chandini and enhanced in Roshini, while 20 mM trehalose application significantly reduced the root P uptake in both flax varieties (Fig. 7).

***Yield attributes*:** Water scarcity decreased 49-60% ($P \leq 0.001$) the number of capsules/plant, number of seeds/plant and total seed weight while 1000 seed weight did not alter by water shortage condition in both flax varieties. Trehalose treatment caused non-significant effect on number of seed/plant and total seed weight in flax. Under water deficit conditions, trehalose application considerably enhanced (40-46%) the number of capsule/plant ($P \leq 0.05$) and number of seeds/plant ($P \leq 0.01$) in Roshini and reduced in Chandini. In both flax varieties, trehalose considerably ($P \leq 0.05$) improved (7-10%) the 1000 seeds weight. Number of capsules/plant improved ($P \leq 0.001$) in Chandini and reduced in Roshini by 10 mM level of trehalose while 20 mM trehalose application reduced the number of capsules/plant in both flax varieties (Fig. 8).

## DISCUSSION

In our experimentation, water scarcity diminished the shoot fresh weight, shoot dry weight and shoot length of flax. Water shortage reduced the cell elongation, cell expansion, turgor pressure, water holding capacity while enhance the production of reactive oxygen species (Dawood and Sadak, 2014) and alter the physiological and chemical processes as a result growth parameters decreased (Zulfiqar *et al*., 2021). Trehalose application (20 mM) enhanced the shoot fresh weight in Chandini. Similarly, growth attributes increased in maize (Zeid, 2009; Ali and Ashraf, 2011), sunflower (Kosar *et al*., 2018), rice (Shahbaz *et al*., 2017), cowpea (Khater *et al*., 2018), radish (Akram *et al*., 2016) and quinoa (Elewa *et al*., 2017) by application of trehalose. Foliarly applied trehalose (5 mM) was beneficial to lessen the consequences of water shortage and enhanced the growth attributes of flax varieties, Azur and Sakha-1 (Sadak *et al*., 2019). Trehalose enhanced the shoot length in both flax varieties, similar consequences were observed in *Brassica* species (Alam *et al*., 2014), maize (Rohman *et al*., 2019) and *Catharanthus roseus*

(Chang *et al*., 2014). Trehalose application protects the plants from stress situations by improving the carbohydrates accumulation and water uptake (Queiroz and Cazetta, 2016). Trehalose enhanced the growth and production of *Arabidopsis thaliana* by scavenging the ROS under water scarcity (Stolker, 2010).

Chlorophyll *a*/*b* ratio increased by 10 mM trehalose and decreased by 20 mM trehalose in Chandini. Trehalose application considerably increased the carotenoids contents in Roshini but decreased in Chandini. Photosynthetic pigments decrease may be due to the disturbance in photosynthetic system that cause reduction in carbon assimilation (Din *et al*., 2011). Early research demonstrated that application of trehalose enhanced the chlorophyll contents in rice (Abdelgawad *et al*., 2014; Shahbaz *et al*., 2017), cowpea (Khater *et al*., 2018), sunflower (Kosar *et al*., 2018), maize (Zeid, 2009), quinoa (Elewa *et al*., 2017), *Brassica* species (Alam *et al*., 2014) and flax (Sadak *et al*., 2019). Under stress conditions, trehalose enhanced the chlorophyll pigments by sustaining the chloroplast's osmotic potential and alleviating the tonoplast (Sadak, 2019).

In our findings, SOD activity decreased in flax due to drought stress and in maize also (Gou *et al*., 2017). Antioxidants like SOD, POD and CAT (Abdelgawad *et al*., 2014) and trehalose application (Stolker, 2010) perform a significant role to scavenge the ROS. Trehalose enhanced the antioxidants (CAT, POD, SOD) activities in both flax varieties under water shortage, similar consequences were observed in cowpea (Khater *et al*., 2018) and sweet basil (Zulfiqar *et al*., 2021). Under water shortage, trehalose enhanced the SOD activity in *Lemna gibba*, cowpea (Khater *et al*., 2018), rice (Nounjan *et al*., 2012), maize (Ali and Ashraf, 2011) and quinoa (Sadak *et al*., 2019). Exogenously applied trehalose activates the genes expressions to increase the production of secondary metabolite that eliminates the effect of various stresses (Aghdasi *et al*., 2008). Moreover, trehalose enhanced the resistance by improving the activities of antioxidants under stress conditions (Aldesuquy and Ghanem, 2015). Antioxidants improves the plants tolerance by detoxifying the ROS under environmental stress (Singh *et al*., 2010). Trehalose application protects the plants from environmental stresses by improving the defense system, antioxidants, secondary metabolites production, development and yield in higher plants (Krasensky and Jonak, 2012). Under water deficit conditions, leaf ascorbic acid concentration increased in Roshini variety of flax which may be beneficial to detoxify the effect of drought stress. Ascorbic acid acts as an antioxidant and reduced the toxic effects of free radicals (Ye *et al*., 2012). Trehalose treatment improved the leaf ascorbic acid in both flax varieties, as was also observed in maize (Ali and Ashraf, 2011). Trehalose increased the leaf ascorbic acid because it acts as a regulatory molecule which activates the functional and chemical processes that are responsible for adaptive mechanisms in plants under stress conditions (Zeid, 2009).

Total phenolic increased in flax under water deficit conditions, Similar findings observed in quinoa (Elewa *et al*., 2017), flax plant (Sadak *et al*., 2019), cowpea (Khater *et al*., 2018) and wheat (Aldesuquy and Ghanem, 2015). Phenolic contents increased due to drought stress because phenolic maintains the oxidative stability of oils of seed (Ali *et al*., 2013), scavenger of free radicals, regulate the metabolic processes that enhance the development, decrease the effect of water stress and fluidity of membranes (Bakry *et al*., 2012). Malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) contents increased in both flax varieties under water shortage conditions. Under water stress, accumulation of MDA measures the damages caused by oxidative stress in plant (Abdelhamid *et al*., 2013). Due to water shortage plants closes their Stomata as a result inside movement of $CO_2$ decreased that increased MDA content as a result electron acceptor decreased in the cell and reduction of $O_2$ takes place instead of $CO_2$ which improved the ROS production (Aldesuquy and Ghanem, 2015) and increased in ROS production abolish the structure of macromolecules (Ashraf, 2009). Under water deficit conditions, trehalose (20 mM) increased the MDA content in Chandini but in case of Roshini, 10 mM trehalose application was effective to enhance the MDA content similar consequences studied in wheat and sweet basil (Ibrahim and Abdellatif, 2016; Zulfiqar *et al*., 2021 respectively). Damage in plants due to oxidative stress measured by the accumulation of MDA content under water stress situation and MDA accumulates due to lipid peroxidation of membrane (Abdelhamid *et al*., 2013). Under water stress, plants close the stomata to reduce the water loss which interrupt the $CO_2$ movement inside the plant as a result $O_2$ reduction start that increase the ROS production which destroys the macromolecules (Aldesuquy and Ghanem, 2015). In our experiment, trehalose increased the MDA content in Chandini and $H_2O_2$ content in both flax varieties. Trehalose application decreased the MDA contents in Roshini same results studied in rice (Shahbaz *et al*., 2017), *Brassica* species (Alam *et al*., 2014), wheat (Sadak, 2019) and quinoa (Sadak *et al*., 2019). Under different stress environment, trehalose plays a crucial role to eliminate the oxidative stress (Nounjan *et al*., 2012) by improving the production of antioxidants and scavenging the ROS (Sadak, 2019).

Trehalose application improved the GB content in Roshini and reduced in Chandini. Previous findings showed that trehalose increased the GB contents in maize, *Brassica campestris*, radish, rice, sunflower and sweet basil (Ali and Ashraf, 2011; Alam *et al*., 2014; Akram *et al*., 2016; Shahbaz *et al*., 2017; Kosar *et al*., 2018; Zulfiqar *et al*., 2021 respectively). Trehalose increased the GB level in sunflower which is helpful to tolerate the water stress (Kosar *et al*., 2018). Trehalose application increased the TSP in Chandini but in case of Roshini, only 20 mM trehalose improved the

TSP, similar consequences were observed by Shahbaz *et al*. (2017) and Zeid (2009) in rice and maize respectively.

Under water scarcity, foliage applied trehalose increased the total free proline in Roshini, similar results were observed in sweet basil (Zulfiqar *et al*., 2021), sunflower (Kosar *et al*., 2018), *Brassica campestris* (Alam *et al*., 2014), radish (Akram *et al*., 2016), cowpea (Khater *et al*., 2018), maize (Zeid, 2009) and flax (Sadak *et al*., 2019). Proline accumulation is a strategy adapted by plants to tolerate the water stress conditions (Sadak *et al*., 2019), because it sustains the osmotic stability via protecting the enzymes, proteins and membrane under water deficit situations (Ashraf and Foolad, 2007) and may eliminate the activity of catabolizing enzymes and proline oxidase (Bakry *et al*., 2012). It also helpful to defends the plants from oxidative stress by detoxify the ROS (Zulfiqar *et al*., 2021). Exogenously applied trehalose increased the osmoprotectants production inside the plants to diminish the oxidative stress and abiotic stresses (Asaf *et al*., 2017).

In both flax varieties, movement and absorption of $Na^+$ ions decreased under water stress condition that minimizes the $Na^+$ level and effect the ionic balance (Zhu, 2003). Shoot $K^+$ increased due to water deficit conditions in flax plant. Potassium is an important element which improves the water holding capacity, defends the membrane from desiccation, maintained the turgor pressure, enzymatic progressions and adjust the osmotic pressure (Zhao *et al*., 2006).

Water shortage reduced the yield and its components (Ghassemi-Golezani *et al*., 2013) reduction in yield depends on developmental stage, intensity and duration of stress (Khater *et al*., 2018). Drought stress decreased the production in maize (Hussain *et al*., 2019), wheat (Aldesuquy *et al*., 2018), flax (Sadak *et al*., 2019) and cowpea (Khater *et al*., 2018). At early reproductive stage, quality and quantity of yield decreased under water shortage situations because plant grain filling period shorten (Shahryari *et al*., 2008). Under water scarcity, trehalose application enhanced the number of seeds per plant and number of capsules per plant in Roshini. Similar consequences were observed in earlier experiments that foliage applied trehalose eliminate the contrary effects of water scarcity and improved the yield in quinoa, cowpea, wheat and flax (Elewa *et al*., 2017; Khater *et al*., 2018; Aldesuquy *et al*., 2018; Sadak *et al*., 2019 respectively). In our findings, trehalose application increased the 1000 seed weight and number of capsules/plant same results were observed in rice (Shahbaz *et al*., 2017) that trehalose application improved the yield attributes. Seeds treatments with osmolytes, vitamins, antioxidants etc helpful to improve the plants performance and production (Dawood and Sadak, 2014).

***Conclusion*:** Trehalose is an osmoprotectant which is helpful to reduce the effects of different abiotic stresses in different plants and improve the production. Integrated effect of exogenously applied trehalose and drought stress considerably improved the antioxidant activity, total free proline and total phenolics in flax which are helpful to diminish the toxic effect of drought stress. Overall, performance of Chandini was better than Roshini under control as well as stress situations, 10 mM trehalose application was more effective to ameliorate the effect of stress situations. Flax seed oil is used in different industries (medicinal, pharmaceutical) and its oil has various beneficial contents, but the effect of different osmolytes including trehalose on the oil content of flax and other crops is limited.

***Conflict of interest:*** The authors declares that they have not conflict of interest.

***Author's contribution statement"***It is declared that each author contributed equally to the article.

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