Fennel outperforms ajwain and anise in the saline environment: physiological response mechanisms in germinating seeds and mature plants

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Highlights

- *Differential salt-induced alterations in ionic status of mature plants of anise, ajwain, and fennel.*
- *Boosted antioxidative enzymes activities in the stressed plants of different species.*
- *Greater grain yield and essential oil of stressed fennel plants compared to anise and ajwain.*
- *Fennel and ajwain mature plants benefit from a stronger antoxidative defense to withstand salinity.*
- *Polyphenols and proline accumulated in the germinating seeds of fennel and ajwain contributes to salt tolerance.*

Abstract

The potential of different medicinal species as alternative crops for saline conditions needs to be explored. Comparative physiological responses of germinating seeds and mature plants of three genotypes of anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Mill.), and ajwain (*Trachyspermum ammi* L.) to salt were studied in a 2-year field experiment using 0 and 100 mcM, and a laboratory experiment using 0, 25, 50, 75, 100, and 125 mM NaCl. Catalase and ascorbate peroxidase activities increased in the salt-stricken plants of all genotypes, but only peroxidase activity of the salt-treated plants of anise genotypes and two of the fennel genotypes increased under field conditions. Chlorophyll and K^+ concentrations of all genotypes decreased, but proline and Na^+ concentrations and Na^+/K^+ increased under saline conditions. Dry mass, grain yield, and essential oil yield decreased in the salt-exposed plants across all genotypes and species. Germination, root, and shoot length were suppressed upon exposure to saline water. Despite the increasing trend of the proline and polyphenol concentrations and catalase and peroxidase activities, ascorbate peroxidase activity of germinating seeds decreased with an increase in NaCl concentration. Smaller adverse effects of salt on fennel germination attributes, grain, and essential oil yields were evident. Moreover, more significant activities of antioxidative enzymes and maintained Na^+ and Na^+/K^+ of salt-stricken fennel plants were observed. These findings indicate that fennel germinating seeds and mature plants have a greater ability to withstand salinity than the other examined species.

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Key words: Germination; polyphenols; salinity; medicinal plant; essential oil.

Contributions: JN, PE, and MHE designed the experiment; JN conducted the experiment, collected, and analysed the data; PE and MHE supervised the study; PE prepared the manuscript. All authors edited the manuscript.

Received for publication: 21 April 2022.
Revision received: 17 June 2022.
Accepted for publication: 17 June 2022.

Italian Journal of Agronomy 2022; 17:2096
doi:10.4081/ija.2022.2096

Introduction

Estimates indicate that over 80% of the population in developing countries rely on herbal medicine for basic healthcare needs (Canter *et al.*, 2005). The health benefits of these species stem from the secondary metabolites produced in different plant organs. In addition, though, many medicinal plants may find applications as spices and food crops. The amount of essential oil (secondary metabolites) and the number of different constituents may vary with the environmental conditions under which the medicinal plant species have been grown (Afshari and Rahimmalek, 2018; Hodaei *et al.*, 2018). Apiaceae (Umbelliferae) is a family of primarily aromatic and economically important species such as ajwain, anise, and fennel. The seeds of these species often produce essential oils containing aromatic compounds. These species are native to semiarid and Mediterranean types of climate prevalent in South-west Asia (Cetin and Celik, 2018) and, hence, they are potential candidates for production under limited water and saline soil conditions.

Salinisation is increasing both in intensity and extent worldwide (Song *et al.*, 2005). The increasing global population has

brought about agricultural malpractices (Khamesi *et al.*, 2020), including the application of low-quality brackish irrigation water, particularly in arid-semiarid climates. In arid regions such as the Middle East, irrigation of arable lands often aggravates salinisation because of evaporation of the groundwater that is naturally high in salts (De la Reguera *et al.*, 2020). Therefore, water shortages and salinity stress related to the use of brackish groundwater for irrigation are prevalent in the arid and semi-arid zones (Ozturk *et al.*, 2018). Nevertheless, the magnitude of soil salinisation is projected to increase in the coming decades due to climate change.

Too often, plants suffer from physiological and biochemical damage caused by exposure to counterproductive conditions, *i.e.*, salt stress (Shavrukov *et al.*, 2010). The results of these injuries are reflected in altered physiological and metabolic processes, leading to the accumulation of reactive oxygen species (ROS) and oxidative stress. Reduced growth and, therefore, lower commercial yield are brought about due to these conditions (Rivero *et al.*, 2001). Phenolic compounds are ubiquitous in plants and known antioxidants, often triggering cellular responses to counter oxidant stress (Shalaby and Horwitz, 2014). Osmolytes, such as free amino acids and soluble carbohydrates, are an adaptive measure against stressful environments and evolved to protect plant organs against a wide range of stresses. The protective and defensive roles of the osmolytes are brought about at least partly by the accumulation of these chemical compounds in the cellular compartments. Oxidative stress is an outcome of a disturbed balance between ROS production and the antioxidative defence of the plant (Yasar *et al.*, 2008). Plant cells possess an antioxidative defence system consisting of non-enzymatic and enzymatic antioxidants, enabling the plant to remove or inactivate ROS. The enzymatic component of the defence includes catalase (CAT), superoxide dismutase (SOD), peroxidases (POX), and ascorbate peroxidase (APX) that are mainly activated in the stressed plants and cooperatively reduce the oxidative state (Khamesi *et al.*, 2020). However, little is known about comparative physiological responses, grain yield, and essential oil yield penalties of different economically important medicinal plant species in saline environments.

In the dry quiescent state, seeds are protected by their seed coat and are exceptionally tolerant to stresses. By contrast, germination is a particularly vulnerable stage in the life history of crops where seeds become highly vulnerable to stresses during germination (Kranner *et al.*, 2010). Tolerance to salt stress during germination is crucially important for establishing and growing plants in saline soils (Saberali and Moradi, 2019). Moreover, even though seed germination and seedling establishment are known as the most sensitive stages of plant development, research on plant salt tolerance is too often focused on mature plants rather than germinating seeds. Time, rate, homogeneity, and synchrony are important aspects of the seed germination process that can be informative when studying the dynamics of this process (Ranal and Santana, 2006). The environmental stresses expect to adversely affect this set of seed germination attributes. Saline water may reduce the seed's ability to absorb water (osmotic stress) and cause an ion imbalance in the seed (ionic stress), eventually disturbing germination and preventing uniform crop establishment (De la Reguera *et al.*, 2020). However, information on the comparative germination behaviour of the different medicinal plant species upon exposure to hostile environments is needed. This lack of sufficient data highlights the necessity of further studies that could offer valuable information on the germinating seeds and seedlings and shed light on possible interrelations to some physiological traits involved in mature plant responses to saline water. A two-year field study was carried out to test the physiological and whole-plant responses under uncontrolled natural conditions, and a laboratory experiment was conducted to shed light on the response of germination and early seedling growth of three fairly common medicinal species of Apiaceae in Iran (*i.e.*, anise, ajwain, and fennel) to irrigation water salinity. These experiments and analyses were designed to understand the performance of the latter medicinal species in saline environments and propose them as sources of high-value alternative crops for marginal saline soils of the arid-semiarid regions.

Materials and methods

Field experiment: effect of salt stress on physiological attributes of mature plants

Experiment setup and plant materials

This experiment was conducted in 2018 and 2019 on fennel, ajwain, and anise, *i.e.*, three medicinal species from Apiaceae, at the Lavark Research Farm in Najaf Abad (32º32´N, 51º23´E, 1630 m above mean sea level, 14.5°C mean annual temperature, and 140 mm mean annual precipitation), Iran. These medicinal species were selected based on recent reports on their salt tolerance (Shafeiee and Ehsanzadeh, 2019) or a recent increase in farmers' interest in their cultivation in the region. Three genotypes of fennel (Shiraz, Yazd, and Kashan), ajwain (Karaj, Nahadjan, and Ghahdrijan), and anise (Isfahan, Marvdasht, and Borazjan) were exposed to 0 mM (control) and 100 mM NaCl (brackish water) irrigation water salinities, in a 3-replicate randomised complete block design in both years. As the soil and water salinities of the marginal lands in the arid regions of central Iran may reach 10 dS m^{-1}, the imposed salinity stress level was chosen to be 100 mM. Furthermore, this salinity level is comparable with reports in the literature for a threshold of meaningful stress in similar species. The soil was a fine loam Typical Haplargid, and according to the chemical analysis of the soil, only 100 kg ha^{-1} of urea fertiliser (*i.e.*, 46% of N) was given to the soil in early-April 2018 and 2019, as P and K macro elements were sufficient. Sufficient seeds were sterilised in a 2% (v/v) sodium hypochlorite (NaOCl) solution, repeatedly washed with distilled water, sown, and pre-germinated in 54×28 cm propagation trays containing coco peat, and grown in a greenhouse in the fourth week of February 2018.

Approximately 5 to 6-leaved seedlings of the genotypes were planted in the field plotsin early April 2018. Each plot comprised four rows 2.5-m long and 0.5 m apart. The seedlings were planted 20 cm apart on each row and watered sufficiently with non-saline irrigation water for one month. In the fourth week after planting, when the seedlings had reached the 8 to 10-leaf stage, the salt treatment was commenced and continued until nearly 60% physiological maturity of the fruits. While the non-saline (control) plots were irrigated with non-saline irrigation water, the salinity plots were watered with the irrigation water containing 100 mM NaCl. The saline water was obtained by dissolving NaCl into the irrigation water. To prevent osmotic shock, the first event of saline irrigation water was applied using a 50 mM salinity. The plots were irrigated by drip tapes of 16 mm diameter that contained drippers and had been installed alongside planting rows. Irrigation events were scheduled to ensure sufficient moisture supply throughout the two growing seasons. The volume of water supplied to each plot, measured by a volumetric counter, was 51 L m^{-2} at each irrigation event. Since there were 16 and 17 irrigation events throughout 2018 and 2019, the total water given to each plot was 4080 and

4335 L, respectively. In the 6th week after the saline water treatment implementation, random leaf samples were obtained from 5 plants in the 2nd row of each plot for measuring physiological responses. Measurements for grain yield, above-ground plant dry mass (SDM), and grain essential oil concentration were carried out at harvest when at least 70% of the plants had reached physiological maturity. The same seeding, germinating, and sowing practices for the ajwain and anise were conducted in March and April 2019. Though, as fennel is a ratoon crop (Akbari-Kharaji *et al.,* 2020), for the 2019 growing season, its plants were allowed to regrow from the stubble of the previous year that remained in the field.

Chlorophyll and carotenoids measurements

Samples of 500 mg from fresh mature leaves were obtained, extracted in acetone (80% v/v), filtered, and centrifuged (5810R, Eppendorf Refrigerated Centrifuge, Germany) at 5000×g for 10 min. Chlorophyll (Chl) *a* and *b* and carotenoid concentrations were determined by reading the absorbencies (spectrophotometer: U-1800 UV/VIS, Hitachi, Japan) at 645, 663, and 470 nm, using 80% acetone as blank. Chlorophyll and carotenoid concentrations were quantified according to Lichtenthaler and Wellburn (1994) and details given by Khamesi *et al.* (2020).

Enzyme extraction and assay

Fresh leaves samples of 500 mg were frozen in liquid nitrogen and ground in 4 mL of a solution containing 50 mM phosphate buffer (pH 7.0), 1% (m/v) polyvinyl polypyrrolidone, and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15 000 *g* for 30 min, and the collected supernatant was used for enzyme assays.

The CAT (EC 1.11.1.6) activity was determined based on the decrease in absorbance at 240 nm for 1 min due to the consumption of hydrogen peroxide (H_2O_2) (Chance and Maehly, 1955). The assay mixture contained 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. One unit of CAT activity was defined as 1 µmol of H_2O_2 decomposed per minute per gram of protein using a coefficient of absorbance of 40 M^{-1} cm^{-1}. The APX (EC 1.11.1.11) activity was determined based on the decrease in absorbance at 290 nm for one min (Nakano and Asada, 1981). The reaction mixture consisted of 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.0), and 0.15 mL of enzyme extract. One unit of APX activity corresponded to 1 nmol of ascorbate oxidised per minute per gram of protein. The POX (EC 1.11.1.7) activity determination was based on the increase in absorbency at 470 nm for 2 min according to Herzog and Fahimi's (1973) procedure. The activity of this enzyme was expressed as units mg^{-1} protein. One unit of POX activity represents the amount of enzyme needed for oxidation of 1.0 µM of guaiacol in 1 min.

The total protein content of the samples was determined based on the method of Bradford (1976). Bradford dye reagent [composing of ethanol (95%), orthophosphoric acid, and Coomassie Brilliant Blue] was diluted and added to test tubes containing the protein extract, and the absorbance was measured at 595 nm and compared to bovine serum albumin (BSA) as a standard. Then, the activities of the enzymes mentioned above were assessed based on the protein content of the samples.

Ion content, above-ground dry mass, grain yield, and essential oil measurements

Samples of 1 g from root tissue were used to quantify the ion concentrations. First, grinding and ashing the samples for 4 h at 550°C was followed by releasing the mineral ions using 2 N HCl. Then, the concentrations of Na^+ and K^+ were determined by a flame photometer (*Corning Flame Photometer 410, Corning Medical and Scientific,* Halstead Essex, UK) as described in Shafeiee and Ehsanzadeh (2019).

After reaching 70% physiological maturity, plant above-ground parts of 2 m^2 of each plot were harvested, air dried for at least 7 days, and weighed to determine the SDM. The grains were separated from the aerial parts, cleaned, and grain yield was expressed as g m^{-2}. A 25-g sample of grains from all plots was powdered and underwent a hydrodistillation process at 100°C in 200 mL of deionised water. The essential oil phase was separated and dried over anhydrous sodium sulphate, and grain essential oil concentration and yield were determined.

Germination experiment: effect of salt stress on seed germination and seedling attributes

Experiment setup and treatments

A 6×6 factorial completely random design experiment in 3 replicates was designed to test the effect of salt stress (0, 25, 50, 75, 100, 125 mM NaCl) on seed germination and seedling growth attributes of two genotypes of anise (*i.e.*, Isfahan and Marvdasht), ajwain (*i.e.*, Nahadjan and Ghahdrijan), and fennel (*i.e.*, Shiraz and Yazd). These NaCl concentrations were equivalent to osmotic potentials of –0.0, –0.12, –0.24, –0.36, –0.48, and –0.60 MPa. The selection of these genotypes was based on the results of the field experiment, where for each of the examined species, the two most tolerant genotypes (*i.e.*, from the three genotypes) were chosen for assessments in the laboratory germination experiment. While distilled water was applied to the non-saline (0 mM) Petri dishes, the five levels of salt stress were created using mixtures of sodium chloride (NaCl) and distilled water. Sufficient seeds of each genotype were disinfected for 2 min in a 0.5% sodium hypochlorite solution and repeatedly washed with distilled water. Then, 50 seeds per replicate were placed on an 85 mm Grade 1 Whatman filter paper in glass Petri dishes and moistened with 5 mL of distilled water or NaCl solution. Since each genotype × solution combination was replicated three times, 150 seeds per genotype × solution combination was assessed. The Petri dishes were tightly covered and wrapped with parafilm to prevent evaporation and were incubated in an incubator (Binder, Model BF400, USA) at 23°C, 60-70% relative humidity, and 16/8 h (light/dark). If the filter paper appeared to be drying or there was no visible solution in the Petri dish, 3 mL of distilled water was added. Germination counts were made daily until no new germination was observed (*i.e.*, until the 14th day after the salt germination commenced) and the final germination percent was determined. A seed was considered germinated if at least 2 mm radicle was present.

Measurement of germination attributes and physiological responses to salinity

On the 14th day, the lengths of roots and shoots of 5 seedlings per Petri dish were measured. In addition, germination percent, seedling vigour index, and germination velocity were determined using Eq. (1), Eq. (2), and Eq. (3), respectively (Zhu and Bañuelos, 2016).

$$\text{Germination percent} = (Ng/Nt) \times 100 \quad (1)$$

where, Ng = total number of seeds germinated during the 14 days, and Nt = total number of seeds in each Petri dish.

$$\text{Vigour index} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination\%} \quad (2)$$

$$\text{Germination velocity} = \Sigma_i[g_i - g_{(i-1)}/i] \quad (3)$$

where, g is the total germination percentage on an incubation day i, minus the total germination percentage on the previous day g (i − 1), and divided by the incubation day i.

Free proline concentration of the seedlings was measured using the method of Bates *et al.* (1973). A 100 mg sample of fresh seedlings was crushed in 10 mL of 3% aqueous sulphosalicylic acid, and the extract was passed through a Whatman filter paper. Two mL of the extract was added into the test tube containing 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid. The reaction mixture was boiled in a water bath at 100°C for 1 h. After cooling the mixture on ice, 4 mL of toluene was added and thoroughly mixed. Finally, the toluene phase was separated, and its absorbance was measured at 520 nm using a spectrophotometer against a blank of toluene.

For polyphenol measurement, a sample of 500 mg from fresh mass (FM) of the seedlings per Petri dish was obtained, ground to a powder, mixed by a magnetic stirrer, and extracted in 100 mL of ethanol 95% at 22°C for 1 h. First, the extract was filtered through Whatman filter paper to remove seedling tissue particles. Then the polyphenol concentration of the extract was determined using the Folin-Ciocalteu reagent following the details given by Singh *et al.* (2002). In this procedure, the extracted sample is mixed with 1 mL of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution, and it is kept at room temperature for at least 1 h. Finally, polyphenol concentration (expressed as mg gallic acid g^{-1} FM) was assessed using the spectrophotometer by reading the absorbencies at 765 nm.

Antioxidative enzymes (*i.e.*, CAT, APX, and POX) assays were conducted according to the details given for the field experiment.

Statistical analyses

For the field experiment, the two years' data were subjected to a combined analysis of variances using the Statistical Analysis Software (SAS Institute Inc., Version 9.4, Cary, NC, USA). For the germination experiment, data were subjected to analysis of variances. The least significant differences (LSD) method was used for the mean comparisons at a 0.05 level of probability. Since most of the studied traits were significantly affected by the interaction effects of genotype × salinity, mean comparisons were only carried out for the latter interactions.

Results

Field experiment

Leaf Chl and carotenoid concentrations and Chl-*a*/*b*, activities of the examined antioxidative enzymes, *i.e.*, CAT, APX, and POX, Na^{+}, and K^{+} concentrations, Na^{+}/K^{+}, SDM, grain yield, and essential oil yield were significantly affected by the salt. However, in the meantime, they were also affected by genotype × salt interaction (Table 1).

Even though salt stress tended to negatively affect the leaf Chl concentration of all of the genotypes of the three medicinal species, these decreases were statistically significant only for two out of the nine genotypes examined, *i.e.*, Kashan and Yazd (Table 2). Salt stress affected the Chl-*a*/*b* in a genotype-specific manner. It was decreased in two of the ajwain genotypes, *i.e.*, Ghahdrijan and Nahadjan, and two of the fennel genotypes, *i.e.*, Shiraz and Yazd, but it increased in the remaining ajwain genotype, *i.e.*, Karaj and decreased non-significantly in all of the anise genotypes. Salt stress led to decreased carotenoid concentration in all genotypes of the three examined species except for genotypes Karaj (ajwain) and Borazjan (anise), where notable changes in the carotenoid concentration were not recorded. The greatest and smallest carotenoid concentrations were observed in the non-stressed plants of genotype Yazd (fennel) and salt-stressed plants of genotype Ghahdrijan (ajwain), respectively. Decreases in the Chl and carotenoid concentration of the stressed anise plants were generally smaller than fennel and ajwain (Table 2). Exposure to brackish irrigation water brought about significant increases in the activities of CAT and APX of all genotypes of the examined species, except for genotype Nahadjan (ajwain), where salt-induced modifications in the activities of these enzymes were not significant (Table 2). Unlike the enzymes mentioned above, the activity of POX was altered in response to the brackish irrigation water in a genotype-specific manner. While POX activity was decreased in two of the ajwain genotypes (Nahadjan and Karaj), it had only a modest increase in the third ajwain genotype (Ghahdrijan). Although POX activity remained unchanged in the Kashan genotype, it increased significantly in the other fennel genotypes (Yazd and Shiraz). Moreover,

Table 1. Analysis of variance (mean squares) for carotenoid and chlorophyll (Chl) concentrations, chlorophyll *a/b* (Chl-*a/b*), catalase, ascorbate peroxidase, and peroxidase activities, root Na^{+} and K^{+} concentrations, Na^{+}/K^{+}, plant above-ground dry mass (SDM), grain yield, and essential oil yield of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at different salinities of irrigation water under field conditions.

Source of variation	df	Carotenoid	Chl	Chl-*a/b*	Catalase	Ascorbate peroxidase	Peroxidase	Na^{+}	K^{+}	Na^{+}/K^{+}	SDM	Grain yield	Essential oil yield
Year (Y)	1	2.65**	0.771**	46.51 ns	1.13 ns	29.53**	23.95**	8.43**	0.07**	4.97**	351232 ns	5086 ns	85.06**
Salt (S)	1	0.064**	0.264**	6.86**	1.29**	109.6**	2.64**	16.73**	3.62**	55.91**	1469975**	63683**	28.76**
Y×S	1	0.014 ns	0.223**	0.12 ns	0.31 ns	2.61 ns	0.39**	1.02**	1.32**	0.25	628935**	29142**	39.81**
Replication (Y×S)	8	0.010	0.023	0.33	0.01	0.52	0.04	0.04	0.02	0.10	12158	235	1.09
Genotype (G)	8	0.034**	0.102**	2.20**	0.05*	16.59**	1.44**	0.18**	0.08**	0.77**	108906**	2164**	15.50**
Y×G	8	0.031**	0.120	0.67	0.03	8.26	1.25	0.07	0.48	1.12	22015 ns	1474**	8.35
S×G	8	0.030**	0.622**	1.63**	0.05*	2.63 ns	0.87**	0.20**	0.14**	1.20**	586241**	5451**	2.24**
Y×S×G	8	0.012 ns	0.039 ns	1.52	0.02 ns	9.22**	0.53**	0.19**	0.25**	0.72**	153557**	2720**	1.88*
Error	64	0.007	0.016	0.29	0.01	1.55	0.08	0.04	0.02	0.19	12136	339	0.40
Coefficient of variation (%)	21.6	11.3	23.70	23.0	24.2	27.8	21.0	15.9	33.4	19.7	19. 3	26.3	

ns, non-significant; Error, within group variance; *P≤0.05; **P≤0.01.

the activity of this enzyme increased by several folds in the examined genotypes of anise. The greatest CAT and POX activities were detected in the salt-stressed plants of genotype Yazd and, to some extent, Shiraz (fennel). The greatest APX activities of the salt-stressed plants belonged to the fennel genotypes, and the smallest ones were found in the anise genotypes. Increases in the activities of the antioxidative enzymes of the salt-stressed anise plants were generally smaller than in fennel and ajwain (Table 2).

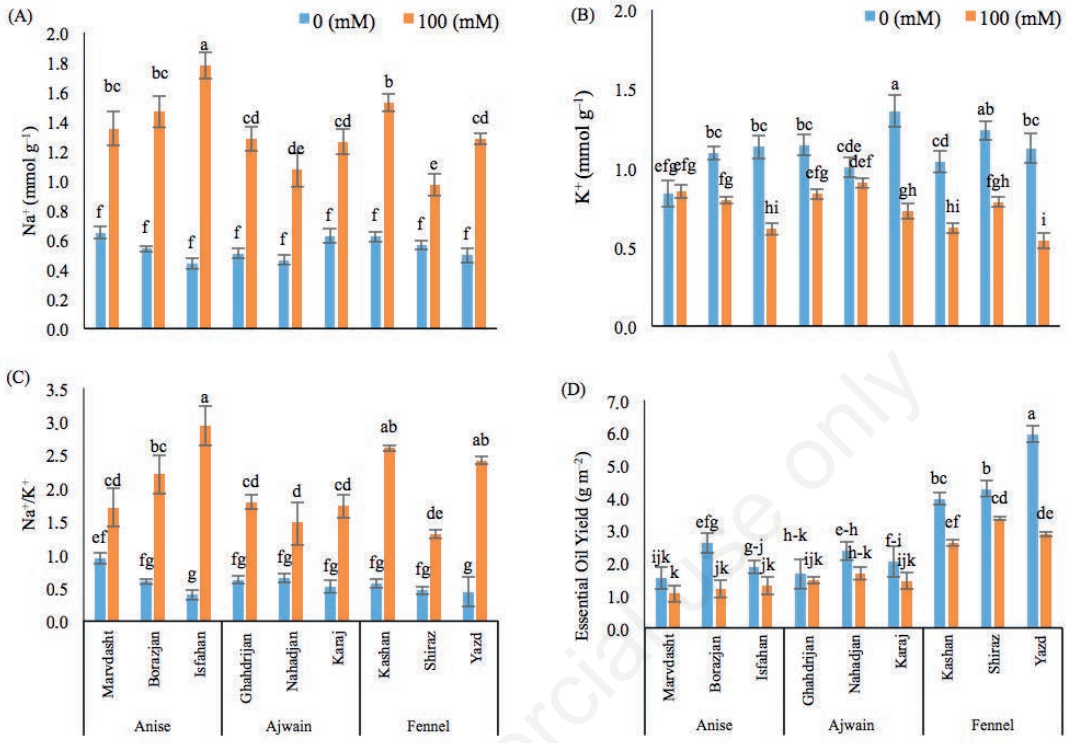

Figure 1. A-D) Mean comparisons of genotype × salinity interaction for root Na^+ and K^+ concentrations, Na^+/K^+, and essential oil yield of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at 0 and 100 mM NaCl salinities under field conditions. Each mean is accompanied by its standard error. Means bearing the same letter are not significantly different according to the least significant difference (LSD) test at P≤0.05.

Table 2. Mean comparisons of genotype × salinity interaction for carotenoid and chlorophyll (Chl) concentrations, chlorophyll *a/b* (Chl-*a/b*), catalase, ascorbate peroxidase, and peroxidase activities, plant above-ground dry mass (SDM), and grain yield of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at different salinities of irrigation water under field conditions.

Species	Genotype	NaCl (mM)	Carotenoid (mg g^{-1} FM)	Chl (mg g^{-1} FM)	Chl-*a/b*	Catalase (unit mg^{-1} protein)	Ascorbate peroxidase (unit mg^{-1} protein)	Peroxidase (unit mg^{-1} protein)	SDM (g m^{-2})	Grain yield (g m^{-2})
Anise	Marvdasht	0	0.41^{abc}	1.08^{cde}	2.7^{b}	0.183^{h}	3.27	0.346^{ef}	402.7^{gh}	101.4^{cd}
		100	0.31^{def}	1.03^{def}	2.1^{b-e}	0.634^{a}	6.52	0.932^{c}	297.7^{h}	53.3^{g}
	Borazjan	0	0.29^{ef}	1.25^{ab}	1.7^{ef}	0.199^{h}	2.31	0.242^{f}	444.3^{fg}	117.0^{c}
		100	0.30^{def}	1.04^{def}	1.5^{ef}	0.449^{bcd}	3.48	1.084^{bc}	312.4^{h}	66.1^{efg}
	Isfahan	0	0.38^{cde}	1.20^{abc}	2.6^{bc}	0.264^{gh}	3.02	0.296^{ef}	445.6^{fg}	108.0^{c}
		100	0.29^{def}	1.22^{abc}	2.0^{c-f}	0.393^{c-e}	4.09	1.039^{bc}	302.7^{h}	63.8^{g}
Ajwain	Ghahdrijan	0	0.43^{abc}	1.32^{a}	3.4^{a}	0.265^{gh}	5.02	0.531^{ef}	658.9^{de}	98.6^{cd}
		100	0.27^{f}	1.22^{abc}	1.8^{def}	0.505^{b}	6.48	0.613^{de}	308.6^{h}	65.9^{fg}
	Nahadjan	0	0.45^{abc}	1.29^{ab}	2.6^{bc}	0.418^{b-e}	5.63	1.322^{b}	560.3^{ef}	99.0^{cd}
		100	0.39^{bcd}	1.20^{abc}	1.53^{f}	0.407^{b-f}	6.50	0.973^{c}	287.3^{h}	58.9^{g}
	Karaj	0	0.46^{abc}	1.22^{abc}	2.6^{bc}	0.281^{gh}	4.31	1.677^{a}	570.8^{ef}	85.6^{def}
		100	0.46^{abc}	1.15^{bcd}	3.6^{a}	0.507^{b}	6.70	1.094^{bc}	292.4^{h}	57.3^{g}
Fennel	Kashan	0	0.48^{ab}	1.19^{abc}	2.4^{bcd}	0.30^{fg}	3.85	1.186^{bc}	849.4^{c}	140.0^{b}
		100	0.39^{bcd}	0.98^{ef}	2.5^{bcd}	0.477^{bc}	7.10	1.189^{bc}	707.0^{d}	87.2^{de}
	Shiraz	0	0.48^{abc}	0.90^{f}	2.4^{bcd}	0.317^{efg}	5.05	0.899^{cd}	984.6^{b}	142.0^{b}
		100	0.42^{abc}	0.90^{f}	1.6^{ef}	0.478^{bc}	6.93	1.641^{a}	716.9^{d}	103.0^{cd}
	Yazd	0	0.50^{a}	1.15^{bcd}	2.5^{bcd}	0.367^{d-g}	4.86	1.002^{c}	1158.9^{a}	186.0^{a}
		100	0.43^{abc}	0.96^{ef}	1.6^{ef}	0.720^{a}	7.65	1.753^{a}	750.6^{cd}	85.5^{def}

[a-h]Values bearing the same letter are not significantly different according to the least significant difference (LSD) test at P≤0.05.

Concentrations of Na^+ (Figure 1A) and K^+ (Figure 1B) were increased and decreased, respectively, in the salt-stressed plants of all genotypes across the examined species, except for genotype Marvdasht (anise), which did not indicate a notable change in K^+ concentration. While the greatest Na^+ concentrations were detected in the salt-stricken plants of the genotypes Isfahan (anise) and Kashan (fennel), the smallest ones were observed in the non-stressed plants of genotypes Nahadjan and Ghahdrijan (ajwain) and Yazd (fennel). Similar to Na^+ concentration, the Na^+/K^+ of all genotypes increased several-fold in the presence of brackish water (Figure 1C). However, the increase in the Na^+/K^+ of the ajwain genotypes was smaller than the fennel and anise genotypes. The greatest Na^+/K^+ ratios were detected in the salt-stricken plants of the genotypes Isfahan (anise) and Kashan (fennel), while the smallest Na^+/K^+ ratios were observed in the non-stressed plants of genotypes Isfahan (anise) and Yazd (fennel). Increases in the Na^+ concentration and $Na^+/K+$ of the stressed anise plants were generally greater than fennel and ajwain.

Concomitant to the salt-induced decreases in the SDM, grain yield (Table 2) and essential oil yield (Figure 1D) of all the genotypes within different species decreased due to salt exposure. The greatest grain yields of the non-stressed plants belonged to the three examined fennel genotypes, but the smallest grain yields of the stressed plants belonged to the three examined ajwain genotypes. The greatest essential oil yields were detected in the non-stressed plants of the fennel genotypes. The essential oil yields of the fennel genotypes were at least twice as much as those of the anise and ajwain genotypes. Albeit, the descending rankings for essential oil yield for both non-stressed and stressed plants tended to be fennel > ajwain > anise (Table 2).

Germination experiment

Salt effects were statistically significant for all the examined traits, *i.e.*, proline and polyphenols concentrations, CAT, POX, and APX activities, germination percent and velocity, root and shoot length, and seedling vigour (Table 3). However, genotype × salt interaction was statistically significant for most of these traits. To minimise the complications in the mean comparisons, only the means for genotype × salt interactions will be given in detail.

Germination percent (Figure 2A) and velocity (Figure 2B) were drastically decreased with an increase in the NaCl concentration of the medium. There were some differences in the magnitude of salt-induced decreases in the germination among the genotypes, leading to the significant genotype × salt interaction. APX activity in the salt-stricken plants of different genotypes of the examined species was decreased (Figure 2C). Albeit genotype Ghahdrijan (ajwain) must be exempted from this generalisation, as its APX activity increased with an increase in NaCl concentration.

Salt-induced significant increases in the polyphenols and proline concentrations of seedlings of the three medicinal species, but the extent of the increase in fennel was greater than those of ajwain and anise (Table 4). Even though CAT and POX activities of the three species (*i.e.*, averaged over genotypes) were increased with higher salt concentrations, the increases for anise and, to some extent, ajwain were smaller than in fennel. Furthermore, averaged over the genotypes, the smallest CAT and POX activities were detected in anise compared to fennel and ajwain. Averaged over genotypes, root and shoot length and seedling vigour of the examined medicinal species were decreased when germinated under saline conditions, but the extent of these decreases was smaller for fennel. When averaged over genotypes, anise suffered more from the saline medium, as its germination attributes and seedling growth characteristics were more drastically decreased compared to those of fennel and ajwain (Table 4).

Discussion

The seeds are germinated in the most published pot and field studies on salt stress responses, and seedlings are allowed to establish in a non-saline medium before salt exposure. However, in such procedures, conclusions and/or inferences made on plant salt tolerance suffer from uncertainty around the salt response at the very earliest stage of plant development, *i.e.*, germination and seedling establishment. In the present study, we have tackled this uncertainty by examining these medicinal species at the germinating seed (*i.e.*, germination experiment) and mature plant (field experiment) levels.

Germinating seeds of anise are more salt-sensitive than ajwain and fennel

Germination is the most critical phase in the plant life cycle. Hence, obtaining rapid and uniform germination and seedling emergence is crucial for successful crop production. To successfully germinate, a seed must absorb water from the medium through the seed coat. As salinity leads to lowering the medium's water potential, it slows the absorption of water by the seed, causing osmotic stress. Moreover, the salt brings about ionic stress which alters the enzymatic activities and seed and plant metabolism. Thus, the saline condition induces a delay in the germination process and a reduction in the germination percent (Pujol *et al.*, 2000).

Moreover, with salinities beyond the tolerance limits of a plant species, complete inhibition of germination may occur. As confirmed by all genotypes of the medicinal species of the present

Table 3. Analysis of variance (mean squares) for germination percent, germination velocity, seedling vigour, root length, shoot length, proline concentration, polyphenol concentration, catalase, ascorbate peroxidase, and peroxidase activities of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at different salinities under laboratory conditions.

Source of variation	df	Germination	Velocity	Seedling vigour	Root length	Shoot length	Proline	Polyphenol	Catalase	Ascorbate peroxidase	Peroxidase
Salt	5	11,619**	45.7**	229**	117**	35.9**	4.43**	7.79**	0.104**	26.1**	7.52**
Genotype	5	298**	5.16**	46.9**	76.3**	28.3**	2.77**	27.1**	0.083*	21.4**	4.58**
Salt × Genotype	25	42.3**	5.190**	5.92**	3.56**	0.819 ns	1.10**	2.67**	0.028*	3.67**	0.682*
Error	70	9.79	0.159	0.295	1.00	0.329	0.197	0.324	0.009	0.449	0.167
Coefficient of variation (%)	8.6	16.2	14.6	20.3	20.6	21.9	13.2	30.8	19.1	34.4	

ns, non-significant; Error, within group variance; *P≤0.05; **P≤0.01.

study, the overall effect of the salt-induced osmotic and ionic stresses is a delay and depression in seed germination (De la Reguera *et al.*, 2020; Dhima *et al.*, 2021). This salt-induced delay and depression of germination were more severe in anise than in fennel and ajwain. In agreement with our findings, salt treatment negatively affected the germination percentage of oregano (*Origanum compactum*) (Laghmouchi *et al.*, 2017) and induced dormancy in seeds of four halophyte species from Southeastern Spain due to a decrease in osmotic potential (Pujol *et al.*, 2000). Germination vigour is an outcome of interplays of several biochemical and molecular variables. The data of the polyphenol and proline concentrations, CAT and POX activities (Table 4), and germination vigour index (Figure 2B) of the three analysed medicinal species reveal a meaningful proportionality between the antioxidative capacity and the germination vigour, particularly under saline conditions. When a germinating seed is challenged by osmotic stress, its growth is slowed, and embryonic programs are reinitiated, presumably due to an increase in biosynthesis and accumulation of ABA, preventing the plant from entering the vulnerable seedling state (Rajjou *et al.*, 2012). The tendency to decrease the activity of APX in the stressed germinating seeds of some genotypes in the present study (Figure 2C) is in accord with those of Dash and Panda (2001), as they also found the salt-induced decrease in germination attributes of black gram (*Phaseolus mungo*) concomitant with an increase in proline concentration and decreases in the activities of some antioxidative enzymes. As abscisic acid synthesis and accumulation and developing seed dormancy are found to be accompanied by a higher level of proline in the germinating seeds under stress conditions (Thakur and Sharma, 2005), proline might be involved in the acclimation of germinating

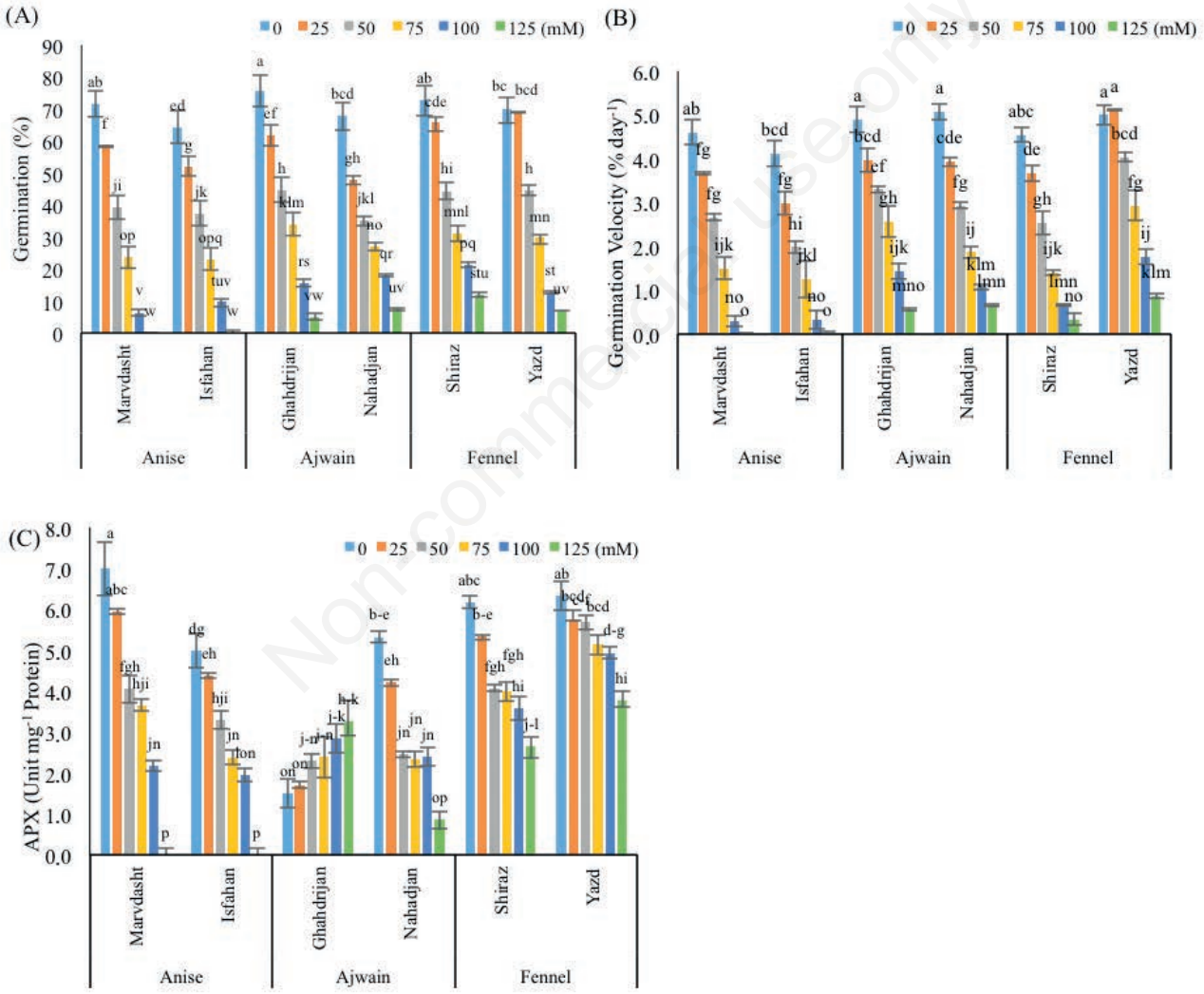

Figure 2. A-C) Mean comparisons of genotype × salinity interaction for germination percent, germination velocity, and ascorbate peroxidase activity of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at 0 to 125 mM NaCl salinities under laboratory conditions. Each mean is accompanied by its standard error. Means bearing the same letter are not significantly different according to the least significant difference (LSD) test at P≤0.05.

seeds. It is, in fact, suggested that the synthesis of proline may be an adaptive strategy toward preventing germination under stressful environments, thus ensuring an effective seedling establishment when conditions are more conducive to seedling establishment. Moreover, an improved germination percentage of salt-exposed seeds of rapeseed was accompanied by increases in proline content due to altered transcription of genes involved in proline metabolism (Kubala *et al.*, 2015). The latter report supports our data (Table 4), as enhanced proline concentrations in fennel and ajwain germinating seeds were associated with greater germination performance compared to anise.

Polyphenol compounds are an acclimation mechanism against an array of stresses; their accumulation under stressful conditions stems either from activating the biosynthesis or inhibiting their oxidation (Rivero *et al.*, 2001). Phenolics are known to be two types. The preformed phenolics are synthesised during normal plant growth and are present in moderate amounts. The induced phenolics are, on the other hand, those that are produced and accumulated upon exposure of plants to stresses. Interestingly, the greater suppression of the germination attributes was proportionate to a smaller accumulation of polyphenol compounds (Table 4). Our data indicate 35-50% increases in the phenolic compounds of seedlings of the examined medicinal species, presumably due to an induced production of these compounds in the stressed seedlings. 100-150 percent increases in buckwheat (*Fagopyrum esculentum*) seedling phenolic compounds and carotenoids, and hence antioxidative activities, under salt stress, have been reported (Lim *et al.*, 2012). The antioxidant activity of polyphenol compounds stems from their capability to inactivate ROS. The ROS undergoes neutralisation when the phenolic compound transfers its electron and/or hydrogen atom to the radical molecule (Olszowy, 2019). Three metallothioneins-encoding genes, which are cysteine-rich small proteins involved in ROS scavenging, are highly expressed in germinating seeds of sacred lotus (*Nelumbo nucifera*) and Arabidopsis (*Arabidopsis thaliana*) (Zhou *et al.*, 2012). They are significantly upregulated in the presence of high salinity and oxidative stresses, indicating their significant role in improving antioxidant activity at an early developmental stage and hence seedling vigor. Even though we did not conduct such gene expression analyses, measurements on the proline, ROS scavenging polyphenol, and antioxidative enzymes (Table 4) are consistent with the above proposal. Furthermore, a comparison of the enzymatic antioxidative defence mechanisms in the germinating seeds to those in the field-grown plants revealed that these defensive mechanisms of salt tolerance in germinating seeds translate more or less into similar defensive strategies in the mature plants.

Salinity alters photosynthetic pigments of anise, ajwain, and fennel

Salinity affects all physiological and morphological parameters, leading to decreases in the growth and productivity of different plant species (Jaleel *et al.*, 2008; Di Mola *et al.*, 2018). Even though decreases in Chl concentration of the salt-stressed plants were common among the three examined species, these decreases were not severe (Table 2). Chlorophyll breakdown often leads to diminished light absorption by the photosystem II. In fact, the light-harvesting complex of photosystem II often varies with environmental conditions (Shafeiee and Ehsanzadeh, 2019). According to a study on sunflowers (Santos, 2004), chlorophyllase activity and hence Chl degradation are not strongly affected by salinity conditions. Instead, 50 to 100 mM NaCl might inhibit the synthesis of 5-aminolaevulinic acid (ALA), a precursor of Chl. As this acid is the precursor of all tetrapyrroles, it is a protochlorophyllide precursor. The latter molecule is converted to Chl upon exposure of the seedlings to light. Glutamate is a precursor of ALA and is known to decrease in salt-stressed plants of certain species. Therefore, the salt stress affects Chl synthesis more drastically (*i.e.*, through decreasing ALA synthesis) than its chlorophyllase-mediated degradation. Salt damage to the photosynthetic components, envisaged by a decrease in Chl and carotenoid concentrations of pearl millet genotypes, is reported in the literature (Makarana *et al.*, 2019). As it has been found with the green bean genotypes, reduction in the concentration of the photosynthetic pigments under high salinity conditions is known to be graver in

Table 4. Mean comparisons of species × salinity interaction for root length, shoot length, seedling vigour, catalase and peroxidase activities, polyphenol and proline concentrations of anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and ajwain (*Trachyspermum ammi*) genotypes at different salinities under laboratory conditions.

Species	NaCl (mM)	Root length (cm)	Shoot length (cm)	Seedling vigour	Catalase (unit mg^{-1} protein)	Peroxidase (unit mg^{-1} protein)	Polyphenol (µmol g^{-1} FM)	Proline (µmol g^{-1} FM)
Anise	0	5.95^{de}	3.58^{c}	6.48^{c}	0.180^{i}	0.21^{hi}	2.87^{i}	1.51^{ik}
	25	4.79^{ef}	2.85^{def}	4.21^{d}	0.228^{ghi}	0.38^{ghi}	3.15^{hi}	1.90^{f-i}
	50	2.90^{gh}	1.90^{gh}	1.86^{f}	0.269^{e-i}	0.63^{gh}	3.57^{gh}	2.06^{e-h}
	75	2.05^{hi}	1.43^{hi}	0.80^{gh}	0.374^{b-f}	1.18^{ef}	3.80^{fg}	2.56^{b-e}
	100	1.40^{i}	1.07^{i}	0.19^{hi}	0.382^{bcd}	1.74^{cd}	4.28^{ef}	1.13^{k}
	125	0.00^{j}	0.00^{j}	0.00^{i}	0.000^{j}	0.00^{i}	0.00^{j}	0.00l
Ajwain	0	6.77^{cd}	4.46^{b}	8.00^{b}	0.283^{d-h}	0.39^{ghi}	3.87^{ef}	1.35^{jk}
	25	7.25^{c}	3.64^{c}	5.94^{c}	0.332^{c-g}	0.77^{fg}	4.23^{ef}	1.39^{jk}
	50	5.47^{e}	2.98^{cde}	3.38^{e}	0.370^{b-f}	1.53^{cde}	4.48^{de}	2.24^{d-g}
	75	3.98^{fg}	2.30^{efg}	1.92^{f}	0.468^{ab}	1.86^{bcd}	4.93^{cd}	2.46^{b-e}
	100	3.03^{gh}	1.83g^{h}	0.81^{gh}	0.534^{a}	2.52^{a}	5.28^{bc}	2.83^{abc}
	125	2.13^{hi}	1.37^{hi}	0.22^{hi}	0.380^{b-e}	1.97^{bc}	3.93^{efg}	2.75^{a-d}
Fennel	0	10.08^{b}	6.46^{a}	11.79^{a}	0.190^{hi}	0.47^{ghi}	4.51^{de}	1.62^{h-k}
	25	11.92^{a}	6.02^{a}	12.03^{a}	0.266^{f-i}	1.20^{ef}	4.95^{cd}	1.84^{g-j}
	50	9.42^{b}	4.61^{b}	6.27^{c}	0.298^{c-h}	1.37^{de}	5.35^{bc}	2.21^{efg}
	75	5.88^{de}	3.46^{cd}	2.82^{e}	0.313^{c-g}	1.74^{cd}	5.65^{ab}	2.38^{c-f}
	100	3.60^{g}	2.20^{fg}	0.98^{g}	0.371^{b-f}	2.28^{ab}	5.85^{ab}	2.89^{ab}
	125	3.65^{fg}	2.23^{fg}	0.55^{ghi}	0.410^{bc}	2.68^{a}	6.20^{a}	3.24^{a}

[a-l]Values bearing the same letter are not significantly different according to the least significant difference (LSD) test at P≤0.05.

the salt-sensitive genotypes than in the salt-tolerant ones (Yasar *et al.*, 2008). Alteration in the Chl-*a*/*b* ratio under salinity stress is confirmed in different plant species, *e.g.*, Cape periwinkle (Jaleel *et al.*, 2008). In the process of Chl synthesis, Chl *a* oxygenase catalyses the conversion of Chl *a* to Chl *b* (Chen *et al.*, 2010). Chl *a* oxygenase might be upregulated under certain environmental constraints such as salinity (Chen *et al.*, 2010) and low light (Tanaka *et al.*, 1998). The tendency towards a decrease in Chl-*a*/*b* of the salt-stricken plants of anise, ajwain, and fennel genotypes found in the present study (Table 2) supports the notion of stress-associated enhancement in the activity of the Chl *a* oxygenase. The carotenoid concentration of the stressed anise plants suffered less than the ajwain and fennel plants (Table 2). The role of carotenoids in photosynthesis is two-fold: they participate in the chloroplastic light-harvesting processes and in the meantime, may protect the photosynthetic machinery from photo-oxidative damage (Kim *et al.*, 2012). High carotenoid content enhances tolerance to environmental stresses such as salt by scavenging ROS. The quenching of singlet oxygen brings about the ROS scavenging of carotenoid. The commonality of salt-associated decreases in the carotenoid concentration of ajwain and fennel (Table 2) suggests that the stress has altered the roles mentioned above of carotenoids in the photosynthesis of the three examined medicinal species. From the smaller decreases in the Chl and carotenoid concentration of their stressed plants, it seems that the salt-induced alteration in Chl and carotenoid function in photosynthesis of anise was smaller than fennel and ajwain.

More serious ionic disturbances in stressed mature plants of anise than fennel and ajwain

Salt-induced ionic disturbances are common in plants. One of the most remarkable alterations in the ion condition of salt-stricken plants is an increase in Na^+ concentration at the expense of K^+ in plant cells, tissues, and organs. Excess Na^+ often competes with K^+ ions in binding to some cytosolic enzymes and proteins, disrupting cellular metabolism. It is postulated that ionic stress is greatly related to Na^+-induced hindrance in K^+ functions in plant cells, from stomatal functioning to activities of enzymes of different types (Khamesi *et al.*, 2020). From the notable increases and decreases in the Na^+ (Figure 1A) and K^+ (Figure 1B) concentrations of the salt-stricken plants, respectively, it could be inferred that all genotypes of the three examined medicinal species have potentially experienced this type of ionic disturbance. Though, the increase in Na^+ concentration and, hence, the proposed ion alteration tended to be greater in anise.

Furthermore, from the most significant increases in the Na^+ concentration and hence Na^+/K^+ (Figure 1C) of the salt-stricken plants of the anise genotypes, it may be inferred that the lack of a Na^+ exclusion mechanism has led to hindered ion homeostasis across different genotypes of this medicinal species. An increase in Na^+ concentration of three *Echinacea* species was accompanied by decreases in the photosynthetic attributes, including Chl concentration, stomatal conductance, and net photosynthetic rate (Sabra *et al.*, 2012), which corroborates our results. Partial salt tolerance of coneflower (*Echinacea purpurea*) was related to its ability for Na^+ exclusion and increased activity of the antioxidative enzymes (Sabra *et al.*, 2012). However, it must be noted that exploiting salt exclusion and compartmentalisation to handle excess Na^+ is comparatively relative rather than an absolute mechanism. Thus, not all the ionic behaviour differences among the present three species and genotypes are necessarily due to differential ion exclusion capabilities. Our results are consistent with those of other researchers (Grewal *et al.*, 2010), who found that Na^+/K^+ in wheat, barley, canola, and chickpea increases with an increase in salt concentration. Differential Na^+/K^+ ratios have also been found by some researchers working on salt response in wheat with different ploidy levels (Shavrukov *et al.*, 2010). Our data from the field experiment (Table 2, Figure 1A and C) and the results reported by the literature mentioned above, taken together, urge us to surmise that increased Na^+ concentration and hence Na^+/K^+ rendered mature plants of anise to be less salt-resilient than fennel and ajwain plants.

Persistency of antioxidative enzymes roles in salt tolerance from germinating seeds to mature plants

We did not attempt to measure the ROS, but an indication of an incidence of oxidative stress was the increased activity of the tested antioxidative enzymes (Tables 2 and 4). All genotypes of the examined species behaved similarly when it came to salt-associated increases in the CAT and POX activities of both germinating seeds and mature plants. Despite the general tendency towards an increase in APX activity of the mature plants of all genotypes of the three medicinal species, a dissimilar pattern existed in APX activity in the germinating seeds. Activities of the three antioxidative enzymes, taken together, are suggestive of the greatest reliance on enzymatic defence in fennel genotypes (particularly genotype Yazd) in countering the salt-imposed stress compared to the other examined species. Salt-driven enhancement in the activities of antioxidative enzymes in the more stress-resilient fennel genotypes has been documented (Shafeiee and Ehsanzadeh, 2019). In line with our findings, salt stress led to increases in osmolytes such as proline and antioxidant enzymes in rice seedlings (Roy *et al.*, 2019). A greater level of salt tolerance in a chickpea genotype was ascribed to the greater activities of the CAT and POX antioxidative enzymes and the ability to maintain ion homeostasis (Khamesi *et al.*, 2020), which supports the idea of the existence of interrelationships among these traits in the Yazd fennel genotype. Despite certain dissimilarities in the activities of the antioxidative enzymes, either among these medicinal species or between the germinating seeds (Figure 2C) and mature plants, our data indicate that these antioxidative enzymes function to counter salt stress both in germinating seeds and mature plants.

Salt-induced penalties of plant dry mass, grain yield, and essential oil of fennel are smaller than ajwain and anise

Our results on salt-induced severe SDM and grain yield penalties (Table 2) and germination suppression of anise and ajwain (Figure 2A) suggest that similar to other medicinal species (*e.g.*, thyme) (Al-Tabbal *et al.*, 2020), anise and ajwain are inappropriate medicinal species to being irrigated with brackish water either in early growth stages or as mature plants. The low SDM, grain yield, and essential oil yield (Figure 1D) of anise and ajwain were further lowered by salinity, rendering them inappropriate for cost-effective production in saline conditions. The fact that anise genotypes show sensitivity to salt stress at both germination and mature plant stages seems mostly related to a lack of sufficient antioxidative defence, ion homeostasis, and osmoprotective measures throughout the life cycle of this species. According to Bustan *et al.* (2005), brackish irrigation water may improve quality despite a decreased fruit and grain yield. Salt stress also increased total phenolic, flavonoid content, and antioxidant activity of myrtle (*Myrtus communis* L.) (Vafadar Shoshtari *et al.*, 2017), and alkaloid content of Cape periwinkle plants was increased under saline soil conditions

(Jaleel *et al.*, 2005). The commonality of the salt-induced increase in the seed essential oil concentration in the examined genotypes across the three medicinal species (data not shown) reconfirms the previous reports (Afshari and Rahimmalek, 2018; Hodaei *et al.*, 2018) on the stress-induced increase in the secondary metabolites of diverse plant species. The salt-induced increase in seed essential oil concentration in the fennel genotypes was greater than in the other species. Moreover, the greater SDM, grain yield, and essential oil yield of the stressed fennel plants justify its use as an alternative crop of choice for extension of medicinal plant production into marginal saline soils.

Conclusions

Understanding the comparative salt response of medicinal species from the very early stage of germination to mature crop is critical to developing informed farm management strategies in arid-semiarid regions. This study aimed to determine: i) comparative responses of seed germination in anise, fennel, and ajwain in a controlled environment experiment; and ii) the physiological and yield responses of the mature field-grown plants of these species to saline irrigation water. Overall, the more promising germination patterns and smaller growth, SDM, and grain yield penalties of fennel and, to some extent, ajwain were explained by the greater activity of the antioxidative enzymes and increased concentrations of polyphenol and proline under saline conditions. The fact that fennel shows modest tolerance to brackish irrigation water demonstrates that it is worthwhile for further examination for adoption to tackle the ever-increasing drought and salinity episodes predicted in the coming decades. This research provides the ground for assessing the economic feasibility of adopting fennel as an alternative medicinal crop for salt-prone arid climates.

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