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Three-Step Screening for Salinity Tolerance in Ajowan (*Trachyspermum ammi* L.) Sprague ex Turrill)

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ABSTRACT

Ajowan is an important medicinal plant that grows mainly in arid and semi-arid regions of the world. To evaluate salinity tolerance of 25 Iranian ajowan ecotypes, three-step screening was conducted at germination, seedling, and adult plant growth stages using 0, 50, 100 and 150 mM of NaCl. The significant effects of salinity were observed at the three studied growth stages of ajowan ecotypes. Germination percentage, seed vigor, and biomass dry weight of investigated ecotypes decreased with the increase in NaCl levels. Different responses were observed among ajowan ecotypes in terms of activity of antioxidant enzymes of catalase and peroxidase, with the increasing salinity stress levels .Under the salinity stress, the proline content increased in the majority of the investigated ecotypes. Salinity stress had adverse effects on single plant seed yield and yield components. Results of the calculated correlation coefficient and path coefficient analysis showed that activity of catalase antioxidant enzyme and 1,000-seed weight were the most important characteristics that can be suggested as selection criteria for seed yield of ajowan under salinity stress conditions. The overall results suggest that nine Iranian ajowan ecotypes including Arak, Felaverjan, Ghoom, Hamedan, Karaj, Ghaen, Tehran, Yazd, and Shiraz were the salinity-tolerant ecotypes.

Keywords: Antioxidant enzymes, Medicinal plant, Saline soil, Salinity stress, Selection criteria.

INTRODUCTION

Salinity stress is one of the most important abiotic stresses in arid and semi-arid regions of the world. Saline soils (Salt concentration $\geq$ 40 mM) can disrupt the normal growth of crops (Munns and Tester, 2008). Breeding, engineering the plants, for salinity tolerance is the most effective way to improve plant productivity in saline environments (Arzani and Ashraf, 2016). However, plant genotypes may respond differently to salt stress at different growth stages, which creates complicated situations for screening of tolerant genotypes (Arzani, 2008). Plant growth and development can be influenced by salinity stress at any time during the life cycle, but the extent of damage and impact on performance depends on the developmental stage at which it is subjected to stress (Arzani, 2008). Seed germination is the most critical phase of plant life cycle and is greatly influenced by drought and salinity stresses (Ashraf and Foolad, 2007; Llanes *et al*., 2016). Generally, there is no correlation between salinity tolerance at the germination stage and other developmental stages such as seedling stage and adult plant. This maybe because of entirely different processes that

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control cell expansion during different growth stages. Therefore, tolerance assessment during germination, emergence, vegetative growth, flowering, and maturity will be more valuable (Arzani, 2008).

Interest in medicinal herbs has greatly increased due to less side effects (Ashraf and Orooj, 2006; Soltani Howyzeh *et al*., 2018; Tohidi *et al*., 2017). One of these important medicinal plants, namely, ajowan (*Trachyspermum ammi* L.), is an annual and diploid plant belonging to *Apiaceae* family and is used in raw or processed forms in traditional medicine or modern pharmaceutical industry (Noori *et al*., 2017). Ajowan seeds contain an essential oil with about 50% content of thymol, which has analgesic and anti-microbial properties (Ashraf and Orooj, 2006; Tohidi *et al*., 2017). In addition to biological properties and toxic-inhibitory effects on various microorganisms —anti-microbial, bactericide, fungicide, insecticide, and nematicide effects— antioxidant activity of the ajowan essential oil has been reported (Dashti-Rahmatabadi *et al*., 2007; Gandomi *et al*., 2014). Ajowan is mainly grows in arid and semi-arid regions of central Europe, India, Egypt, Iran, Iraq, Afghanistan, and Pakistan (Ashraf and Orooj, 2006; Moosavi *et al*., 2015). One of the major problems for agriculture in arid and semi-arid regions of the world is the salinity of soil (Sadat Noori and McNeilly, 2000). Several studies have assessed the effect of salinity stress on agronomic and physiological traits, as well as essential oil yield in ajowan (Ashraf and Orooj, 2006; Omer *et al*., 2014; Yogita *et al*., 2014). The aims of the present study were: (i) To investigate the effect of salinity stress on different growing stages of ajowan, and (ii) To find salinity-tolerant Iranian ajowan ecotypes based on three-step screening.

MATERIALS AND METHODS

Plant Materials and Salinity Stress Induction

Three independent experiments were conducted to assess the effect of salinity stress on 25 Iranian ajowan ecotypes. All experiments were conducted at Agricultural College of Aburaihan, University of Tehran, in 2014-2015. The Research Institute of Forests and Rangelands of Iran provided the seeds. Information of ajowan ecotypes used in the present study is presented in Table 1.

Assessment of Salinity Tolerance in Germination Stage

The experiment was carried out as factorial, arranged in a Randomized Complete Block Design (RCBD), with three replications (as petri dishes). The two factors used in this study were plant ecotypes (25 Iranian ajowan ecotypes) and NaCl concentrations (0, 50, 100, and 150 mM).

The healthy seeds of each ecotype were sterilized by submerging in sodium hypochlorite solution (1.5%, v/v) for 3 min and rinsing three times with sterile distilled water. Twenty-five sterilized seeds were placed on sterilized filter paper on plastic petri dishes (9×1.5 cm) (three petri dishes as replications for each treatment) and then 10 mL of NaCl solution (with the aforementioned concentrations) was added to each petri dish. Cultures were kept at 26±2^{o}C for two weeks. Plant characteristics evaluated at this stage included Germination Percentage (GP), Seed Vigor (SV), and Biomass Dry Weight (BDW). Germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds sown. Seed vigor was measured according to the following equation (Dhanda *et al*., 2004):

$$SV = (RL+SL)\times GP\ (\%) \quad (1)$$

Where, SV is the Seed Vigor, RL is the Root Length, SL is the Shoot Length, and GP is the Germination Percentage (%). To calculate BDW, at the end of experiment (the fourteenth day), plant materials of each Petri dish were placed in oven at 50^{o}C for 48 hours and then BDW of each treatment was recorded.

Assessment of Salinity Tolerance at Seedling Stage

A factorial experiment, based on randomized complete block design with three replications, was conducted to

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Table1. The geography profile of origins of twenty-five Iranian ajowan ecotypes.

No	Province	City	Geographical state	No	Province	City	Geographical state
1	Tehran	Tehran	Center	14	Yazd	Shahedie 1	East
2	Alborz	Karaj	Center	15	Yazd	Sadoogh1	East
3	Ardabil	Ardabil 1	North West	16	Fars	Shiraz	South
4	Yazd	Yazd	East	17	Khorasan Razavi	Sabzevar	North east
5	South Khorasan	Birjand 1	East	18	Ardabil	Ardabil 3	North West
6	Kerman	Rafsanjan 1	East	19	Hamedan	Hamedan	Center
7	Golestan	Gorgan	North East	20	Ardabil	Ardabil 4	North West
8	Fars	Marvdasht	South	21	Yazd	Sadoogh 2	East
9	Ardabil	Ardabil 2	North West	22	Esfahan	Felaverjan	Center
10	South Khorasan	Birjand 2	East	23	South Khorasan	Ghaen	East
11	Markazi	Arak	Center	24	South Khorasan	Sarbishe	East
12	Kerman	Rafsanjan 2	East	25	Yazd	Shahedie 2	East
13	Ghoom	Ghoom	Center				

investigate the effects of different levels of salinity stress (0, 50, 100, and 150 mM NaCl) on 25 ajowan ecotypes at seedling stage. Seeds were sown in plastic pots (5 cm diameter and 10 cm depth) containing sand: perlite-cocopeat (1:1). Pots were transferred to a growth chamber with controlled conditions (6,000 Lux light intensity and temperature of 25-31°C). Regular irrigation with distilled water was given for one month to establish healthy seedlings. When seedlings had 5-6 leaves, salinity stress (at different levels) was applied on each pot through irrigation with saline water for 24 days, with five days intervals. Then, physiological traits predominantly associated with stress, such as Proline Content (PC), and activity of antioxidant enzymes including Catalase (CAT) and Peroxidase (POX) were estimated in treated plants. Proline content was extracted and measured by following the method of Bates *et al*. (1973).

For enzyme extraction, 0.1 g of fresh sample was homogenized in chilled 2 mL tubes and then 20 mg of PolyVinylPyrrolidone (PVP) was added to each sample. Then, 1 mL of extraction buffer [potassium phosphate (50 mM)+sodium sulfite (1 mM)] was added to samples and after vortexing, samples were incubated at 4 ^{o}C for 30 min and centrifuged in 12,000 rpm (Sudhakar *et al*., 2001). After centrifuging, supernatant was isolated to measure antioxidant enzymes.

The kinetic activity of catalase enzyme was measured using Aebi (1984) method based on the rate of Hydrogen peroxide (H_2O_2) decomposition. Decomposition of H_2O_2 with catalase enzyme was recorded by the reduction of absorbance at 240 nm, through a spectrophotometer (Perkin Elmer, Lambda 25 UV.VIS Spectrometer). Finally, enzyme activity was calculated according to the following equation (Bergmeyer and Grassl, 1983):

$$\text{Activity (U } \mu l^{-1}) = 240\ A\Delta \times L \times Vt \times Df \times € \times I \times T \times Vs \quad (2)$$

Where, U is the enzyme Unit, 240 AΔ is the difference in Absorption of the reaction mixture at the beginning and the end of the reaction, L is the Length of light passing through the reaction mixture (1 cm), Vt is the Volume of reaction mixture, Df is the Dilution factor, € is the extinction coefficient (39.4 mM^{-1} cm^{-1}), I is the hydrogen peroxide coefficient, T is the reaction Time, and Vs is the Volume of enzyme sample.

Peroxidase enzyme activity was measured using Chance and Maehly (1955) method. The assay mixture including 67 µL H_2O_2 (70 mM) soluble in 100 mM potassium phosphate+934 µL distilled water+40 µL of

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extracted enzyme was prepared and then enzyme uptake was measured using a spectrophotometer at a wavelength of 470 nm. Finally, enzyme activity was calculated using Equation (2).

Assessment of Salinity Tolerance in Adult Plant Stage

In this experiment, the effect of salinity stress was assessed on yield and yield components of ajowan plants. Factorial experiment, based on RCBD design with three replications, was conducted with 25 ecotypes of ajowan and 0, 50, 100, and 150 mM of NaCl. Seeds of ajowan ecotypes were planted separately at the bottom of perforated plastic pots (25 cm diameter and 30 cm depth) filled with river sand: farm soil (3:1) and kept in a greenhouse with average temperature of 25±2 ^{o}C and 50-54% relative humidity. Pots were irrigated with tap water until 50% of plants reached flowering stage. Thereafter, salinity stress was applied on each ecotype by irrigation with salt water containing different concentrations of NaCl, with five days intervals. Salinity stress was continued until the physiological maturity of seeds. At the end of growing season, Biological Yield (BY), Single Plant Seed Yield (SPSY), along with yield component traits such as Number of Umbels Per Plant (NUPP), Number of Seeds Per Umbel (NSPU), and 1,000-Seed Weight (TSW) were measured. To calculate biological yield, at the end of experiment (harvesting time), survived plants were cut from the ground level and sun dried for 3 days, then weighed for recording BY.

Statistical Analyses

Statistical analyses such as Analysis Of Variance (ANOVA), mean comparisons analysis, simple correlation analysis, and path analysis were conducted using SAS® software (SAS Institute Inc., Carry, NC). Normality test was conducted with SAS software before the analysis of variance. Tukey's HSD test at 5% probability level was used for mean comparisons analysis. The pooled data of germination, seedling, and adult plant experiments were used for correlation, path coefficient, and cluster analyses. Matrix of correlation between independent variables (R_{XX}), vector of correlation between independent variables and dependent variable (R_{XY}), and estimated coefficient of regression (p_{xy}) were used for path analysis. Cluster analysis was done with Ward's method (Ward, 1963) using SPSS software version 16.0. The distance between clusters was determined by the Lance and William's recurrence formula (Lance and Williams, 1967). The cluster quality was verified through Cophenetic correlation coefficient (CPCC) (Sokal and Rohlf, 1962).

RESULTS AND DISCUSSION

Effect of Salinity at Germination Stage

The results of ANOVA showed significant genetic variation among Iranian ajowan ecotypes for salinity tolerance at germination stage (data not shown). Mean comparisons analysis, using Tukey's HSD test, showed adverse effect of salinity stress on germination percentage, seed vigor, and biomass dry weight of all investigated ajowan ecotypes (Table 2). The greatest mean of germination percentage was observed in Yazd ecotype followed by Ghoom, Sadoogh 2, and Karaj ecotypes. Similarly, the order of superior ecotypes for seed vigor index was Shiraz, Ghoom, Sadoogh 2, Yazd, and Arak. Corresponding order in case of biomass dry weight was Yazd, Hamedan, Shiraz, Ghoom, and Arak (Table 2). Therefore, Yazd, Ghoom, Sadoogh 2, Karaj, Shiraz, Arak, and Hamedan ecotypes were the superior ecotypes at germination stage.

Germination and early growth parameters are usually affected by salinity stress in many of plant species (Zhang *et al*., 2015).

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Table 2. Effect of different levels of salinity stress on percent germination, seed vigor and biomass dry weight in twenty-five Iranian ajowan ecotypes. [a]

Ajowan ecotypes	Germination percentage (%)					Seed vigor (%)					Biomass dry weight (mg)				
	Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)				
	0	50	100	150	Mean±SE	0	50	100	150	Mean±SE	0	50	100	150	Mean±SE
Arak	100.0	80.0	44.0	35.6	64.9±6.0^{efg}	51.7	40.8	34.2	9.5	34.0±3.5^{bc}	8.3	7.3	5.7	4.2	6.4± 0.3^{c}
Ardebil 1	85.0	66.3	46.3	31.3	57.2±4.6^{kl}	55.3	39.4	25.8	6.5	31.7±4.1^{cd}	8.6	6.3	4.8	2.3	5.5±0.5^{d-f}
Ardebil 2	86.0	72.6	66.6	42.6	66.9±3.6^{b-f}	56.6	43.5	22.1	3.6	31.4±4.6^{cd}	6.7	5.6	4.2	2.4	4.7±0.3^{hi}
Ardebil 3	85.3	74.6	48.0	27.6	58.9±5.2^{i-l}	45.7	33.2	21.9	6.2	26.7±3.3^{gh}	7.1	6.5	4.6	2.6	5.2±0.4^{fg}
Ardebil 4	97.3	72.6	64.6	37.3	67.9±4.9^{b-e}	58.4	44.0	28.4	3.6	33.6±4.6^{bc}	7.6	5.6	3.5	1.4	4.5±0.5^{ij}
Birjand 1	96.0	81.3	62.0	26.6	66.4±6.0^{c-f}	46.8	32.8	24.5	5.5	27.4±3.4^{f-h}	8.1	6.2	4.9	2.5	5.4±0.4^{ef}
Birjand 2	97.3	62.6	46.0	22.6	57.1±6.2^{kl}	56.0	40.2	25.9	2.4	31.1±4.5^{cd}	7.1	5.2	3.7	2.3	4.6±0.4^{h-j}
Felaverjan	93.3	72.0	65.3	35.3	66.4±4.7^{c-f}	49.3	35.8	21.5	3.3	27.5±3.9^{f-h}	5.3	4.6	2.8	0.8	3.4±0.3^{m}
Ghaen	82.0	76.6	61.0	30.0	62.4±4.6^{f-j}	41.8	32.2	29.1	5.6	27.2±3.0^{f-h}	6.6	4.7	3.4	1.6	4.1±0.4^{kl}
Ghoom	90.0	84.0	73.3	41.3	72.1±4.3^{b}	56.4	42.8	35.2	9.4	36.0±3.9^{ab}	8.7	7.1	6.4	4.1	6.5±0.3^{c}
Gorgan	83.3	53.3	43.3	22.3	50.5±5.0^{mn}	47.9	33.5	20.6	7.5	27.4±3.4^{f-h}	3.4	7.4	5.7	3.5	5.0±0.3^{gh}
Hamedan	86.6	79.3	65.3	30.6	65.4±4.9^{e-g}	51.9	42.5	20.8	8.5	30.9±3.9^{c-e}	9.8	8.5	7.4	5.9	7.9±0.3^{a}
Karaj	96.6	83.3	66.6	36.6	70.8±5.1^{b-d}	44.5	36.4	27.3	6.3	28.6±3.2^{e-h}	8.2	6.6	4.6	2.8	5.5±0.4^{d-f}
Marvdasht	95.3	88.0	63.3	26.6	68.3±6.1^{b-e}	40.2	32.8	23.9	7.5	26.1±2.8^{h}	6.4	5.7	3.7	1.2	4.2±0.4^{jk}
Rafsanjan 1	81.3	74.00	42.0	20.6	54.4±5.6^{lm}	54.0	36.4	22.4	2.5	28.8±4.3^{d-h}	8.4	5.5	4.7	2.3	5.2±0.5^{fg}
Rafsanjan 2	73.3	51.3	43.3	24.0	47.9±4.0^{no}	52.8	40.5	26.5	7.3	31.8±3.9^{cd}	6.6	4.4	3.3	2.4	4.2±0.3^{jk}
Sabzevar	80.0	74.6	68.0	32.0	63.6±4.3^{e-i}	52.0	31.9	28.1	6.6	29.7±3.7^{d-g}	8.5	7.4	5.2	4.6	6.4±0.3^{c}
Sadoogh 1	71.3	48.0	35.3	23.3	44.4±4.1^{o}	45.8	35.9	28.1	7.4	29.3±3.2^{d-h}	8.4	7.4	5.1	1.7	5.6±0.5^{e-g}
Sadoogh 2	98.6	91.3	62.6	33.3	71.4±5.9^{bc}	60.5	51.9	25.5	5.1	35.8±5.0^{b}	7.6	6.5	5.4	3.3	5.7±0.3^{de}
Sarbishe	85.3	72.6	62.6	22.6	60.8±5.4^{h-k}	47.0	32.9	20.3	6.5	26.7±3.4^{gh}	7.3	6.2	5.2	3.2	5.5±0.3^{d-f}
Shahedie 1	85.3	66.6	60.6	22.0	58.6±5.3^{i-l}	52.1	41.8	22.6	4.5	30.2±4.2^{d-f}	5.6	4.3	3.3	1.3	3.6±0.3^{lm}
Shahedie 2	72.6	47.3	38.6	28.6	46.8±3.7^{no}	53.5	46.5	28.8	5.8	33.7±4.2^{bc}	8.3	6.4	5.2	3.5	5.8±0.4^{d}
Shiraz	77.3	74.0	67.3	38.3	64.2±3.5^{d-g}	65.4	45.4	35.6	9.8	39.1±4.6^{a}	9.5	8.2	6.4	4.1	7.1±0.4^{b}
Tahran	91.0	83.3	46.6	35.3	64.0±5.4^{e-h}	44.1	34.4	25.8	6.7	27.7±3.1^{e-h}	7.1	6.2	4.5	3.2	5.3±0.3^{e-g}
Yazd	100.0	96.0	81.3	48.0	81.3±4.7^{a}	60.7	43.7	30.9	7.5	35.7±4.4^{b}	10.5	8.8	7.4	6.0	8.2±0.3^{a}
Mean±SE	87.6±1.7^{a}	73.0±2.5^{b}	56.9±2.4^{c}	31.0±1.4^{d}		51.6±1.2^{a}	38.8±1.0^{b}	26.2±0.8^{c}	6.2±0.4^{d}		7.6±0.3^{a}	6.3±0.2^{b}	4.8±0.2^{c}	2.9±0.2^{d}	

[a] (a-o) Treatments that have letters in common do not differ significantly from one another at the 0.05 significance level according to Tukey's HSD.

Zhu and Bañuelos (2016) also reported decrease in germination percentage of guayule (*Parthenium argentatum* A. Gray) with increase in salinity levels.

Effect of Salinity at Seedling Stage

The results of ANOVA showed significant effects of ecotype, salinity, and their interaction (ecotype × salinity) on peroxidase, catalase, and proline content of ajowan at 1% probability level (data not shown). According to mean comparisons analysis, using Tukey's HSD test at 5% probability level, peroxidase activity of investigated ajowan ecotypes was increased by increasing salinity stress levels (Table 3). Corresponding order in case of activity of peroxidase antioxidant enzyme was Ardabil 4, Rafsanjan 1, Karaj, Yazd, Sadoogh 1, and Ghoom (Table 3). The activity of catalase antioxidant enzyme was decreased with the increasing levels of salinity stress (Table 3). Tehran ecotype had the greatest mean of catalase activity followed by Sarbishe, Birjand 2, Ardabil 4, and Rafsanjan 2 ecotypes (Table 3). Salinity is very often accompanied by oxidative stress, due to generation of Reactive Oxygen Species (ROS) (Rout and Shaw, 2001; Isayenkov, 2019). Dash and Panda (2001) reported that increase in concentration and duration of salinity stress (NaCl) led to decrease in activity of catalase, peroxidase, and polyphenol oxidase enzymes in Blackgram (*Phaselous mungo* L.). Ghassemi *et al*. (2019) reported increased activities of antioxidant enzymes, such as Ascorbate Peroxidase (APX), CAT, peroxidase, and PolyPhenol Pxidase (PPO), in ajowan under different levels of water limitation.

The proline content showed an increased trend with the increasing levels of salinity stress (Table 3). Corresponding order in case of proline content was Arak, Ghaen, Birjand 1, Hamedan, Ardabil 1, Rafsanjan 2, and Felaverjan (Table 3). In Ardabil 4, Birjand 1, Hamedan, and Sabzevar ecotypes; the proline content was first increased with increasing in NaCl levels and then decreased at severe salinity (150 mM NaCl) (Table 3). Shiraz ecotype was another exception whose proline content decreased with increasing levels of NaCl (Table 3), and it is interesting that this ecotype showed the greatest mean of seed vigor index in germination experiment (Table 2). These findings are consistent with previous studies that found both significant effect (Akrami and Arzani, 2018) and insignificant effect (Arabbeigi *et al*., 2019) of proline on plant growth under salinity stress conditions. Recently, Arabbeigi and coworkers (2019) have proposed three hypotheses to account for the non-significant effects of salinity on proline accumulation as well as on proline synthesis via *P5CS* gene transcripts: (i) Species dependency of proline accumulation under salinity stress; (ii) The higher role of proline under low to moderate salinity stress; and (iii) A predominant phase of "ion specific" (Na^+ and Cl^-) salinity stress (hypo-osmotic stress) at severe salinity stress. Plants employ various mechanisms to cope with salinity stress (Arzani and Ashraf, 2016). Osmotic adjustment is one such mechanism for plant defense against salinity stress that can be achieved through synthesis of intracellular solutes (Serrano *et al*. 1999). Proline is one of the intracellular solutes that accumulate in common responses to water deficit and saline environments (Yazici *et al*., 2007). Many roles such as osmo-protectant, protective agent for cytosolic enzymes and cellular organelles, a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules, and a free radical scavenger have been reported for proline in plant responses to salinity stress (Bohnert *et al*., 1995; Fedina, 1996; Jain *et al*., 2001; Yazici *et al*., 2007). Based on the results of this experiment, Ardabil 1, Ardabil 4, Rafsanjan 1, Rafsanjan 2, Ghoom, Karaj, Yazd, Sadoogh 1, Tehran, Sarbishe, Birjand 1, Birjand 2, Arak, Ghaen, Hamedan, and Felaverjan were the superior ecotypes at seedling stage.

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Table 3. Effect of different levels of salinity stress on activity of peroxidase and catalase antioxidant enzymes and proline content in twenty-five Iranian ajowan ecotypes.

Ajowan ecotypes	Peroxidase (U g^{-1} Plant)					Catalase (U g^{-1} Plant)					Proline content (µmol g^{-1})				
	Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)				
	0	50	100	150	Mean± SE	0	50	100	150	Mean± SE	0	50	100	150	Mean±SE
Arak	1.60	1.90	3.00	1.70	2.00± 0.001^{j}	2.80	2.60	1.70	3.20	2.80±0.10^{j}	9.57	10.40	14.55	17.97	13.12±0.77^{a}
Ardebil 1	1.60	1.60	1.40	2.00	1.20±0.10^{kl}	2.40	3.20	2.50	2.10	2.40±0.10^{ijk}	3.25	7.02	8.41	15.68	8.59±1.04^{ij}
Ardebil 2	0.50	2.00	1.10	0.80	0.70±0.10^{j-m}	1.50	1.30	3.50	2.50	1.50±0.20^{no}	4.590	12.98	14.27	16.39	12.05±1.03^{c}
Ardebil 3	1.60	1.00	1.80	4.40	2.30±30^{gh}	1.50	1.60	1.50	1.80	1.50±0.00^{no}	7.84	8.71	10.44	15.29	10.57±0.66^{ef}
Ardebil 4	1.10	6.70	8.10	5.50	5.40±0.60^{a}	5.30	4.20	3.60	2.30	5.30±0.30^{cd}	4.55	8.66	5.66	5.90	6.19±0.34^{m}
Birjand 1	1.90	1.30	1.20	1.00	1.40±0.10^{j}	4.40	2.30	1.20	2.90	4.40±0.30^{ef}	10.42	12.42	17.30	10.60	12.68±0.64^{b}
Birjand 2	1.30	2.40	2.50	3.00	2.30±0.10^{gh}	5.60	3.10	2.50	3.70	5.60±0.30^{c}	8.32	10.26	12.34	14.97	11.47±0.57^{de}
Felaverjan	1.30	5.00	5.20	3.40	3.70±0.40^{c}	1.20	3.20	5.40	3.40	1.20±0.30^{p}	9.52	10.14	12.18	14.94	11.69±0.48^{de}
Ghaen	1.50	1.70	5.40	4.90	3.40±0.40^{ef}	1.20	3.20	4.10	4.30	1.20±0.30^{p}	7.73	11.61	14.32	16.77	12.60±0.77^{b}
Ghoom	1.20	4.90	6.10	3.00	3.80±0.40^{de}	2.20	1.70	3.10	2.80	2.20±0.10^{j-l}	6.36	7.46	9.34	11.91	8.76±0.48^{i}
Gorgan	1.00	1.40	1.70	6.30	2.60±0.50^{fg}	1.70	1.80	2.40	1.70	1.70±0.10^{n}	3.61	4.95	10.85	12.69	8.02±0.88^{j}
Hamedan	2.40	2.80	2.10	0.20	1.90±0.20^{i}	2.10	4.20	3.50	4.10	2.10±0.20^{m}	16.97	15.44	9.42	7.13	12.24±0.94^{bc}
Karaj	1.90	2.60	5.10	7.40	4.30±0.50^{bc}	4.80	2.60	2.40	2.40	4.80±0.20^{de}	4.10	5.49	11.45	17.65	9.67±1.24^{g}
Marvdasht	1.50	1.60	3.40	5.60	3.00±0.40^{fg}	1.40	4.20	0.70	2.30	1.40±0.30^{o}	5.56	9.26	13.54	14.46	10.70±0.82^{e}
Rafsanjan 1	2.40	4.20	5.30	7.50	4.90±0.40^{b}	2.30	2.20	1.60	1.50	2.30±0.10^{ijk}	4.83	8.84	12.28	16.29	10.56±0.97^{ef}
Rafsanjan 2	1.80	2.30	3.70	6.90	3.70±0.50^{c}	5.00	3.70	3.00	1.70	5.00±0.30^{d}	6.74	10.50	14.45	16.15	11.96±0.84^{d}
Sabzevar	1.40	2.40	4.10	1.90	2.40±0.20^{gh}	2.50	5.30	4.30	3.60	2.50±0.20^{i}	4.14	13.43	7.91	6.66	8.03±0.78^{j}
Sadoogh 1	1.40	3.20	4.10	6.80	3.90±0.50^{de}	4.60	4.60	2.80	2.00	4.60±0.30^{e}	2.64	7.42	9.73	11.53	7.83±0.76^{k}
Sadoogh 2	1.70	1.80	3.20	5.40	3.00±0.30^{fg}	3.70	2.40	2.20	1.40	3.70±0.20^{g}	3.65	6.93	8.93	10.08	7.39±0.56^{l}
Sarbishe	1.10	2.20	3.00	4.90	2.80±0.30^{gh}	5.70	3.40	3.00	2.60	5.70±0.30^{b}	2.47	8.93	12.69	12.44	9.13±0.95^{h}
Shahedie 1	1.00	4.00	4.60	3.10	3.20±0.30^{ef}	3.50	3.30	2.20	1.60	3.50±0.20^{gh}	1.24	4.17	7.33	9.58	5.58±0.72^{n}
Shahedie 2	1.20	2.40	4.60	5.80	3.50±0.40^{cd}	4.40	3.20	2.50	1.50	4.40±0.20^{ef}	5.22	6.67	10.48	12.16	8.63±0.64^{i}
Shiraz	1.60	3.30	3.60	5.40	3.50±0.30^{cd}	3.10	7.40	3.20	2.70	3.10±0.40^{hi}	9.57	8.58	7.67	5.15	7.74±0.37^{kl}
Tahran	1.30	2.10	3.60	7.60	3.60±0.60^{cd}	6.10	5.60	4.80	3.50	6.10±0.20^{a}	2.30	6.81	8.42	14.79	8.08±1.03^{j}
Yazd	3.20	3.60	6.60	3.40	4.20±0.30^{bc}	3.70	1.40	1.60	2.40	3.70±0.20^{g}	2.25	2.76	4.79	7.43	4.30±0.47^{o}
Mean± SE	1.50± 0.10^{d}	2.70±0 .20^{c}	3.8±0. 30^{b}	4.2±0. 40^{a}		3.30± 0.30^{a}	3.30± 0.20^{a}	2.80± 0.20^{b}	2.60±0. 10^{c}		5.89±0. 70^{d}	8.79±0. 60^{c}	10.75± 0.61^{b}	12.58± 0.77^{a}	

[a](a-p) Treatments that have letters in common do not differ significantly from one another at the 0.05 significance level according to Tukey's HSD.

Effect of Salinity at Adult Plant Stage

The results of ANOVA showed significant effects of ecotype, salinity, and their interaction (ecotype×salinity) on number of umbels per plant, number of seeds per umbel, 1000-seeds weight, single plant seed yield, and biological yield ($P< 0.01$) (data not shown). Based on the mean comparisons analysis using Tukey's HSD test at 5% probability level, all investigated yield components characteristics, including number of umbels per plant, number of seeds per umbel, 1,000- seeds weight, single plant seed yield, and biological yield decreased in 25 Iranian ajowan ecotypes with the increasing levels of salinity stress (Table 4). Under control condition (0 mM NaCl), the greatest mean of NSPU was related to Ardabil 1 and Ardabil 2 ecotypes, whereas under severe salt stress at 150 mM of NaCl, the greatest means of NSPU were achieved by Arak and Hamedan ecotypes (Table 4). In Shiraz ecotype, SPSY in 50 mM of NaCl treatment was more than 0 mM NaCl, which indicates that moderate soil salinity is more favorable for this Iranian ecotype of ajowan. However, higher concentrations of NaCl (100 and 150 mM of NaCl) led to the decrease of single plant seed yield of this ecotype (Table 4). The greatest means of BY under the 150 mM of NaCl were obtained in Shiraz, Hamedan and Arak ecotypes (Table 4). The greatest mean of NUPP was observed in Hamedan followed by Shiraz, Arak, and Tehran ecotypes. Corresponding order of superior ecotypes in case of NSPU was Hamedan, Arak, and Shahedie 1 ecotypes. The greatest mean of TSW was observed in Shiraz followed by Hamedan, and Ghoom ecotypes. Corresponding order of superior ecotypes in case of SPSY was Shiraz, Ghoom, and Arak, ecotypes. Similarly, the greatest mean of BY was observed in Felaverjan followed by Ghaen, and Shiraz ecotypes (Table 4). Therefore, Hamedan, Shiraz, Arak, Ghoom, Felaverjan, Ghaen, Shahedie 1, and Tehran were the superior ecotypes at adult plant stage. The adverse effect of salinity stress on ajowan seed yield and its components has been reported in previous studies (Ashraf and Orooj, 2006; Ramezani *et al*., 2012). Hassanzadehdelouei *et al*. (2013) applied different levels of salinity stress, from 2 to 11 dS m^{-1}, in cumin (*Cuminum cyminum* L.) and reported that increase in salinity stress level led to significant decrease in all vegetative and reproductive characteristics, such as number of seeds in each plant, number of seeds in umbrella, number of umbrellas in plant, weight of thousand seeds, seed yield, height of plant, and biological yield. They reported that adverse effect on photosynthesis resulting from salinity stress can lead to negative effect on the seed production organs and thus decrease in seed yield.

Correlation of Traits under Different Levels of Salinity Stress

Pooled data of germination, seedling, and adult plant experiments were used for correlation analysis. The pooled data represented a wider range of information, at different stages of life cycle, and were beneficial to test the stability of responses of investigated ecotypes in this study. Correlation analysis of the pooled data, obtained from different experiments, has also been reported in other studies (Kenzhebayeva *et al*., 2017; Li *et al*., 2018). Researchers can choose from several methods for analysis of yield components, depending on the objective of the project. Techniques such as analysis of variance, simple correlation coefficient, multiple regression and path analysis are usually used to analyze yield components (Fraser, 1983). One of the simplest methods for better understanding of yield components that assists in effective selection is correlation coefficient analysis (Mishra *et al*., 2015)

Determination of salinity tolerance-associated traits is very important for their implementation in breeding programs (Akrami and Arzani, 2018). Correlation

[DOR: 20.1001.1.16807073.2021.23.2.17.2]

coefficients calculated for 11 studied characteristics of ajowan ecotypes are presented in Table 5. There was no significant correlation between germination percentage and other investigated traits. Seed vigor had positive and significant correlation with biomass dry weight, number of umbels per plant and single plant seed yield at 5% probability level (Table 5). The highest positive correlation of seed vigor was observed with BDW($r= 0.47^{*}$). Biomass dry weight had positive and significant correlation with NSPU and SPSY at 5% probability level and with TSW at 1% probability level, respectively (Table 5). Antioxidant enzymes of CAT and POX and proline content, measured in seedling stage, did not show significant correlations with other investigated traits of ajowan under different levels of salinity stress, except catalase that had positive and significant correlation with single plant seed yield ($r= 0.43^{*}$). Number of umbels per plant had positive and significant correlation with NSPU at 5% probability level ($r= 0.42^{*}$), and with TSW and SPSY at 1% probability level. Number of seeds per umbel had positive and significant correlation with TSW at 1% probability level (Table 5). Simple correlation of 1000-seed weight and SPSY was significant at 1% probability level. Dalkani *et al*. (2012) used simple correlation analysis to find interrelationship between seed yield and some agro-morphological traits in different Iranian ajowan populations and reported positive and significant correlation between single plant seed yield and aerial parts dry weight (r= 0.67). Ghanshyam *et al*. (2015) reported positive and significant genotypic and phenotypic correlations between number of umbellets in an umbel and seed yield in Indian ajowan germplasm.

Path Coefficient Analysis for Seed Yield under Different Levels of Salinity Stress

Path analysis was done for independent variables that had the greatest simple correlations with single plant seed yield. In this step, using correlation between independent variables, correlation between independent variables and dependent variable, and coefficient of regression, direct and indirect effects of independent variables on dependent variable were calculated. The greatest positive direct effect of independent variables was related to catalase antioxidant enzyme (Table 6), which was in agreement with the results of simple correlation analysis (Table 5). The second positive direct effect on SPSY was related to TSW. The greatest positive indirect effect on SPSY was related to BDW through 1,000-seed weight (Table 6). The greatest negative indirect effect on SPSY was made by NUPP through BDW. Seed is the most consumable part of ajowan medicinal plant. Seed yield improvement in ajowan, through a better understanding of relationship between seed yield and its components, is one of the most important goals of any breeding program. Direct selection for improving a quantitative trait, like seed yield, is not effective, so, indirect selection via simple morphological traits can be more helpful. Path analysis can measure the direct influence of a predictor variable on the dependent variable (Bahmani *et al*., 2015). From the results of the present study, it can be found that activity of catalase antioxidant enzyme is the most important trait that affects the seed yield of ajowan under different salinity stress levels. Thousand-seed weight is the second important trait that can be used as selection criterion for seed yield of ajowan under salinity stress.

Classification of Ecotypes under Different Levels of Salinity Stress

Cluster analysis was conducted based on the evaluated characteristics of twenty-five investigated ajowan ecotypes at germination, seedling, and adult plant growing stages, under different concentration of NaCl (pooled data).Three groups of ecotypes were achieved with dendrogram cutting in distance 10. The first cluster was the biggest

[DOR: 20.1001.1.16807073.2021.23.2.17.2]

Table 4. Effect of different levels of salinity stress on yield and yield components traits in twenty-five Iranian ajowan ecotypes.

Ajowan ecotypes	Peroxidase (U g^{-1} Plant)					Catalase (U g^{-1} Plant)					Proline content (µmol g^{-1})				
	Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)				
	0	50	100	150	Mean± SE	0	50	100	150	Mean± SE	0	50	100	150	Mean±SE
Arak	72.66	61.66	55.33	44.66	58.58±2.34^{bc}	125.00	82.66	76.88	67.66	88.05±5.08^{b}	0.84	0.71	0.62	0.55	0.68±0.02^{d}
Ardebil 1	74.33	63.33	52.33	36.66	56.66±3.22^{e}	133.33	86.66	67.66	45.44	83.27±7.48^{d}	0.73	0.58	0.53	0.44	0.57±0.02^{fg}
Ardebil 2	71.22	60.50	44.00	33.00	52.18±3.40^{hi}	133.33	85.33	64.66	44.44	81.94±7.62^{de}	0.64	0.53	0.45	0.22	0.46±0.04^{pq}
Ardebil 3	70.00	60.00	43.00	32.00	51.25±3.40^{jk}	130.33	84.33	65.66	45.33	81.41±7.26^{e}	0.65	0.58	0.44	0.23	0.48±0.04^{op}
Ardebil 4	71.00	61.00	42.00	31.00	51.25±3.62^{k}	13.66	84.66	65.44	42.33	51.52±6.12^{s}	0.72	0.53	0.45	0.24	0.48±0.04^{op}
Birjand 1	71.33	61.33	42.33	30.33	51.33±3.69^{j}	110.12	100.36	61.66	32.44	76.15±7.17^{g-i}	0.65	0.53	0.45	0.21	0.46±0.04^{pq}
Birjand 2	70.33	60.66	41.33	30.33	50.66±3.63^{l}	110.44	97.55	60.44	34.33	75.69±6.96^{i}	0.66	0.52	0.44	0.23	0.47±0.04^{p}
Felaverjan	70.66	60.33	45.00	33.66	52.41±3.27^{h}	78.55	74.55	64.66	36.33	63.52±3.81^{r}	0.78	0.56	0.46	0.39	0.55±0.03^{hi}
Ghaen	70.33	62.66	53.00	32.00	54.50±3.32^{g}	101.33	73.66	59.22	36.55	67.69±5.43^{p}	0.80	0.55	0.48	0.41	0.56±0.03^{g}
Ghoom	72.33	62.00	51.00	43.33	57.17±2.54^{d}	113.11	94.33	85.33	56.00	87.19±4.76^{bc}	0.93	0.68	0.63	0.55	0.70±0.03^{c}
Gorgan	71.66	61.66	42.66	36.33	53.08±3.28^{g}	97.66	64.33	59.33	32.66	63.50±5.34^{r}	0.77	0.53	0.44	0.22	0.49±0.05^{no}
Hamedan	74.00	67.00	51.33	44.67	59.25±2.72^{a}	121.22	88.33	86.11	60.67	89.08±4.97^{a}	0.96	0.72	0.68	0.52	0.72±0.04^{b}
Karaj	71.44	60.66	44.66	34.66	52.86±3.28^{gh}	97.50	74.55	60.33	30.12	65.63±5.64^{pq}	0.82	0.57	0.41	0.28	0.52±0.05^{lm}
Marvdasht	67.00	50.66	47.00	25.00	47.42±3.46^{m}	118.66	70.99	63.66	34.00	71.83±7.02^{k}	0.80	0.50	0.43	0.33	0.52±0.04^{lm}
Rafsanjan 1	69.66	45.66	51.33	37.33	51.00±2.74^{kl}	118.50	68.55	63.44	31.55	70.51±7.19^{lm}	0.67	0.55	0.44	0.21	0.47±0.04^{p}
Rafsanjan 2	74.33	65.66	58.33	30.33	57.16±3.81^{d}	119.33	70.00	64.22	31.77	71.33±7.23^{kl}	0.68	0.55	0.47	0.24	0.49±0.04^{no}
Sabzevar	72.66	42.66	32.66	30.33	44.58±3.89^{n}	120.33	72.66	62.55	34.55	72.52±7.14^{j-k}	0.67	0.51	0.41	0.21	0.45±0.04^{q}
Sadoogh 1	72.66	62.66	52.00	33.66	55.25±3.34^{fg}	111.33	91.33	75.00	34.66	78.08±6.51^{fg}	0.76	0.60	0.41	0.35	0.53±0.04^{jk}
Sadoogh 2	72.33	61.33	51.33	33.00	54.50±3.34^{f}	110.20	72.33	61.44	33.55	69.38±6.35^{n}	0.75	0.59	0.42	0.27	0.51±0.04^{n}
Sarbishe	74.33	61.33	55.33	33.66	56.16±3.39^{ef}	123.77	74.44	62.66	35.33	74.05±7.39^{j}	0.96	0.75	0.55	0.52	0.69±0.04^{cd}
Shahedie 1	70.33	65.33	56.00	29.66	55.33±3.62^{fg}	108.89	101.88	80.19	56.33	86.82±4.74^{c}	0.79	0.73	0.51	0.43	0.61±0.03^{e}
Shahedie 2	70.00	64.66	52.33	27.33	53.58±3.80^{g}	105.00	84.55	70.55	54.66	78.69±4.27^{f}	0.78	0.71	0.41	0.20	0.53±0.05^{j}
Shiraz	75.00	61.66	57.67	41.33	58.91±2.77^{b}	108.33	87.00	83.66	53.77	83.19±4.49^{d}	0.92	0.75	0.75	0.55	0.74±0.03^{a}
Tahran	75.00	68.66	55.66	33.33	58.16±3.68^{c}	97.33	82.77	63.00	33.66	69.19±5.51^{no}	0.74	0.64	0.52	0.35	0.56±0.03^{g}
Yazd	70.33	60.33	42.66	34.66	52.00±3.25^{hi}	105.12	84.32	60.33	34.22	71.00±6.12^{l}	0.85	0.61	0.44	0.28	0.54±0.05^{i}
Mean± SE	71.80± 0.39^{a}	60.54± 1.18^{b}	48.81± 1.29^{c}	34.09± 1.00^{d}		108.49± 4.69^{a}	82.08± 2.04^{b}	67.52± 1.69^{c}	41.29± 2.15^{d}		0.77±0. 01^{a}	0.60± 0.01^{b}	0.49± 0.01^{c}	0.34± 0.02^{d}	

^{a}(a-p) Treatments that have letters in common do not differ significantly from one another at the 0.05 significance level according to Tukey's HSD.

Continued….

Continued of Table 4. Effect of different levels of salinity stress on yield and yield components traits in twenty-five Iranian ajowan ecotypes.

Ajowan ecotypes	Single plant seed yield (g)					Biological yield (g)				
	Salinity stress levels (mM NaCl)					Salinity stress levels (mM NaCl)				
	0	50	100	150	Mean± SE	0	50	100	150	Mean± SE
Arak	1.02	0.94	0.80	0.44	0.80±0.05^{d}	0	50	100	150	Mean± SE
Ardebil 1	0.95	0.71	0.66	0.28	0.65±0.06^{i}	9.28	8.40	7.44	5.64	7.69±0.31^{cd}
Ardebil 2	0.94	0.70	0.41	0.30	0.59±0.06^{l}	7.46	5.57	5.00	3.76	5.45±0.311
Ardebil 3	0.95	0.71	0.44	0.30	0.60±0.06^{jk}	7.51	5.44	5.20	2.74	5.22±0.39^{m}
Ardebil 4	0.96	0.70	0.43	0.30	0.60±0.06^{jk}	7.42	5.11	5.32	2.71	5.14±0.39^{o}
Birjand 1	1.04	0.55	0.46	0.30	0.59±0.06^{l}	7.49	5.44	5.55	2.14	5.16±0.44^{n}
Birjand 2	1.02	0.54	0.45	0.30	0.58±0.06^{mn}	8.32	5.42	5.55	2.14	5.36±0.51^{lm}
Felaverjan	1.26	0.88	0.75	0.46	0.84±0.07^{c}	8.11	5.87	4.48	2.12	5.15±0.50^{no}
Ghaen	1.01	0.64	0.67	0.32	0.66±0.06^{i}	11.56	9.24	6.86	5.62	8.32±0.53^{a}
Ghoom	1.17	0.95	0.86	0.65	0.91±0.04^{b}	12.62	8.55	6.93	4.65	8.19±0.67^{b}
Gorgan	1.11	0.59	0.42	0.30	0.61±0.07^{j}	7.83	7.27	7.03	5.27	6.85±0.22^{ef}
Hamedan	1.48	0.68	0.40	0.27	0.71±0.11^{gh}	7.45	6.52	5.11	4.32	5.85±0.28^{j}
Karaj	1.54	0.75	0.45	0.32	0.77±0.11^{ef}	9.26	7.51	6.37	6.80	7.49±0.25^{d}
Marvdasht	0.97	0.75	0.42	0.35	0.62±0.06^{ij}	7.85	6.85	5.41	2.11	5.56±0.50^{k}
Rafsanjan 1	0.88	0.71	0.43	0.30	0.58±0.05^{n}	11.64	7.75	6.96	4.70	7.76±0.58^{c}
Rafsanjan 2	0.89	0.74	0.44	0.30	0.59±0.05^{l}	8.12	6.11	4.11	2.72	5.27±0.47^{lm}
Sabzevar	1.02	0.65	0.49	0.17	0.58±0.07^{mn}	8.10	6.22	4.57	3.69	5.65±0.39^{k}
Sadoogh 1	1.11	0.75	0.65	0.42	0.73±0.06^{g}	11.25	5.89	4.42	3.12	6.17±0.71^{h}
Sadoogh 2	1.00	0.74	0.62	0.41	0.69±0.05^{gh}	10.19	8.35	5.32	3.38	6.81±0.61^{f}
Sarbishe	1.11	0.73	0.68	0.57	0.77±0.05^{e}	10.02	7.52	4.32	2.38	6.06±0.68^{hi}
Shahedie 1	1.15	0.95	0.44	0.17	0.68±0.09^{h}	12.55	6.13	4.89	3.29	6.72±0.81^{fg}
Shahedie 2	1.02	0.74	0.42	0.14	0.58±0.08^{mn}	9.38	8.45	6.32	4.42	7.14±0.44^{e}
Shiraz	1.55	1.83	0.86	0.48	1.18±0.12^{a}	9.05	8.41	5.32	3.24	6.51±0.54^{g}
Tahran	1.75	0.83	0.70	0.43	0.92±0.11^{b}	12.02	8.49	5.58	5.98	8.02±0.59^{bc}
Yazd	1.65	0.74	0.52	0.34	0.81±0.12^{cd}	7.86	6.97	5.64	2.84	5.83±0.44^{j}
Mean± SE	1.14±0.04^{a}	0.78±0.04^{b}	0.55±0.03^{c}	0.34±0.02^{d}		11.04	5.41	4.32	2.04	5.70±0.77^{jk}

[a](a-p) Treatments that have letters in common do not differ significantly from one another at the 0.05 significance level according to Tukey's HSD.

Table 5. Correlation coefficients of morpho-physiological and yield components attributes studied in ajowan at germination, seedling, and adult plant stages under different concentrations of NaCl.[a]

Pearson Correlation	GP (X1)	SV (X2)	BDW (X3)	POX (X4)	CAT (X5)	PC (X6)	NUPP (X7)	NSPU (X8)	TSW (X9)	SPSY (X10)	BY (X11)
X2	0.146^{ns}										
X3	0.142^{ns}	0.47^{*}									
X4	-0.07^{ns}	0.08^{ns}	-0.18^{ns}								
X5	0.04^{ns}	0.12^{ns}	0.14^{ns}	0.25^{ns}							
X6	0.09^{ns}	-0.27^{ns}	-0.03^{ns}	-0.30^{ns}	-0.19^{ns}						
X7	-0.07^{ns}	0.42^{*}	0.34^{ns}	-0.006^{ns}	0.23^{ns}	-0.01^{ns}					
X8	-0.05^{ns}	0.31^{ns}	0.49^{*}	-0.25^{ns}	-0.20^{ns}	0.17^{ns}	0.42^{*}				
X9	0.22^{ns}	0.39^{ns}	0.51^{**}	-0.007^{ns}	0.24^{ns}	-0.06^{ns}	0.74^{**}	0.53^{**}			
X10	0.32^{ns}	0.41^{*}	0.42^{*}	0.21^{ns}	0.43^{*}	-0.18^{ns}	0.59^{**}	0.22^{ns}	0.74^{**}		
X11	0.16^{ns}	0.08^{ns}	0.08^{ns}	0.09^{ns}	-0.16^{ns}	0.11^{ns}	0.30^{ns}	0.22^{ns}	0.65^{**}	0.52^{**}	

[a] BDW: Biomass Dry Weight; BY: Biological Yield; CAT, Catalase; GP: Germination Percentage; NSPU: Number of Seeds Per Umbel; NUPP: Number of Umbels Per Plant; PC, Proline Content; POX, Peroxidase; SPSY: Single Plant Seed Yield; SV: Seed Vigor; TSW: 1000-Seed Weight. *,**: Significant at 5 and 1% probability level, respectively; ns: Not significant.

Table 6. Path coefficient analysis showing direct and indirect effects of different characters on seed yield under different concentrations of NaCl stress (pooled data); as well as Pearson's correlation coefficients between variables in ajowan.

Characteristics [a]	Seed vigor	Biomass dry weight	Catalase	Number of umbels per plant	1000-Seed weight	Correlation with single plant seed yield
SV	**0.45**[b]	-0.12	-0.02	-0.03	0.13 [c]	0.41
BDW	0.32	**-0.35**	0.12	-0.04	0.37	0.42
CAT	-0.03	-0.07	**0.70**	-0.05	0.12	0.43
NUPP	0.28	-0.21	0.26	**-0.06**	0.32	0.59
TSW	0.29	0.04	-0.07	-0.03	**0.51**	0.74

[a] BDW: Biomass Dry Weight; CAT, Catalase; NUPP: Number of Umbels Per Plant; TSW: 1000-Seed Weight. [b] The numbers on the diameter are the direct effect of independent variables on dependent variable. [c] Underlined numbers are the highest indirect effect of independent variables on dependent variable.

cluster with 13 ecotypes including, Yazd, Sadoogh 2, Sabzevar, Ardabil 4, Sarbishe, Tehran, Ardabil 3, Birjand 2, Rafsanjan 2, Sadoogh 1, Ardabil 2, Ardabil 1, and Shahedie 1 (Figure 1). Some of these ecotypes, including Yazd, Sadoogh 2, Ardabil 1, Ardabil 4, Rafsanjan 1, Rafsanjan 2, Tehran, Sarbishe, Birjand 1, and Birjand 2 were superior in terms of GP, SV, BDW, CAT, POX, PC, and BY characteristics. Birjand 1, Ghaen, Gorgan, Marvdasht, Rafsanjan 1, and Shahedie 2 ecotypes were located at the second cluster (Figure 1). Rafsanjan 1, Ghaen, and Birjand 1 ecotypes were superior in terms of POX and PC. According to clustering results, cluster 3 had ecotypes (Arak, Felaverjan, Karaj, Hamedan, Ghoom, and Shiraz) that were superior in terms of GP, SV, BDW, POX, PC, and seed yield components. These ecotypes showed increasing trends in activities of antioxidant enzymes of catalase and peroxidase and proline content with increasing salinity stress levels (Table 3). Ward dendrogram had Cophenetic Correlation Coefficient (CPCC) of 0.82. CPCC is one of the most popular measures of agreement between the original distances

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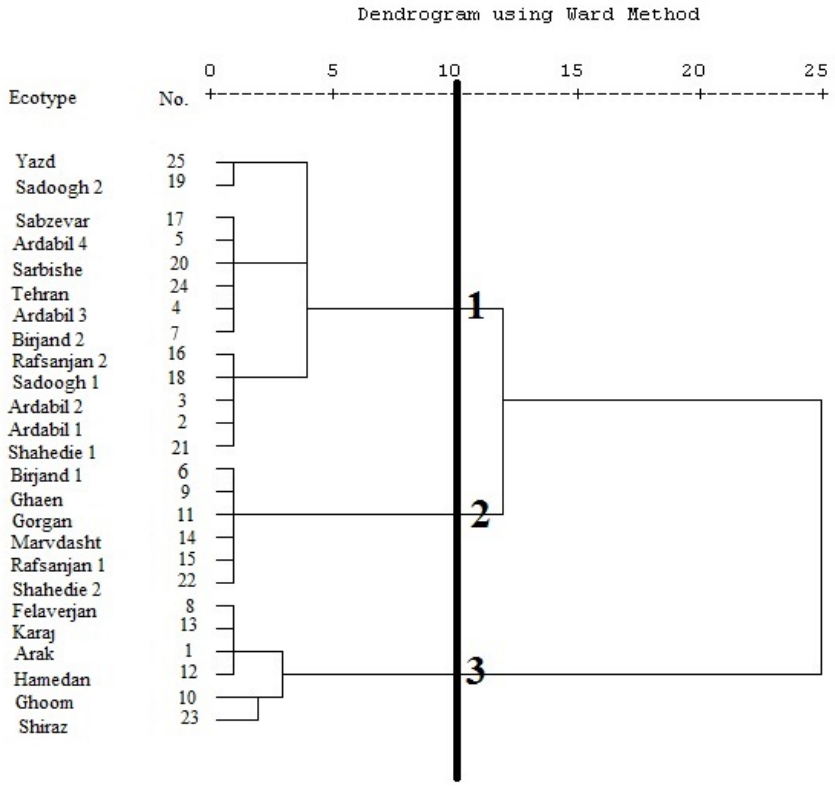

Figure 1. Cluster analysis of twenty-five Iranian ajowan ecotypes using Ward's method under salinity stress (pooled data).

and the distances in dendrogram, which can be used as an indicator for the strength of subgroup differentiation. It is recommended that CPCC should be very high (CPCC ≥ 0.9) for a dendrogram to be useful for taxonomic applications (Rohlf, 1992). However, CPCC ≥ 0.8 indicates the presence of reliable population structure in the data (Odong *et al*., 2011).

CONCLUSIONS

Increasing global food requirements, ever-increasing human population, and drought stress-soil salinization are the serious challenges of modern agriculture and future food security. Breeding and screening of plant germplasms for salt tolerance is a complex task because of different phenotypic responses of plants at different growth stages, different physiological mechanisms, complicated genotype×environment interactions, and variability of the chemical and physical compositions of the salt-affected soils.

Salinity tolerance enhancement is an important breeding objective in ajowan, as a valuable medicinal plant. As a seed-producing medicinal plant, tolerance of salinity is obviously necessary at the complete life cycle of ajowan. In the present study, three-step screening was applied in order to find salinity-tolerant Iranian ajowan ecotypes. Adverse effects of salinity were observed at germination, seedling, and adult plant stages of the investigated ecotypes. Ajowan plants responded to different levels of salinity stress by increasing and decreasing the activities of antioxidant enzymes of peroxidase and catalase, respectively. Therefore, POX and PC were not correlated in most of ecotypes investigated. With respect to the assessed

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parameters in germination, seedling, and adult plant stage, especially activities of antioxidant enzymes of catalase and peroxidase, percent reduction due to progressive increase in salinity stress was minimum in the Felaverjan, Karaj, Arak, Hamedan, Ghoom, Ghaen, Tehran, Yazd, and Shiraz ecotypes. Therefore, these ecotypes were considered as the most salinity-tolerant Iranian ajowan ecotypes. Results of the present study, with further assessments at molecular and cellular levels, could be applicable in screening and breeding programs of ajowan and other valuable seed-producing medicinal plants of *Apiaceae* family.

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ارزیابی سه مرحله‌ای تحمل به تنش شوری در زنیان (*Trachyspermum ammi* L.) Sprague ex Turrill

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چکیده

زنیان یک گیاه دارویی مهم است که عمدتاً در برخی مناطق خشک و نیمه خشک جهان رشد می کند. ارزیابی سه مرحله ای از طریق آزمایش های مستقل در مراحل رشدی جوانه‌زنی، گیاهچه، و گیاه بالغ جهت شناسایی اکوتیپ‌های ایرانی متحمل به تنش شوری از میان ۲۵ اکوتیپ مختلف با استفاده از غلظت های ۰، ۵۰، ۱۰۰، و ۱۵۰ میلی مولار NaCl انجام شد. اثر متقابل معنی دار اکوتیپ × شوری برای تمام صفات مورد بررسی در مراحل مختلف رشدی در هر سه آزمایش مستقل مشاهده شد. درصد جوانه‌زنی، قوه نامیه بذر، و بیوماس وزن خشک تمامی اکوتیپ‌های مورد بررسی با افزایش سطوح شوری کاهش یافت. با فزایش سطوح تنش شوری اکوتیپ های زنیان پاسخ های متفاوتی از لحاظ فعالیت آنزیم‌های آنتی اکسیدانی کاتالاز و پراکسیداز نشان دادند. تحت شرایط تنش شوری محتوای پرولین بیشتر اکوتیپ های مورد بررسی افزایش یافت. اثر مضر تنش شوری بر صفات عملکرد بذر و سایر اجزای عملکرد در ارزیابی مرحله گیاه بالغ مشاهده

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شد. تجزیه همبستگی ساده صفات و تجزیه ضرایب مسیر نشان داد که صفات کاتالاز و وزن هزار دانه صفاتی هستند که می توان از آنها به عنوان معیار انتخاب غیرمستقیم برای عملکرد بذر زنیان تحت شرایط تنش شوری استفاده نمود. بر اساس غربالگری سه مرحله‌ای در مراحل ذکر شده، شش اکوتیپ ایرانی زنیان شامل اکوتیپ‌های اراک، فلاورجان، قم، همدان، کرج، و شیراز به عنوان اکوتیپ‌های متحمل به تنش شوری شناسایی شدند.

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