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Proceeding Paper

Investigation of the Physiological and Biochemical Responses of *Echinacea purpurea* under Salinity Stress †

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Abstract: *Echinacea purpurea* is an important medicinal plant that contains valuable medicinal compounds that have a tremendous effect on stimulating the body's immune system to fight off viral and bacterial agents. To evaluate salinity stress tolerance in *Echinacea purpurea*, an experiment was conducted using a diverse population. The seeds used in this experiment were the result of selecting superior genotypes in terms of chicoric acid content and drought tolerance. Considering the medicinal value of *Echinacea purpurea* and the high area of saline soils in Iran, the purpose of this study was to investigate the possibility of cultivating this plant in saline soils. In this experiment, salinity stress at two levels of 0 and 60 mM of NaCl started when the plant was at the six-leaf stage and continued for 14 days. The results showed a significant decrease in the amounts of photosynthetic pigments and potassium under salinity stress. Under saline conditions, the amount of sodium ions in the shoots, ion leakage, and total phenols increased, but there was no significant change in the amount of proline, antioxidant capacity, and chlorophyll fluorescence parameters. It seems that among the genotypes under salinity stress, based on the results obtained under stress, Genotypes 34, 46, 90, 89, 79, and 165 have high levels of proline and phenolic compounds, and strong antioxidant properties. These genotypes were in a better position in terms of these parameters and were placed in a separate cluster in cluster analysis, so these can be selected as tolerant genotypes.

Keywords: salinity stress; proline; polyphenol compounds; antioxidant capacity; elements

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1. Introduction

Medicinal and aromatic plants are valuable products. The natural products of these plants are small in volume but very valuable and have many applications in various industries such as food, beverages, food supplements, perfumery, cosmetics, and medicine [1].

Purple coneflower (*Echinacea purpurea* L.) is a perennial and herbaceous medicinal plant belonging to the family Asteraceae. All parts of *Echinacea purpurea*, including the leaves, flowers, and roots, are widely used in the preparation of pharmaceutical products. These products are used to stimulate the immune system and for treating respiratory disorders and viral infections [2]. In this sense, this medicinal plant is famous for its effects on the immune system (2). The use of echinacea products has dramatically increased: sales in 2013 increased by 94.7% over those in 2012, making it the eighth most commonly sold herb in the United States [3]. By 2014, sales of echinacea had increased by 79% from 2013 and it was the third most commonly sold herb in the United States, with sales surpassing $50 million [4].

Salinity stress results in excessive generation of ROS [5]. Elevated CO_2 mitigates the oxidative stress caused by salinity through reduced ROS generation and better maintenance of redox homeostasis as a consequence of higher assimilation rates and lower photorespiration [6]. Extensive investigation through cellular, metabolic, and physiological analysis

has cleared that among various salinity responses, mechanisms or strategies controlling ion uptake, transport and balance, osmotic regulation, hormone metabolism, antioxidant metabolism, and stress signaling to play decretive roles in plant adaptation to salinity stress [7]. The leaf area in *Echinacea purpurea* decreases significantly due to high NaCl concentrations. Increasing concentrations of NaCl lead to an increase in the levels of shoot phenol, shoot flavonoids, and proline content [8]. Increases in Na^+ and Cl^- during salt stress have resulted in decreased levels of N, P, K^+, Ca^{2+} and Mg^{2+} in fennel, *Trachyspermum ammi*, peppermint, lemon verbena, *Matricaria recutita*, and *Achillea fragratissima* [1,9,10]. Both chlorophyll a and b, along with the total chlorophyll content, were decreased in centaury, *Teucrium polium*, *Thymus vulgaris*, *Zataria multiflora*, *Ziziphora clinopodioides* and *Satureja hortensis* [11]. A decrease in protein content under salt stress was reported in *Catharanthus roseus* [12].

Considering the medicinal value of *Echinacea purpurea* and the high area of saline soils in Iran, the purpose of this study was to investigate the possibility of cultivating this plant in saline soils.

2. Materials and Methods

The seeds used in this experiment were the result of selecting superior genotypes in terms of chicoric acid content and drought tolerance [13]. Uniformly sized seedlings reaching a height of 10–12 cm (60 days after germination) were transplanted into 10 L pots. To guarantee optimal nutritional support, plants were regularly fed with a diluted nutrition solution (fertigation) during the experiment [14].

Analysis of variance of the morphological and phytochemical data was performed based on the relevant experiments. At the 6-leaf stage, salinity stress started at 2 levels, namely the control (no salinity) and 60 mM of NaCl, and continued for 14 days. Data analysis of the physiological and biochemical properties of different genotypes of *Echinacea purpurea* was performed using Minitab V.14 software.

2.1. Cholorophyll Assays

The content of chlorophyll and carotenoids were measured by Arnon's [15] method, and the adsorption rate was read by a spectrophotometer at wavelengths of 663, 645, and 470 nm.

2.2. Total Polyphenol Content

TPC was measured according to the method of Stankovic [16].

2.3. DPPH Radical Scavenging Assay

A DPPH (2,2-diphenyl-1-picrylhyrazyl) radical scavenging assay was performed according to the method of Stankovic [16].

2.4. Relative Water Content

The method of Cameron et al. [17] was used to measure the relative water content of the leaves.

2.5. Chlorophyll Fluorescence

All chlorophyll fluorescence parameters (F_0, F_m, F_v/F_m) were measured by a portable chlorophyll fluorescence meter (handyPEA, Hansatech Instruments, King's Lynn, UK).

2.6. Ion Leakage

Ion leakage was measured based on the method of Sullivan and Ross [18].

2.7. Proline

Free proline was measured according to the method of Bates et al. [10], and the absorbance was read spectrophotometrically at 520 nm.

2.8. Measurement of Elements

Ca^{2+}, Na^{-}, K^{+}, and Cl^{-} ions were measured based on the method of Tahmasebi [19].

3. Results and Discussion

The analysis of variance results showed that there was a significant difference between the salinity treatment and the control in terms of chlorophyll a and b, total chlorophyll, carotenoids, total polyphenol, ion leakage, and sodium and potassium ions (Table 1).

Table 1. Mean of echinacea genotypes at two levels of control and salinity in terms of physiological and biochemical traits.

Traits	*t*-Value	*p*-Value	Salinity Level	Control Level	Difference
Chlorophyll a (mg/g fw)	12.33	0.00	0.40	1.28	0.87
Chlorophyll b (mg/g fw)	12.48	0.00	0.32	1.05	0.73
Total chlorophyll (mg/g fw)	13.39	0.00	0.72	2.34	1.61
Carotenoid (mg/g fw)	2.32	0.024	0.081	0.14	0.056
TPC (mg/g fw)	2.85	0.006	329	235	93.70
DPPH (%)	1.91	0.06	11.98	14.01	202
RWC (%)	0.33	0.744	66.70	67.60	0.93
Fv/Fm	0.35	0.729	0.70	0.68	0.02
Ion leakage (%)	6.53	0.00	88.30	56	32.30
Proline (mg/g fw)	1.87	0.07	52.40	41.10	11.36
Ca^{2+} (%)	0.12	0.91	4.66	4.72	0.07
Na^{-} (%)	4.54	0.00	0.21	0.10	0.11
K^{+} (%)	2.20	0.034	0.79	0.51	0.27
Cl^{-} (%)	1.75	0.89	3.84	3.17	0.67

3.1. Cholorophyll Assays

Based on results of the genotypes under salinity stress, the highest amounts of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll were observed in Genotype 34; Genotypes 27, 34, and 83; Genotypes 95 and 89; and Genotype 34, respectively, and the lowest amounts of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll were observed in Genotypes 25, 161, 163, 32, 164, 36, 37, 141, 142, and 147; Genotype 49; Genotypes 25, 161, 163, 32, 36, 37, 155, and 159; and Genotypes 161, 163, 37, 38, 169, 142, 36, and 40, respectively. The results showed that the amount of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll under salinity stress decreased by 68.75, 69.52, 42.14, and 69.23%, respectively, compared with the control treatment. Sabra [19] showed that the application of 50 mM NaCl to *Echinacea purpurea* decreased chlorophyll a, chlorophyll b, and carotenoids by 16.67, 23.01, and 14.77%, respectively.

3.2. Total Polyphenol Content

Based on the results of the genotypes under salinity stress, the highest amounts of TPC were observed in Genotypes 46 and 165, and the lowest amounts of TPC were observed in Genotypes 161, 42, 155, and 159. The results showed that the TPC under salinity stress increased by 40.00% compared with the control treatment. Cappellari [20] showed that the application of 75 mM NaCl to *Mentha piperita* increased TPC by 17.39%.

3.3. DPPH Radical Scavenging

Based on the results of the genotypes under salinity stress, the highest levels of antioxidant activity were observed in Genotype 137, and the lowest levels of antioxidant activity were observed in Genotypes 34, 41, 43, 155, 150, and 156. The results show that the amount of antioxidant activity under salinity stress decreased by 14.49% compared with the control treatment. Khorasaninejad [8] showed that the application of 75 mM NaCl to *Echinacea purpurea* increased antioxidant activity by 56.2%.

3.4. Relative Water Content

Based on the results of the genotypes under salinity stress, the highest levels of relative water content were observed in Genotype 156, and the lowest levels of relative water content were observed in Genotypes 81, 165, 41, 47, 40, 161, 140, and 141. The results show that the amount of RWC under salinity stress decreased by 1.33% compared with the control treatment. Zrig [21] showed that the application of 100 mM NaCl to *Thymus vulgaris* decreased RWC by 31.00% after 2 weeks of treatment.

3.5. Chlorophyll Fluorescence

Based on the results of the genotypes under salinity stress, the highest quantum efficiency of Photosystem II was observed in Genotypes 50 and 166, and the lowest quantum efficiency of Photosystem II was observed in Genotype 80. The results show that the amount of Fv/Fm under salinity stress increased by 2.94% compared with the control treatment.

3.6. Ion Leakage

Based on the results of the genotypes under salinity stress, the highest ion leakage percentage was observed in Genotypes 25, 162, 34, 164, 35, 36, 50, 159, and 169, and the lowest ion leakage percentage was observed in Genotypes 140 and 154. The results show that the percentage of ion leakage under salinity stress increased by 32.3% compared with the control treatment. Sabra [22] showed that the application of 50 mM NaCl to *Echinacea purpurea* increased ion leakage by 8.00%.

3.7. Proline Content

Based on the results of the genotypes under salinity stress, the highest proline content was observed in Genotypes 34, 79, 89, and 90, and the lowest proline content was observed in Genotypes 82, 137, and 33. The results show that the proline content under salinity stress increased by 27.49% compared with the control treatment. Zrig [21] showed that the application of 100 mM NaCl to *Thymus vulgaris* increased proline content by 188.46% after 4 weeks of treatment.

3.8. Element Content

Based on the results of the genotypes under salinity stress, the highest amounts of calcium, sodium, potassium, and chlorine were observed in Genotypes 79, 49, 48, and 84, respectively, and the lowest amounts of calcium, sodium, potassium, and chlorine were observed in Genotypes 39, 160, 42, 47, 142, 144, 159, 150, 154, and 147; Genotypes 27, 161, 43, 135, and 169; Genotypes 43 and 135; and Genotypes 37 and 93, respectively. Sabra [20] showed that the application of 50 mM NaCl to *Echinacea purpurea* increased sodium, potassium, and chlorine content by 7.72, 1.60, and 30.20%, respectively.

4. Conclusions

Applying abiotic stresses is one of the ways to change the amount and components of the active ingredients of medicinal plants, on which much research has been carried out. In general, on the basis of the physiological and biochemical results, it can be said that echinacea has the ability to adapt to salinity stress conditions. By selecting genotypes that are tolerant to salinity stress in breeding programs, the conditions for cultivating this plant in areas with saline water and soil can be provided. It seems that among the genotypes under salinity stress, based on the results obtained from the histograms and cluster analysis of the genotypes under stress, Genotypes 34, 46, 90, 89, 79, and 165 have high levels of proline and phenolic compounds, and strong antioxidant properties. These genotypes are in a better position in terms of these parameters and were placed in a separate cluster in cluster analysis, so these can be selected as tolerant genotypes.

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