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Impact of Water-deficit and Salinity stress on Seed Germination and Seedling Growth of *Capsicum annuum* 'Solan Bharpur'

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Abstract

In both natural and agricultural conditions plants are frequently exposed to environmental stresses. The aim of present study is to determine the adverse effects of abiotic stresses viz. water and salinity stress on the growth of Capsicum annuum 'Solan Bharpur' *at the germination and seedling growth stage using polyethylene glycol 6000 (5% PEG and 10% PEG) and NaCl (50 mM NaCl and 100 mM NaCl). Proline, MDA and chlorophyll content were also assessed. Seed germination and seedling growth reduced with increasing concentrations of PEG or NaCl. However, PEG induced water stress caused more growth inhibition compared to NaCl induced salinity stress. Water and salinity stress caused increase in the level of proline and MDA of both shoots and roots. The total chlorophyll content decreased with an increase in water or salinity stress.*

Key words: Stress, Polyethylene glycol, NaCl, Proline, MDA, Chlorophyll content.

Introduction

The term stress is often used with various meanings. The definition and appropriate term for stress are referenced as responses to different situations. The flexibility of normal metabolism allows the development of responses to environmental changes; those fluctuate regularly and predictably over daily and seasonal cycles[1]. Abiotic stresses including drought and salinity stress are currently the major factors which reduce crop productivity worldwide. Excessive amount of salts in soil severely reduced the seed germination and further seedling growth[2]. Water deficit, is defined as the absence of adequate moisture necessary for a plant to grow normally[3]. When a plant is exposed to high salinity and water stress, its major processes such as lipid peroxidation, photosynthesis and protein synthesis are affected.

India is the second largest producer of vegetables in the world but production of vegetables is much less than the daily requirement of increasing population of India. Efforts are to increase the production of vegetables at national level. It is noticed in a report of Indian Council of Agricultural Research that the present production of 90.8 million tonnes needs to be raised to 250 million tonnes by 2024-2025[4]. Nutritionally, vegetables are important as they are rich source of vitamins, minerals and dietary fibers[5]. From many years growing vegetables has been the mainstay of rural economy and has emerged as an indispensable component of agriculture[6]. Of the various vegetables capsicum's nutritive value is high as it contains 1.29 mg protein, 11 mg calcium, 870 I.U. vitamin A, 17.5 mg ascorbic acid, 0.6 mg thiamin, 0.003 mg riboflavin and 0.55 mg niacin per 100 g of edible fruit[7]. It is also a good source of ascorbic acid, tocophenols, provitamin A, carotenoids including lutein, β-carotene, capsanthin and violaxanthin which contribute to antioxidative activity[8]. The major objective of present investigation is to study effects of water deficit and salinity stress on germination and growth of seedling, free proline, MDA level and chlorophyll contents in *C. annuum* 'Solan Bharpur' which is selection from cross between california wonder and chilli.

Material and Methods

Mature seeds of *Capsicum annuum* 'Solan Bharpur' were collected from Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. India. Seeds of *C. annuum* 'Solan Bharpur' selected for uniformity were surface sterilized, washed thoroughly under tap water and soaked in water (control), 5% PEG, 10% PEG, 50 Mm NaCl and 100 mM NaCl solution for 24 hours. Thereafter, the seeds were transferred to petriplates lined with three layers of filter papers moistened either only by distilled water (control) or by effect of PEG or NaCl concentrations of same volume. The seeds were then allowed to germinate in BOD incubator at 25 ± 2^0C under continuous illumination provided by fluorescent white light. Emergence of 2-5 mm radical was taken as seed germination. After 25 days of germination, seedling growth was measured in terms of root length; shoot length and seedling fresh weight. Seedlings, 15 days old were shifted to hydroponic culture containing Hoagland nutrient solution and allowed to grow in BOD incubator at 25±2ºC for further growth. Nutrient medium was replaced by fresh one at regular intervals. After 14 days of shifting to BOD the plants were treated with 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl through appropriate addition to the nutrient medium. After 21 days of treatment, determination of proline, lipid peroxidation and chlorophyll contents were done.

Determination of free proline content: Free proline was estimated spectrophotometrically following the method of Bates et al[9]. The leaves / roots weighing 200/150 mg were homogenized with 3 % sulphosalicyclic acid (SSA). The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. Supernatant (2ml) was reacted with 2 ml of freshly prepared ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml glacial acetic acid and 20 ml of 6 molar orthophosphoric acid with warming and stirring) and 2 ml of glacial acetic acid in a test tube and then was kept in a boiling water bath at 100^0C for 1 hour. The reaction was terminated in an ice bath and then shifted to room temperature. Thereafter, the reaction mixture was extracted with 4 ml of toluene, mixed vigorously with test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the calibration curve.

Lipid peroxidation: Lipid peroxidation was estimated from accumulated malondialdehyde (MDA) following the method given by Dhindsa et al[10]. Leaves or roots was used as test material. The leaf / root weighing 200/150 mg were homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected. Supernatant (2ml) was reacted with 4 ml of 0.5 % thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and kept at 95 ^{0}C in a water bath for 30 minutes. The reaction was terminated by cooling the reaction mixture in ice for 5 minutes. Absorbance was read at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600nm. MDA content[11] was determined using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Chlorophyll estimation: Leaf tissue weighing 100 mg was homogenized with 80% acetone. The homogenate was centrifuged at 5000 rpm for 5 min. Final volume was made approximately 5 ml with 80% acetone. The absorbance of extract was read at 663 and 645 nm.

Amount of total chlorophyll, chlorophyll a (chl a) and chlorophyll b (chl b) was estimated by using following equations given by Harborne[12]:

$$\text{Chl a (mg/g)} = \frac{12.3\ A_{663} - 0.86\ A_{645}}{a \times 1000 \times w} \times v$$

$$\text{Chl b (mg/g)} = \frac{19.3\ A_{645} - 3.6\ A_{663}}{a \times 1000 \times w} \times v$$

In these equations; v corresponds to volume (ml), a to length of path of light i.e. 1 cm and w to fresh weight of tissue (g).

Statistical analysis: The experiments had completely randomized design and each experiment was repeated at least thrice. Data are analyzed using T- test at p=0.05 for significance. The standard error is plotted in all graphics.

Results and Discussion

In the present study the effects of water deficit and salinity stress were monitored on seed germination and seedling growth. Seed germination and early seedling growth are the most vulnerable stages of the plant life cycle where plants are frequently subjected to water-deficit during the dry season or grown in salty lands. Salt and drought stresses are two most important abiotic stresses that limit the number of seedlings and seedling growth[13]. *C. annuum* 'Solan Bharpur' seeds were non-dormant and responded differentially to PEG (water deficit) and NaCl concentrations (figures 1-2). Seeds germination in control started on 6th day of incubation and thereafter progressed gradually. Seed germination rate decreased at 5% PEG, 10% PEG and 100 mM NaCl. However, at 50 mM NaCl the germination increased. PEG treatments proved to be inhibitor of germination. But NaCl pre-treatments of seeds increased the germination of seeds at lower concentration. Interestingly, the seed germination initially imposed by NaCl got diminished with lapse of time. Since NaCl was applied only once, it is likely that NaCl concentration got diluted due to the growth of embryo and eventually seedlings.

The seedling growth of *C. annuum* '*Solan* Bharpur' was measured in terms of seedling root length, shoot length and fresh weight as shown in figure 3. Root length of seedling increased at 50 mM NaCl concentration but it decreased at 5% PEG, 10% PEG and 100 mM NaCl. Seedling shoot length and seedling fresh weight decreased in all treatments (5% PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl) compared to control. The root length of *C. annuum* 'Solan Bharpur' in PEG treatments decreased with an increase in concentration. This suggest that *C. annuum* 'Solan Bharpur'can tolerate drought up to a certain degree as root length is an important trait against drought stress in several plant species[14]. But in case of NaCl root length increased at lower concentration (50 mM) but decreased at higher concentration (100 mM). An increased root growth might be due to mild salinity stress. Reduction in root length at higher concentration in response to salinity may be due to Na^+ and Cl^- ions in growing media. The Na^+ and Cl^- affects root permeability due to displacement of Ca^+ ions from the plasma lemma, which inhibits roots growth and root length[15]. Shoot length and seedlings fresh weight decreased with PEG and NaCl treatments which is similar to several earlier reports[16].

In another experiment, physiological / metabolic responses of *C. annuum* 'Solan Bharpur' under water and salinity stress were assessed in hydroponic system. The free proline content was measured in leaves and roots of *C. annuum* 'Solan Bharpur' after treating the plants with PEG and NaCl treatments in hydroponic culture for twenty one days (Figure 4). The proline content in leaves and roots increased significantly with an increase in PEG and NaCl concentration. The free proline content in leaves was 41.62 %, 44.97 %, 38.0 % and 44.1% greater than control at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl treatments, respectively. Likewise, the roots free

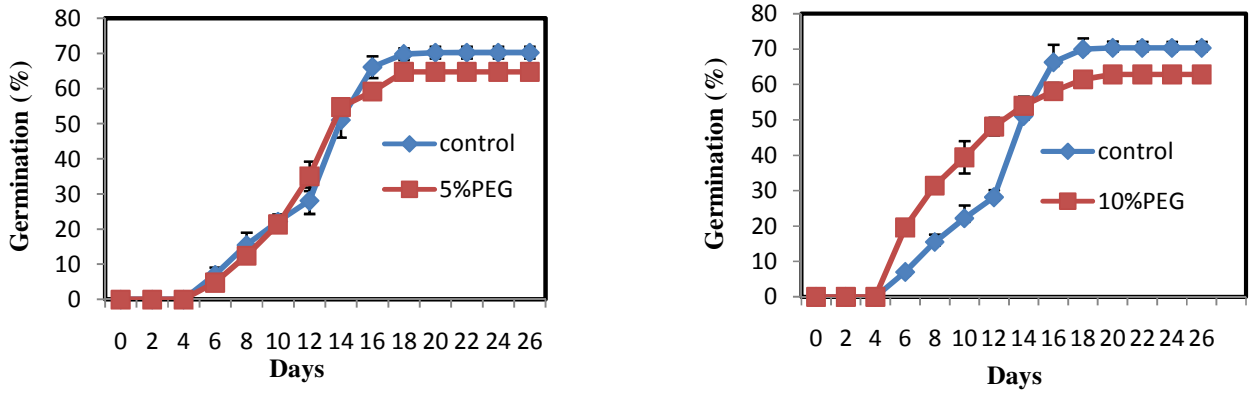
proline content was 22.7 %, 29.16 %, 14.14 % and 22.7 % higher than control at the same treatments. It was observed that the increase in free proline content induced by the PEG treatments was much more than induced by NaCl treatments. It is evident from figure 4 that increase in proline content in leaves was much more as compared to roots at both PEG and NaCl treatments than control. The most prominent function of proline might be to act as an osmoregulator and thus keeping cells turgid. Free proline accumulation in water stressed plants has also been suggested to serve as an index of drought hardness; higher proline accumulation being a characteristic of resistant cultivars[17]. Free proline has also been demonstrated to act as an antioxidant[18].

The MDA content in leaves and roots was measured as an index of lipid per oxidation. The quantitative estimation of MDA content was done in leaves and roots in figure 5. MDA content increased with an increase in concentration of NaCl. It also increased in 10 % PEG treatment more than 5 % PEG treatment. MDA content present in leaves was always higher than control and was 68.8, 83.8, 66.0 and 75.5 n mol g^{-1} FW at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl treatments, respectively. In roots also the MDA content was higher than control and was 9.3, 11.0, 9.0 and 10.0 n mol g^{-1} FW at 5 % PEG, 10 % PEG, 50 mM NaCl and 100 mM NaCl, respectively. But increase in MDA content was more in leaves as compared to roots. Generally, abiotic stresses caused an extensive lipid per oxidation which has been often used as an indicator of stress induced oxidative damage of membrane. The increase in MDA contents might be due to the increased antioxidative enzymes which have scavenged various reactive oxygen species (ROS) produced due to abiotic stresses.

The content of chlorophyll a in leaves decreased with an increase in PEG concentration. Likewise, the content of chlorophyll b in leaves decreased with an increase in PEG concentration. It is evident from figure 6 that chlorophyll a content was higher in leaves as compared to chlorophyll b. The chlorophyll a: b ratio altered marginally with the PEG (5 % or 10 %) and NaCl (50 or 100 mM) treatments. The total chlorophyll content of leaves decreased with an increase in PEG and NaCl concentrations. The magnitude of reduction was more in PEG treated plants than in NaCl treatments (Figure 6). The chlorophyll content was suppressed by both stresses; magnitude of reduction being greater in PEG treatments. Our results of decrease in chlorophyll content corroborated with the findings of [19,20], who also found a decrease in chlorophyll content with NaCl stress in five widely cultivated rice (*Oryza sativa* L.).The loss in chlorophyll content lead to disruption of photosynthetic machinery. Moreover, the high activity of chlorophylls enzyme which activated by salinity lead to chlorophyll reduction[21].

Conclusion

Plants have inbuilt ability to adjust to seasonal environmental variables. Apart from the environmental variables, there may be certain other rapid and predictable disturbances in environment resulting in stressful conditions. Plants are exposed to many stress conditions such as low temperature, salt, drought, flooding and heavy metal toxicity. The capacity for a plant species to adjust these stresses is usually limited and varies from plant to plant. The findings from present study reveal the nature and magnitude of the responses of *C.annuum* 'Solan Bharpur' an important vegetable of mid hill of Himachal Pradesh to water deficit and salinity stress. Stress effects are evident from germination to plant growth. Water deficit and salinity stress seem to involved in observed growth suppression where as proline and MDA accumulation might help plants to stressful conditions to some extent.



(A) (B)

Figure-1
Time–course of germination of *C. annuum* 'Solan Bharpur' seeds as affected by 5 % PEG (A) and 10 % PEG (B) treatments. The values are mean ± S.E

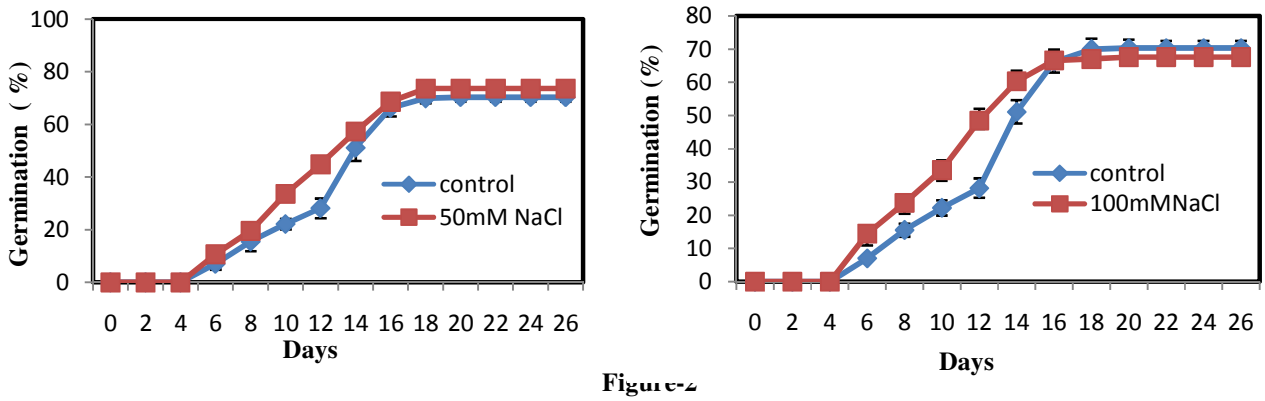


Figure-2

Time–course of germination of *C. annuum* 'Solan Bharpur' seeds as affected by 50 mM NaCl (A) and 100 mM NaCl (B) treatments. The values are mean ± S.E

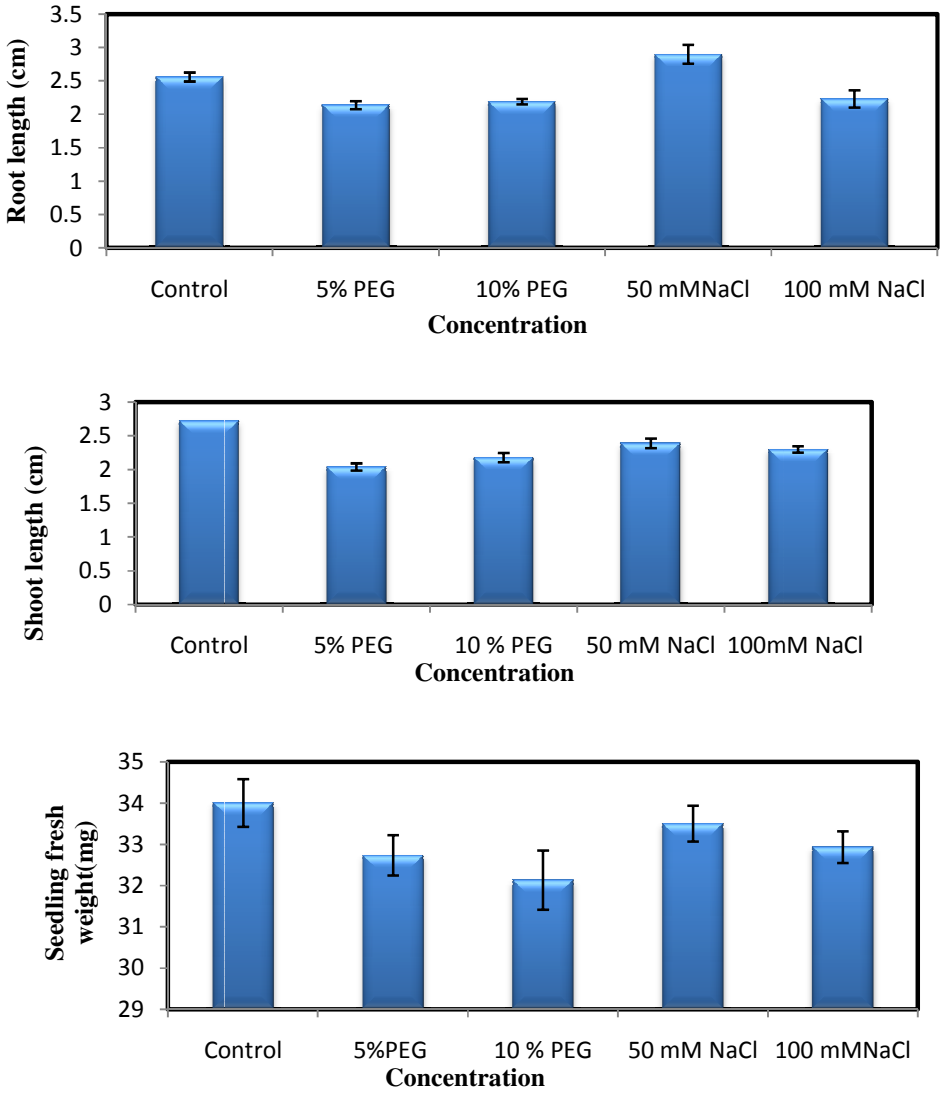


Figure-3

Effect of PEG (%) and NaCl (mM) treatments on root length (A); shoot length (B) and seedling fresh weight (C) Growth measured after 25 days of incubation. Values are mean ±S.E. (n=15)

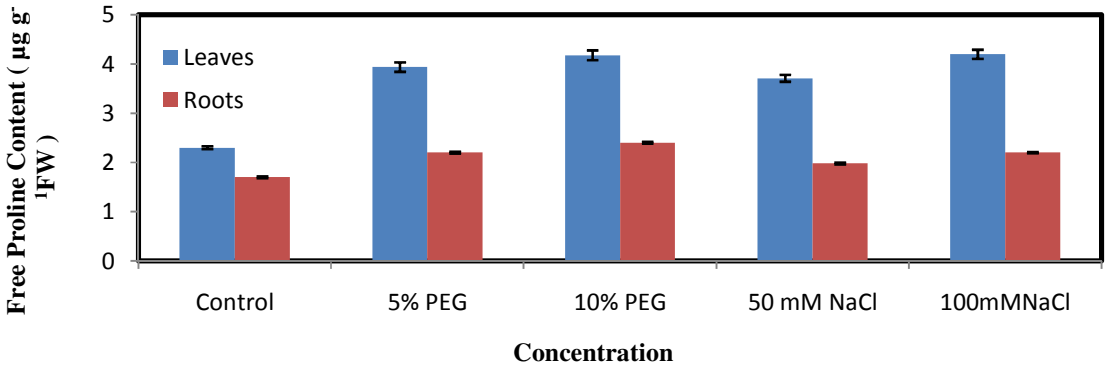


Figure-4
Effect of PEG-6000 and NaCl on free proline content in leaves and roots *C. annuum* 'Solan Bharpur'. Seedlings (15 days old) were grown hydroponically for 14 days and subsequently exposed to stress for 21 days. Values are mean ± S.E.

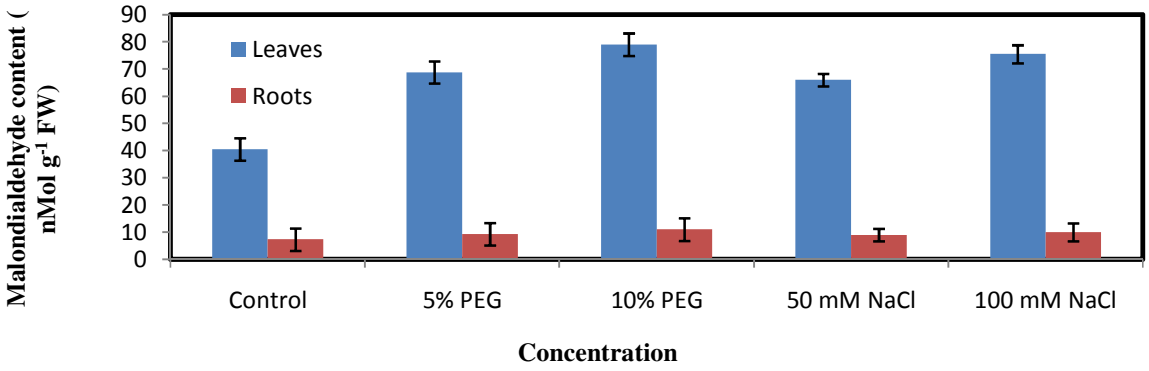
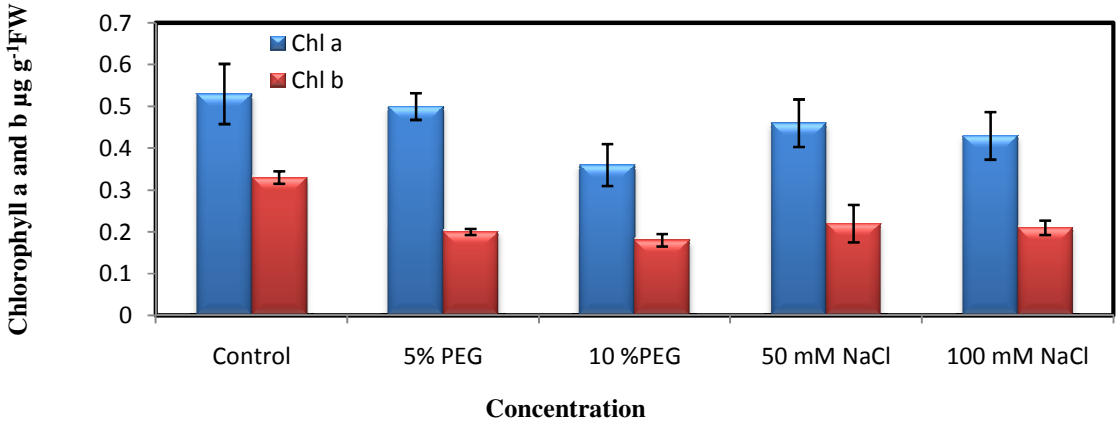


Figure-5
Effect of PEG-6000 and NaCl on MDA contents in leaves and roots of *C. annuum* 'Solan Bharpur'. Seedlings (15 days) old were grown hydroponically for 14 days and subsequently exposed to stress for 21 days. Values are mean ± S.E



(A)