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**Research paper**

# Evaluation of salinity tolerance of cow cockle (*Vaccaria hispanica*) ecotypes at germination and adult plant stages

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**Abstract**
This study aimed to investigate the response of cow cockle (*Vaccaria hispanica* (Mill.) Rauschert) ecotypes from northwest Iran to salinity stress at the germination and adult plant stages via a factorial experiment based on a completely randomized design with three replications in 2021. The results showed significant differences among the ecotypes regarding the germination components and morpho-physiological traits, indicating the existence of genetic diversity among them. Germination percentage and its components decreased with increasing salinity levels. So, at the NaCl concentrations greater than 120 mM, seed germination was inhibited in all studied ecotypes. This indicated that the cow cockle is a salinity-sensitive species and does not tolerate high salt concentrations. At the adult plant stage, salinity decreased plant height, root and stem dry weights, chlorophyll *a* and *b* content, and increased shoot sodium content and sodium to potassium ratio. The estimation of the multi-trait genotype-ideotype distance index (MGIDI) for different ecotypes based on various traits showed that the E3, E8, and E6 ecotypes having the lowest MGIDI values under salt stress conditions at both germination and adult plant stages, were the most tolerant ecotypes. Moreover, the E7 and E1 ecotypes had the highest MGIDI values at both growth stages and showed a higher sensitivity to salt stress. The more tolerant ecotypes selected based on the MGIDI indicator can be used for further research and selection in the breeding programs of this plant.

**Keywords:** cow cockle, factor analysis, MGIDI index, salt stress

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## Introduction

Cow cockle (*Vaccaria hispanica* (Mill.) Rauschert is a native species to Eurasia and is widely distributed in East Asia, Australia, North America, and South America (Meesapyodsuk *et al.* 2007). Cow cockle has been cultivated in rangelands as a medicinal and ornamental plant (Kumar *et al.* 2011). Also, it is grown commercially as a cut flower in California, USA (Koike *et al.* 1999). This plant produces many seeds, which are physically similar to canola but different in composition. Seeds are low in oil and instead have high amounts of starch (Goering *et al.* 1966). The main constituents of its seeds are starch, cyclopeptides, and a significant amount of bisdesmoside triterpenoid saponins (Biliaderis *et al.* 1993; Balsevich *et al.* 2006). In addition, the *V. hispanica* seed extracts have anti-cancer properties (Campbell *et al.* 2002;

Shoemaker *et al.* 2005; Ma *et al.* 2008) and are used as a feed additive to enhance the milk yield (Jumaboev *et al.* 2022). This plant has high commercial importance for local farmers for its forage value (Moradi 2021), starch and saponins production for industrial purposes, cyclic peptides, flavonoids, polysaccharides, and other vital pharmaceuticals used in skin disorder treatment (Efthimiadou *et al.* 2012; Tian *et al.* 2021). Also, it is exported to European countries in the form of dried flowers for decorative uses.

The valuable seed compositions, favorable agronomic characteristics (Ari *et al.* 2022; Solat *et al.* 2022), use of machinery for mechanized production, and reduction of wild cow cockle population in meadows (Thomas *et al.* 2007) have aroused interest in domesticating cow cockle for use as an alternative crop. Although this plant is not widely planted in Iran, its cultivation as a medicinal and ornamental plant is promoted in countries such as Turkey (Ari *et al.* 2022), Uzbekistan (Jumaboev *et al.* 2022), Canada (Koike *et al.* 1999), and USA (Duddu *et al.* 2015). Research is being done on breeding and improving its medicinal properties and seed composition (Ari et al. 2022; Tian *et al.* 2021).

Salinity, one of the major abiotic stresses, causes physiological, morphological, biochemical, and molecular changes in plants with adverse effects on growth and yield (Rodriguez *et al.* 2005). The first effect of salinity on plants is the delay in germination and seedling formation. Salinity tolerance at the seedling stage varies considerably from plant to plant. It has been indicated that salinity stress at the germination stage can be used as a reliable test in evaluating the tolerance of many crop plants (Munns and Tester 2008). Salinity reduces germination percentage and rate and diminishes root and shoot growth (Taghizadeh *et al.* 2018). Sodium ions prevent water and nutrients such as iron, phosphorus, and zinc absorption. Under salinity conditions, sodium uptake competes with potassium and may block specific potassium ion transporters in root cells. Photosynthetic activity, chlorophyll content, and carbon assimilation are decreased under potassium-deficient conditions (Tester and Davenport 2003).

The efforts on cultivation and genetic improvement of cow cockle are scarce in Iran. Therefore, considering the medicinal and industrial importance of this species and the possibility of its domestication and cultivation (Efthimiadou *et al.* 2012), this experiment aimed to collect natural populations of cow cockle from northwest Iran, investigate their genetic variation in response to salinity stress at different growth stages, and select the tolerant ecotype(s) using multi-trait genotype-ideotype distance index (MGIDI). Collection and evaluation of the natural populations are beneficial in germplasm establishment to be utilized in the breeding programs of this

species.

## Materials and Methods

### *Germination stage*

To investigate the salinity response of cow cockle ecotypes at the germination stage, a factorial experiment based on a completely randomized design with three replications was performed at Mohaghegh Ardabili University in 2021. The first factor included different salinity levels (0, 40, 80, 120, 160, and 200 mM NaCl), and the second was eight cow cockle ecotypes collected from different areas of the northwest of Iran (Table 1). Three samples of 30 healthy seeds from each ecotype were disinfected with 5% sodium hypochlorite, soaked in distilled water for two days at 4 °C, and then cultured on filter paper in Petri dishes at 25 °C. Germinated seeds (with root length ≥ 2 mm) were counted daily for 10 days. After this, shoot and root lengths and dry weights were measured. Based on the number of germinated seeds with the daily count, germination components, including germination percentage, germination index, average germination time, germination rate coefficient, and mean daily germination (Hunter *et al.* 1984; Scott *et al.* 1984), were calculated. Also, the seedling vigor indices were calculated as germination percentage (%) × seedling length (cm) or × seedling dry weight (mg) (Abdul-Baki and Anderson 1973).

Table 1. Geographical coordination of the collection sites of cow cockle ecotypes.

| Ecotype | Name of the collection sites | Longitude | Latitude | Altitude (m) |
|---|---|---|---|---|
| E1 | Meshgin Shahr, Ardabil province, Iran | 38° 31' 42.60" | 47° 50' 32.64" | 1126 |
| E2 | Maku, West Azerbaijan province, Iran | 39° 20' 14.28" | 44° 10' 3.00" | 1921 |
| E3 | Tasuj, East Azerbaijan province, Iran | 38° 19' 46.56" | 45° 16' 44.4" | 1384 |
| E4 | Julfa, East Azerbaijan province, Iran | 38° 50' 43.80" | 45° 13' 37.92" | 945 |
| E5 | Horand, East Azerbaijan province, Iran | 38° 34' 57.00" | 47° 13' 24.24" | 1776 |
| E6 | Shabestar, East Azerbaijan province, Iran | 38° 11' 25.26" | 45° 51' 11.16" | 1372 |
| E7 | Sarab, East Azerbaijan province, Iran | 37° 57' 24.48" | 47° 2' 24.00" | 1662 |
| E8 | Urmia, West Azerbaijan province, Iran | 37° 32' 22.92" | 45° 12' 50.04" | 2139 |

### *Adult plant stage*

For the greenhouse experiment, 25 germinated seeds of each cow cockle ecotype were planted in plastic pots (50 × 25 cm) filled with sand, soil, and compost (1: 1: 1 v/v). After the emergence and establishment of the seedlings, 10 healthy plants were kept in each pot. Irrigation of plants was done every other day with tap water up to the 4-5 leaf stage. Then, salinity treatments (control, 5, and 10 dS $m^{-1}$) were applied considering the weight and saturation percentage of the pot's soil. The required NaCl for each salinity level was calculated using Saltcalc software (Hagh Bahari and Seyed Sharifi 2013) and applied gradually with irrigation water in three shifts. Thirty days after the salinity treatment, the traits such as plant shoot and root lengths, plant shoot and root dry weights, and leaf chlorophyll *a*, and *b* contents were measured

through spectrophotometry (Lichtenthaler and Wellburn 1983). The sodium and potassium contents were measured by flame photometry according to the method described by Hamada and El-Enany (1994).

***Statistical analysis***

Analysis of variance was performed for the factorial experiment based on the completely randomized design with three replications, and the means were compared by the LSD test ($p \leq 0.05$), using SPSS software. To better and more efficiently select the salt-tolerant ecotypes using all traits, MGIDI was calculated as described by Olivoto and Nardino (2020) and Solat *et al.* (2022). Briefly, the scale of the data matrix (X) was changed according to the following equation so that all values have a range of 0-100, and the matrix ($rX_{ij}$) was obtained as follows:

$$rX_{ij} = \frac{\eta_{nj} - \varphi_{nj}}{\eta_{oj} - \varphi_{oj}} \times \left(\theta_{ij} - \eta_{oj}\right) + \eta_{nj}$$

where $\eta_{nj}$ and $\varphi_{nj}$ are the maximum (100) and minimum (0) new values for the jth parameter after rescaling, respectively, $\eta_{oj}$ and $\varphi_{oj}$ are the maximum and minimum values of the jth parameter, and $\theta_{ij}$ is the original value of the $j_{th}$ parameter in the $i_{th}$ genotype. The values of $\eta_{nj}$ and $\varphi_{nj}$ were chosen as follows. For parameters whose lower values were desired, $\eta_{nj} = 0$ and $\varphi_{nj} = 100$, and for parameters whose higher values were desired, $\eta_{nj} = 100$ and $\varphi_{nj} = 0$ were used. After rescaling, a two-way table of new values ($rX_{ij}$) was obtained. Each column of $rX_{ij}$ had a range of 0-100. The next step was to do a factor analysis to group the parameters related to each factor and then estimate the scores of each genotype for these factors. Factor scores were obtained only by considering factors with eigenvalues higher than one. Varimax rotation was used to rotate and estimate the final coefficients. Then, the scores of the genotypes along with the ideal genotype for each factor were obtained. By definition, the ideal genotype has the highest new scale value (100) for all analyzed characteristics. In the last step, MGIDI was calculated according to the equation below:

$$\mathrm{MGIDI}_i = \sqrt{\sum_{j=1}^{f} (\gamma_{ij} - \gamma_j)^2}$$

where $\mathrm{MGIDI}_i$ is the multivariate distance index from the ideal genotype for the $i_{th}$ genotype, $\gamma_{ij}$ is the score of the $i_{th}$ genotype in the $j_{th}$ factor (i=1, 2, ..., g and j=1, 2, ..., f), where g is the number of genotypes, f the number of factors, and $\gamma_j$ is the score of the ideal genotype in the $j_{th}$ factor. Based on this index, the genotype with the lowest MGIDI is closer to the ideal genotype. Also, the contribution of each factor in the MGIDI index of the $i_{th}$ genotype, represented by ($\omega_{ij}$), was calculated according to the following equation:

$$w_{ij} = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^{f} \sqrt{D_{ij}^2}}$$

where $D_{ij}$ is the distance between the $i_{th}$ genotype and the ideal genotype for the $j_{th}$

factor. For a given genotype, factors with a lower contribution indicate that this genotype is close to the ideal genotype in terms of important parameters within that factor (Olivoto and Nardino 2020). Factor analysis was performed based on principal component analysis using SPSS software, and MGIDI was calculated using Excel software.

## Results and Discussion

### *Seed germination indices and seedling growth*

Salinity affected germination percentage in the cow cockle ecotypes. So, with increasing the salinity levels, the germination percentage decreased, but this reduction was not the same among different ecotypes (Table 2). In normal conditions, the highest germination percentage was observed in the ecotypes E2, E3, E5, and E6, and the lowest in the ecotype E4. At 40 mM NaCl, the highest germination percentage was observed in the ecotypes E2, E3, and E5, and the lowest one in E4. Ecotypes E3, E5, and E6 also showed a significantly higher germination percentage at 80 and 120 mM NaCl than other ecotypes (Table 2). At 160 and 200 mM NaCl, there was no germination in all of the studied ecotypes. The germination index of cow cockle ecotypes also decreased with increasing salinity levels, but different ecotypes did not show the same response. With increasing salinity levels from 40 to 80 mM and from 80 to 120 mM, the germination index of all ecotypes decreased significantly. At 40 and 80 mM NaCl, the highest germination index was observed in ecotypes E3 and E5 and the lowest in E7. Also, at 120 mM NaCl, the lowest germination index was observed in the ecotype E7, and other ecotypes were not significantly different (Table 2).

The inhibitory effects of high salinity on seed germination have been reported by many researchers (Hosseini and Rezvani Moghadam 2006; Wei *et al.* 2008). Salinity reduces seed germination indices by reducing the water required for imbibing, and the toxicity of sodium and chlorine ions (Zia and Khan 2004; Rodriguez *et al.* 2005; Munns and Tester 2008). Seed germination reduction under salinity conditions is a result of high osmotic potential and impaired absorption of nutrients (Wu *et al.* 2019). Salinity increases the absorption of sodium and chlorine ions, which in turn disrupt the absorption of other nutrients. For example, the competition of sodium ions with potassium, and chlorine with nitrate interferes with the absorption of potassium and nitrate nutrients. This harms the plant's physiological processes and can be the reason for the reduction of germination percentage (Parida and Das 2005). Likewise, increasing salinity levels from 40 to 120 mM NaCl showed a significant increase in the mean germination time of ecotypes. This was especially evident in the case of ecotype E7 at all salinity levels. This ecotype had the highest

Table 2. Analysis of variance and mean comparison among ecotypes of cow cockle for the studied traits in each salinity level at the germination stage.

| Salt | Ecotype | GP | GI | MTG | CVG | MDG | RL | SL | SDW | RDW | SeDW | SeL | SWVI | SLVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | E1 | 74.44$^{bc}$ | 18.81$^{ab}$ | 1.47$^{bc}$ | 0.69$^{a}$ | 18.61$^{bc}$ | 5.53$^{c}$ | 2.86$^{b}$ | 1.35$^{bc}$ | 0.62$^{bc}$ | 1.97$^{c}$ | 8.39$^{c}$ | 1.46$^{b}$ | 6.26$^{de}$ |
| | E2 | 88.89$^{ab}$ | 19.98$^{ab}$ | 1.87$^{abc}$ | 0.54$^{b}$ | 22.22$^{ab}$ | 6.54$^{b}$ | 2.53$^{b}$ | 1.26$^{c}$ | 0.77$^{ab}$ | 2.03$^{bc}$ | 9.06$^{b}$ | 1.81$^{ab}$ | 8.03$^{bc}$ |
| | E3 | 82.22$^{abc}$ | 21.35$^{a}$ | 1.48$^{bc}$ | 0.70$^{a}$ | 20.56$^{abc}$ | 7.75$^{a}$ | 3.52$^{a}$ | 1.56$^{abc}$ | 0.92$^{a}$ | 2.48$^{ab}$ | 11.27$^{a}$ | 2.06$^{a}$ | 9.19$^{ab}$ |
| | E4 | 54.45$^{d}$ | 14.22$^{c}$ | 1.32$^{c}$ | 0.77$^{a}$ | 13.61$^{d}$ | 6.25$^{b}$ | 2.69$^{b}$ | 1.78$^{a}$ | 0.89$^{a}$ | 2.67$^{a}$ | 8.94$^{b}$ | 1.46$^{b}$ | 4.81$^{e}$ |
| | E5 | 93.33$^{a}$ | 20.80$^{ab}$ | 1.83$^{abc}$ | 0.55$^{b}$ | 23.33$^{a}$ | 5.18$^{c}$ | 2.36$^{b}$ | 1.72$^{ab}$ | 0.67$^{bc}$ | 2.38$^{abc}$ | 7.54$^{c}$ | 2.22$^{a}$ | 7.06$^{cd}$ |
| | E6 | 97.78$^{a}$ | 21.03$^{a}$ | 1.94$^{ab}$ | 0.52$^{b}$ | 24.44$^{a}$ | 5.84$^{c}$ | 2.77$^{b}$ | 1.72$^{ab}$ | 0.63$^{bc}$ | 2.35$^{abc}$ | 8.61$^{b}$ | 2.31$^{a}$ | 8.43$^{abc}$ |
| | E7 | 75.56$^{bc}$ | 10.70$^{d}$ | 2.23$^{a}$ | 0.45$^{b}$ | 18.89$^{bc}$ | 5.17$^{c}$ | 2.81$^{b}$ | 0.79$^{d}$ | 0.27$^{d}$ | 1.05$^{d}$ | 7.98$^{c}$ | 0.80$^{c}$ | 9.90$^{a}$ |
| | E8 | 76.67$^{bc}$ | 17.92$^{b}$ | 1.33$^{c}$ | 0.76$^{a}$ | 16.67$^{cd}$ | 6.07$^{bc}$ | 2.59$^{b}$ | 1.53$^{abc}$ | 0.56$^{c}$ | 2.09$^{bc}$ | 8.67$^{bc}$ | 1.39$^{b}$ | 5.74$^{de}$ |
| 40 mM | E1 | 54.44$^{d}$ | 14.06$^{d}$ | 1.34$^{b}$ | 0.75$^{a}$ | 14.44$^{c}$ | 3.54$^{c}$ | 2.04$^{b}$ | 1.12$^{bc}$ | 0.62$^{de}$ | 1.73 | 5.58$^{c}$ | 0.96$^{d}$ | 3.07$^{e}$ |
| | E2 | 83.33$^{ab}$ | 20.20$^{bc}$ | 1.60$^{b}$ | 0.63$^{ab}$ | 20.83$^{ab}$ | 5.63$^{b}$ | 2.39$^{b}$ | 1.35$^{b}$ | 0.87$^{b}$ | 2.22$^{bc}$ | 8.01$^{b}$ | 1.89$^{bc}$ | 6.76$^{bc}$ |
| | E3 | 91.11$^{a}$ | 23.15$^{a}$ | 1.58$^{b}$ | 0.63$^{ab}$ | 22.78$^{a}$ | 8.01$^{a}$ | 3.29$^{a}$ | 1.94$^{a}$ | 1.13$^{a}$ | 3.07$^{a}$ | 11.30$^{a}$ | 2.79$^{a}$ | 10.29$^{a}$ |
| | E4 | 37.78$^{e}$ | 9.89$^{e}$ | 1.40$^{b}$ | 0.75$^{a}$ | 9.45$^{d}$ | 4.40$^{bc}$ | 2.58$^{b}$ | 1.28$^{b}$ | 0.81$^{bc}$ | 2.09$^{cd}$ | 6.98$^{bc}$ | 0.81$^{d}$ | 2.69$^{e}$ |
| | E5 | 88.89$^{a}$ | 20.86$^{ab}$ | 1.69$^{b}$ | 0.59$^{b}$ | 22.22$^{a}$ | 4.43$^{bc}$ | 2.12$^{b}$ | 1.88$^{a}$ | 0.78$^{bc}$ | 2.67$^{ab}$ | 6.55$^{bc}$ | 2.38$^{ab}$ | 5.82$^{bc}$ |
| | E6 | 72.22$^{bc}$ | 19.03$^{bc}$ | 1.38$^{b}$ | 0.74$^{a}$ | 18.06$^{bc}$ | 5.08$^{b}$ | 2.41$^{b}$ | 1.95$^{a}$ | 0.67$^{cd}$ | 2.62$^{ab}$ | 7.49$^{b}$ | 1.91$^{bc}$ | 5.52$^{cd}$ |
| | E7 | 61.11$^{cd}$ | 5.75$^{f}$ | 3.24$^{a}$ | 0.31$^{c}$ | 15.28$^{c}$ | 5.19$^{b}$ | 2.62$^{b}$ | 0.87$^{c}$ | 0.33$^{f}$ | 1.20$^{e}$ | 7.81$^{b}$ | 0.73$^{d}$ | 7.23$^{b}$ |
| | E8 | 68.89$^{bcd}$ | 17.58$^{c}$ | 1.38$^{b}$ | 0.73$^{a}$ | 17.22$^{bc}$ | 4.78$^{b}$ | 2.44$^{b}$ | 1.86$^{a}$ | 0.47$^{ef}$ | 2.33$^{bc}$ | 7.23$^{bc}$ | 1.64$^{c}$ | 4.98$^{d}$ |
| 80 mM | E1 | 35.56$^{b}$ | 7.44$^{c}$ | 1.82$^{bc}$ | 0.50$^{c}$ | 8.89$^{b}$ | 2.39$^{cd}$ | 1.30$^{c}$ | 0.85$^{b}$ | 0.43$^{bc}$ | 1.28$^{bc}$ | 3.69$^{de}$ | 0.47$^{c}$ | 1.36$^{c}$ |
| | E2 | 37.78$^{b}$ | 8.03$^{c}$ | 1.78$^{bcd}$ | 0.58$^{bc}$ | 9.44$^{b}$ | 1.65$^{d}$ | 1.23$^{c}$ | 0.74$^{b}$ | 0.39$^{cd}$ | 1.13$^{bc}$ | 2.88$^{de}$ | 0.45$^{b}$ | 1.17$^{c}$ |
| | E3 | 64.44$^{a}$ | 17.50$^{a}$ | 1.27$^{d}$ | 0.79$^{a}$ | 16.11$^{a}$ | 5.07$^{a}$ | 3.09$^{a}$ | 1.54$^{a}$ | 0.68$^{a}$ | 2.22$^{a}$ | 8.15$^{a}$ | 1.46$^{a}$ | 5.30$^{a}$ |
| | E4 | 26.67$^{b}$ | 6.39$^{c}$ | 1.55$^{cd}$ | 0.65$^{b}$ | 6.67$^{b}$ | 4.29$^{ab}$ | 1.70$^{c}$ | 1.00$^{b}$ | 0.58$^{ab}$ | 1.58$^{b}$ | 5.99$^{bc}$ | 0.42$^{b}$ | 1.61$^{c}$ |
| | E5 | 78.89$^{a}$ | 15.44$^{a}$ | 2.03$^{bc}$ | 0.50$^{c}$ | 18.06$^{a}$ | 4.95$^{a}$ | 2.24$^{b}$ | 1.82$^{a}$ | 0.53$^{abc}$ | 2.35$^{a}$ | 7.19$^{ab}$ | 1.85$^{a}$ | 5.73$^{a}$ |
| | E6 | 71.11$^{b}$ | 12.14$^{b}$ | 2.25$^{b}$ | 0.45$^{c}$ | 17.78$^{a}$ | 3.23$^{bc}$ | 1.23$^{c}$ | 1.80$^{a}$ | 0.53$^{abc}$ | 2.33$^{a}$ | 4.46$^{cd}$ | 1.67$^{a}$ | 3.27$^{b}$ |
| | E7 | 24.45$^{b}$ | 2.00$^{d}$ | 3.83$^{a}$ | 0.26$^{d}$ | 6.11$^{b}$ | 1.61$^{d}$ | 0.62$^{d}$ | 0.65$^{b}$ | 0.23$^{d}$ | 0.88$^{c}$ | 2.23$^{e}$ | 0.22$^{b}$ | 0.56$^{c}$ |
| | E8 | 68.89$^{a}$ | 11.78$^{b}$ | 2.25$^{b}$ | 0.45$^{c}$ | 17.22$^{a}$ | 5.59$^{a}$ | 2.47$^{b}$ | 1.72$^{a}$ | 0.70$^{a}$ | 2.42$^{a}$ | 8.06$^{a}$ | 1.67$^{a}$ | 5.51$^{a}$ |
| 120 mM | E1 | 15.00$^{bc}$ | 2.67$^{ab}$ | 1.96$^{c}$ | 0.54$^{a}$ | 6.39$^{ab}$ | 0.32$^{cd}$ | 0.40$^{bc}$ | 0.33$^{ab}$ | 0.08$^{a}$ | 0.40$^{bc}$ | 0.72$^{b}$ | 0.07$^{a}$ | 0.12$^{a}$ |
| | E2 | 8.89$^{c}$ | 2.00$^{ab}$ | 1.67$^{c}$ | 0.50$^{ab}$ | 3.89$^{ab}$ | 0.18$^{d}$ | 0.21$^{c}$ | 0.26$^{b}$ | 0.10$^{a}$ | 0.36$^{bc}$ | 0.39$^{b}$ | 0.03$^{a}$ | 0.04$^{a}$ |
| | E3 | 24.45$^{abc}$ | 5.11$^{a}$ | 1.83$^{c}$ | 0.57$^{a}$ | 6.11$^{ab}$ | 0.80$^{bcd}$ | 0.96$^{a}$ | 0.66$^{a}$ | 0.19$^{a}$ | 0.85$^{ab}$ | 1.76$^{ab}$ | 0.22$^{a}$ | 0.45$^{a}$ |
| | E4 | 11.11$^{c}$ | 2.33$^{ab}$ | 1.74$^{c}$ | 0.61$^{a}$ | 3.33$^{b}$ | 1.05$^{abcd}$ | 0.69$^{abc}$ | 0.15$^{b}$ | 0.11$^{a}$ | 0.26$^{c}$ | 1.74$^{ab}$ | 0.03$^{a}$ | 0.20$^{a}$ |
| | E5 | 32.22$^{a}$ | 4.69$^{a}$ | 2.62$^{b}$ | 0.38$^{b}$ | 8.06$^{a}$ | 1.90$^{a}$ | 1.18$^{a}$ | 0.67$^{a}$ | 0.18$^{a}$ | 0.85$^{ab}$ | 3.08$^{a}$ | 0.28$^{a}$ | 1.03$^{a}$ |
| | E6 | 30.00$^{ab}$ | 4.36$^{ab}$ | 2.77$^{b}$ | 0.36$^{b}$ | 7.50$^{ab}$ | 1.79$^{ab}$ | 0.90$^{ab}$ | 0.70$^{a}$ | 0.20$^{a}$ | 0.90$^{a}$ | 2.69$^{a}$ | 0.28$^{a}$ | 0.84$^{a}$ |
| | E7 | 20.00$^{bc}$ | 1.44$^{b}$ | 4.42$^{a}$ | 0.23$^{c}$ | 5.00$^{ab}$ | 0.40$^{bcd}$ | 0.22$^{c}$ | 0.37$^{ab}$ | 0.11$^{a}$ | 0.48$^{abc}$ | 0.62$^{b}$ | 0.09$^{a}$ | 0.12$^{a}$ |
| | E8 | 27.78$^{ab}$ | 4.33$^{ab}$ | 2.61$^{b}$ | 0.39$^{b}$ | 6.95$^{ab}$ | 1.70$^{abc}$ | 1.10$^{a}$ | 0.68$^{a}$ | 0.16$^{a}$ | 0.84$^{ab}$ | 2.80$^{a}$ | 0.27$^{a}$ | 0.81$^{a}$ |
| Salinity (S) | $F_{(3,64)}$ | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Ecotype (E) | $F_{(7,64)}$ | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| E × S | $F_{(21,64)}$ | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| CV (%) | | 16.01 | 14.17 | 15.62 | 13.99 | 16.48 | 18.75 | 16.37 | 17.30 | 19.42 | 15.90 | 15.54 | 27.29 | 23.00 |

GP: Germination percentage, GI: Germination index, MTG: Mean germination time, CVG: Coefficient of velocity of germination, MDG: Mean daily germination, RL: Root length, SL: Shoot length, SDW: Shoot dry weight, RDW: Root dry weight, SeDW: Seedling dry weight, SeL: Seedling length, SLVI: Length index of seed vigor, and SWVI: Weight index of seed vigor; *, **: Significant at 5% and 1% probability levels, respectively in the analysis of variance; Different letters in each column show significant differences ($p \leq 0.05$) among ecotypes at each salinity level using the LSD test.

mean germination time at all salinity levels (Table 2). Also, the lowest coefficient of germination rate was observed in the ecotype E7 at all salinity levels. At the highest salinity

level (120 mM NaCl) after the E7 ecotype, E8, E5, and E6 had the lowest coefficient of germination rate (Table 2). Both weight and length indices of seed vigor also decreased with increasing the salinity level. Ecotypes E3, E5, and E6 at the salinity levels of 40 and 80 mM had the highest values of seed vigor indices, and the lowest was observed in ecotypes E7 and E4. However, under 120 mM salinity treatment, no significant difference was observed among the studied ecotypes regarding the seed vigor indices (Table 2). In compliance with our results, Hashemi *et al.* (2016) also concluded that increasing salinity concentration decreased germination percentage, germination rate, and seed vigor in *Plantago ovata* Forsk.

The results also showed that with increasing salinity levels in the cow cockle ecotypes, shoot, root, and seedling lengths decreased. However, the intensity of this reduction was not the same in different ecotypes. At the control, and 40 and 80 mM NaCl, the longest shoot and root were observed in the ecotype E3. Also, under 80 and 120 mM NaCl, the ecotypes E3, E4, E5, E8, and E6 had the longest root, shoot, and seedling lengths (Table 2). On the other hand, in the control conditions, the highest shoot and root dry weights were seen in the ecotypes E4 and E3 and the lowest in the ecotype E7. The ecotypes E3, E5, E8, and E6 at the salinity levels of 40 and 80 mM had the highest shoot dry weight, and the lowest shoot dry weight at these levels was observed in the ecotypes E1, E7, and E4. Although at the 120 mM NaCl, the highest shoot and seedling dry weights were observed in the ecotypes E3, E5, E6, and E8, no significant differences in root dry weight were observed among the studied ecotypes (Table 2). One of the causes for the reduction in the root and shoot length under salinity stress is the slower decomposition of endosperm materials and the consequent reduction of nutrients' transfer from the seed storage tissues to the embryo (Soltani *et al.* 2006; Wu *et al.* 2019). Fallahi *et al.* (2009) in sage reported that the seedling length decreased with increasing salinity levels. Also, Mostafavi and Heidarian (2012) and Li *et al.* (2020) reported that in sunflowers, along with germination percentage and rate, the root, shoot, seedling lengths, and fresh and dry weights were also decreased with increasing salinity due to slowing water uptake.

***Growth characteristics at the adult plant stage***

The analysis of variance showed that the main effect of salinity was significant for shoot length, root and shoot dry weight, chlorophyll *a* and *b*, $Na^+$, and $Na^+/K^+$, and the main effect of ecotype was significant for all traits. The interaction of these factors was only significant for the root and shoot dry weights (Table 3). At the average salinity levels, the highest root and

shoot lengths were observed in ecotype E6 and then in ecotypes E7 and E8 (Table 3). The highest root dry weight at the 5 dS $m^{-1}$ salinity level, was observed in ecotypes E8, E3, and E6, however, other ecotypes showed the lowest values without significant differences among each other. Also, the ecotypes E3, E6, and E8 showed the highest, and the ecotypes E1, E2, E4, and E7 had the lowest root dry weight under the 10 dS $m^{-1}$ salinity. Under the salinity level of 5 and 10 dS $m^{-1}$, the highest shoot dry weight was observed in the ecotype E6 and the lowest in the ecotypes E1, E4, and E7 (Figure 1).

Salinity decreases leaf, shoot, and root growth due to the reduced ability of plants to

Table 3. Results of the analysis of variance and mean comparison among ecotypes of cow cockle and the salinity levels for the studied traits at the adult plant stage.

| | Levels | PRL | PSL | PRDW | PSDW | Chl *a* | Chl *b* | $K^+$ | $Na^+$ | $Na^+/K^+$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Ecotype | E1 | $10.04^{a}$ | $14.48^{cd}$ | $11.86^{c}$ | $144.09^{f}$ | $14.36^{b}$ | $3.91^{b}$ | $53.86^{d}$ | $50.80^{c}$ | $0.97^{a}$ |
| | E2 | $10.21^{a}$ | $15.03^{bcd}$ | $12.83^{c}$ | $194.95^{d}$ | $14.50^{b}$ | $5.48^{ab}$ | $64.86^{cd}$ | $56.61^{bc}$ | $0.91^{ab}$ |
| | E3 | $11.15^{a}$ | $15.24^{bcd}$ | $18.11^{a}$ | $195.85^{d}$ | $13.47^{b}$ | $3.85^{b}$ | $64.55^{cd}$ | $60.24^{bc}$ | $0.96^{a}$ |
| | E4 | $9.79^{a}$ | $15.70^{bc}$ | $12.66^{c}$ | $159.69^{e}$ | $16.30^{b}$ | $5.09^{ab}$ | $71.16^{bc}$ | $57.55^{bc}$ | $0.81^{ab}$ |
| | E5 | $10.48^{a}$ | $16.09^{ab}$ | $14.96^{b}$ | $204.32^{c}$ | $14.01^{b}$ | $4.49^{ab}$ | $79.89^{abc}$ | $58.58^{bc}$ | $0.76^{bc}$ |
| | E6 | $9.90^{a}$ | $17.06^{a}$ | $17.43^{a}$ | $252.53^{a}$ | $22.41^{a}$ | $6.01^{a}$ | $87.30^{a}$ | $52.67^{c}$ | $0.63^{b}$ |
| | E7 | $10.64^{a}$ | $15.25^{bcd}$ | $15.41^{b}$ | $207.8^{c}$ | $16.02^{b}$ | $4.01^{b}$ | $78.94^{abc}$ | $70.59^{a}$ | $0.90^{ab}$ |
| | E8 | $11.11^{a}$ | $14.02^{d}$ | $18.89^{a}$ | $226.1^{b}$ | $21.71^{a}$ | $6.02^{a}$ | $81.06^{ab}$ | $63.94^{b}$ | $0.80^{b}$ |
| Salinity (dS $m^{-1}$) | 0 | $10.18^{a}$ | $18.67^{a}$ | $14.25^{c}$ | $226.30^{a}$ | $19.62^{a}$ | $6.06^{a}$ | $74.91^{a}$ | $46.88^{c}$ | $0.66^{c}$ |
| | 5 | $10.49^{a}$ | $15.18^{b}$ | $16.58^{a}$ | $195.19^{b}$ | $17.27^{b}$ | $5.02^{b}$ | $68.18^{a}$ | $52.75^{b}$ | $0.80^{b}$ |
| | 10 | $10.58^{a}$ | $12.23^{c}$ | $15.10^{b}$ | $173.04^{c}$ | $12.91^{c}$ | $3.49^{c}$ | $75.03^{a}$ | $76.99^{a}$ | $1.06^{a}$ |
| Salinity $F_{(2,48)}$ | | ns | ** | ** | ** | ** | ** | ns | * | ** |
| Ecotype $F_{(7, 48)}$ | | ns | ** | ** | ** | ** | * | ** | ** | ** |
| E × S $F_{(14, 48)}$ | | ns | ns | ** | ** | ns | ns | ns | ns | ns |
| CV (%) | | 13.29 | 8.99 | 9.93 | 3.64 | 18.74 | 22.91 | 20.27 | 15.55 | 20.33 |

PRL: Plant root length, PSL: Plant shoot length, PRDW: Plant root dry weight, PSDW: Plant shoot dry weight, Chl *a*: Leaf chlorophyll *a*, Chl *b*: Leaf chlorophyll *b*, $K^+$: Potassium content, $Na^+$: Sodium content, $Na^+/K^+$: Sodium/potassium ratio; ns, *, **: Non-significant, and significant at 5% and 1% probability levels, respectively in the analysis of variance; Different letters in each column and each factor show significant differences ($p \leq 0.05$) among ecotypes and salinity levels using the LSD test.

absorb and transfer water. If stress is continued due to the entry of excessive amounts of salt into the plant, the roots transport excess ions to the vacuole of the aged leaf cells and accumulate in the cytoplasm. As a result, the cells and the old leaves die, eventually. This leads to a gross reduction in growth in the sensitive genotypes than in the more tolerant genotypes (Munns and Tester 2008). In many studies, the dry weight of leaf, stem, and root are is one of the criteria for determining the salinity tolerance of genotypes. Salinity reduces plant height by reducing cell division and elongation. It also causes a

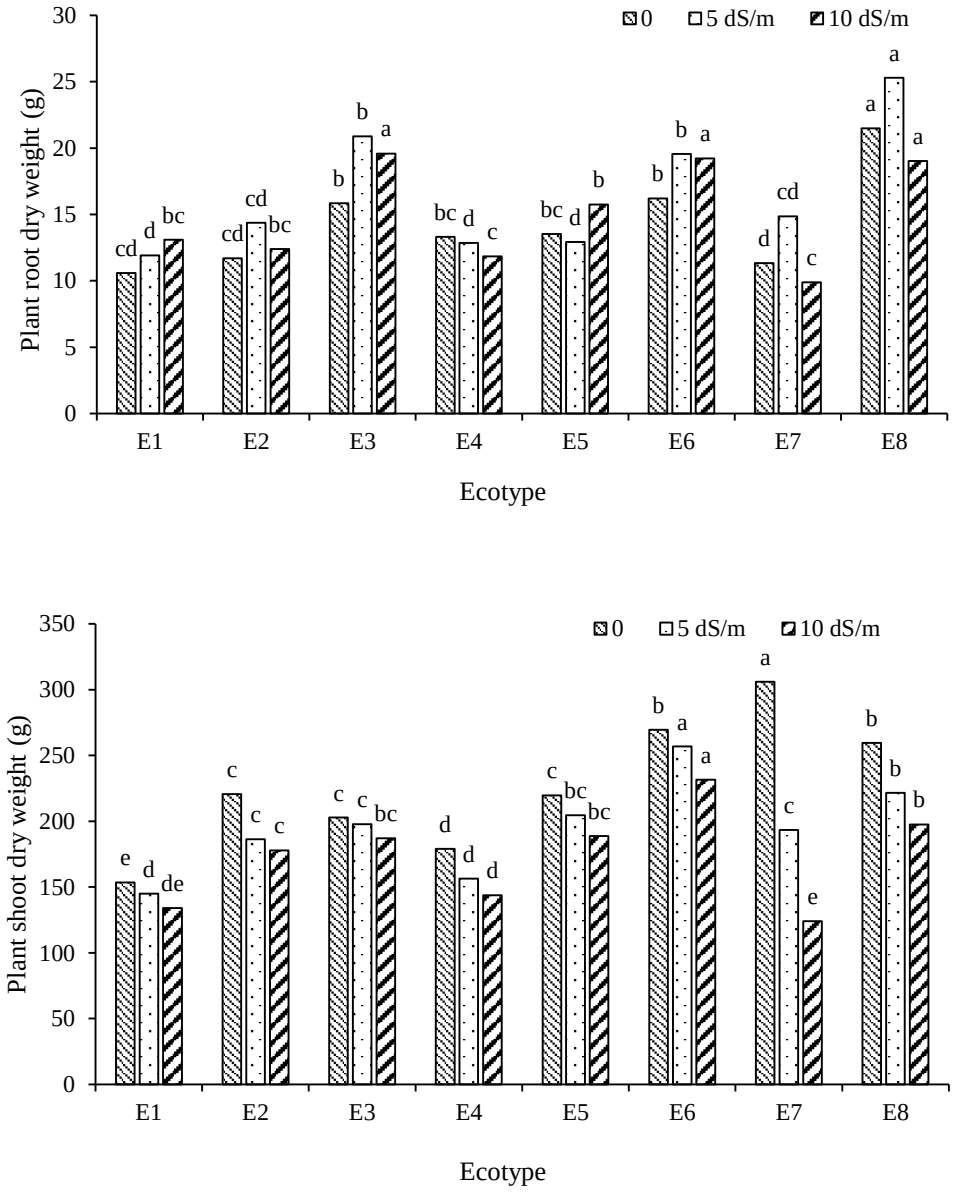


Figure 1. Means of cow cockle ecotypes at different salinity levels regarding plant root (a) and shoot (b) dry weights. Dissimilar letters indicate a significant difference ($p \leq 0.05$) among ecotypes at each salinity level using the LSD test.

decrease in shoot height and shoot and root weight and disrupts all plant metabolic activities due to ion toxicity and loss of ion and osmotic balance (Jamil *et al.* 2006).

Salinity decreased chlorophyll *a* and *b* of the cow cockle ecotypes with increasing salinity levels (Table 3). At the average of salinity levels, the highest chlorophyll *a* was observed in the ecotypes E6 and E8 having a significant difference from other ecotypes (Table 3). The ecotypes E1, E3, and E8 had the lowest chlorophyll *b* content (Table 3). Photosynthesis is the main process that determines the growth and yield of plants and

the ability to maintain yield under environmental stresses. Accumulation of sodium in the leaves is associated with the closure of the stomata and a decrease in the total chlorophyll content, which subsequently reduces the photosynthetic products (Parida and Das 2005). Chlorophyll stability, as an indicator of plant tolerance to salinity stress, seems to vary among different species and cultivars. Although a decrease in the chlorophyll content is seen under the salinity stress, tolerant plants show higher chlorophyll content (Öncel *et al.* 2000). Variation in response to the NaCl stress for germination components, morphophysiological characteristics, and chlorophyll content have been reported in various ecotypes of flax (*Linum usitatissimum* L.) and alfalfa (Mahdavi and Alasvandyari 2018; Hosseini-Boldaji *et al.* 2020).

The $Na^+$ content and $Na^+/K^+$ ratio increased in the cow cockle ecotypes with increasing the salinity level (Table 3). On the average salinity levels, the highest amount of $K^+$ was observed in the ecotypes E5, E6, and E8, and the lowest amount was observed in the ecotypes E1, E2, and E3. Also, the highest amount of sodium was seen in E7. On the other hand, ecotype E6 showed the lowest $Na^+/K^+$ ratio (Table 3). Although the molecular and biological mechanisms of salinity tolerance in plants are not well understood, it has been suggested that it is related to the $Na^+$ content in tissues (Omielan *et al.* 1991). In this regard, tolerant plants evolved mechanisms such as lower absorption of $Na^+$ in the roots, less transfer to the shoots, and its accumulation in some specified cells (Shannon and Grieve 1999). Chen *et al.* (2005) found that salinity increases the $Na^+$ content and $Na^+/K^+$ ratio in plants, as also observed in our study. $K^+$ concentration in the root zone decreases due to competition with $Na^+$ under salinity conditions. Many plants, especially those with low salinity tolerance, retain their $K^+$ selectivity even at high salinity levels and prefer to have more $K^+$ than $Na^+$ in their vacuoles under low to medium stress conditions (Cramer *et al.* 1990). In the present study, under a salinity of 10 dS $m^{-1}$, with increasing $Na^+$, some ecotypes could absorb more $K^+$ ions while others lacked this ability. Although $Na^+$ concentration in the leaves may help maintain leaf turgescence, it cannot be a proper alternative for $K^+$, because $K^+$ is essential for protein synthesis and enzyme activity (Parida and Da 2005).

***MGIDI index***

Factor analysis based on the principal component analysis at the germination stage showed that at the normal conditions, the first three factors and at the salinity stress conditions, the first two factors with eigenvalues greater than 1 explained 94.62% and 92.20% of the total variance of the studied

traits, respectively (Table 4). Under normal conditions, the first factor explained 48.58% of the total variance. Variables with the highest positive factor coefficients were the germination index, germination percentage, mean daily germination, and seed vigor indices after varimax rotation. The second factor explained 33.34% of the total variance in which root, shoot, and seedling lengths and root dry weight had the highest positive coefficients. The third factor, with 12.70% of the total variance, had the high and positive factor coefficients for the germination index, average germination time and germination rate, and the shoot, root, and seedling dry weights.

Under normal conditions at the germination stage, the MGIDI index of the studied ecotypes based on the factor scores for these three factors are shown in Table 5. According to the MGIDI index, the lower the MGIDI value of the target ecotype, the less distant from the ideal genotype and the closer to the desired genotype. In contrast, the higher the MGIDI value for an ecotype, the greater the distance from the ideal genotype, and would not be selected (Pour-Aboughadareh *et al.* 2021).

According to the MGIDI index, under normal conditions at the germination stage, the E3 ecotype had the lowest value and was considered a desirable ecotype, followed by the E2 and E6 ecotypes. However, E5 and E7 had the highest MGIDI in this condition and were undesirable ecotypes (Table 5).

Under the salt stress conditions, the first factor explained 74.60% of the total variation. The highest positive coefficients for the first

Table 4. Factor loading with varimax rotation at the germination stage under normal and salinity conditions.

| Trait | Normal conditions | | | Salinity conditions (on average of salt levels) | |
|---|---|---|---|---|---|
| | Factor 1 | Factor 2 | Factor 3 | Factor 1 | Factor 2 |
| GI | 0.613 | 0.354 | 0.610 | 0.852 | 0.438 |
| MTG | -0.635 | 0.393 | 0.603 | 0.096 | 0.929 |
| CVG | -0.713 | 0.397 | 0.526 | 0.005 | 0.987 |
| GP | 0.996 | -0.048 | 0.029 | 0.981 | -0.041 |
| MDG | 0.996 | -0.047 | 0.029 | 0.961 | -0.011 |
| RL | -0.017 | 0.957 | 0.166 | 0.780 | 0.551 |
| ShootL | 0.024 | 0.888 | 0.294 | 0.715 | 0.540 |
| SDW | 0.015 | 0.088 | 0.973 | 0.936 | 0.177 |
| RDW | -0.040 | 0.611 | 0.686 | 0.535 | 0.814 |
| SeDW | -0.009 | 0.317 | 0.930 | 0.906 | 0.363 |
| SeL | -0.004 | 0.973 | 0.215 | 0.766 | 0.552 |
| SWVI | 0.681 | 0.214 | 0.697 | 0.972 | 0.196 |
| SLVI | 0.798 | 0.588 | 0.123 | 0.924 | 0.287 |
| Eigenvalue | 6.32 | 4.33 | 1.65 | 9.70 | 2.29 |
| % of variance | 48.58 | 33.34 | 12.70 | 74.60 | 17.61 |
| Cumulative variance (%) | 48.58 | 81.92 | 94.62 | 74.60 | 92.20 |

GI: Germination index, MTG: Mean germination time, CVG: Coefficient of velocity of germination, GP: Germination percentage, MDG: Mean daily germination, RL: Root length, SL: Shoot length, SDW: Shoot dry weight, RDW: Root dry weight, SeDW: Seedling dry weight, SeL: Seedling length, SLVI: Length index of seed vigor, and SWVI: Weight index of seed vigor.

factor were related to the germination index, germination percentage, mean daily germination, seed vigor indices, root, shoot, and seedling lengths, and root, shoot, and seedling dry weights (Table 4). The second factor explained 17.61% of the total variance, in which the average germination time and germination rate had the highest positive coefficients (Table 4).

Under salt stress at the germination stage, the E3 ecotype had the lowest MGIDI value followed by the E8 and E5 ecotypes. So, these ecotypes could be considered salt-tolerant ecotypes at the germination stage. On the other hand, Ecotypes E7 and E4 had the highest amount of MGIDI and these ecotypes were considered sensitive to salt stress (Table 5).

Factor analysis at the adult plant stage showed that at both normal and salt-stress conditions, the first four factors with eigenvalues greater than one explain 94.83% and 91.47% of the total variance, respectively (Table 6). Under normal conditions, the first factor explained 37.25 % of the total variance with the highest positive factor coefficients for root length and root and shoot dry weights. The second factor explained 23.43% of the total variance in which $K^+$ content and $Na^+/K^+$ ratio had the highest positive factor coefficients. The third factor contributing to 18.90% of the total variance, had a high and positive factor coefficient for chlorophyll *a* and *b*. The fourth factor explained 15.24% of the total variance in which the plant length had the highest positive, and $Na^+$ content had the highest negative factor coefficients (Table 6).

According to the MGIDI index under normal conditions at the adult plant stage, the E6, E3, and E8 ecotypes had the lowest value and were considered desirable ecotypes. On the other hand, the E1 and E7 ecotypes had the highest amount of MGIDI in this condition (Table 5).

Under the salt stress conditions at the adult plant stage, the first factor explained 30.69% of the total variance with the highest positive factor coefficients for the plant length and $Na^+$ content and the highest negative factor coefficient for $K^+$. The second factor explained 27.50% of the total variance in which root length and $Na^+/K^+$ ratio had the highest positive and negative factor coefficients, respectively. The third factor showing 16.81% of the total variance, had a high and positive factor coefficient for chlorophyll *a* and *b*. The fourth factor explained 16.48% of the total variance in which plant root and shoot dry weight had the highest positive factor coefficients (Table 6).

Based on the MGIDI index, E6 and E8 had the lowest values and were considered desirable ecotypes under salt stress conditions at the adult plant stage. However, the E7 and E1 ecotypes had the highest amount of MGIDI

Table 5. The MGIDI index of different ecotypes at the germination and adult plant stages under normal and salinity conditions.

| Conditions | Ecotype | Germination stage | | | | | Adult plant stage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MGIDI | Rank | $\omega_{i1}$ | $\omega_{i2}$ | $\omega_{i3}$ | MGIDI | Rank | $\omega_{i1}$ | $\omega_{i2}$ | $\omega_{i3}$ | $\omega_{i4}$ |
| Normal | E1 | 2.429 | 4 | 0.297 | 0.533 | 0.170 | 4.370 | 8 | 0.398 | 0.137 | 0.304 | 0.161 |
| | E2 | 1.860 | 2 | 0.015 | 0.592 | 0.394 | 2.796 | 4 | 0.268 | 0.340 | 0.313 | 0.079 |
| | E3 | 0.759 | 1 | 0.312 | 0.184 | 0.504 | 2.218 | 2 | 0.288 | 0.451 | 0.020 | 0.241 |
| | E4 | 3.117 | 6 | 0.505 | 0.441 | 0.054 | 3.707 | 6 | 0.409 | 0.238 | 0.213 | 0.140 |
| | E5 | 3.193 | 7 | 0.051 | 0.854 | 0.095 | 3.574 | 5 | 0.240 | 0.208 | 0.311 | 0.241 |
| | E6 | 2.308 | 3 | 0.206 | 0.753 | 0.042 | 2.065 | 1 | 0.691 | 0.046 | 0.014 | 0.249 |
| | E7 | 3.982 | 8 | 0.139 | 0.419 | 0.442 | 4.083 | 7 | 0.289 | 0.524 | 0.122 | 0.065 |
| | E8 | 2.695 | 5 | 0.401 | 0.501 | 0.098 | 2.772 | 3 | 0.004 | 0.309 | 0.683 | 0.004 |
| On the average of salinity levels | E1 | 2.389 | 6 | 0.745 | 0.255 | | 4.029 | 7 | 0.126 | 0.126 | 0.265 | 0.484 |
| | E2 | 1.945 | 5 | 0.688 | 0.312 | | 3.447 | 6 | 0.079 | 0.318 | 0.305 | 0.298 |
| | E3 | 0.205 | 1 | 0.603 | 0.397 | | 2.727 | 3 | 0.030 | 0.137 | 0.336 | 0.497 |
| | E4 | 2.504 | 7 | 0.909 | 0.091 | | 3.154 | 4 | 0.028 | 0.228 | 0.070 | 0.674 |
| | E5 | 1.369 | 3 | 0.036 | 0.964 | | 3.402 | 5 | 0.163 | 0.026 | 0.511 | 0.301 |
| | E6 | 1.445 | 4 | 0.286 | 0.714 | | 1.598 | 1 | 0.058 | 0.153 | 0.677 | 0.111 |
| | E7 | 3.554 | 8 | 0.398 | 0.602 | | 4.052 | 8 | 0.305 | 0.098 | 0.216 | 0.380 |
| | E8 | 1.149 | 2 | 0.326 | 0.674 | | 2.336 | 2 | 0.163 | 0.402 | 0.093 | 0.343 |

MGIDI (Multi-trait genotype–ideotype distance index) for each ecotype; $\omega_{i1}$, $\omega_{i2}$, $\omega_{i3}$, and $\omega_{i4}$, the share of the first, second, third, and fourth factors in the MGIDI index for each ecotype, respectively.

Table 6. Factor loadings after factor analysis with varimax rotation at the adult plant stage under normal and salinity conditions.

| Trait | Normal conditions | | | | Salinity conditions (on average of salt levels) | | | |
|---|---|---|---|---|---|---|---|---|
| | Fac1 | Fac2 | Fac3 | Fac4 | Fac1 | Fac2 | Fac3 | Fac4 |
| PRL | 0.951 | -0.086 | -0.029 | -0.006 | -0.148 | 0.902 | -0.103 | 0.254 |
| PSL | 0.017 | 0.333 | 0.140 | 0.884 | 0.796 | -0.105 | 0.044 | 0.409 |
| PRDW | 0.700 | 0.127 | -0.482 | 0.374 | -0.208 | 0.570 | 0.153 | 0.631 |
| PSDW | 0.896 | 0.333 | 0.248 | 0.073 | -0.004 | 0.050 | 0.030 | 0.998 |
| Chl *a* | -0.063 | 0.069 | 0.974 | -0.041 | 0.000 | 0.034 | 0.925 | 0.275 |
| Chl *b* | 0.129 | -0.383 | 0.909 | 0.069 | 0.078 | 0.114 | 0.935 | -0.141 |
| $K^+$ | 0.132 | 0.893 | -0.068 | 0.411 | -0.837 | -0.377 | -0.223 | 0.251 |
| $Na^+$ | -0.133 | 0.038 | 0.121 | -0.946 | 0.921 | -0.160 | -0.081 | -0.206 |
| $Na^+/K^+$ | 0.084 | 0.973 | -0.156 | -0.026 | -0.195 | -0.935 | -0.262 | 0.115 |
| Eigenvalue | 3.35 | 2.11 | 1.70 | 1.37 | 2.76 | 2.48 | 1.51 | 1.48 |
| % of variance | 37.25 | 23.43 | 18.90 | 15.24 | 30.69 | 27.50 | 16.81 | 16.48 |
| Cumulative variance (%) | 37.25 | 60.69 | 79.59 | 94.83 | 30.69 | 58.18 | 74.99 | 91.47 |

PRL: Plant root length, PSL: Plant shoot length, PRDW: Plant root dry weight, PSDW: Plant shoot dry weight, Chl *a*: Leaf chlorophyll *a*, Chl *b*: Leaf chlorophyll *b*, $K^+$: Potassium content, $Na^+$: Sodium content, $Na^+/K^+$: Sodium/potassium ratio.

in this condition (Table 5).

Considering that each ecotype is closer to the ideal genotype in terms of traits within factors in which it shows a smaller share (Olivoto and Nardino 2020; Olivoto *et al.* 2021), it can be said that at the germination stage, the E3 tolerant ecotype, which under salinity stress conditions had the lowest value for the second factor, was close to the ideal genotype for the traits such as average

germination time, germination rate, and root dry weight which had the highest coefficients for this factor. At the same time, the ecotype E8 had the lowest value for the first factor under salt stress conditions and was close to the ideal genotype for all traits except for average germination time and germination rate. Likewise, under salt stress conditions at the adult plant stage, E6 was considered the most tolerant ecotype, having the lowest value for the first and fourth factors. So, it was close to the ideal genotype for traits such as plant height, $Na^+$ and $K^+$ contents, and plant root and shoot dry weights (Table 5). The MGIDI index was proposed by Olivoto and Nardino (2020) to facilitate the selection of the desired genotypes based on multi-trait information. Recently Olivoto *et al.* (2021) used this index to select the ideal strawberry genotypes. The MGIDI index was also used to accelerate the screening of barley genotypes for salinity stress in the early stages of growth (Pour-Aboughadareh *et al.* 2021).

## Conclusions

Overall, the estimation of the MGIDI for different ecotypes based on all traits showed that the E3, E8, and E6 ecotypes with the lowest MGIDI value under salt stress conditions at both germination and adult plant stages were the most tolerant ecotypes and can be used for further research and selection in breeding programs of this plant. Also, the ecotypes E7 and E1 had the highest MGIDI values in both growth stages and were the most sensitive ecotypes to salt stress. The MGIDI indicator may be used in breeding programs as an efficient index to select the desired genotypes.

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## Conflicts of interest

The authors declare no conflicts of interest.

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# ارزیابی تحمل شوری اکوتیپ‌های جغجغک (*Vaccaria hispanica*) در مراحل جوانه‌زنی و گیاه بالغ

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**چکیده**

این تحقیق با هدف بررسی واکنش اکوتیپ‌های جغجغک (*Vaccaria hispanica* (Mill.) Rauschert) شمال غرب ایران به تنش شوری در مراحل جوانه‌زنی و گیاه بالغ به‌صورت آزمایش فاکتوریل بر پایه طرح کاملاً تصادفی با سه تکرار در سال ۱۴۰۰ انجام شد. نتایج نشان داد که بین اکوتیپ‌ها از نظر مولفه‌های جوانه‌زنی و صفات مورفوفیزیولوژیک تفاوت معنی داری وجود دارد که نشان‌دهنده وجود تنوع ژنتیکی در بین آن‌ها است. با افزایش شدت تنش، درصد جوانه‌زنی و شاخص‌های آن کاهش یافت، به طوری که در غلظت بیش از ۱۲۰ میلی‌مولار NaCl، جوانه‌زنی بذر در تمامی اکوتیپ‌های مورد مطالعه متوقف شد. این نتایج نشان داد که جغجغک گونه‌ای نسبتاً حساس به شوری است و غلظت بالای نمک را تحمل نمی‌کند. در مرحله گیاه بالغ، شوری باعث کاهش ارتفاع بوته، وزن خشک ریشه و ساقه، محتوای کلروفیل a و b و افزایش میزان سدیم اندام هوایی و نسبت سدیم به پتاسیم شد. برآورد شاخص فاصله ژنوتیپ-ایدئوتیپ چند صفتی (MGIDI) برای اکوتیپ‌های مورد استفاده بر اساس صفات مختلف نشان داد که اکوتیپ‌های E3، E8 و E6 با داشتن کمترین مقادیر MGIDI در شرایط تنش شوری در هر دو مرحله جوانه‌زنی و گیاه بالغ متحمل‌تر بودند. علاوه بر این، در هر دو مرحله رشدی، اکوتیپ‌های E7 و E1 با بالاترین مقادیر MGIDI، حساسیت بیشتری به تنش شوری نشان دادند. از اکوتیپ‌های متحمل‌تر انتخاب شده بر اساس شاخص MGIDI می‌توان برای تحقیقات و گزینش بیشتر در برنامه‌های اصلاحی این گیاه استفاده کرد.

**واژه‌های کلیدی**: تجزیه به عامل‌ها، تنش شوری، جغجغک، شاخص MGIDI