



RESPONSE OF *CHENOPODIUM ALBUM* L. TO VARYING LEVELS OF WATER STRESS: EFFECTS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

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Abstract

The frequency and intensity of drought is increasing in recent years in many parts of the world, affecting the growth and yield of plants. *Chenopodium album* L. is known to have high nutritional value. The present work focuses on studying the effect of water stress on morphological parameters such as shoot and root length, leaf and branch number and biomass and physiological as well as biochemical parameters such as relative water content (RWC), chlorophyll content, ascorbic acid and free proline content of *Chenopodium album* L. Plants were grown under artificially simulated drought stress conditions with moisture stress ranging from mild to severe stress. Results showed that severe water stress significantly reduced the relative water content by 28.28% plant height by 28.31 and biomass by 44.58%. Leaf number and branch number was also reduced. However, root length was increased by 48.32%. Chlorophyll b was more affected than a and was reduced by 47.16% at moderate water stress. Ascorbic acid acts as an important antioxidant whereas proline provides protection under stressful conditions and stabilizes the enzymes and proteins. Ascorbic acid was reduced while free proline content showed an increasing trend with increasing stress. The results suggest that *C. album* can easily survive under low and moderate stress. However, *C. album* is susceptible to prolonged severe water stress.

Key words: *Chenopodium album*, Chlorophyll content, Free proline content, Relative Water content, Water stress.

Introduction

Increase in global climate change is a major concern worldwide that is increasing the frequency of various meteorological events. Drought, being an extreme meteorological event is greatly affecting the agriculture production as well as the social and economic development of a country (Wilhite *et al.*, 2014). In plants water stress effects different physiological as well as biochemical processes like respiration, ion uptake, photosynthesis, translocation as well as nutrient metabolism (Azadeh *et al.*, 2014). The response of plants to drought stress depends upon the species type, growth stage, intensity and duration of exposure of stress. Relative water content (RWC) is one of the major indicator of water status that shows the balance between supply of water and its transpiration rate from the plant leaf (Lugojan and Ciulca, 2011). However, stomatal closure is the first reaction of plant under water stress to prevent water loss (Nemeskeri *et al.*, 2015). Closure of stomata

thus reduces photosynthesis. Antioxidants such as ascorbic acid play a key role in detoxification of reactive oxygen entities in plants (Qian *et al.*, 2014). To counteract stressful environment such as water stress plants often accumulate osmolytes or osmoprotectants (Tanveer *et al.*, 2019). Proline is one such important osmolyte. Proline in plants under different environmental stresses is involved in protection of membrane integrity, stabilization of various enzymes and detoxification of reactive oxygen species (Ashraf, 2009).

Chenopodium album L. belongs to Chenopodiaceae family, commonly known as Bathoua. It is an annual herb and cosmopolitan in nature. Due to high nutritional value it is grown as a subsidiary food crop in the north-western Himalayan region in mixed farming system (Partap and Kapoor, 1987). In Asia, in recent years interest in *C. album* as a valuable food source has renewed not only due its versatility but also its universal adaptability to grow in different stressful conditions such as low rainfall, hot and cold conditions as well as sub-freezing temperatures

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(Poonia and Upadahiya, 2015). Thus, the main objective of the present study was to determine the effects of various intensities of water stress on the morphology, physiology and biochemistry of *C. album*.

Materials and methods

Plant material

Seeds of *Chenopodium album* L. were collected from the plants growing wild in periphery of Industrial area of Panipat (Haryana) India. The collected seeds were dried for 2 weeks at 25°C and then stored at 5°C until use. Further, to remove the debris and other plant materials, the seeds were sieved and viability test was done by using 1% tetrazolium chloride solution before experimental trial (Lakon, 1949).

Experimental Pot design

To evaluate the effects of water stress on physiological parameters of *Chenopodium album* L. a pot experiment was carried out in a completely randomized block design. Morphologically uniform healthy seeds of *C. album* were sown in earthen pots of 25 cm height under 16:8 h light/dark cycle at a temperature regime of 25-32°C with 60-80% relative humidity for a period of 45 days in green house of the Institute of Environmental Studies, Kurukshetra University, Kurukshetra, India. The pots were filled with 5 kg soil with 1kg of manure in 5:1 proportion. The seeds were watered with distilled water every day till fifth day followed by thinning of seedlings to maintain uniform spacing amongst them.

Water stress treatments

The pot experiment on *C. album* was performed in a complete randomized block design, divided with 4 water stress treatments. Each treatment had 3 replicates. Weighing method was used to maintain the soil moisture level as per the experimental requirements. The details of the treatments used:

1. Control (C) with soil moisture maintained upto 75-85% of field moisture capacity
2. Mild water stress (WI) with soil moisture maintained at 50-60% of field moisture capacity
3. Moderate water stress (WII) with soil moisture maintained at 40-50% of field moisture capacity and;
4. Severe water stress (WIII) with soil moisture maintained at 30-40% of field moisture capacity. Matured 45 days plants were harvested and the leaves of the test plants were used for estimating different parameters.

Physiological and biochemical estimations

Plant height and root length were determined by using a centimeter scale. For biomass estimation, plants were

removed from the pots, separated into root and shoot and weighed immediately for fresh weight followed by oven drying at 80°C for 48 h. and reweighed for dry weight.

Relative water content (RWC) was measured according to Weatherley (1950) method by using the formula:

$$\text{RWC (\%)} = \frac{\text{DM} - \text{FM}}{\text{DM} - \text{TM}} \times 100$$

Here,

DM: dry weight of the leaf disc

FM: the fresh weight

TM: the turgid weight.

Chlorophyll (Chl a, b and total chl) was determined by Arnon, (1949) method. The absorbance of the extract was read at 645 and 663nm using Systronics UV-VIS spectrophotometer 117 and expressed as mg.g⁻¹ fresh weight.

Ascorbic acid concentration was determined as per the method of Bajaj and Kaur, (1981). 0.5g of dried plant material was extracted with 10 ml of oxalic acid-EDTA solution for overnight at room temperature. The extract was filtered through filter paper. To 2.5 ml aliquot, 2.5 ml of oxalic acid-EDTA solution, 0.5 ml of metaphosphoric acid-acetic acid solution, 0.1 ml of sulfuric acid solution and 2 ml of ammonium molybdate reagent were added. The solvent was made up to 25 ml with distilled water and read at 760 nm. Ascorbic acid was expressed as µg.g⁻¹ fresh weight.

Free proline was estimated as per the method given by Bates *et al.*, (1973). 0.05 g dry weight of leaves was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and centrifuged at 1500 g for 10 min. 2 ml of supernatant was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin (1.25 g of ninhydrin and 30 ml of glacial acetic acid, adding in 20 ml of 6 M phosphoric acid) followed by boiling in sealed test tubes at 100°C for 1 h. and then terminating the reaction on ice. 4 ml toluene was added, supernatant was aspirated and the proline content read at 520 nm against toluene as control and L-proline as standard solution on spectrophotometer. Proline was expressed as µg.g⁻¹ dry weight.

Statistical analysis

The results were analyzed by using MS Excel, 2007 and expressed as means of standard error of 3 replicates. One way ANOVA was done using SPSS software package (IBM SPSS Statistics v 20) and expressed at $p < 0.05$.

Table 1: Effect of water stress on physiological parameters of *C. album*

Treat-ment	Shoot length (cm)	Root length (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Leaf no.	Leaf fresh wt. (g)	Branch no.	Total fresh weight (g)	Relative water content (%)
C	53.01 ^c ±1.30	13.92 ^a ±0.97	9.33 ^a ±0.62	1.51 ^a ±0.14	62.33 ^c ±6.23	11.31 ^c ±1.16	10.34 ^c ±3.01	22.16 ^c ±0.93	93.09 ^c ±2.93
WI	51.2±1.14	16.91 ^a ±1.39	7.35 ^a ±0.64	2.2 ^b ±0.30	56.33 ^b ±5.20	9.28 ^b ±0.69	10.01 ^c ±2.56	18.83 ^{bc} ±1.30	88.71 ^c ±2.34b
WII	48.4 ^b ±1.65	23.30 ^b ±2.41	5.41 ^b ±0.55	2.96 ^c ±0.08	34.66 ^b ±4.48	7.24 ^b ±0.71b	9.58 ^{cb} ±2.24	15.61 ^{ab} ±0.61	79.04 ^b ±3.47
WIII	44±1 ^a 2.09	26.94 ^c ±1.34	4.60 ^c ±0.47	3.37 ^c ±0.15	31.30 ^a ±4.35	4.31 ^a ±0.65	9.14 ^{cb} ±2.11	12.28 ^a ±1.07	66.76 ^a ±3.68

Here, C=Control; WI=mild stress; WII=moderate stress, WIII=severe water stress. Values are means of ± S.E of three independent replicates. Different letters are significant at $p<0.05$ by Duncan's multiple range test.

Results and Discussion

Effect of water stress on morphological, physiological and biochemical parameters of *Chenopodium album* L.

Plants facing different stresses such as salinity and water stress often show reduction in different morphological features such as shoot height, leaf number, number of branches, biomass etc. that ultimately leads to reduction in yield. In the present study, water stress noticeably reduced the leaf number and biomass of *C. album* with respect to its control. Shoot length decreased with increase in stress. However, root length showed enhancement with increase in water stress (Table 1). The moderate and severe water stress reduced the shoot length by 8.69 and 18.81% and root length enhanced by 40.25 and 48.32% in comparison to its control. The results are in conformity with the findings of Mukami *et al.*, (2019). Shoot dry weight reduced and root dry weight increased with increasing water stress. Shoot dry mass was reduced by 42.01 and root dry mass increased by 48.98% at moderate water stress. Leaf number decreased significantly with increasing stress. The leaf number was reduced by 49.48 and biomass by 44.58% as compared to control at severe water stress. Similar reductions in plant biomass has been obtained by Sarker and Oba, (2018) where water stress reduced biomass in *A. tricolor*.

Decrease in relative water content is correlated with the degree of water loss in plants (Soltys-Kalina, 2016). Relative water content is one of the easiest and basic methods calculated by the gravimetric method (Silva *et al.*, 2007). Water stress treatment significantly affected the relative water content in leaves of *C. album*. In control plants, RWC were maintained between 91-95% whereas in water stressed plants RWC continued to decrease with increasing stress. At moderate water stress, RWC was decreased by 15.09%, which further decreased by 28.28% at severe water stress (Table 1). The results are in conformity with the findings of Hemmati *et al.*, (2018); Mukami *et al.*, (2019). The decrease in RWC shows

loss of turgor pressure resulting in *restricted* amount of water required for cell expansion (Katerji *et al.*, 1997).

Effect on photosynthetic pigment of *C. album* under water stress

Among the various metabolic processes, photosynthesis is one of the important processes directly affected by drought stress. The present study shows decreased in chlorophyll content of *C. album* with increasing water stress. The study also highlights more significant ($p<0.05$) reduction in chl b than chl a indicating that chl b was more sensitive to increasing water stress. Chl a was reduced by 14.20% and chl b by 27.16% at severe water stress which further reduced with increasing stress (Fig. 1). Also, total chlorophyll content decreased with increasing water stress. The results are in conformity with the findings of Mafakheri *et al.*, (2010). Similar reduction in chlorophyll content was observed in faba bean (Siddiqui *et al.*, 2015). The decline in chlorophyll content under water stress is an indication of photooxidation and degradation of chlorophyll molecules (Anjum *et al.*, 2011). Further, chlorosis in *C. album* was observed at severe water stress.

Effects on ascorbic acid and free proline content of *C. album* under water stress

Ascorbic acid is considered as an important

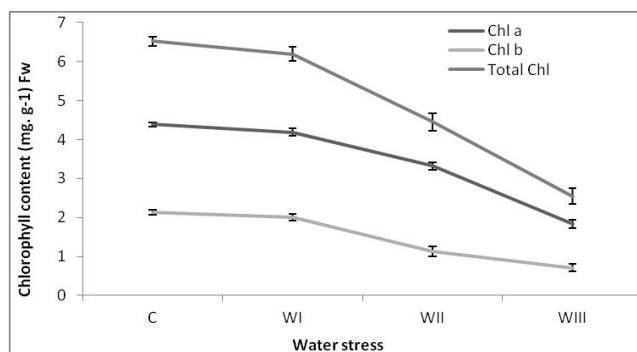


Fig. 1: Effect of water stress on photosynthetic pigments (mg g⁻¹) of *C. album*. Here, C=Control; WI=mild stress; WII=moderate stress, WIII=severe water stress. Values are means of ± S.E of three independent replicates.

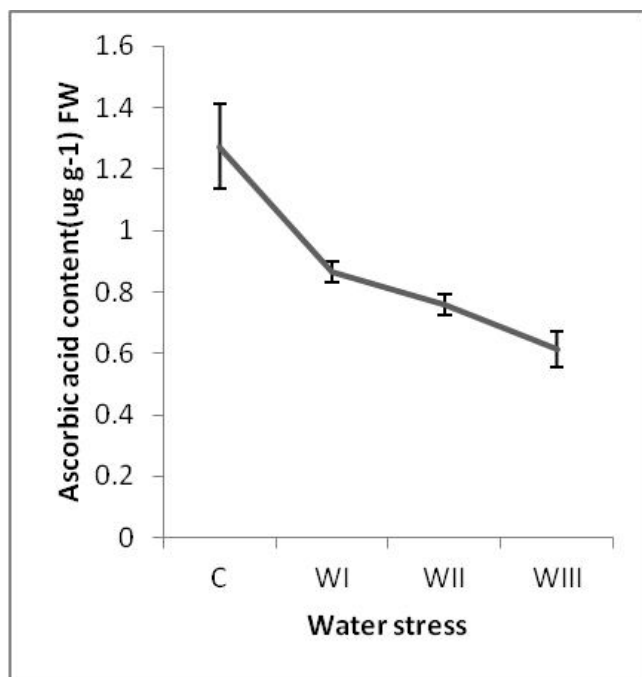


Fig. 2: Effect of water stress on ascorbic acid content ($\mu\text{g g}^{-1}$) of *C. album*. Here, C=Control; WI=mild stress; WII=moderate stress, WIII=severe water stress. Values are means of \pm S.E of three independent replicates.

antioxidant in plants. The present study showed noticeable decline in ascorbic acid content in *C. album* with increasing water stress. A decline of 40.94% was observed at moderate water stress in comparison to its control that continued to decline with increasing water stress (Fig. 2). This shows a negative correlation of ascorbic acid with the intensity of stress. The results are in conformity with the findings of Dolatabadian *et al.*, (2009) in maize; Murshed *et al.*, (2013) in tomato and (Sarkar *et al.*, 2016) in mandarin. The decrease in ascorbic acid content is credited to scavenging of O_2^- and H_2O_2 . (Du *et al.*, 2012; Smirnoff, 2018). Similarly reduction in ascorbic acid in *C. album* may be due to decline in antioxidant capacity resulting in damaging of membrane system. Also, in rice seedlings defect in biosynthesis of ascorbic acid showed increase in membrane damage (Liu *et al.*, 2013).

Proline is one of the most important solute and its high concentration in plants protects them from different environmental stresses. It is involved in membrane integrity and also stabilizes various antioxidant enzymes (Ozden *et al.*, 2009). The present study shows increase in free proline content with increase water stress. At moderate water stress, the proline content increased by 66.67% that further increased at severe water stress (Fig.3). However, the most significant increase was observed at moderate water stress treatment in comparison to control.

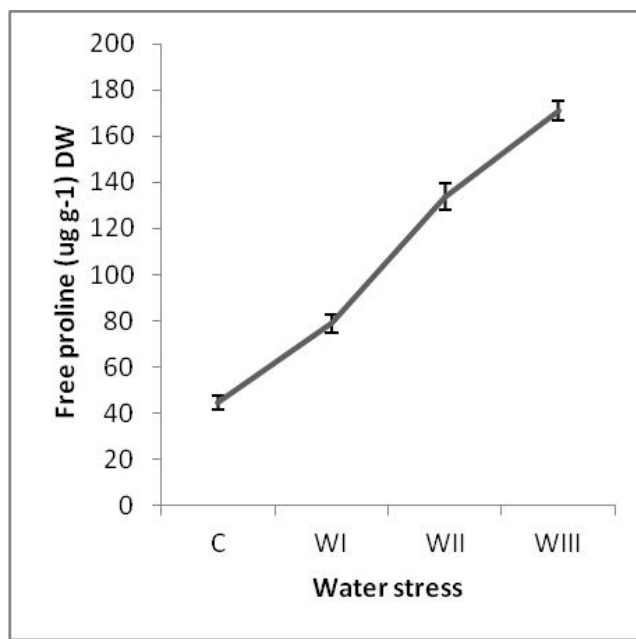


Fig. 3. Effect of water stress on free proline content ($\mu\text{g g}^{-1}$) of *C. album*. Here, C=Control; WI=mild stress; WII=moderate stress, WIII=severe water stress. Values are means of \pm S.E of three independent replicates.

The results of the present study are consistent with the findings of Pandey *et al.*, (2016); Mbinida *et al.*, (2018); Rahimi *et al.*, (2019) that showed that increased proline content was consistent with increasing water stress. The high levels of proline in plants under various stressful conditions, helps the plant to maintain low water potentials that buffers the instant effects of water scarcity within the organism (Moussa and Abdel, 2008). Thus, in the present study the high levels of proline in *C. album* indicated that it was able to osmoregulate as well as adapt itself under water stress conditions.

Conclusion

In natural environment plants are exposed to a variety of stresses such as salinity, light, heat, water etc. that affect the morphological, physiological and biochemical status of plants. The resilience of *Chenopodium album* L. was different under different stress intensities. Water stress significantly decreased plant growth, leaf number, biomass, relative water content and ascorbic acid with enhancement in root length. Chlorophyll b was more affected than chl a. However, free proline content showed a positive correlation with increasing water stress. The plant has high nutritional food value. *C. album* can be grown on moderate water deficient soil. With increase in water stress, the plant showed compensation to partial compensation effect.

Acknowledgement

The authors acknowledged Institute of Environmental Studies, Kurukshetra University for providing Laboratory and Library facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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