http://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 33, Number 1, Pages 13-26 (2024)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1386462

Research Article; Received: November 05, 2023; Accepted: December 25, 2023

DRABA VERNA L. (BRASSICACEAE/CRUCIFERAE): A SALT AVERSE TAXON

GİZEM SAYGIN[1], İNCİ BAHAR ÇINAR[2], GÜL NİLHAN TUĞ[1]

[1] Department of Biology, Faculty of Science, Ankara University, Ankara, TÜRKİYE
[2] Department of Environmental Protection and Technologies, Suluova Vocational School, Amasya University, Amasya, TÜRKİYE

ABSTRACT. In this study, it was aimed to determine whether *Draba verna* L. (=*Erophila verna* (L.) Chevall. subsp. *verna* (L.) DC.), which is a relative of model organism *Arabidopsis thaliana* (L.) Heynh. and distributing around saline areas, is a halophyte or not and to research the salinity tolerance during germination period. *D. verna* seeds were germinated at distilled water and different NaCl concentrations (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mM) at 8ºC/16ºC 12/12 h photoperiodism (light intensity 12000 lux ±%10) for 20 days. The NaCl concentrations and the germination percentages were as follows distilled water 100%, 100 mM NaCl 83%, 200 mM NaCl 2%, 300 mM NaCl 1% and no germination over 300 mM NaCl. Un-germinated seeds were taken into recovery and show 87.3% mean germination, and the ones still ungerminated were tested for viability. Increase in salinity, cause decrease in germination rate which means that *D. verna* is not resistant to salinity but salinity has important pressure on germination. The tolerance of *D. verna* seeds to salinity, although it has a wide distribution area at saline areas, is result of decrease in soil salinity during germination period. It can be concluded that *D. verna* is not a halophyte it is a salt avoider.

1. INTRODUCTION

Plants have adaptations that enable them to survive in very variable and extreme ecosystems ranging from habitats with wide distribution and favourable living conditions such as temperate forests, steppes, meadows to tundras, and deserts and saline areas. Plants are categorized in different ways, and when it is based on soil salinity they are seperated in two main groups; halophytes that tolerate salinity and glycophytes that avoid salinity [1]. Seeds of halophytic and glycophytic plants exhibit a response to salt stress similar to each other, and their germination process is delayed under salt stress conditions [2,3].

Halophytes possess distinctive adaptation mechanisms that enable them to germinate, thrive, and accomplish their life cycles in high-salinity environments, where majority of plants fail to survive [4, 5]. Despite such adaptations, they display superior germination abilities under unsaline or less saline conditions [6-9]. However, an increase in salinity levels leads to a decline in the germination rate and ratio and/or retards germination [10-17, 18-21].

Keywords. Brassicaceae/Cruciferae, *Draba verna*, germination, salinity

gizemsaygin1991@gmail.com- 0009-0003-9992-0009
inci.cinar@amasya.edu.tr- Corresponding author; 0000-0002-1983-0261
tug@science.ankara.edu.tr- 0000-0002-2702-2387

The majority of halophyte seeds that fail to germinate due to salinity are able to germinate after salt is removed from the environment and when stress conditions are reduced [22], reflecting the physiological response of plants under high stress [23]. Under field conditions, seed germination of halophytes occurs after rain or floods, which provide moisture to the soil and cause salt to leach into the lower layers of the soil. The germination stage of most species occurs during this period [24].

Seed germination in saline conditions typically occurs in the spring or during seasons with excessive rainfall when the soil salt levels decrease [23]. Salinity has a drought-like effect on plants, known as ‘physiological drought’. It obstructs the plants' osmotic activities and hinders root water and mineral uptake from the soil. While annual plants are more vulnerable to salt, perennial plants are more adaptable to saline conditions [25].

Brassicaceae/Cruciferae, commonly known as the mustard family, is a widely distributed monophyletic group consisting of 338 genera and 3709 species. Among them, *Arabidopsis thaliana* (L.) Heynh. is the most well-known species and serves as a model organism for flowering plants [26].

Draba verna L. is an annual, small, and delicate plant belonging to the Brassicaeae/Cruciferae family. It grows along roadsides, in swamps and fields, as well as in forest clearings where the vegetation cover is not dense. Typically, this taxon colonizes these areas following light or moderate disturbances. Thus, throughout the growing season from autumn to early spring, the plant faces minimal competition from larger plants and benefits from ample access to sunlight throughout its life cycle [27]. This widely distributed species was first described from Europe. In Türkiye, it can be found in various regions including Edirne, Istanbul, Çankırı, Sinop, Samsun, Trabzon, Artvin, Izmir, Ankara, Niğde, Muğla, Muş, Mardin, Konya, and Gaziantep [28, 29]. *D. verna* is a plant with multiple seeds, each ranging from 0.3-0.5 mm in size.

D. verna was chosen for this study due to its relation to *A. thaliana*, a model organism for seed plants that grows in saline environments. The aim was to ascertain whether the *D. verna* is a halophyte or salt-averse taxon.

2. MATERIALS AND METHODS

2.1. Study Area

Draba verna seeds were collected from Bolluk Lake in Konya-Cihanbeyli (N 38º 32ˈ 59.8ˮ-E 32º 55ˈ 33.6ˮ) in May of 2014 (Figure 1). Bolluk Lake, located west of Tuz Lake, contains high levels of sodium sulphate, making it a saline water body. The lake is encompassed by infertile hills and steppe lands. Surface currents and a sulfurous water spring in the north provide the lake’s main sources of water.

FIGURE 1. General view of Bolluk Lake

2.2. Collection of Seeds and Germination Trials

Draba verna seeds (Figure 2 and Figure 3) were kept at +4ºC until they were examined. The dimensions of the seeds were evaluated by BAB stereo binocular microscope and BAB image processing and analysis system (Bs200Pro). Mean weight of seeds were measured. Randomly selected one hundred seeds were randomly grouped in 500 to weighed using a precision balance for 5 times. Then the average weight of a *D. verna* seed was measured as $3.0\times10^{-5}\pm0.0008$ g.

To prevent fungal infection, the seeds underwent treatment with a 0.1% sodium hypochlorite solution for 3 minutes. Subsequently, they underwent three rounds of washing with sterile distilled water to ensure no contamination was present. Then four replicates of 25 seeds were placed in petri dishes on two layers of Whatman No.1 filter paper dampened with 4 ml of distilled water. The petri dishes were sealed with parafilm and then incubated at daily temperatures of 6/18°C, 8/20°C and 10/22°C in light (12-hour daily photoperiod) and continuous darkness (petri dishes were kept in black bags) for 20 days. Germination was monitored every two days for the light trial and at the end of the trial for the dark treatment. The temperatures recorded represent the mean maximum and minimum daily temperatures of the distribution area during the germination period in March and April. Germination is regarded as the emergence of radicle. The rate of germination in light was calculated using the modified Timson index of germination rate [12].

FIGURE 2. *Draba verna* (Photograph by Dr. İsa Başköse)

FIGURE 3. *Draba verna* seeds

Twenty-five seeds with 4 replicates were incubated at optimum light and temperatures for 20 days at the following NaCl concentrations: distilled water, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mM NaCl solutions. Seeds that failed to germinate during the salinity trials were washed using distilled water and then were incubated at optimum temperature for another 20 days with 4 ml of distilled water. The viability of the non-germinated seeds was determined with the TTC (Triphenyl Tetrazolium Chloride) test [13].

The germination index was calculated using the formula ΣG/t, where G is the percentage of seed germination at 2-day intervals and t is the total germination time. The maximum value obtained with this equation is 50 and indicates a high germination velocity [30].

Recovery percentage was calculated with the formula: [(a−b) / (c−b)] x 100 here a: total number of germinated seeds tested (all the seeds germinated in NaCl treatment and germinated after recovery); b: number of seeds germinated under saline conditions; c: total number of seeds tested [31]. The last germination was calculated with the formula: (a / c) x 100 [13]. Seed viability was calculated with the formula: [(a + d) / c] x 100. Here d is the number of embryos that were stained pink in the TTC solution [13].

Ungerminated seeds were treated with a 1% tetrazolium solution for 24 hours at 30°C. The viability of the seeds was then observed under a binocular microscope [32, 33]. Red staining of the seed was considered a positive indicator of viability as it has activity at the cellular level. In contrast, TTC does not react with non-viable seeds and therefore they do not stain [34].

Decreasing germination percentage (DGP) gives information about salinity tolerance and calculated by using the following formula:

DGP = [(Germination percentage at distilled water – Germination percentage at salinity) / (Germination percentage at distilled water)] x 100 [35].

2.3. Statistical Analysis

All the data were arcsin transformed and then evaluated by SPSS (IBM SPSS Statistics Version 25) with One Way ANOVA for the comparison of the influence of trials. T test was used for importance control ($p < 0.05$).

2.4 Bioclimatic Analysis of the Distribution Area of the Taxon

In order to determine the climatic characteristics of Bolluk Lake, which is the research region, the meteorological data of Cihanbeyli district, which is the closest station to the region, were used.

Precipitation and temperature data for Cihanbeyli were obtained from the General Directorate of Meteorology, Ministry of Forestry and Water Affairs. Bioclimatic interpretations were made according to Emberger (1955) [36, 37]. Ombrothermic diagrams of the study area were constructed according to Gaussen (1955) [38].

2.5 Soil Analysis of the Distribution Area of the Taxon

Soil samples of 1-2 kg were taken from appropriate root depths (rhizosphere) in the Konya-Cihanbeyli Bolluk Lake area where *D. verna* is distributed. All the soil analyses were accomplished at the 'General Directorate of Agricultural Research Central Soil, Fertilizer, and Water Resources Research Institute (Ankara)'.

3. RESULTS AND DISCUSSION

3.1. Germination Features

Temperature, salinity and alkalinity are some of the factors that affect the initial and maybe the most important life cycle stage of plants; germination [18,20]. The response of seeds to salinity, whether salinity cause death of seeds or prevent germination, depends on species. Halophyte plants, like glycophyte plants, show the highest germination rate in distilled water, but the percentage of germination decreases for both halophytes and glycophytes as the salt concentration increases, but halophyte seeds have higher salt resistance compared to glycophytes [2, 3, 6-17, 39].

D. verna is a widespread taxon and its range includes the vicinity of saline lakes. Being related to *Arabidopsis thaliana*, which is the model organism of seed plants, and its presence in saline areas makes it a suitable candidate for the study of salt resistance mechanisms at molecular level. The restricted distribution of this plant to saline areas requires further investigation into whether it is a halophyte or an ecotype suited to halophytic areas. Halophytes refer to plants that can germinate in NaCl solutions of 200 mM concentration and beyond [13, 20].

Based on the temperature experiments conducted, the germination percentages were observed to be 100% at 6 ºC/18ºC, 92% at 8ºC/20ºC and 95% at 10ºC/22ºC. Considering these germination percentages, it was determined that the most suitable temperature conditions for the germination of *D. verna* seeds are 6ºC/18ºC (12/12 h photoperiodism) (Figure 4). The impact of varying salt concentrations (distilled water, 100, 200, 300, 400, 500, 500, 600, 700, 800, 900, 1000 mM NaCl) on the sprouting of *D. verna* seeds was evaluated by subjecting them to 12/12 h photoperiod and 6ºC/18ºC temperature conditions. All the germination results, germination percentages, germination rates, last germination percentages, viability percentages after TTC test DGP ratios at changing salinities were given at Table 1.

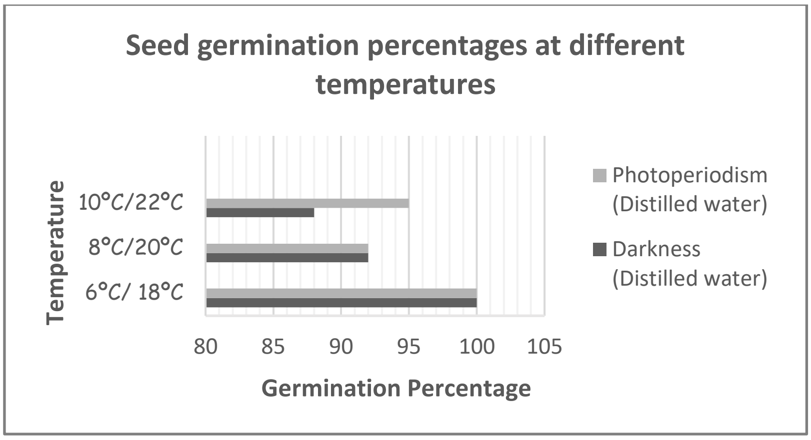

FIGURE 4. Seed germination percentages of *Draba verna* taxon at different temperatures

TABLE 1. Germination results of *D. verna*

	Germination (%)	Germination rate	Recovery (%)	Last germination (%)	Viability (%)	DGP
Darkness (Distilled water)	**100**	-	-	-	-	-
Photoperiodism (Distilled water)	**100[a]**	**34.85**	-	**100**	**100**	-
100 mM NaCl	**83[b]**	**23.95**	**47.06**	**91**	**94**	**16.16**
200 mM NaCl	**2[c]**	**0.45**	**93.88**	**94**	**95**	**97.98**
300 mM NaCl	**1[c]**	**0.1**	**97.97**	**98**	**98**	**98.99**
400 mM NaCl	**0[c]**	**0**	**97**	**97**	**97**	**100**
500 mM NaCl	**0[c]**	**0**	**89**	**89**	**89**	**100**
600 mM NaCl	**0[c]**	**0**	**95**	**95**	**95**	**100**
700 mM NaCl	**0[c]**	**0**	**89**	**89**	**89**	**100**
800 mM NaCl	**0[c]**	**0**	**93**	**93**	**93**	**100**
900 mM NaCl	**0[c]**	**0**	**84**	**84**	**84**	**100**
1000 mM NaCl	**0[c]**	**0**	**87**	**87**	**87**	**100**

D. verna showed the highest germination in distilled water like other taxa (Table 1). However, even in *D. verna*, which boasted a 100% germination rate in distilled water, the percentage of germinating seeds rapidly declined with increasing salt concentrations. At concentrations of 400 mM NaCl and beyond, no seeds germinated.

After conducting the improvement experiments, it was found that *D. verna* seeds exhibited an average germination rate of 87.3%. The results demonstrate that these seeds are capable of successful germination even after exposure to salt stress. The seeds germinate when environmental conditions improve, particularly when the salt level reduces.

The average percentage of viability of mature seeds of *D. verna* individuals was 92.36%. Such a high percentage of viability of the seeds and high germination percentages in the result of the recovery experiment after exposure to salt indicate that the low germination in salinity trials is due to the increased salt concentration.

Zhang et al. (2015) established the concept of 'Decreasing Germination Percentage (DGP)' and examined the germination patterns of 12 halophytes. Their study concluded that the highest reduced germination percentage meant the lowest salt tolerance. The results of this study have supported the idea that salt tolerance decreases with increasing salinity levels (Table 1).

Seeds stayed non-germinated at the end of the salinity trials first taken into recovery and if they did not germinate, then the viability test was conducted [13]. The color change observed in Figure 5 was an indicator of the viability.

According to Moore's (1972), Delouche's (1976), and Grabe's (1976) classification, seeds are considered viable if their embryos are entirely light pink or bright red without any milky white or yellowish staining at the end of the radicle [40-42].

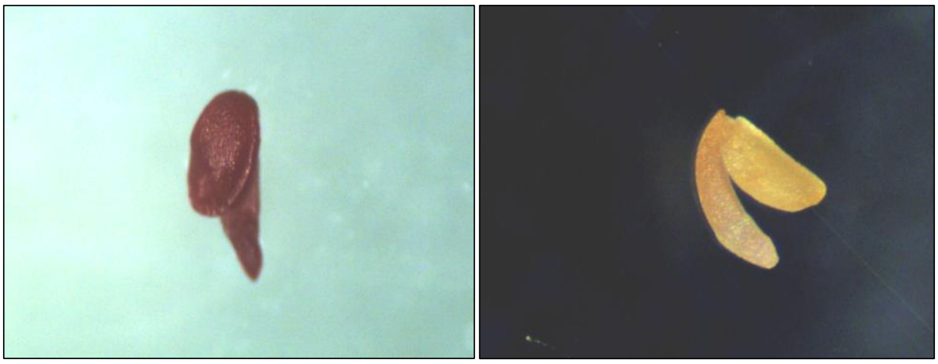

FIGURE 5. Viable seed (left-completely satined red) and un-viable seed (right-not stained red) after TTC test

As outlined in Parretio-de Guzman et al. (2011) study, the distinctions between viable and non-viable seeds post-TTC treatment were categorised in the following manner: The seeds were deemed viable if the entire embryo was stained or if there were minimal unstained regions in the plumule. Conversely, seeds were considered non-viable if more than the tip of the radicle was unstained, more than half of the radicle was unstained, the entire radicle and the junction of the plumule and radicle axis were unstained, the entire radicle and more than half of the plumule were unstained, the radicle and more than half of the plumule were unstained and greenish in colour, or the entire embryo was unstained [43, 44].

3.2. Bioclimatic Features

Bioclimatic analysis of the study area was performed according to Emberger method (Table 2). Ombrothermic diagrams of the study areas were created according to Gaussen method (Figure 6) [38]. The dry periods determined in the ombrothermic diagram start with May in Cihanbeyli and continue until the beginning of October.

TABLE 2. Bioclimatic analysis of the study area

Stations / Parameters	Cihanbeyli (Konya)
P (mm)	326.5
M (°C)	30.7
m (°C)	-4.9
Q	32.04
PE (mm)	40.1
S	1.31
Rainfall regime	Sp.W.F.Su. (Eastern Mediterranean Type 2)
Bioclimatic layers	Semi-arid, very cold in winter, Mediterranean climate

P: Mean total annual rainfall (mm), **M:** Mean maximum. temperature of the warmest month (°C), **m:** Mean mininum temperature of the coldest month (°C), **Q:** Rainfall-temperature coefficient, **PE:** Summer rainfal total (mm), **S:** Drought index, **Sp:** Spring, **W:** Winter, **F:** Fall, **Su:** Summer [36].

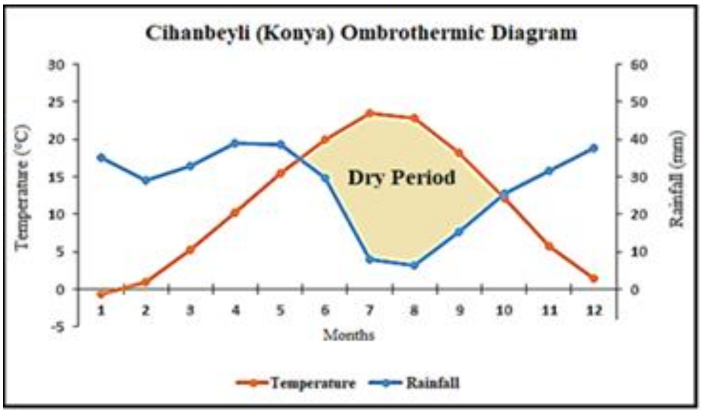

FIGURE 6. Cihanbeyli Ombrothermic Diagram

3.3. Soil Features

The salinity tolerance of certain species determines their habitat types and range [45]. Germination is typically the most vulnerable stage of a plant's life cycle as it defines the conditions that the plant must face in the soil [46].

The results of the analyses of the soil samples taken from the area of distribution of the target taxon in Bolluk Lake are presented in Table 3. The salt concentration in the soil is determined by the electrical conductivity of the saturated solution. The electrical conductivity of a solution is proportional to the salinity of the soil. Accordingly, the EC (electrical conductivity) is below the lower limit of 4 dS/m during the germination period in the distribution area. This value can be interpreted as non-saline or slightly saline soil [47]. In addition, when the total salt content of the soil is examined, it can be said that the soil is very low in salt.

TABLE 3. Analysis of soil samples from the research area

Soil samples	**Texture**	**Electrical conductivity (dS/m)**	**Salt (%)**
I	CL (Clay loam)	1.439	0.062
II	CL (Clay loam)	1.482	0.064

4. CONCLUSIONS

As a result, a statistically significant difference was determined between distilled water and 100 mM NaCl ($p<0.05$). The same situation was observed between 200 mM NaCl and higher salt concentrations ($p<0.05$). A value between 100-200 mM NaCl can be considered as the threshold value for *D. verna* seeds. *D. verna*, an ephemeral taxon like *Holosteum umbellatum* L., avoids salt by germinating during the period of decreasing salt concentration, when the salt in the soil is washed away by snowmelt and spring rains, and by completing its development before the dry summer months when salt concentration increases. Seed germination at low salinity also coincides with climatic conditions. The germination period coincides with the period when the salt is washed out of the soil as a result of snowfall and rain, and the plant completes its development bear and shed seeds before the start of the summer season when the salt rises again. In this case, the taxon *D. verna* is not halophyte, but it can complete its life cycle in saline areas with an ecological adaptation that allows it to avoid salt, so it can be classified as salt averse taxon.

Acknowledgement The authors would like to thank to Prof. Dr. Ahmet Emre Yaprak and Dr. İsa Başköse for their kind help.

Author Contribution Statements Experimental design: G.N.T., G.S., Experimental performance: G.S., İ.B.Ç., Data analysis and interpretation: G.S., İ.B.Ç., G.N.T., Manuscript writing: G.N.T., İ.B.Ç., G.S. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest. This paper is a summary of a Master's thesis titled "*Erophila verna* (L.) Chevall. subsp. *verna* (Brassicaceae)'nın Çimlenme Dönemi Tuz Direncinin Araştırılması" submitted to Ankara University.

REFERENCES

[1] Ungar, I.A., Ecophysiology of Vascular Halophytes, CRC Press, Boca Raton, 1991. https://doi.org/10.1201/9781003418269

[2] Almansouri, M., Kinet, J.M., Lutts, S., Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil*, 231 (2001), 243–254. https://doi.org/10.1023/A:1010378409663

[3] Keiffer, C.H., Ungar, I.A., The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *American Journal of Botany*, 84 (1) (1997), 104–111. https://doi.org/10.2307/2445887

[4] Estrelles, E., Biondi, E., Galiè, M., Mainardi, F., Hurtado, A., Soriano, P., Aridity level, rainfall pattern and soil features as key factors in germination strategies in salt-affected plant communities, *Journal of Arid Environments*, 117 (2015), 1–9. https://doi.org/10.1016/j.jaridenv.2015.02.005

[5] Gul, B., Ansari, R., Flowers, T.J., Khan, M.A., Germination strategies of halophyte seeds under salinity, *Environmental and Experimental Botany*, 92 (2013), 4–18. https://doi.org/10.1016/j.envexpbot.2012.11.006

[6] Seneca, E.D., Germination response to temperature and salinity of four dune grasses from the outer banks of North Carolina, *Ecology*, 50 (1969), 45–53. https://doi.org/10.2307/1934661

[7] Onnis, A., Bellettato, R., Dormienza e alotolleranza in due specie spontanee di *Hordeum* (*H. murinum* L. e *H. marinum* Huds), *G. Bot. Ital*, 106 (1972), 101–113. https://doi.org/10.1080/11263507209426542

[8] Breen, C.M., Everson, C., Rogers, K., Ecological studies on *Sprorobolus virginicus* (L.) Kunth with particular reference to salinity and inundation, *Hydrobiologia*, 54 (1977), 135–140. https://doi.org/10.1007/BF00034987

[9] Dietert, M.F., Shontz, J.P., Germination ecology of a Maryland population of saltmarsh bulrush (*Scirpus robustus*), *Estuaries*, 1 (1978), 164–170. https://doi.org/10.2307/1351458

[10] Ungar, I. A., Halophyte seed germination, *The Bot. Rev*, 44 (1978), 233–264. https://doi.org/10.1007/BF02919080

[11] Huiskes, A.H.L., Stienstra, A.W., Koutataal, B.P., Markusse, M.M., Van Soelen, J., Germination ecology of *Salicornia dolichostachya* and *S. brachystacya*, *Acta Bot. Neerl*, 34 (4) (1985), 369–380. https://doi.org/10.1111/j.1438-8677.1985.tb01943.x

[12] Khan, M.A., Ungar, I.A., Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions, *Am. J. Bot*, 84 (2) (1997), 279–283. https://doi.org/10.2307/2446089

[13] Baskin, C.C., Baskin, J.M., Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination, Academic Press, San Diego, 1998.

[14] Khan, M.A., Gul, B., High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*, *International Journal of Plant Sciences*, 159 (1998), 826–832. https://doi.org/10.1086/297603

[15] Khan, M.A., Gul, B., Weber, D.J., Temperature and high salinity effects in germinating dimorphic seeds of *Atriplex rosea*, *Western North American Naturalist*, 164 (2) (2004), 193–20.

[16] Sekmen, A.H., Ozdemir, F., Türkan, İ., Effects of salinity, light and temperature on seed germination in an Turkish endangered halophyte, *Kalidiopsis wagenitzii* (Chenopodiaceae), *Israel Journal of Plant Sciences*, 52 (2004), 21–30.

[17] Wang, L., Huang, Z., Baskin, C.C., Baskin, J.M., Dong, M., Germination of dimorphic seeds of desert annual halophyte *Suaeda aralocaspica* (Chenopodiaceae), a C4 plant without kranz anatomy, *Ann. Bot*, 102 (2008), 757–769. https://doi.org/10.1093/aob/mcn158

[18] Khan, M.A., Gul, B., Weber, D.J., Seed germination in the Great Basin halophyte *Salsola iberica*. *Canadian Journal of Botany*, 80 (2002), 650–655. https://doi.org/10.1139/b02-046

[19] Woodell, S.R.J., Salinity and seed germination patterns in coastal plants, *Vegetatio*, 61 (1985), 223–229. https://doi.org/10.1007/BF00039828

[20] Ungar, I.A., Seed germination and seed bank ecology in halophytes. In seed development and seed germination, Edited by J. Kigel and G. Galili, Marcel Dekker, (1995), 599–628, New York. https://doi.org/10.1201/9780203740071-23

[21] Wei, Y., Dong, M., Huang, Z.Y., Tan, D.Y., Factors influencing seed germination of *Salsola affinis* (Chenopodiaceae) a dominant annual halophyte inhabiting the deserts of Xinjiang, China, *Flora*, 203 (2008), 134–140. https://doi.org/10.1016/j.flora.2007.02.003

[22] Ungar, I.A., Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). *American Journal of Botany*, 83 (5) (1996), 604–607. https://doi.org/10.1002/j.1537-2197.1996.tb12745.x

[23] Pujol, J.A., Calvo, J.F., Ramirez-Diaz, L., Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Annals of Botany*, 85 (2) (2000), 279–286. https://doi.org/10.1006/anbo.1999.1028

[24] Khan, M.A., Ungar, I.A., Influence of salinity and temperature on the germination of *Haloxylon recurvum* Bunge ex. Boiss. *Annals of Botany*, 78 (5) (1996), 547–551. https://doi.org/10.1006/anbo.1996.0159

[25] Bojović, B., Đelić, G., Topuzović, M., Stanković, M., Effects of NaCl on seed germination in some species from families Brassicaceae and Solanaceae, *Kragujevac Journal of Science*, 32 (2010), 83–87.

[26] Al-Shehbaz, I.A., Beilstein, M.A., Kellogg, E.A., Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. *Plant Systematics and Evolution*, 259 (2006), 89–120. https://doi.org/10.1007/s00606-006-0415-z

[27] Baskin, J.M., Baskin, C.C., The light factor in the germination ecology of *Draba verna*. *American Journal of Botany*, 59 (7) (1972), 756–759. https://doi.org/10.1002/j.1537-2197.1972.tb10149.x

[28] Davis, P.H., Cullen, J., Coode, M.J.E., Flora of Turkey and East Aegean Islands, Vol. 1, Edinburgh University Press, Edinburgh, 1965.

[29] Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. Türkiye bitkileri listesi. Damarlı Bitkiler, Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını (2012), 262.
[30] Khan, M.A., Ungar, I.A., The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *American Journal of Botany*, 71 (4) (1984), 481–489. https://doi.org/10.1002/j.1537-2197.1984.tb12533.x
[31] Gul, B., Weber, D.J., Effect of salinity, light, and temperature on germination in *Allenrolfea occidentalis*. *Canadian Journal of Botany*, 77 (2) (1999), 240–246. https://doi.org/10.1139/b98-204
[32] Grabe, D.F., Tetrazolium testing handbook for agricultural seeds. Association of Official Seed Analysts, Lan- sing, 62, Michigan, USA, 1970.
[33] Williams, S., Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness, *Ecol Appl*, 11 (2001), 1472–1488. https://doi.org/10.1890/1051-0761(2001)011[1472:RGDIET]2.0.CO;2
[34] França-Neto, J., Krzyzanowski, F., Tetrazolium: An important test for physiological seed quality evaluation, *Journal of Seed Science*, 41 (2019), 359–366. https://doi.org/10.1590/2317-1545v41n3223104
[35] Zhang H., Zhang G., Lü X., Zhou D., Han, X., Salt tolerance during seed germination and early seedling stages of 12 halophytes, *Plant Soil*, 388 (2015), 229–241. https://doi.org/10.1007/s11104-014-2322-3
[36] Emberger, L., Une classification biogéographique des climats, Rec. Trav, Faculté des sciences de Montpellier Botanique, 7 (1955), 3–43.
[37] Akman, Y., Climate and Bioclimate, Palme Publication, Ankara, 2010.
[38] Gaussen, H., Determination des climatspar la methode des courbes ambrothermiques, Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences, 240(6) (1955), 642–643.
[39] Terzi, H., Yıldız, M., Altuğ, Ü., Halofit *Salsola crassa* tohum çimlenmesi üzerine tuzluluk, sıcaklık ve ışığın etkileri, *AKÜFEMÜBİD*, 17 (2017), 1–9. https://doi.org/10.5578/fmbd.52763
[40] Moore, R.P., Interpretation of color differences in tetrazolium testing, *Seed Technologist News*, 44 (3) (1972), 22–24.
[41] Delouche, J. C., Standardization of vigor tests, *Journal of Seed Technology*, 1 (2) (1976), 75–85.
[42] Grabe, D. F., Manual do teste de tetrazólio em sementes, Brasília (1976): Agiplan.
[43] de Andrade Melo, L.D.F., Junior, J.L.D.A.M., Ferreira, V.M., de Araújo Neto, J.C., Viability *Mimosa bimucronata* (DC.) O. Kuntze. seeds by the tetrazolium test and oil content. *Diversitas Journal*, 5 (3) (2020), 1575–1587. https://doi.org/10.17648/diversitas-journal-v5i3-846
[44] Parretio-de Guzman, L.E., Zamora, O.B., Borromeo, T.H., Cruz, P.C.S., Seed Viability and Vigor Testing of *Jatropha curcas* L. *Philippine Journal of Crop Science* (PJCS), 36 (3) (2011), 10–18.
[45] Soriano, P., Moruno, F., Boscaiu, M., Vicente, O., Hurtado, A., Llinares, J.V., Estrelles, E., Is salinity the main ecologic factor that shapes the distribution of two endemic Mediterranean plant species of the genus Gypsophila?, *Plant and Soil*, 384 (2014), 363–379. https://doi.org/10.1007/s11104-014-2218-2

[46] Ungar, I.A., Germination ecology of halophytes. In: Sen, D.N., Rajpurohit, K.S. Editors. *Contributions to The Ecology of Halophytes. Tasks for Vegetation Science*, Springer, Dordrecht, (1982), 143−154. https://doi.org/10.1007/978-94-009-8037-2_10

[47] Richards, L.A., Diagnosis and improvement of saline and alkali soils (No. 60), US Government Printing Office, Washington DC, 1954.