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EFFECTS OF LIGHT AND SALINITY ON THE GERMINATION OF CLOSELY RELATED THREE *SALSOLA* TAXA

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

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

ABSTRACT. Three closely related *Salsola* taxa (*Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila, *Salsola boissieri* Botsch. subsp. *boissieri*, *Salsola turcica* Yıldırımlı (halophytic ecotype), *Salsola turcica* Yıldırımlı (gypsicole ecotype)) from different edaphic conditions were studied according to changing light and salinity conditions. Seeds of target taxa were collected in 2017 and their weights were determined. The perianth segments were removed before the experimental trials and all the trials were conducted at 9°C/22°C which is the mean night and day temperatures of germination season. For the determination of the influence of light, one set of seeds for distilled water trial were kept at complete darkness. Different NaCl concentrations (distilled water, 100, 200, 300 mM NaCl) were used to evaluate the effects of salinity on germination. Viability of the seeds were determined by Triphenyl Tetrazolium Chloride (TTC) test which was applied to the seeds that did not germinate during the trials. As a result, it was found out that light stimulates germination of the taxa and *Salsola* seeds showed better germination ratio at light. The most tolerant taxa against salinity are the halophytic and gypsicole ecotypes of *S. turcica*, and the most susceptible one is *S. boissieri* subsp. *serpentinicola*. Both of the species show reduced germination ratios with increasing salinity. Salinity tolerance of *S. boissieri* subsp. *serpentinicola* and *S. boissieri* subsp. *boissieri* are very low, according to the Decreasing Germination Percentage (DGP) values. Although they show different germination response against increasing salinity, there is not any statistically meaningful difference between these three taxa according to germination percentages at different salinities, germination rates, last germination ratios and seed viabilities (F=1.818 p>0.05) (One Way ANOVA, SPSS 25).

Keyword and phrases. *Amaranthaceae/Chenopodiaceae, germination, Gypsicole, halophyte, salinity, Salsola, serpentine*

inci.cinar@amasya.edu.tr-Corresponding author; tug@science.ankara.edu.tr
0000-0002-1983-0261; 0000-0002-2702-2387

1. INTRODUCTION

Soil salinity became an important problem as a result of global warming, lack of precipitation and also wrong irrigation policies, especially in arid and semi-arid areas increase [1] so halophytes became an important plant group whose biology and germination ecology attracts attention in last few decades [2-6]. Halophytes have some special adaptation mechanisms that make them germinate, survive and complete their life cycles at high salinities where the most of the plants can not survive [5, 7]. Although they have these adaptation they show better germination abilities at non-saline or less saline conditions [8-20], and increase in salinity cause decrease in their germination rate and ratio and/or retard germination [3, 12-19, 21-23].

The genus *Salsola* L. is represented by 18 species and 23 taxa in Turkey [24]. The members of the genus are generally adapted to saline or semi-saline areas of arid regions [25, 26]. Although *Salsola turcica* Yıldırımlı, *Salsola boissieri* Botsch. subsp. *boissieri* and *Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila are phylogenetically closely related, they are adapted to different edaphic conditions and became habitat specialists which make them more vulnerable against habitat loss and degradation [27].

S. turcica is endemic to Turkey and has two ecotypes spread over gypsaceous and saline steppes between altitudes 950 m and 1000 m [28]. The subspecies of *S. boissieri* are geographically separated from each other. *S. boissieri* subsp. *boissieri* prefers rocky and stony slopes at 900-2500 m altitudes around Kahramanmaraş and Sivas provinces [25]. *S. boissieri* subsp. *serpentinicola* is also endemic to Turkey and, prefers serpentine rocks and rock cracks between 1600 and 2000 m around Muğla and Burdur provinces [29]. If populations of a species is adapted to local conditions and have genetically preserved morphological and physiological differences, they are called as ecotypes [30]. *S. turcica* has a wide distribution area and populations from both halophytic and gypsicole areas were studied and because of this differentiation they may have different physiological adaptations. The germination trials of these ecotypes can provide some information about the differentiations of them.

Most of the *Salsola* taxa are salt tolerant during the germination phase of their life cycles, as supported by several studies on different *Salsola* species like: *S. kali* L. [21], *S. baryosma* (Schult.) Dandy [31], *S. villosa* Del. ex Roem. et Schult [32], *S. iberica* Sennen & Pau [3], *S. chandharyi* L. [33], *S. imbricata* Forssk. [34, 35], *S.affinis* C.A.Mey. ex Schrenk [23], *S. vermiculata* L. [26], *S. ferganica* Drobow [36], *S. grandis* Freitag, Vural & N. Adıgüzel [37].

In this study, three closely related *Salsola* taxa adapted to different edaphic conditions were studied to understand the influence of their salinity tolerance on their distribution and also the germination characteritics of them under different NaCl concentrations. These taxa are *S. boissieri* subsp. *serpentinicola*, *S. boissieri* subsp. *boissieri*, *S. turcica* (halophytic ecotype), *S. turcica* (gypsicole ecotype).

2. MATERIALS AND METHODS

2.1 Collection of fruits and seeds

During vegetation period of 2017, populations were evaluated at their natural distribution areas and, fruits and seeds were collected according to the current population sizes of the taxa and at least 10 individuals were sampled. Until the start of experiments specimens were stored at 4°C. All the fruit and seed specimens were evaluated by BAB stereo binocular microscope and BAB image processing and analysis system (Bs200Pro). Mean weight of fruits and seeds were measured. The locations and collection numbers of specimens were given in Table 1.

TABLE 1. Taxa with their subspecies and ecotype information, collection number of specimens and localities

Taxon	**Collection number of specimen (IBÇınar)**	**Locality**
***S. boissieri* subsp. *serpentinicola* (serpentinicole subspecies)**	**1139**	Burdur, Altınyayla, Dirmil pasture, serpentine soils, 1669 m
***S. boissieri* subsp. *boissieri* (glycophyte subspecies)**	**1141**	Sivas, Yıldızeli, Yusufoğlan village, 1400 m
***S. turcica* (halophytic ecotype)**	**1142**	Konya, Cihanbeyli, Bolluk Lake, saline alkaline areas, 943 m
***S. turcica* (gypsicole ecotype)**	**1143**	Ankara, Beypazarı, about 14 km west of Beypazarı, on the right side of Beypazarı-Nallıhan highway, 948 m

2.2 Germination trials

Periant segments of seeds were removed before the trials and seeds were sterilized with 0,1% sodium hypochlorite. During the trials, the mean night and day temperatures of the germination period of the distribution areas were used, 9°C / 22°C with 12 h photoperiodism (light intensity 12000 lux ± %10). For the determination of the influence of salinity on germination distilled water and 100, 200, 300 mM NaCl solutions were used. At each trial, 25 seeds with 4 replicates were monitored for 10 days and the emergence of radicula was accepted as germination. Ungerminated seeds were taken into TTC test for detection of their viability under binocular microscope [38-39]. Stained red at TTC test is the indicator of viability which detects the cellular level respiratory activity [40]. Red stained seeds were accepted as viable and the ones stained green were accepted as unviable [15].

After the trials germination percentages, germination rates (Timson index) [41] and viability percentages were calculated.

Germination rate: $\Sigma G/t$ [41]
Last germination: $(a / c) \times 100$
Seed viability: $[(a+d) / c] \times 100$ [15]
ΣG: sum of the germination percentages of every 2 days
t: total germination period.
a: total germinated seed number at the end of the trials (number of germinated seeds after recovery + number of germinated seeds at salinity)
c: Total number of seeds
d: seeds stained red after test- viable seeds

DGP: [(Germination percentage at distilled water − Germination percentage at salinity) / Germination percentage at distilled water] × 100 [42].

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 ($p < 0.05$).

3. RESULTS AND DISCUSSION

Mean weight and sizes of all the taxa were given at Table 2 and the examples of fruits and seeds are provided in Figure 1 and 2.

TABLE 2. Taxa, collection number of specimens and localities

Taxon	**Mean weight of a fruit (g)**	**Mean weight of a seed (g)**	**Mean diameter of a fruit (mm)**	**Mean diameter of a seed (mm)**
***S. boissieri* subsp. *serpentinicola* (serpentinicole subspecies)**	0.615 ± 0.029	0.297 ± 0.008	5.53 ± 0.74	1.40 ± 0.18
***S. boissieri* subsp. *boissieri* (glycophyte subspecies)**	0.389 ± 0.019	0.225 ± 0.025	4.22 ± 0.64	1.23 ± 0.15
***S. turcica* (halophytic ecotype)**	0.862 ± 0.039	0.415 ± 0.016	4.34 ± 0.54	1.50 ± 0.15
***S. turcica* (gypsicole ecotype)**	0.583 ± 0.028	0.362 ± 0.035	4.63 ± 0.75	1.42 ± 0.17

Halophytic ecotype of *S. turcica* has the heaviest fruits and seeds according to the mean weights, and the lightest fruits and seeds belong to *S. boissieri* subsp. *boissieri*. *S. boissieri* subsp. *serpentinicola* has the largest mean fruit diameter and *S. boissieri* subsp. *boissieri* has the smallest. However, in general there is no big difference between the weights and diameters of fruits and seeds. Even though the values were close to each other; the largest seed diameter was measured at halophytic ecotype of *S. turcica* and the smallest at *S. boissieri* subsp. *boissieri.*

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 3 and Figure 2.

TABLE 3. Germination results of taxa

S. boissieri* subsp. *serpentinicola					
	Germination percentage	**Germination rate**	**Last germination (%)**	**Viability (%)**	**DGP**
Darkness (Distilled water)	59	-	-	-	-
Photoperiodism (Distilled water)	65	12.8	73	73	-
100 mM NaCl	46	12.7	52	52	29.2
200 mM NaCl	40	10.1	43	43	38.4
300 mM NaCl	33	12.9	33	33	49.2
S. boissieri* subsp. *boissieri					
	Germination percentage	**Germination rate**	**Last germination (%)**	**Viability (%)**	**DGP**
Darkness (Distilled water)	76	-	-	-	-
Photoperiodism (Distilled water)	100	24.9	100	100	-
100 mM NaCl	59	17.3	60	60	41.0
200 mM NaCl	57	16.2	65	65	43.0
300 mM NaCl	55	13.8	59	59	45.0
***S. turcica* (halophytic ecotype)**					
	Germination percentage	**Germination rate**	**Last germination (%)**	**Viability (%)**	**DGP**
Darkness (Distilled water)	69	-	-	-	-
Photoperiodism (Distilled water)	77	22.9	80	80	-
100 mM NaCl	74	22.5	82	82	3.8
200 mM NaCl	70	21.6	84	84	9.1
300 mM NaCl	45	12.5	55	55	41.5
***S. turcica* (gypsicole ecotype)**					
	Germination percentage	**Germination rate**	**Last germination (%)**	**Viability (%)**	**DGP**
Darkness (Distilled water)	90	-	-	-	-
Photoperiodism (Distilled water)	96	35.4	96	96	-
100 mM NaCl	85	32.4	89	89	11.4
200 mM NaCl	88	29.3	91	91	8.3
300 mM NaCl	83	25.2	86	86	13.5

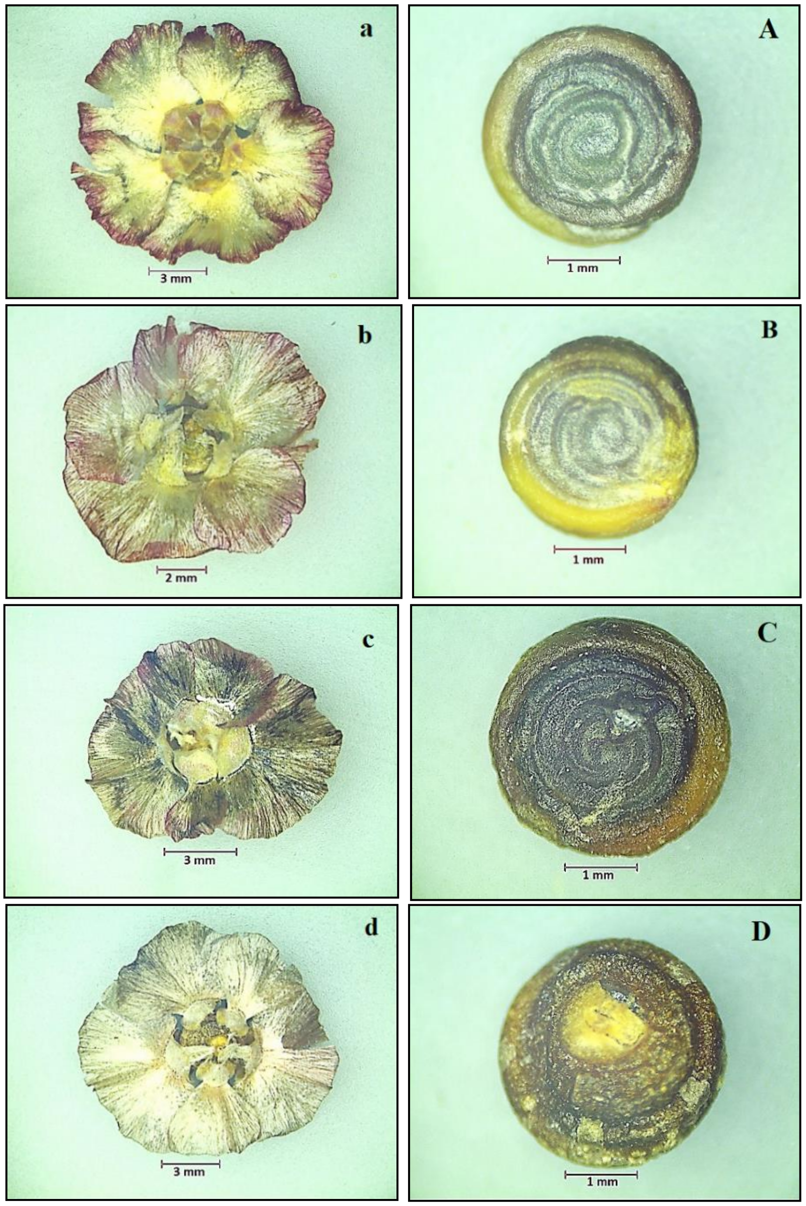

FIGURE 1. Fruits of analysed taxa (**a.** *S. boissieri* subsp. *serpentinicola*, **b.** *S. boissieri* subsp. *boissieri*, **c.** *S. turcica* (halophytic ecotype), **d.** *S. turcica* (gypsicole ecotype)) and seeds of taxa (**A.** *S. boissieri* subsp. *serpentinicola*, **B.** *S. boissieri* subsp. *boissieri*, **C.** *S. turcica* (halophytic ecotype),**D.** *S. turcica* (gypsicole ecotype))

All of the previous studies about halophyte germination showed that the seeds showed better germination ratios at distilled water [8-20]. Our results are consistent with the former results and we also found better germination ratios at distilled water (Figure 2, Table 3); and increasing NaCl concentrations inhibited the seed germination [18, 26, 37, 43-44].

Also, when the results of complete darkness were evaluated, it was found that light stimulates the germination and for this reason 12/12 h photoperiodism was used during the germination trials. The comparisons of the germination percentages at light and complete darkness and also changing NaCl conditions can be seen in Figure 2.

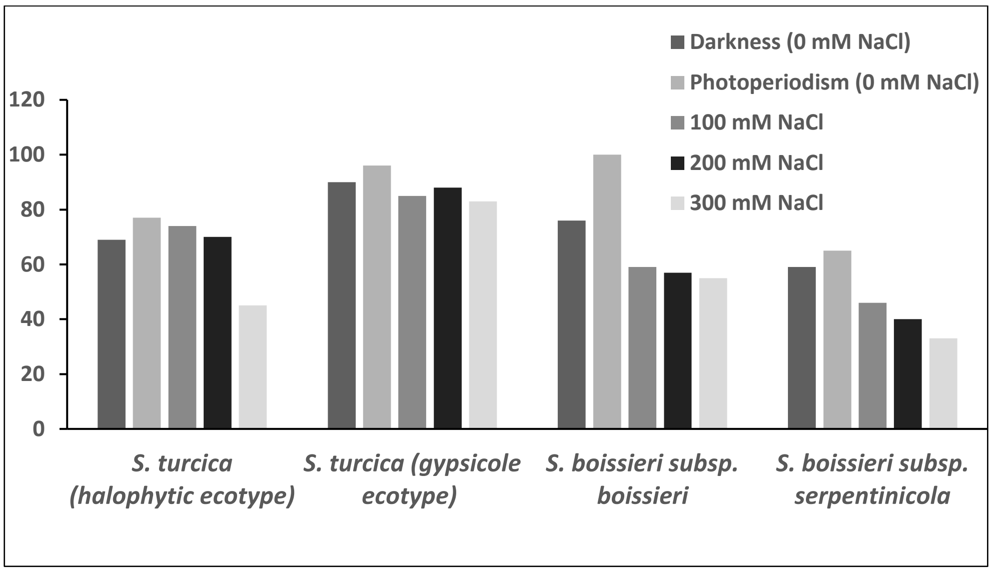

FIGURE 2. Germination percentages of each taxa at distilled water and at changing NaCl concentrations

The presence of halophyte taxa at their habitats is shaped by their salinity tolerance [45] and germination phase of their life cycles defines edaphic conditions that they are facing with throughout their life span [46] so salinity, light, photoperiodism etc. influence germination ability at time and space [6].

According to our results, not only the germination percentages and rates but also the seed viabilities of all taxa decreased with increasing salinity (Table 3). Likewise, there is a negative correlation between last germinations and increasing salinities.

Halophytes show species specific salinity tolerance at germination and seedling phases [42] but for our study this specificity is not so obvious which may be caused by very close relations of the studied taxa. Formulize DGP (decreasing germination percentage) to quantify the negative correlation between salinity tolerance and germination percentages, higher the DGP lower the salinity tolerance.

According to the statistical analysis, even though these closely related taxa prefer different edaphic conditions, there is not any statistically important difference between germination percentages at different salt concentrations, last germination percentages, germination rates and seed viabilities (F=1.818 p>0.05)(One Way ANOVA, SPSS 25).

Seeds did not germinate after salinity treatments were taken into viability test. At the end of TTC test colour change denotes viability as can be seen from Figure 3. According to the classification used by [47-51] seeds stained totally red are accepted as viable.

FIGURE 3. Un-viable (partially stained) and viable seeds (completely satined red) after TTC

According to our results, we concluded that light stimulates germination better than complete darkness at both of the taxa. The highest salinity tolerance among these three taxa was observed at both halophytic and gypsicole ecotypes of *S. turcica* and according to salinity tolerance the most vulnerable one is the *S. boissieri* subsp. *serpentinicola*. Both of the examined taxa showed better germination at distilled water which is consistent with the general knowledge about salinity tolerance mechanism of halophytes [12, 52-53]. Even though these three taxa are closely related, their salinity tolerances are different from each other, especially according to calculated DGP values. The studied taxa are phylogenetically close to each other [25, 28-29] but because of their habitat specifications they evolved some physiological adaptations at germination phase and ecotypes of *S. turcica* are more tolerant than the subspecies of *S. boissieri* are glycophytes.

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Conflict of interest The authors declare that they have no conflict of interest.

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