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Physiological responses of fennel seedling to four environmental stresses

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is widely cultivated for its edible, strongly-flavored leaves, fruits, seeds and medicinal uses. As the wild and cultivated plants are influenced by environmental stresses, the effects of salt (25, 50 and 75 mM NaCl), nitrogen and iron deficiency (0 N, 0.5 N of Hoagland's solution, 0 Fe and 0.5 Fe of Hoagland's solution), 2°C cold (2, 3 and 4 h) and drought (3, 5 and 7 days) stresses on growth, pigment content, and antioxidant activity of fennel at the seedling stage were evaluated. All four stresses resulted in a significant reduction of fresh and dry weights, and chlorophyll *a* and *b* and β-carotene content of seedlings. The antioxidant activity of seedling extracts also increased significantly ($P \leq 0.05$) in all stresses except for 5 and 7 days of drought.

Keywords*:* Antioxidant activity; cold stress; drought; Iron deficiency Nitrogen deficiency; salinity

1. Introduction

Fennel (*Foeniculum vulgare* Mill.; Apiaceae), a perennial plant that is native to the Mediterranean areas, has a long history of herbal use [1, 2]. Roots, young shoots, leaves, flowering stems, mature inflorescences and fully ripened and dried seeds are commonly used for homemade remedies, being useful in the treatment of a variety of complaints [3]. In addition, the bulb, foliage, and seeds of the fennel plant are widely used in traditional medicine of the world. Fennel is considered indigenous to the shores of the Mediterranean, but grows wild in India, Pakistan, France, Iran and Russia [4]. Fennel is widely cultivated, both in its native range and elsewhere for its uses, and is influenced by environmental stresses. Plants are constantly confronted with various biotic and abiotic stress factors such as low or high temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity [5-7]. Environmental stresses, irrespective of their nature, lead to a series of molecular, biochemical, physiological and morphological changes that adversely affect plant growth and productivity [8]. They can enhance the formation of reactive oxygen species (ROS) [9], thereby activating both protective mechanisms and cellular damage [10]. To reduce ROS-induced damage, plants have evolved an intricate antioxidative system, including antioxidative enzymes, as well as low-molecular mass secondary metabolites i.e., ascorbate, glutathione, tocopherols, carotenoids and phenolic compounds [11]. Phenolics are low molecular compounds found in all tissues of higher plants having a pronounced effect on plant development. The synthesis and release of phenolics depend on the plant organ, the age of the plant and the cultivation conditions and are induced by various biotic and abiotic factors [12, 13]. The presence of phenolic compounds in medicinal plants is responsible for their antioxidant and anti-inflammatory activities [14]. Antioxidants have gained importance on account of their positive involvement as health promoters in conditions such as cardiovascular problems, and atherosclerosis, as well as for treatment of many forms of cancer and aging processes. They have been identified as free radicle scavengers [15]. Therefore, much attention has been focused on the use of antioxidants, especially natural antioxidants to protect from damage due to free radicals [16, 17]. Antioxidant activity has been reported in *F. vulgare* seeds [18, 19], leaves [20] and fruits [21]. Oktay et al. (2003) reported a high phenolic content of both aqueous and ethanolic fennel extracts, which exhibited strong antioxidant activity.

As plants are exposed to various environmental stresses and some endogenous plants increase under

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stress conditions [22], this experiment was designed to improve our knowledge about fennel cultivation under environmental stresses by investigation of physiological responses.

2. Materials and methods

2.1. Preparation of plant samples

Fennel seeds (provided from Zeitoon Talaee Kavir Co., Esfahan, Iran) were surface sterilized with a mixture of water and bleach (95:5) for 10 min and were placed on sterilized filter paper in 6-cm Petri dishes to germinate. Three-day-old seedlings were sown in plastic pots filled with perlite. Each pot, into which 15 seedlings were sown, was 14 inches in diameter, 8 cm in height, and contained 150 g perlite. Hoagland's solution [23] was used to irrigate the pots (Table 1). Since the pots did not have drainage, the nutrient solution was applied only on the first day. The pots were weighed after the first irrigation, and the same mass was maintained by irrigating with distilled water daily for two weeks. Hoagland's solution was applied every 2 weeks. Samples were maintained in a growth chamber with a 16-h photoperiod (25°C during the day and 15°C at night) at a light intensity of 8000 lux.

2.2. Salt stress

Salt (NaCl) at 0, 25, 50 and 75 mM in Hoagland's solution was prepared. The irrigation schedule was as described in 2.1., also for 2 weeks.

2.3. Nitrogen and iron deficiency stresses

The elements Fe and N were omitted or reduced separately to 1:2 in Hoagland's solution. Changes in the solutions are shown on Table 1. The irrigation schedule was as described in 2.1., also for 2 weeks.

Table 1. Hoagland solution with necessary modifications which were used

Stock solution (1M)	Dose for 1L	0N	0.5 N	0 Fe	0.5 Fe
$Ca(NO_3)_2$	5 mL	-	3.75 mL	5 mL	5 mL
KNO_3	5 mL	-	-	5 mL	5 mL
$MgSO_4$	2 mL	2 mL	2 mL	2 mL	2 mL
KH_2PO_4	1 mL	1 mL	1 mL	1 mL	1 mL
Micronutrients: (H_3BO_3, $MnCl_2$-$4H_2O$, $ZnSO_4$-$7H_2O$, $CuSO_4$, H_2MoO_4-H_2O)	1 mL	1 mL	1 mL	1 mL	1 mL
$CaCl_2$	-	5 mL	1.25 mL	-	-
KCl	-	5 mL	5 mL	-	-
Fe	0.03 g	0.03 g	0.03 g	-	0.015 g

2.4. Cold stress

Seedlings which had adapted for 14 days in the growth chamber were exposed daily to 0, 2, 3 and 4 h of cold stress (2°C) for a total of 6 days. After each cold-stress treatment, pots were quickly returned to the growth chamber. The irrigation schedule was as described in 2.1., also for 20 days.

2.5. Drought stress

14-day-old seedlings were exposed to 0, 3, 5 and 7 days water deficit. During the first two weeks, the irrigation schedule was as described in 2.1. but the samples were not irrigated during the two further

weeks (the stress period).

2.6. Extraction from shoots

Shoots from each seedling grown under salt, nutrient deficiency, cold and drought stress treatments were separately air-dried and powdered. Twenty grams of each powdered sample was extracted with 200 mL ethanol (96%) on a shaking water bath for 24 h at room temperature. The extracts were separated from solids by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 40°C using a rotary vacuum evaporator.

2.7. Determination of antioxidant activity

The antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) method as described by Benzie and Strain [24]. In this method the antioxidant power of the sample was measured by its ability to reduce the complex of Fe^{3+} with 2,4,6-tri(2-pyridyl)-s-triazine (Fe(III)-TPTZ) to Fe(II)-TPTZ. Fe(II)-TPTZ has a high absorbance at 595 nm and is measured spectrophotometrically.

2.8. Measurement of Chl and β-carotene

Fresh leaves of each seedling (200 mg) was mixed with acetone (80%) and crushed in a porcelain mortar. The solution volume was made up to 25 mL by adding acetone (80%). The solution was centrifuged (4800×*g*) for 20 min; the supernatant was isolated and absorbance was measured at 412, 431, 460 and 480 nm. Chl *a* and *b* and β-carotene content were evaluated according to these formulae [25, 26].

Chl *a* (µg/mL)=$-1.709(A_{412})+11.970(A_{431})-2.993(A_{460})-5.708(A_{480})$

Chl *b* (µg/mL)=$-0.171(A_{412})-0.23(A_{431})+11.871(A_{460})-13.248(A_{480})$

β-carotene (mg/mL)=$-0.43(A_{412})+0.251(A_{431})-4.376(A_{460})+13.216(A_{480})$

2.9. Measurement of fresh and dry weights

The fresh weight (FW) of the shoots and roots of samples were determined separately while dry weight (DW) was determined after placing seedlings in an oven at 50°C for 72h.

2.10. Statistical analysis

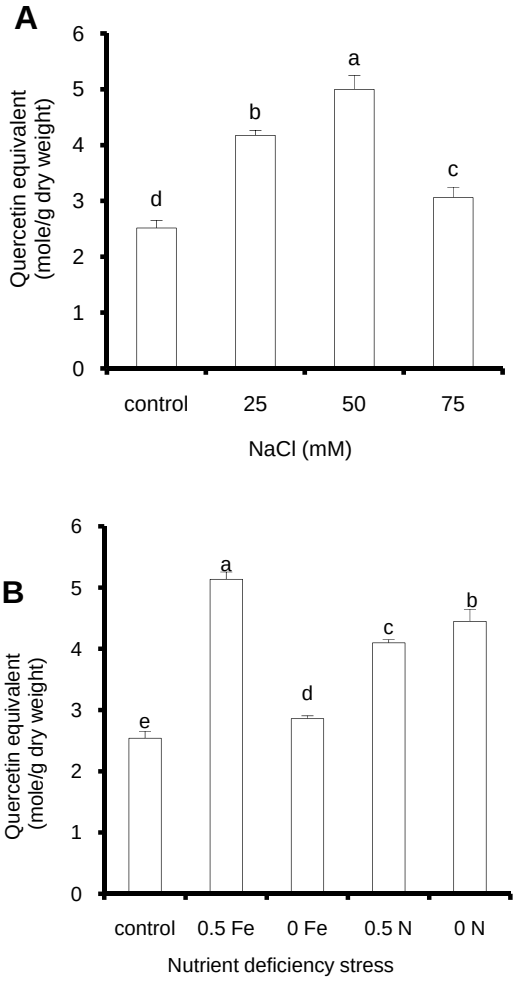
The experiment was arranged in a complete randomized design with four replications per treatment. Data were analyzed using SPSS v. 17.0 and mean comparisons were made with the LSD test at $P \leq 0.05$.

3. Results

3.1. Antioxidant activity assay

The antioxidant potential, which was evaluated by the FRAP method, illustrated that fennel seedlings have antioxidant activity. As shown in Figs. 1A-D, growing seedlings under all applied stresses increased antioxidant activity in comparison with the control samples.

Different concentrations of NaCl caused a significant increase in antioxidant activity of fennel seedling extracts, maximum at 50 mM NaCl (Fig. 1A). The deficiency or lack of Fe and N increased the antioxidant activity of the samples significantly (Fig. 1B). Chilling stress at 2°C significantly enhanced the antioxidant power (Fig. 1C). Drought stress for 3 days increased the antioxidant potential significantly, while 5 or 7 days decreased it (Fig. 1D).



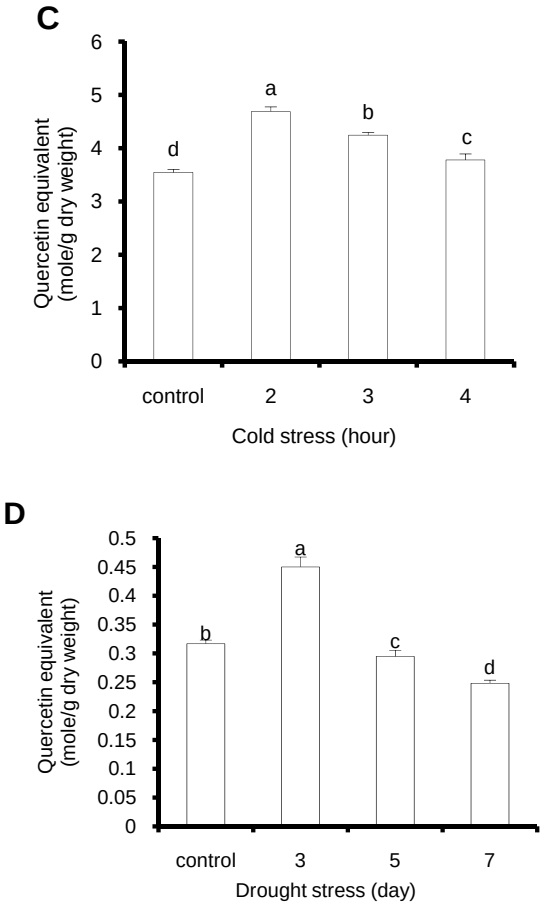


Fig. 1. Antioxidant activity of fennel seedling extract under the effect of salt (A), nutrient (B), cold (C) and drought (D) stresses (different letters show significant difference at $P \leq 0.05$) (0.5: reduction of N or Fe to half that of the original nutrient solution, 0: omission of N or Fe in the nutrient solution)

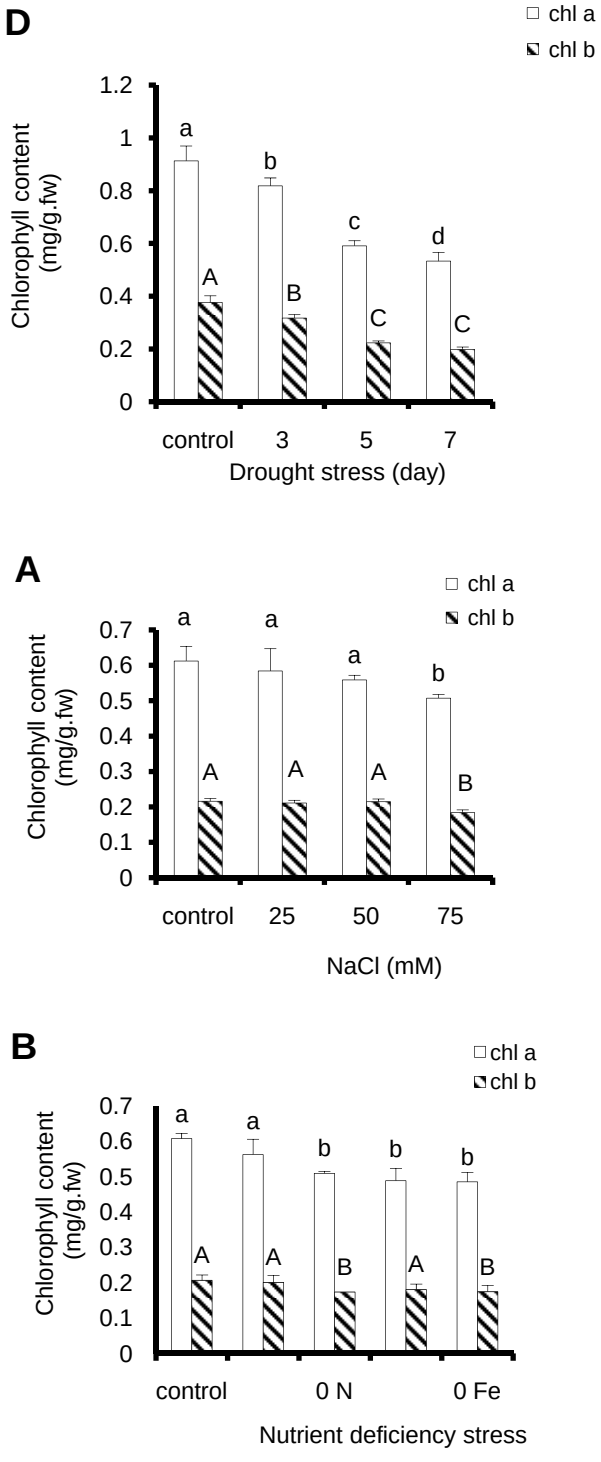
3.2. Content of Chl and β-carotene

Chlorophyll *a* and *b* decreased significantly at 75 mM NaCl (Fig. 2A). Nutrient stress had a significant negative effect on Chl (Fig. 2B). Chlorophyll content was significantly reduced under cold (Fig. 2C) and drought (Fig. 2D) stresses. At 50 and 75 mM NaCl (Fig. 3A), in Fe-deficient samples (Fig. 3B), cold stress (Fig. 3C) and 5 and 7 days of water deficiency (Fig. 3D) β-carotene was significantly decreased.

3.3. Fresh and dry weight of seedlings

Figures 4 and 5 show the effect of salt, nutrient deficiency, cold and drought stresses on FW and DW of roots and shoots.

Fifty and 75 mM NaCl significantly reduced the FW and DW of seedlings (Figs. 4A and 5A). As shown in Fig. 4B and 5B, N and Fe deficiency had a significant negative effect on FW and DW. Root FW of samples under cold stress was significantly reduced (Fig. 4C). Cold stress for 3-4 h also resulted in a significant reduction in shoot DW (Fig. 5C). Drought stress decreased seedling FW and DW significantly (Figs. 4D and 5D).



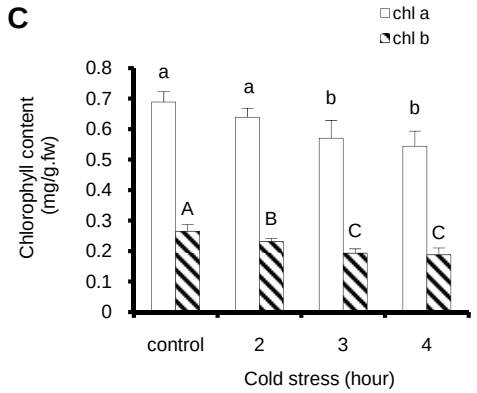


Fig. 2. Chlorophyll content of fennel seedling under the effect of salt (A), nutrient (B), cold (C) and drought (D) stresses (different lower-case letters and different capital letters show a significant ($P \leq 0.05$) reduction in Chl a and b content, respectively.) (0.5: reduction of N or Fe to half that of the original nutrient solution, 0: omission of N or Fe in the nutrient solution)

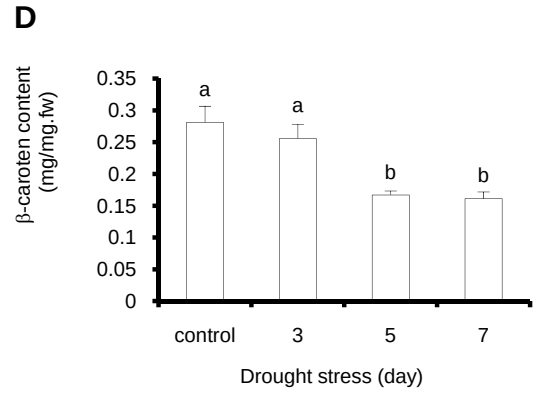
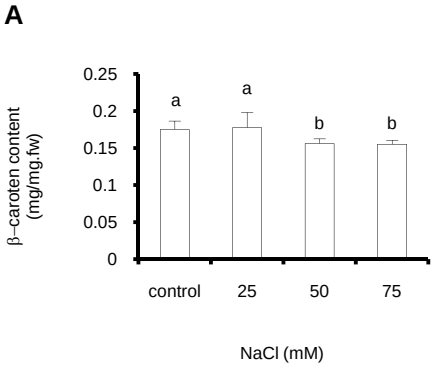
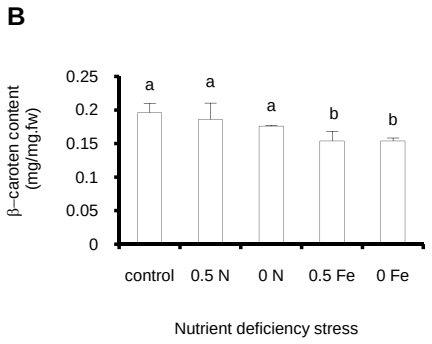
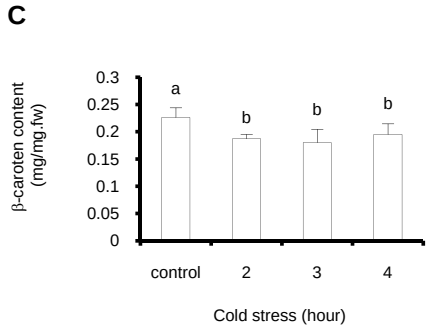
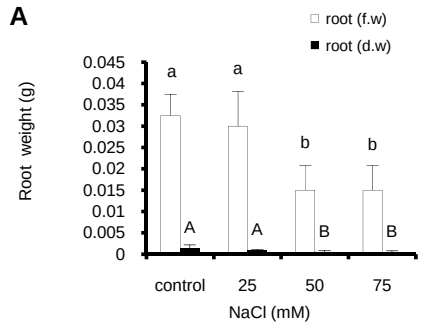


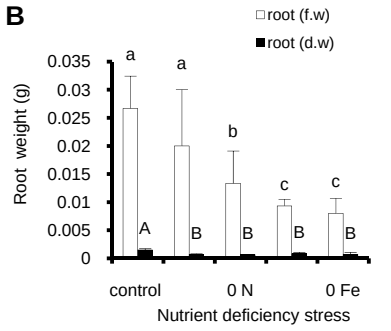
Fig. 3. β-carotene content of fennel seedling under the effect of salt (A), nutrient (B), cold (C) and drought (D) stresses (different letters show significant difference at $P \leq 0.05$) (0.5: reduction of N or Fe to half that of the original nutrient solution, 0: omission of N or Fe in the nutrient solution)

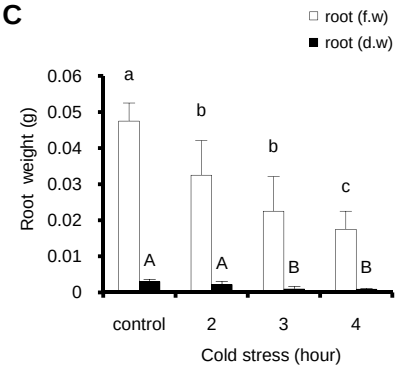












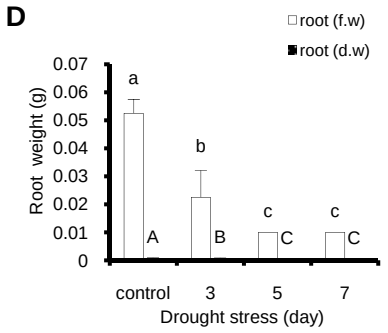
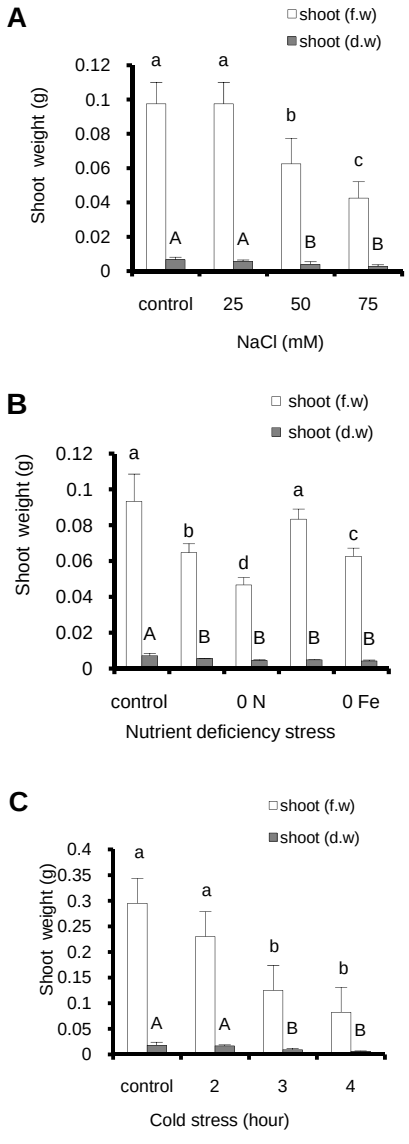


Fig. 4. Root weight of fennel seedling under the effect of salt (A), nutrient (B), cold (C) and drought (D) stresses (different small letters and different capital letters show significant ($p \leq 0.05$) reduction in FW and DW of root, respectively.) (0.5: reduction of N or Fe to half that of the original nutrient solution, 0: omission of N or Fe in the nutrient solution)



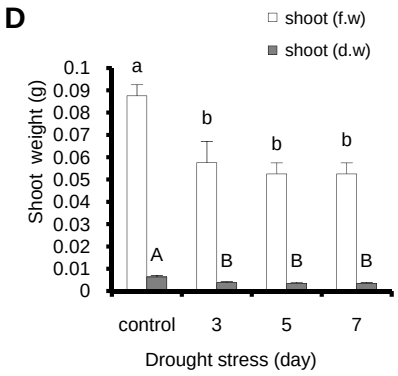


Fig. 5. Shoot weight of fennel seedling under the effect of salt (A), nutrient (B), cold (C) and drought (D) stresses (different small letters and different capital letters show significant ($p \leq 0.05$) reduction in FW and DW of shoot, respectively.) (0.5: reduction of N or Fe to half that of the original nutrient solution, 0: omission of N or Fe in the nutrient solution)

4. Discussion

4.1. Effect of abiotic stresses on antioxidant activity

The results showed that salt, nutrient deficiency, cold and drought stresses had a significant ($p \leq 0.05$) effect on antioxidant activity of fennel seedling extract.

Main antioxidants in higher plants include glutathione, ascorbate, tocopherol, proline, betaine, and others, which are also information-rich redox buffers and important redox signaling components that interact with biomembrane-related compartments [27]. Sources of natural antioxidants primarily are plant phenolic that may occur in all plant parts [16, 17]. Phenylalanine ammonia-lyase (PAL) is a key enzyme in the phenylpropanoid pathway and an important regulatory of many phenolic compounds. Studies with several different species have shown that PAL activity was induced by various biotic and abiotic stresses, such as low levels of nutrient, light, wounding, chilling, heavy metal and infection by viruses, bacteria or fungi [28-30]. Besides, reactive oxygen species induced by stresses trigger accumulation of several secondary metabolites, including phenolics, to mediate these stresses [31-33].

Although there are no similar studies on fennel, there are several investigations on the antioxidant activity or phenolic content of other species. Our results are in agreement with similar studies on other plants. Leaf phenolic content of cardoon (*Cynara cardunculus* L.) was significantly increased at 25-50 mM NaCl and the antioxidant scavenging activity was stimulated by salt treatment with a maximum at 150mM [34]. Total phenolic

content and PAL activity increased in N-deficient *Matricaria chamomilla* L. leading to enhancement of antioxidative status for plant protection against oxidative stress [30]. As the results of Fe-deficiency showed (Fig. 1B), the antioxidant activity increased significantly ($P \leq 0.05$) compared to control. Enhancement of antioxidant enzymes such as lipid peroxidase in borage (*Borago officinalis*) was also evident under severe Fe-deficiency [35]. Iron deficiency can modulate the content of glutathione (GSH) level, which was significantly increased in Fe deficiency treatment compared to Fe-sufficient treatment, and activity of superoxide dismutase (SOD) was increased under Fe deficiency treatment [36]. In this study, the enhancement of the antioxidant activity was higher in 0.5 Fe compared to 0 Fe but more investigation and information are needed to discuss the reason for this.

Cold treatment (4°C) for three days increased accumulation of phenolic compounds in pepper (*Capsicum annuum* L.) seedlings, which was positively related to antioxidant capacity [37]. Phenolic compounds which have antioxidant, anti-inflammatory and anti-clotting properties, may explain the cardiovascular health of some consumers of plants which contain phenols [15]. As the fennel is a medicinal plant, this experiment moved forward the information of fennel phenols, increasing by study the antioxidant activity of seedlings in response to four stresses. It seems that adapting the fennel under stress may convert this medicinal plant to a more powerful antioxidant in comparison with wild type.

4.2. Effect of abiotic stresses on pigments content and seedling growth

The results of this study illustrated that Chl *a* and *b* and β-carotene content, FW and DW of seedlings decreased significantly ($p \leq 0.05$) under salt, nutrient deficiency, cold and drought stresses.

Other researchers have also shown that irrigation schedule had significant ($p \leq 0.05$) effects on fennel plant growth characters. Water stress imposed by restricting the number of irrigations at 21-day intervals, commencing 21 days after sowing, significantly decreased plant height, branch number and fruit and oil yield [38]. Salt stress at 25, 50, 75 and 100 mM NaCl decreased FW and DW of both the shoots and roots of fennel. Maximum reduction in biomass was observed at 100 mM NaCl. Salt stress also caused a marked reduction in seed yield per plant [39]. Leaf growth (leaf biomass, length and number) of cardoon (*Cynara cardunculus* L.) was severely reduced at 150 mM NaCl [34]. Severe Fe-deficiency induced yellowing and typical Fe deficiency symptoms in borage (*Borage officinalis*), while Chl was inhibited [35].

The detrimental effects of salts on plants are the consequence of both a water deficit that results from the relatively high solute concentrations as well as a stress specific to Cl^- and Na^+, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, protein synthesis and nucleic acid metabolism [40-42]. One possibility is that salinity reduced photosynthesis, which in turn limited the supply of carbohydrate needed for growth. A second possibility is that salinity reduced shoot and root growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium. Third, a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentrations of specific ions in the growth medium, might have directly affected growth [36, 43]. The decrease in Chl content under salt stress is a commonly reported phenomenon in various studies, because of its adverse effects on membrane stability [44]. The primary effect of cooling plants below their optimum temperature range is the reduction of rates of growth and metabolic processes. Chilling stress causes a phase change (liquid to soilid) in membrane lipids leading to inactivation of membrane-bound enzymes and denaturation of enzymes [45]. Low temperature is one of the most important factors that may limit photosynthetic activity. A decrease in photosynthesis induced by low temperatures is a well-known response of chilling stress [46]. Chlorophyll *a* and *b* content decreased in cucumber (*Cucumis sativus* L.) and pea (*Pisum sativum* L.) when subjected to chilling at 5°C for 12 hours [47]. A decrease in the Chl content could be a typical symptom of oxidative stress [37]. Reactive oxygen species induced by chilling stress trigger a series of deleterious processes, such as lipid peroxidation and degradation of proteins and nucleic acids leading to the death of the cells [31, 33].

Drought is the most significant factor restricting plant growth and crop productivity [48, 49]. It inhibits the photosynthesis of plants, causes changes in Chl contents and components and damage to the photosynthetic apparatus [50]. In addition, it inhibits the photochemical activities and decreases the activities of enzymes in the Calvin Cycle in photosynthesis [51]. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and the expansion growth of cells [52-54]. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure [27].

Mineral nutrition of higher plants influences both primary and secondary metabolism [55]. N deficiency leads to coordinate repression of the

majority of genes assigned to photosynthesis and Chl synthesis [56]. One of the general aspects of N deficiency is a reduced root hydraulic conductivity leading to the inability of leaves to maintain adequate turgor [30]. It also induces decreases of Chl [57, 58]. When the plants were treated with no nitrogen, $Ca(NO_3)_2$ and KNO_3 could not be used in Hoagland's solution, and other types of salts must be replaced. In this study, chloridric salt of potassium and calcium were used, but the results which have been obtained for nitrogen deficiency might be related to the toxicity of Cl. Potassium and calcium sulfate can also induce sulfur toxicity. Too much chloride in plants results in symptoms that are similar to typical cases of salt damage. Sometimes in chloride toxicity, the leaf margins scorch and abscission is excessive, but these symptoms were not seen.

Iron (Fe) is considered as an essential nutrient for plant growth and plays a central role in the overall physiology of plants. When plants subjected to Fe deficiency stress, a range of deleterious effects including inhibition of photosynthesis processes, pigment synthesis and other metabolic disturbances were established [59, 60]. The lower Chl concentration can be explained, at least in part, by the role of Fe in the formation of **δ**-aminolevulinic acid and protoporphyrin, precursors of the Chl molecule [61].

This experiment developed knowledge of fennel cultivation under environmental stresses by investigating the physiological changes. Studying the effect of stresses on different plant stages and evaluation of the influence of these stresses on the composition and biological properties of seedling extract and seed essential oil will be necessary.

Acknowledgments

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References

[1] Anwar, F., Ali, M., Hussain, A. I. & Shahid, M. (2009). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. *Flavour Frag J*, *24*(4), 170-176.

[2] Piccaglia, R. & Marotti, M. (2001). Characterization of some Italian types of wild fennel (*Foeniculum vulgare* Mill.). *J Agri Food Chem*, *49*(1), 239-244.

[3] Barros, L., Heleno, S. A., Carvalho, M. & Ferreira, I. R. (2009). Systematic evaluation of antioxidant potential of different parts of *Foeniculum vulgare* Mill. From Portugal. *Food Chem Toxicol*, *47*(10), 2458-2464.

[4] Duke, J. A. (1985). CRC handbook of medicinal herbs. Boca Raton, FL: CRC Press. PP. 424-426.

[5] Mahajan, S. & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Biochem Biophys*, *444*(2), 139-158.

[6] Achuo, E. A., Prinseh, E. & Hofte, M. (2006). Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidium neolycopersici*. *Plant Pathol*, *55*(2), 178-186.

[7] Jaleel, C. A., Manivannan, P., Sankar, B., Kishorekumar, A. & Gopi, R. (2007). Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf B Biointerfaces*, *60*(2), 110-116.

[8] Ait, B. & Audran, J. C. (1987). Response of champenoise grapevine to low temperatures: Changes of shoot and bud proline concentrations in response to low temperatures and correlations with freezing tolerance. *J Hortic Sci Biotech*, *72*(4), 577–582.

[9] Blokhina, O., Virolainen, E. & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot*, *91*(2), 179-194.

[10] Sgherri, C., Cosi, E. & Navari-Izzo, F. (2003). Phenols and antioxidative status of *Raphanus sativus* grown in copper excess. *Plant Physiol*, *118*(1), 21–28.

[11] Posmyk, M. M., Kontek, R. & Janas, K. M. (2009). Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol Environ Safety*, *72*(2), 596-602.

[12] Roda, A. L., Oldham, N. J., Svatos, A. & Baldwin, I. T. (2003). Allometric analysis of the induced flavonols on the leaf surface of wild tobacco (*Nicotiana attenuate*). *Phytochem*, *62*(1), 527-536.

[13] Joachim, H. J. R., Ndakidemi, M. & Ndakidemi, P. A. (2007). Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. *Afric J Biotech*, *6*(12), 1358-1368.

[14] Surh, Y. (1999). Molecular mechanism of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutation Res*, *428*(1), 305-327.

[15] Shaharuddin, N. A., Kawamura, F., Sulaiman, O. & Hashim, R. (2008). *Evaluation on antioxidant activity, antifungal activity and total phenolic of selected commercial Malaysian timbers*. International Conference on Environmental Research and Technology. PP. 970-974.

[16] Du, G., Li, M., Ma, F. & Liang, D. (2009). Antioxidant capacity and relashionship with polyphenol and vitamin C in *Actinidia* fruits. *Food Chem*, *113*(15), 557-562.

[17] Mathew, S. & Abraham, T. (2006). *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem Toxicol*, *44*(2), 198-206.

[18] Oktay, M., Gulcin, I. & Kufrevioglu, I. (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT Food Sci Tech*, *36*(1), 263-271.

[19] Surveswaran, S., Cai, Y. Z., Corke, H. & Sun, M. (2007). Systematic evaluation of natural phenolic

antioxidants from 133 Indian medicinal plants. *Food Chem*, *102*(5), 938-953.
[20] Heinrich, M. (2005). Understanding local Mediterranean diets: a multidisciplinary pharmacological and ethnobotanical approach. *Pharmacol Res*, *52*(1), 353-366.
[21] Marino, S. D., Gala, F., Borbone, N., Zollo, F., Vitalini, S., Visioli, F. & Iorizzi, M. (2007). Phenolic glycosides from *Foeniculum vulgare* fruit and evaluation of antioxidative activity. *Phytochem*, *68*(1), 1805-1812.
[22] Boush, S. M., Schwrz, K. & Alegre, L. (1999). Enhanced formation of α-tocopherol and highly oxidize diterpenes in water-stressed Rosemary plants. *Plant Physiol*, *121*(3), 1047-1052.
[23] Epstein, E. (1972). *Mineral nutrient of plants: principles and perspectives*. New York, Wiley.
[24] Benzie, I. F. F. & Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of ‘‘antioxidant power the FRAP assay. *Anal Biochem*, *239*(1), 70-76.
[25] Chu, T. M., Aspinall, D. & Paleg, L. G. (1976). Stress metabolism. VII, salinity and proline accumulation in barley. *Aust J Plant Physiol*, *3*(2), 219-228.
[26] Kingsbury, R. W. (1986). Salt sensivity in wheat. *Plant Physiol*, 80, 651-654.
[27] Shao, H. B., Chu, L. Y., Shao, M. A., Jaleel, C. A. & Mi, H. M. (2008). Higher plant antioxidants and redox signaling under environmental stresses. *C R Biolo*, *331*(1), 433-441.
[28] Taiz, L. & Zeiger, E. (2002). *Plant Physiology*. Sunderland. Sinauer Associates Inc., Publishers.
[29] MacDonald, M. J. & D’Cunha, G. B. (2007). A modern view of phenylalanine ammonia-lyase. *Biochem Cell Biol*, *85*(3), 273-282.
[30] Kovaeik, J., Klejdus, B., Baekor, M. & Repeak, M. (2007). Phenylalanine ammonia-lyase activity and phenolic compounds accumulation in nitrogen-deficient *Matricaria chamomilla* leaf rosettes. *Plant Sci*, *172*(2), 393-399.
[31] Fridovich, I. (1978). The biology of oxygen radical. *Science*, *201*(2), 875-580.
[32] Christie, P. J., Alfenito, M. R. & Walbot, V. (1994). Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta*, *194*(4), 541-549.
[33] Apel, K. & Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol*, *55*(1), 373-379.
[34] Hanen, F., Ksouri, R., Megdiche, W., Trabelsi, N., Boulaaba, M. & Abdelly, C. 2008. Effect of salinity on growth, leaf-phenolic content and antioxidant scavenging activity in *Cynara cardunculus* L. *Biosaline Agri High Salinity Tolerance*, *3*(1), 335-343.
[35] Mohamed, A. A., Aly & A. A. (2004). Iron deficiency stimulated some enzymes activity, lipid peroxidation and free radicals production in *Borage officinalis* induced *in vitro*. *Int J Agri Biol*, *6*(1), 179-184.
[36] Zhu, J. K. (2002). Salt and drought stress signal transduction in plants. *Ann J Plant Biol*, *14*(2), 267-273.
[37] Koc, E., Islek, C. & Ustun, A. S. (2010). Effect of cold on protein, proline, phenolic compounds and chlorophyll content of two pepper (*Capsicum annuum* L.) varieties. *J Sci*, *23*(1), 1-6.
[38] Mohamed, M. A. H. & Abdu, M. (2004). Growth and oil production of fennel (*Foeniculum vulgare* Mill): Effect of irrigation and organic fertilization. *Biol Agri Hotic*, *22*(1), 31-39.
[39] Ashraf, M. & Akhtar, N. (2004). Influence of salt stress on growth, ion acuumulation and seed oil content in sweet fennel. *Biol Plantarum*, *48*(3), 461-464.
[40] Rajest, A., Arumugam, R. & Venkatesalu, V. (1998). Growth and photo-synthesis characteristics of *Ceriops roxburghiana* under NaCl stress. *Photosynthetica*, *35*(2), 285-287.
[41] Sairam, R. K., Rao, K. V. & Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentrations. *Plant Sci*, *163*(1), 1037-1046.
[42] Trinchant, J. C., Boscari, A., Spennato, G., Van De Sype, G. & Le Rudulier, D. (2004). Proline Betaine accumulation and metabolism in alfalfa plants under sodium chloride stress. Exploring its compartmentalization in nodules. *Plant Physiol*, *135*(3), 1583-1594.
[43] Lazof, D. B. & Bernstein, N. (1998). The NaCl induced inhibition of shoot growth: the case for disturb nutrition with special consideration of calcium. *Adv Bot Res*, *29*(1), 113-189.
[44] Ashraf, M. Y. & Bhatti, AS. (2000). Effect of salinity on growth and chlorophyll content in rice. *Pak J Sci Ind Res*, *43*(2), 130-131.
[45] Cherry, J. H. (1987). *Environmental stress in plants, biochemical and physiological mechanisms*. London, Springer-Verlag.
[46] Yadegari, L. Z., Heidari, R. & Carapetian, J. (2007). The influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (*Glycine max*) seedlings. *J Biol Sci*, *7*(8), 1436-1141.
[47] Wise, R. R. & Naylor, A. W. (1987). The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiol*, *83*(4), 272-277.
[48] Tas, S. & Tas, B. (2007). Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkiye. *World J Agri Sci*, *3*(2), 178-183.
[49] Mohsenzadeh, S., Malboobi, M. A., Razavi, K. & Farrahi-aschtiani, S. (2006). Physiological and molecular responses of *Aeluropus lagopoides* (poaceae) to water deficit. *Environ Exper Bot*, *56*(3), 314-322.
[50] Nayyar, H. & Gupta, D. (2006). Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. *Environ Exper Bot*, *58*(2), 106-113.

[51] Monakhova, O. F. & Chernyadev, I. I. (2002). Protective role of kartolin-4 in wheat plants exposed to soil drought. *Appl Environ Microbiol*, *38*(4), 373-380.
[52] Anjum, F., Yaseen, M., Rasul, E., Wahid, A. & Anjum, S. (2003). Water stress in barley (*Hordeum vulgare* L.). I. Effect on morphological characters. *Pak J Agric Sci*, *40*(1), 43-44.
[53] Bhatt, R. M., Srinivasa Rao, N. K. (2005). Influence of pod load response of okra to water stress. *Indian J Plant Physiol*, *10*(1), 54-59.
[54] Kusaka, M., Ohta, M. & Fujimura, T. (2005). Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. *Physiol Plantarum*, *125*(1), 474-489.
[55] Aerts, R. & Chapin, F. S. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res*, *30*(2), 1-67.
[56] Scheible, W. R., Morcuende, R., Czechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., Schindelasch, D., Thimm, O., Uvardi, M. K. & Stitt, M. **(**2004). Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiol*, *136*(2), 2483-2499.
[57] Ciompi, S., Gentili, E., Guidi, L. & Soldatini, G. F. (1996). The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. *Plant Sci*, *118*(2), 177 184.
[58] Huang, Z. A., Jiang, D. A., Yang, Y., Sun, J. W. & Jin, S. H. (2004). Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica*, *42*(1), 357-364.
[59] Terry, N. (1980). Limiting factors in photosynthesis. 1. Use of iron stress to control photochemical capacity *in vivo*. *Plant Physiol*, *65*(1), 114-120.
[60] Manthey, J. A. & Crowley, D. E. (1997). Leaf and root responses to iron deficiency in avocado. *J Plant Nutr*, *20*(1), 683-693.
[61] Marschner, H. (1986). Mineral Nutrition of Higher Plants. Orlando, Florida, USA: Academic Press