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WATER DEFICIT AND SALINITY STRESS INDUCED ALTERATIONS IN MEMBRANE STABILITY INDEX, BIOMASS PRODUCTIVITY AND RELATIVE WATER CONTENT OF OENOTHERA BIENNIS L. GROWN IN WESTERN HIMALAYAN REGION

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ABSTRACT

In both natural and agricultural conditions plants are frequently exposed to environmental stress. The productivity of plants is greatly affected by various environmental stresses that cause delayed germination, high seedling mortality, stunted growth, poor crop stands and lower yields. The O. biennis L,a medicinal plant was subjected to abiotic stresses, namely water deficit (-0.01 M Pa, -0.03 M Pa, -0.05 M Pa, -0.07 M Pa) and NaCl (25 mM, 50 mM, 75 mM and 100 mM) for long term. So, the present study aimed at determining the impact of water and salinity stress on plant fresh and dry weight, relative water content and membrane stability index after an interval of 30 days i,e. 30, 60, 90,120, 150,180 and 210 days after the induction of water and salinity stress. Increase in fresh weight and dry weight were noticed during the vegetative stage, earlier flowering stage after that biomass productivity decreased both in untreated and treated plants. It is evident from the results that with an increase in the intensity of stress a progressive decrease in the relative water content of leaves and membrane stability Index were noticed under both stress conditions and maximum decrease was noticed at 100 mM NaCl.So,these findings render Oenothera a sensitive plant at higher stress condions. However, changes in characteristics were realistic upto moderate stresses concentrations.

Keywords: Biomass Productivity, Membrane Stability Index, Oenothera Biennis L., Salinity Stress, Water Stress

1. INTRODUCTION

Environmental factors affect almost all functions of plants viz. the characters, composition, growth, development a nd productivity of individuals and plant communities. When any environmental factor exceeds the optimum tolerance of a plant, the result is stress to that plant Lawlor (2002), Jaleel (2007). Environmental stresses caused by the global warming and climate changes are one of the biggest challenges, significantly lowering the yield and productivity Jalil and Ansari (2020), Chaudhry and Sidhu (2021). Plants, because of their immovable nature, are the foremost organisms which always face several environmental stresses such as drought, water logging and salinity etc., which affect growth and productivity Mohammadkhani and Heidari (2008). Drought is considered one of the most severe

environmental stresses due to unpredictable factors such as fluctuating temperature dynamics, light intensity and unpredictable rainfall pattern Seleiman et al. (2021). It has been established that drought stress is an important limiting factor at initial phase of plant growth and establishment. It affects both elongation and expansion growth Jaleel et al. (2009). High salt contents reduce the growth by affecting physiological processes Munns (1993). Salinity and drought stresses generally occur concurrently Hu and Schmidhalter (2005). The increase in dry land areas is often associated with the consumption of low-quality irrigation water. Thereby, the continuous progression of drought and salinity stress results in a decrease in the available arable land Abdelraheem et al. (2019). Little is known about the combined effect of salinity and drought stress on the growth performance of 0. biennis. Taking this context into account, the current study was carried out to unravel the effects of salinity and drought stress on 0. biennis L. a medicinal plant.

Oenothera biennis L. is commonly called evening primrose because flowers close partially during the day and open in the evening Duncan et al. (1975). The evening primrose oil is used in various disorders such as eczema, pre-menstrual syndrome, benign breast pain, rheumatoid arthritis Deng et al. (2001) multiple sclerosis and sjorens syndrome as well as in psychiatric conditions such as hyperactivity, dementia and schizophrenia Roizen and Fleisher (1997). Young roots are eaten like a vegetable (with a peppery flavor) and shoots as salad. It is also considered to be effective in healing asthmatic cough, gastrointestinal disorder and whooping cough. From O. biennis roots, gallic acid is isolated which showed an antifungal activity against Fusarium semitactum, F. fusiformis and Alternaria alternae Shukla (1999), Singh (2012).

The objective of this study is to investigate the effect of water stress and salinity stress on the growth parameters including plant shoot and root fresh weight, shoot and root dry weight, Relative Water Content (RWC) and Membrane Stability Index of O. biennis L.

2. MATERIALS AND METHODS

The seeds of Oenothera biennis L. were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP). Experiments were conducted in the Botany laboratory and nursery area of Shoolini University of Biotechnology and Management Sciences, Solan.

2.1. SEEDLING GROWTH ASSAYS

Surface sterilization of seeds of Oenothera biennis L. was done with 0.1% HgCl2, rinsed three times with distilled water prior to sowing. Before sowing, damaged and insect infected seeds were discarded, and the empty ones were eliminated using floating method in distilled water. Seeds were sown in nursery beds (10×1m) during the month of September 2012 in the Herbal Garden of Shoolini University, Solan (latitude 30°51'N, longitude 77°07 'E and altitude 1195 m), where the average annual rainfall was 1315.6 mm. The average maximum and minimum temperatures were 32°C and 2°C, respectively. When the first three leaves appeared, the seedlings were transferred to the ceramic pots of fixed capacity (25 cm diameter). The pots were filled with 7 kg uniform soil mixture containing soil: sand in 2:1 ratio. Three seedlings per pot were transplanted but after some days the seedlings were thinned to two seedlings per pot. Plants were irrigated daily with Hoagland's solution for one month before carrying the experiment and during

experiment solution was given weekly. For each treatment 60 plants were selected. Destructive and non-destructive sampling was done for analyses. For non-destructive observations pots were marked randomly within a treatment. Destructive observations were taken in triplicate at an interval of 30, 60, 90,120,150,180 and 210 days. Each experiment was replicated thrice. The pots were arranged in a completely randomized design and the position of the pots was changed weekly to avoid position effect.

2.2. INDUCTION OF WATER AND SALINITY STRESS

Water-deficit and salt stress were induced after one month of transplanting the plants to pots, when the plant adapted in pots. Water-deficit experiment was conducted by gravimetric method in which initially soil water potential was maintained by tensiometer. Soil water potential was restored everyday by watering plants daily in the morning hours for seven months. For restoring soil water potential to the pots, water was added to the surface of the pots as well as to the bottom of the pots through already inserted polytubes in growing medium. The water potential was maintained at -0.01, -0.03, -0.05 and -0.07 M Pa. For salt stress, potted plants were given 50 ml of fixed quantity of solution (25 mM, 50 mM, 75 mM and 100 mM NaCl) after one week. After an interval of 30, 60, 90, 120, 150, 180 and 210 days of applying stresses (water and salt), the plants were analysed. Untreated and stress-treated plants were kept under natural photoradiation.

2.3. BIOMASS AND PRODUCTIVITY 2.3.1. FRESH AND DRY WEIGHT

Fresh and dry weight (g/plant) of plant was recorded to determine the effect of abiotic stresses (water-deficit and salinity stress) at different growth stages i.e., at 30, 60, 90, 120, 150, 180 and 210 days after applying stresses.

2.3.2. RELATIVE WATER CONTENT (RWC)

The fresh weight of top 5th and 6th leaves from each treatment was recorded. The leaves were then immersed in distilled water in beakers and left for 24 h. Thereafter, fully turgid leaves were weighed again. The leaves were dried in oven for 72 h at 70 $^{\circ}$ C, until constant weight of leaves was obtained. Relative water content (RWC) of leaves was calculated according to Wheatherly (1950).

$$RWC = \frac{[(fresh mass - dry mass)]}{(saturated mass - dry mass)} \times 1$$

2.4. MEMBRANE STABILITY INDEX (MSI)

Membrane stability index (MSI) was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 and 100°C Sairam (1994). Leaf samples (0.1 g) of top 5th and 6th leaves were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 min and another set at 100°C in boiling water bath for 15 min and their respective electric conductivity's C1 and C2 were measured by conductivity meter.

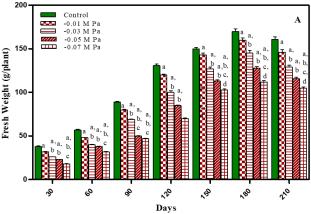
Membrane stability index =
$$\left[1 - \left(\frac{C_1}{C_2}\right)\right] \times 100$$

3. RESULTS

3.1. TOTAL FRESH WEIGHT

Plant fresh weight under water-deficit and salinity stress was observed after an interval of 30 days of growth i.e., 30, 60, 90, 120, 150, 180 and 210 days in treated and untreated plants. The results are shown in Figure 1 and Table 1 Increase in total fresh weight of plants was noted during vegetative growth stage upto earlier flowering stage. After pod formation during ripening of seeds fresh weight decreased in both treated and untreated plants. After 180 days of growth the treated plants decreased in total fresh weight in comparison to untreated plants by 5.8% in -0.01 M Pa; by 14.7% in -0.03 M Pa; by 24.7% in -0.05 M Pa and by 34% in -0.07 M Pa. In salt stressed plants the total fresh weight decreased by 8.2% in 25 mM; by 17.6% in 50 mM NaCl; by 25.8% in 75 mM NaCl and by 35.2% in 100 mM NaCl. After 210 days total fresh weight decreased by 9.3% in -0.01 M Pa; by 19.8% in -0.03 M Pa; by 27.9% in -0.05 M Pa; by 36% in -0.07 M Pa; by 11.8% in 25 mM; by 21.7 % in 50 mM NaCl; by 29.1% in 75 mM NaCl and by 39% in 100 mM NaCl.





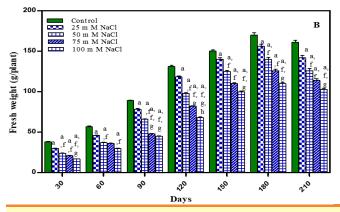


Figure 1 Fresh Weight of O. Biennis L. Under Two Stress Conditions I.E., Water Deficit and Salinity Stress. Values Are Mean \pm SE; N=6. Ap<0.05 Vs Control, Bp<0.05 Vs -0.01 Mpa, Cp <0.05 Vs -0.03 Mpa, Dp <0.05 Vs -0.05 M Pa, Ep<0.05 Vs -0.07 Mpa, Fp <0.05 Vs 25 Mm Nacl, Gp <0.05 Vs 50 Mm Nacl, Hp<0.05 Vs 75 Mm; Ip<0.05 Vs 100 Mm Nacl; Analysed by One Way ANOVA Followed by Tukey's Multiple Comparison Test.

Table 1 Table 1 Biomass Productivity, Relative Water Content and MSI in O.biennis L. at Different Growth Stages Under Water Deficit and Salinity Stress; Values are mean±S.E. n=6 30 38.0±0.3 32.0±1.2 26.0±0.2 23.0±0.2 18.0±0.2 30.0±0.3 24.0±0.2 21.0±0.3 17.0±0.2 60 57.0±0.8 48.0±1.5 40.0 ± 0.4 38.0 ± 0.4 32.0 ± 0.4 46.0±0.4 37.0±0.4 36.0±0.4 30.0±0.3 Fresh weight(g) 90 89.0±1.2 80.0±2.0 69.0±0.7 50.0±0.7 47.0±0.5 78.3±0.8 66.0±0.9 48.0±0.4 45.0±0.7 131.0±2.1 120.0±2.1 100.0±1.0 85.0±1.0 70.0±0.8 118.2±1.3 68.0±1.2 120 98.0±1.6 82.0±0.6 150.0±2.9 143.0±5.0 100.0±2.4 150 127.0±1.5 113.0±1.5 103.0±1.2 140.0±2.3 125.0±3.2 110.0±0.9 180 170.0±3.1 160.0±4.1 145.0±1.8 128.0±1.8 112.0±1.8 156.0±3.1 140.0±4.0 126.0±1.2 110.0±3.7 210 161.0±5.0 146.0±5.0 129.0±2.0 116.0±2.0 105.0±2.0 142.0±2.8 126.0±3.0 114.0±2.7 103.0±1.5 30 18.0±0.3 15.0±0.2 12.3±0.1 8.8±0.1 5.4±0.08 14.8±0.03 12.0±0.05 8.0±0.09 4.4±0.08 60 21.0±0.5 19.0±0.3 16.0±0.2 12.3±0.2 9.2 ± 0.1 18.2±0.05 15.0±0.1 11.8±0.2 8.2 ± 0.1 Dry weight (g) 90 27.0±0.6 24.0±0.9 18.0±0.4 15.2±0.3 12.1±0.2 23.7±0.3 17.8±0.3 15.0±0.3 11.1±0.3 34.4±0.7 28.0±1.0 21.2±0.5 17.8±0.4 15.1±0.3 2 120 27.4±0.4 20.3±0.4 16.0±0.5 14.2±0.5 .43.0±0.9 38.0±1.4 22.0±0.8 150 29.0±0.6 26.1±0.5 24.0±0.4 37.0±0.5 28.0±0.7 25.0±1.0 180 56.0±1.5 49.0±1.6 37.0±0.8 34.0±0.6 30.0 ± 0.5 48.0±0.7 36.0±0.8 33.0 ± 1.2 28.0±0.9 50.0±1.2 42.1±1.3 32.0±0.5 29.0±0.4 27.0±0.3 41.1±0.4 32.0±0.8 27.0±1.1 26.0±0.6 210 83.0±0.9 77.1±1.8 73.0±2.1 68.0±2.3 66.0±1.9 71.3±2.1 70.0±2.0 67.0±1.9 63±1.9 30 Leaf relative water 60 85.0±1.3 75.0±1.6 72.0±2.0 67.3±2.2 65.0±1.5 70.0±2.0 69.5±1.8 66.2±1.7 62.1±2.0 86.0±1.2 73.0±1.5 70.0±1.8 66.2±2.1 64.2±1.4 69.0±1.7 68.4±1.7 65.1±1.6 61.0±1.6 90 content 87.0±1.5 72.0±1.4 69.0±1.6 60.0±1.3 120 65.1±1.7 63.0±1.1 68.0±1.5 66.8±1.6 64.4±1.5 88.0±2.0 70.0±1.3 150 67.0±1.4 64.5±1.5 62.4±0.9 67.0±1.2 66.0±1.5 63.0±1.1 59.2±1.2 69.0±1.2 180 88.7±2.5 66.0±1.3 63.0±1.1 62.0±1.1 66.0±1.1 65.3±1.3 62.0±1.0 59.0±1.1 210 86.1±2.1 68.0±1.0 65.0±1.2 62.0±0.9 60.0±1.0 65.0±1.0 64.1±1.1 61.1±0.9 58.0±1.0 30 86.3±0.9 80.2±0.8 77±0.9 75.0±1.0 66.0±0.6 72.0±1.1 71± 1.0 67.1±2.1 63.0±2.1 Membrane Stability 60 87.1±2.0 75.0±0.9 72±1.0 70.0±1.1 62.0±0.9 70.0±1.2 67.0±0.9 63.0±2.0 60.0±1.5 % 88.0±2.2 90 73.0±1.0 68±1.2 66.0±1.2 60.0±1.0 72.6±1.3 63.0±1.2 60.0±1.9 57.0±1.4 Index 120 88.2±1.4 70.1±1.1 65±1.3 63.0±1.3 57.0±1.1 68.0±1.4 60.0±1.4 57.0±1.7 54.0±1.2 150 88.1±0.6 68.0±1.2 62±1.4 58.1±1.5 53.0±1.2 64.0±1.5 58.3±1.2 53.0±1.6 51.0±1.1

3.2. TOTAL DRY WEIGHT

56.0±1.5

52.0±1.7

51.6±1.3

50.2±1.4

59±1.5

55±1.7

Increase in total dry weight of plants was noted during vegetative growth stage. As the plant flowered dry weight decreased in treated and untreated plants as evident from Figure 2 and Table 1 After 180 days water-deficit treated plants showed decrease in total dry weight in comparison to untreated plants by 12.5% in -0.01 M Pa; 33.6% in -0.03 M Pa; by 39.2% in -0.05 M Pa; by 43.4% in -0.07 M Pa. In case of salt stress conditions total dry weight decreased by 14.2% in 25 mM; by 35.7% in 50 mM NaCl; by 41% in 75 mM NaCl and by 46.6% in 100 mM NaCl. After 210 days total dry weight decreased by 15.8% in -0.01 M Pa; by 33.6% in -0.03 M Pa; by 42% in -0.05 M Pa; by 46% in -0.07 M Pa; by 18% in 25 mM; by 34% in 50 mM NaCl; by 46% in 75 mM NaCl and by 48% in 100 mM NaCl.

62.6±0.9

61.0±0.5

53.0±1.1

50.0±0.9

52.1±1.4

50.0±1.2

180

210

88.0±1.2

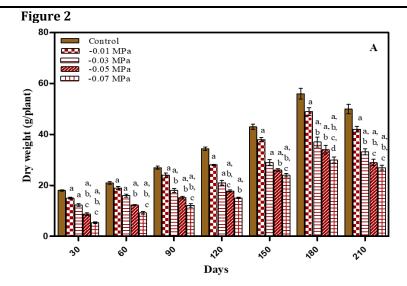
88.2±.09

64.0±1.3

62.2±1.5

50.0±1.0

46.0±0.9



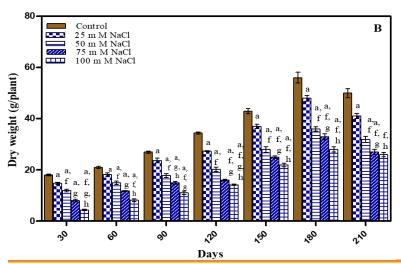
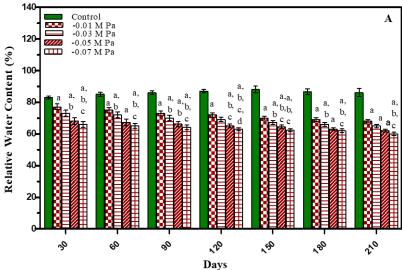


Figure 2 Total Dry Weight of O. Biennis L. Plants on Different Days Under Two Stress Conditions I.E., Water Deficit (A) and Nacl (B). Values Are Mean ± SE; N=6. Ap<0.05 vs Control, Bp<0.05 Vs-0.01 M Pa, Cp<0.05 Vs-0.03 M Pa,Dp<0.05 vs -0.05 M Pa, Ep<0.05 Vs-0.07 M Pa, Fp<0.05 vs 25 Mm Nacl, Gp<0.05 vs 50 Mm Nacl, Hp<0.05 vs 75 Mm Nacl, Ip<0.05 vs 100 Mm Nacl; Analysed by Two-Way ANOVA Followed by Bonferroni's Multiple Comparison Test.

3.3. RELATIVE WATER CONTENT

The results of relative water content (RWC) are presented in Figure 3 and in Table 1 It is evident from the results that with an increase in the concentration of stress there was a progressive decrease in the relative water content of leaves. After 210 days RWC in water stress plants decreased by 23.4% in -0.01 M Pa (minimum decrease); by 26.8 % in -0.03 M Pa; by 30.1% in -0.05 M Pa; by 32.4% in -0.07 M Pa. In salinity stress plants it decreased by 25.6 % in 25 mM NaCl; by 27.8% in 50 mM NaCl; by 31.3% in 75 mM NaCl and by 34.6% in 100 mM NaCl (maximum decrease).





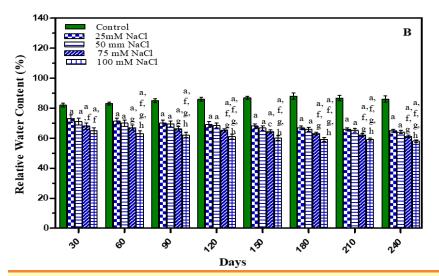
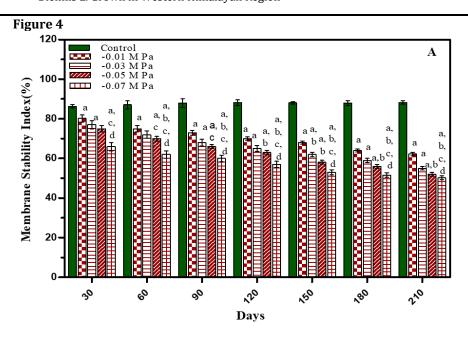


Figure 3 Leaf Relative Water Content of O. Biennis Plants on Different Days Under Two Stress Conditions I.E., Water Deficit (A) And Nacl (B). Values Are Mean ± SE; N=6. Ap<0.05 Vs Control, Bp<0.05 Vs -0.01 M Pa, Cp<0.05 Vs -0.03 M Pa, Vs -0.05 M Pa,Ep<0.05 Vs -0.07 M Pa, Fp<0.05 Vs 25 Mm Nacl, Gp<0.05 Vs 50 Mm Nacl,Hp<0.05 Vs 75 Mm Nacl, Ip<0.05 Vs 100 Mm Nacl; Analysed by Two-Way ANOVA Followed by Bonferroni's Multiple Comparison Test.

3.4. MEMBRANE STABILITY INDEX (MSI)

Membrane stability index (MSI) of O. biennis L leaves was measured after 30, 60, 90, 120, 150, 180 and 210 days of growth in water-deficit and salinity stress plants and results presented in Figure 4 and Table 1 Membrane stability index decreased with an increase in concentration of treatments in both the stress studied. It was observed that after 210 days membrane stability index in water stressed plants decreased by 29.4% in -0.01 M Pa (minimum decrease); by 37.6% in -0.03 M Pa; by 41% in -0.05 M Pa and by 42.4% in -0.07 M Pa under water deficit. In salinity stress membrane stability decreased by 30.8% in 25 mM NaCl; by 39.9% in 50 mM NaCl; by 43.3% in 75 mM NaCl and by 47.8% in 100 mM NaCl (maximum decrease).



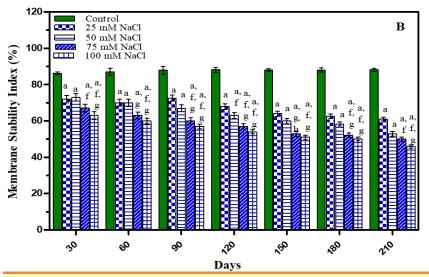


Figure 4 Membrane Stability Index of O. Biennis Plants on Different Days Undertow Stress Conditions I.E., Water Deficit (A) And Nacl (B). Values Are Mean ±SE; N=6. Ap<0.05 Vs Control, Bp<0.05 Vs-0.01 M Pa,Cp<0.05 Vs-0.03 Mpa, Dp<0.05 Vs-0.05 M Pa, Ep<0.05 Vs-0.07 M Pa, Fp<0.05 Vs 25 Mm Nacl, Gp<0.05 Vs 50 Mm Nacl, Hp<0.05 Vs 75 Mm Nacl, Ip<0.05 Vs 100 Mm Nacl Analysed by Two-Way ANOVA Followed by Bonferroni's Multiple Comparison Test.

Statistical Analysis

Data were analyzed using ANOVA (two ways) statistical program. Mean + SE values calculated for Oenothera biennis L. samples and the significance of the data was tested using Tukey's multiple comparison test / Bonferroni's multiple comparison test (P < 0.05).

4. DISCUSSION AND CONCLUSION

In O. biennis the reduction in fresh weight was noticed under both stress conditions and reduction was more severe with an increase in intensity of stresses Figure 1. It has been reported that restriction of water supply from the soil reduced plant biomass Ogbonnaya et al. (2003). Similarly decrease in fresh weight were noticed in, Ocimum sanctum Azhar et al. (2011), in Pennisetum glaucum and Abelmoschus esculentum Khalid (2006), Azhar et al. (2011). The fresh weight decrease under drought may be due to the suppression of cell expansion and cell growth which in turn is attributed to low turgor pressure Sankar et al. (2007). The reduction in shoot biomass in the plants may be due to the chlorosis and necrosis of the leaves that reduced the photosynthetically active area De Herralde et al. (1998). In Glycine max L. fresh weight of shoot and root tissues were significantly inhibited by higher salinity concentration compared to low or no salinity Dogan (2011). Similar results were reported in Triticum aestivum plants by Datta et al. (1998), in Echium amoenum Ramezani et al. (2011), in Echinacea purpurea Miri and Mirjalili (2013). It appears that the decreased root and shoot weight is due to the reduced water absorption, which in turn causes a reduction in the amount of water in plant tissue Sharma et al. (2004), Ramezani et al. (2011).

In present study the dry weight of the plant decreased with stress treatments. Similarly, dry weight decreased in Silybum marianum Ahmadian et al. (2012), in Populus species Jaleel et al. (2009), in Beta vulgaris genotypes Mohammadian et al. (2005). Both salinity and soil drying significantly affected root dry weight. Yet, unlike shoots, root growth was more sensitive to salt than to soil drying. Moreover, drought significantly exacerbated the adverse effect of salinity on root growth Slama et al. (2008). Shoot and root dry weight of Rosmarinus officinalis was significantly decreased by salinity stress Hejazi Mehrizi et al. (2012). A moderate stress tolerance in terms of shoot dry mass was noticed in Oryza sativa Lafitte et al. (2007). In Andrographis paniculata, applied NaCl led to a decrease in dry weight of the plant in salted medium as compared to control medium Talei et al. (2012). Similar results were found in Kochia prostrata Karimi et al. (2005), Brassica juncea L. Joshi et al. (2011), in Ricinus communis Janmohammadi et al. (2012). This might be the consequence of diversion of metabolic energy and metabolites from the processes involved in alleviating the toxic effects of salinity stress.

Relative water content (RWC) is the measure of health and sturdiness in a plant and in stress growing plants it is lower Bhardwaj and Yadav (2012). Relative leaf water is also considered a reliable and widely used indicator for defining the sensitivity of plants to dehydration Rampino et al. (2006), Kavas et al. (2013). Relative water content decreased with time in 0. biennis growing in water and saline stress conditions Figure 3. Relative water content had been used as one of the potential parameters for accessing intra-specific variation for salt tolerance in a number of crops such as Triticum aestivum Pier and Berkowitz (1987), Zea mays Premachandra et al. (1990) and Vigna radiata Nandwal et al. (1998). Salt treatment induced a reduction in relative water content in the leaves of Beta vulgaris, which indicated a loss of turgor that resulted in limited water availability for cell extension process Mane et al. (2011). In Kochia prostrata, no significant difference at lower stress concentrations was observed for relative water content, but it significantly decreased at 200 mM NaCl Karimi et al. (2005). In Ricinus communis L. the relative water content of leaf decreased from 96.4 percent in control to 66.5 percent at 200 mM NaCl treatment Janmohammadi et al. (2012).

In the present study, under both stress conditions membrane stability decreased with an increase in stress treatments in Oenothera biennis Figure 4. Cell membrane stability is an indicator of stress tolerance and membrane stability/injury and is also related to tolerance/susceptibility of plants Khan and Naqvi (2010). Cell membrane integrity undergoes diverse changes such as increase in penetrability and decrease in sustainability under drought stress Blokhina et al. (2003), Anjum et al. (2012). It has been suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS Heidari and Jamshidi (2011). Increased H2O2 accumulation and lipid peroxidation due to salinity stress resulted in significant decrease in membrane stability index Sairam et al. (2002). Farooq and Azam (2006) reported an increased cell membrane injury under salt stress in different Triticum aestivum varieties.

So, findings of present study can be used as a base for further study in plant breeding programs. The plant has potential to tolerate water and salinity stress conditions. The need is to verify its tolerance ability under field conditions.

CONFLICT OF INTERESTS

None.

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