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In vitro germination and vegetative growth characteristics of *Gypsophila pilulifera* (*Caryophyllaceae*) seeds grown under abiotic stress conditions

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Abiyotik stres koşullarında yetiştirilen *Gypsophila pilulifera* (*Caryophyllaceae*) tohumlarının in vitro çimlenme ve vejetatif büyüme özellikleri

Abstract: *Gypsophila pilulifera* Boiss. & Heldr. is an economically important critically endangered (CR) endemic species of Turkey. This species is a Mediterranean element and type sample is located in square C3 (Antalya). Plants are much more sensitive to abiotic stress factors, especially during germination and seedling development stages. In this study, *in vitro* germination and seedling growth properties of *G. pilulifera* Boiss. & Heldr. (*Caryophyllaceae*) were investigated under salt and drought stress. The seeds were cultured in hormone-free MS media containing increasing doses of NaCl (50, 100, 150, 200 mM) and PEG 6000 (5%, 10%, 15% and 20%). Germinated seeds were counted every three days for the first week and every day for the second week. The root and hypocotyl length, number of leaves, fresh weight and vigor index of the plants were determined at the end of 21 days. A negative correlation was determined between increased salt and drought stress and all vegetative growth parameters. Although germination was obtained on MS medium with the highest level of drought (20%), there was no germination on MS medium containing 200 mM NaCl. As a result, it was determined that the plant was more sensitive to salinity stress than drought and high salt concentrations inhibited the germination by 100%.

Keywords: Drought, *Gypsophila pilulifera*, salinity, vegetative growth, vigor index.

Özet: *Gypsophila pilulifera* Boiss. & Heldr. Türkiye'de ekonomik açıdan önemli, nesli tükenmekte olan (CR) endemik bir türdür. Bu tür bir Akdeniz elementidir ve tip örneği C3 karesinde (Antalya) bulunmaktadır. Bitkiler, özellikle çimlenme ve fide gelişme aşamalarında abiyotik stres faktörlerine çok daha duyarlıdır. Bu çalışmada *in vitro* koşullarda *G. pilulifera* Boiss. & Heldr. (*Caryophyllaceae*) 'in tuz ve kuraklık stresi altında çimlenme ve fide büyüme özellikleri incelendi. Tohumlar, artan NaCl (50, 100, 150, 200 mM) ve PEG 6000 (%5, %10, %15 ve %20) dozlarını içeren hormonsuz MS ortamında kültüre alındı. Çimlenen tohumlar ilk hafta üç günde bir ve ikinci hafta için her gün sayıldı. Bitkilerin kök ve hipokotil uzunluğu, yaprak sayısı, taze ağırlık ve canlılık indeksi 21 gün sonunda belirlendi. Artan tuz ve kuraklık stresi ile incelenen tüm bitkisel büyüme parametreleri arasında negatif bir korelasyon belirlendi. Ayrıca kuraklık oranı en yüksek olan MS besiyerinde (%20) çimlenme elde edilmesine rağmen 200 mM NaCl içeren MS besiyerinde çimlenme elde edilemedi. Sonuç olarak bitkinin tuzluluk stresine kuraklığa göre daha duyarlı olduğu ve yüksek tuz konsantrasyonlarının çimlenmeyi %100 engellediği belirlenmiştir.

Anahtar Kelimeler: *Gypsophila pilulifera*, kuraklık, tuzluluk, vejetatif büyüme, vigor indeks.

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1. Introduction

In plants faced with abiotic and biotic environmental stresses, especially abiotic stresses such as drought, salinity, and extreme temperature affect all stages of growth and development. The stages where plants are most susceptible to abiotic stress are seed germination, seedling development, and flowering stages (Patade et al., 2011; Partheeban et al., 2017). Abiotic stress, which reduces the average crop yield in cultivated plants by more than 50%, is the primary cause of crop yield loss worldwide (Wang et al., 2004). As a result of stress, biological tension which is explained as a change in plant metabolism and morphology and a decrease in growth is occurred (Salisbury and Ross, 1992). Plants react to this tension with some physiological and metabolic changes and in this way they try to overcome stress conditions with the least damage (Kalefetoğlu and Ekmekçi, 2005). Understanding the response of plants to drought and salinity stress, which is a common problem in many parts of the world, has therefore become an important issue (Jamil et al., 2011; Korkmaz and Durmaz, 2017).

Gypsophila species, which are found as annual, biennial or perennial, belong to the *Caryophyllaceae* family of the order Caryophyllales. The name 'Gypsophila' has been given to a group of plants that adapt to gypsum environments (Williams, 1989). Species belonging to this genus distribute in arid areas around Anatolia in Turkey. (Davis et al., 1988). The roots of species belonging to the genus *Gypsophila* are rich in triterpenoid saponins. Saponins are commercially important substance used commercially in the detergent, adjuvant and cosmetic industry because of their emulsifying and foaming properties. In addition, phytochemicals, including saponins, have the potential to be used against various diseases such as cancer. Therefore, it is also used for medical purposes (Mujeeb et al., 2014; Arslan et al., 2012; Gevrenova et al., 2010).

In Turkey, sixty three *Gypsophila* species are known and 41 of these species are endemic (Özçelik and Özgökçe, 2021) and according to IUCN, *G. pilulifera* Boiss. & Heldr.

appears to be in the Critically Endangered (CR) category (Ekim et al., 2000).

In addition, the species is very rich in saponins (Arslan et al., 2012) and has great commercial importance. In the study, it was aimed to investigate the *in vitro* germination and vegetative growth tolerance of *G. pilulifera*, a Mediterranean element is endemic to Turkey and is in the CR category under drought and salinity stress. In this way, besides contributing to the protection of the natural populations of the species, it would also be contributed to determine suitable conditions for the production of this species, which also has economic and medicinal value. As a result of climatic and ecological changes, the survival of the species, which is already potentially endangered, will be severely restricted due to possible drought and salinity stress in the future. In this context, by researching the production potential of this species against possible stresses and ensuring its production by creating the determined optimum conditions, will be prevented from extinction from nature, and its use in landscaping and medicine will be increased.

2. Materials and Method

2.1. Material

In the study, the seeds of *G. pilulifera* which grown in Lara locality of Antalya province were collected in October 2019. The experiments were carried out in the Organic Agriculture Laboratory of Vocational School of Technical Sciences of Akdeniz University, in June 2020.

2.2. Sterilization of Seeds

The seeds dried on blotting papers were packed and stored at + 4 ° C in the dark. The seeds were washed with detergent before surface sterilization and kept under running tap water for 15 minutes. For surface sterilization, the seeds were kept in 20% sodium hypochlorite (NaOCl) solution for 20 minutes and then kept in 70% ethyl alcohol for 2 minutes. Then, sterilization was completed by rinsing in sterile distilled water 3 times for 5 minutes.

2.3. Germination and Vegetative Development Parameters

In order to determine the effects of salt and drought on germination and vegetative growth parameters, hormone-free MS medium containing NaCl (50, 100, 150, 200 mM) and PEG 6000 (5%, 10%, 15% and 20%) at different concentrations were used. MS medium (Murashige and Skoog, 1962) without NaCl and PEG 6000 was used as the control medium. After adding 30 g/L sucrose to the medium as a carbon source, the pH was adjusted to 5.7 and it was autoclaved after adding 7 g/L agar. PEG 6000 was added to sterilized media after sterile filtering.

The trials were set up in three duplications with 10 seeds per replicate according to the randomized plot design. Germination percentages of the seeds was determined at the end of the first week and at the end of the second week. Radicle and hypocotyl lengths, fresh weights was measured and, survival times and vigor index were determined at the end of the third week. In accordance with the International Seed Testing Association (ISTA) rules, the trials for germination tests were completed on the 14th day (ISTA, 2007). The number of germinated seeds was determined by counting at approximately the same hour every day, based on the emerge of the radicle from the testa starting from the day the seeds were cultured. Percentage of germination and vigor index were determined according to Gosh et al. (2014) and Hu et al. (2005) respectively.

Germination percentage (%) = Number of seeds germinated / Total number of seeds placed in jars x 100 (Gosh et al., 2014)

Vigor index = [Germination percentage x (radicle length + hypocotyl length)] (Hu et al., 2005)

2.4. Analysis of Data

In both trials, the data were subjected to statistical evaluation with ANOVA test after testing their suitability to a normal distribution, and the differences between vegetative growth parameters were statistically determined by multiple comparison tests (TUKEY, DUNCAN). Pearson Correlation was used to evaluate the relationships between growth parameters, germination, and survival times.

3. Results

In the study, as a result of the statistical analysis, it was determined that there was a significant difference between drought and salt doses in terms of germination ($p < 0.001$). When the effects of salt and drought doses on germination rate were examined, it was found that stress increased with each dose increase compared to control. Germination occurred in MS medium containing 20% PEG, the highest dose used in drought stress trials, but the germination percentage decreased from 93% (in control group) to 43%. In salt stress trials, the germination percentage decreased significantly at each dose, and germination was not observed in seeds at a dose of 200 mM Nacl (Figure 1,2).

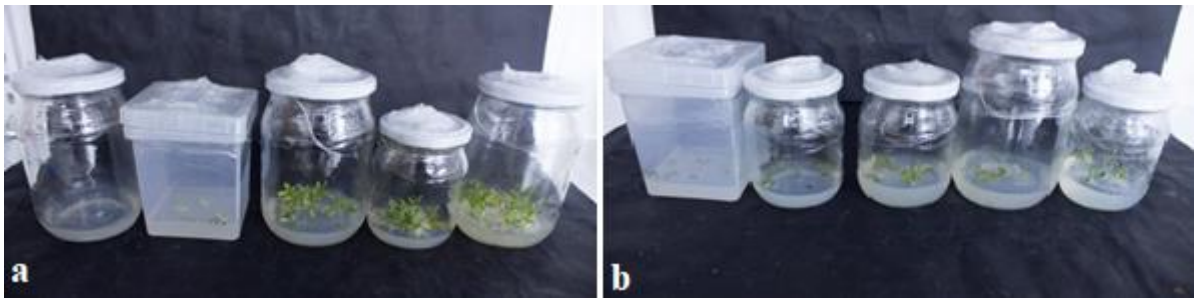

Figure 1. The appearance of the seeds at the end of the 2nd week in MS mediums containing different doses of drought and salinity (a. Drought: From right to left 0, 5%, 10%, 15%, 20% PEG 6000; b. Salinity: From right to left 0, 50, 100, 150, 200 mM NaCl

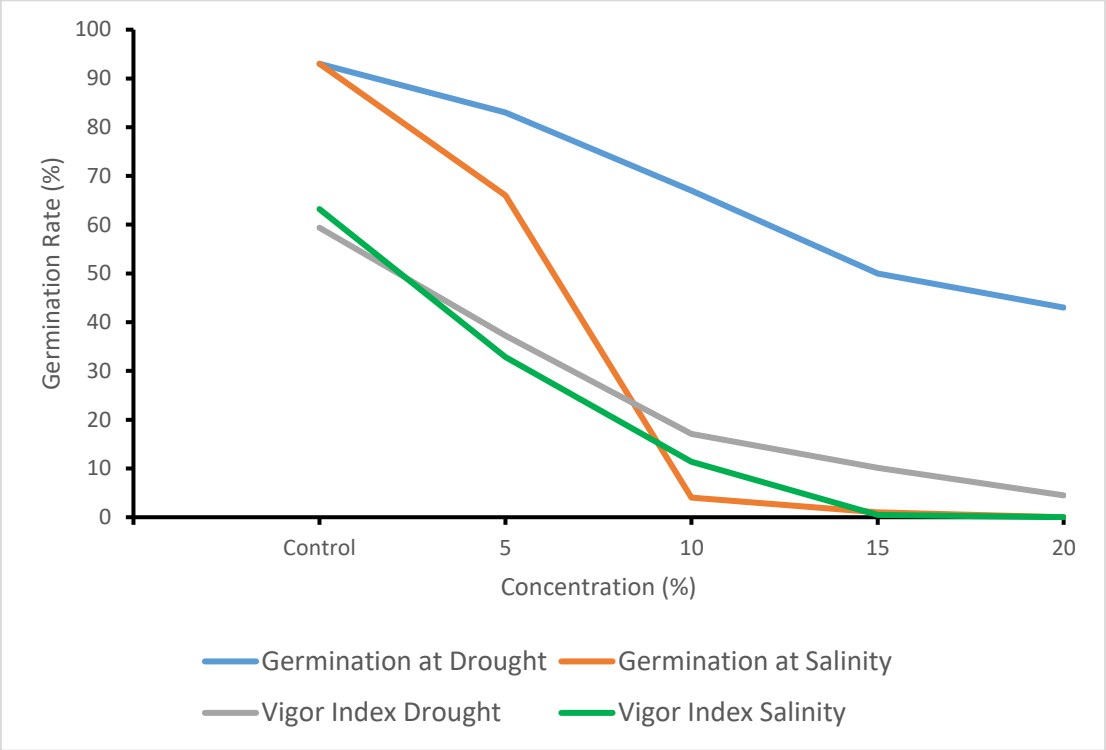

Figure 2. The germination percentages of seeds at the end of the 2nd week and vigor index at the end of the 3rd week in MS medium containing different doses of drought and salinity

A strong negative correlation was determined between increased salt and drought stress and radicle length, hypocotyl length, leaf number, plant fresh weight and survival time (Figure 3). On the other hand, there was a negative correlation between increased salt stress and germination time, while a positive correlation was found between increased drought stress and germination time.

Increasing drought stress did not make a statistically significant difference in terms of germination times. On the other hand, the length of the radicle, the length of the hypocotyl, the number of leaves, the fresh weight of the plant and the survival time decreased with the increase of drought ($r_{\text{Radicle Length}} = -0.802$; $p < 0.001$, $r_{\text{Hypocotyl Length}} = -0.816$; $p < 0.001$, $r_{\text{Number of Leaves}} = -0.786$; $p < 0.001$, $r_{\text{Fresh Weight}} = -0.793$; $p < 0.001$, $r_{\text{Germination Time}} = 0.168$; $p > 0.05$,

$r_{\text{Survival Time}} = -0.350$; $p < 0.001$). Besides, increased salt stress decreased the length of the radicle, hypocotyl length, number of leaves, fresh weight of the plant and the survival time, however, it caused a prolongation of the germination period ($r_{\text{Radicle Length}} = -0.801$; $p < 0.001$, $r_{\text{Hypocotyl Length}} = -0.799$; $p < 0.001$, $r_{\text{Number of Leaves}} = -0.793$; $p < 0.001$, $r_{\text{Fresh Weight}} = -0.783$; $p < 0.001$, $r_{\text{Germination Time}} = -0.553$; $p < 0.001$, $r_{\text{Survival Time}} = -0.693$; $p < 0.0001$).

Also, with increasing drought stress, the differences in terms of radicle length ($F = 83.286$; $df_1 = 4$; $df_2 = 145$;

$p < 0.001$), hypocotyl length ($F = 94.099$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), number of leaves ($F = 77.913$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), plant fresh weight ($F = 83.417$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$) and survival time ($F = 5.213$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$) were significant in all concentrations. There was no statistical difference between increased drought stress and germination time (Table 1). On the other hand, with increasing salinity stress, the differences in terms of radicle length ($F = 97.618$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), hypocotyl length ($F = 94.765$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), number of leaves ($F = 109.154$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), plant fresh weight ($F = 83.417$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$), survival time ($F = 35.074$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$) and germination time ($F = 18.239$; $df_1 = 4$; $df_2 = 145$; $p < 0.001$) were significant (Table 2).

Vigor indexes of *G. pilulifera* seeds showed a negative correlation at all concentrations of increased salt and drought stress. In drought stress, the vigor index, which was 59.38 in the control group, decreased to 4.49 at 20% PEG concentration. A sharp decline was seen in salt stress conditions. The vigor index, which was 63.19 in the control group, almost halved at 50 mM NaCl concentration and reached 32.89. It decreased to 0 at 200 mM NaCl concentration (Fig. 2).

4. Discussions

Turkey is the gene center of the *Gypsophila* genus, which has high economic value due to the saponins they contain (Özçelik and Muca, 2010). The use of most of the species belonging to this genus from nature without culturing causes to be extinct of these species (Özçelik and Yıldırım, 2011). *G. pilulifera* Boiss. & Heldr. (Caryophyllaceae) is also not cultivated and the areas where it is spread are open to anthropogenic effect. This situation may cause to decrease the population density of the species and eventually to extinction. Researching the growing conditions and tolerance to abiotic factors of this endemic species, which also has medical-economic value, is important for its culture and production. In addition, increasing the agricultural production by selecting plants tolerant to stress factors has become an inevitable necessity in today's world where abiotic stress factors are showing more and more effects.

Table1. Vegetative growth values of *G. pilulifera* at different drought concentrations

	Doses (mM)	Mean±SE			N
			Tukey HSD[a]	Duncan[a]	
Radicle Length	0	31.833±1.616	d	d	30
	5	20.633±1.732	c	c	30
	10	9.533±1.265	b	b	30
	15	5.567±1.042	ab	a	30
	20	2.833±0.612	a	a	30
	Total	14.080±1.055			150
	ANOVA	**F=83.286; df_1=4; df_2=145; p<0.0001**			
Hypocotyl Length	0	27.767±1.407	d	d	30
	5	17.400±1.470	c	c	30
	10	7.700±1.028	b	b	30
	15	4.567±0.859	ab	ab	30
	20	1.700±0.369	a	a	30
	Total	11.827±0.922			150
	ANOVA	**F=94.099; df_1=4; df_2=145; p<0.0001**			
Number of Leaves	0	11.400±0.622	d	d	30
	5	7.567±0.657	c	c	30
	10	3.067±0.437	b	b	30
	15	1.933±0.365	ab	ab	30
	20	1.133±0.266	a	a	30
	Total	5.020±0.385			150
	ANOVA	**F=77.913; df_1=4; df_2=145; p<0.0001**			
Fresh Weight	0	0.265±0.014	d	d	30
	5	0.170±0.015	c	c	30
	10	0.086±0.012	b	b	30
	15	0.030±0.006	a	a	30
	20	0.030±0.006	a	a	30
	Total	0.116±0.008			150
	ANOVA	**F=83.417; df_1=4; df_2=145; p<0.0001**			
Germination Time	0	6.400±0.361	a	a	30
	5	6.767±0.615	a	a	30
	10	6.600±0.739	a	a	30
	15	5.667±0.920	a	a	30
	20	5.333±0.926	a	a	30
	Total	6.153±0.330			150
	ANOVA	**F=0.700; df_1=4; df_2=145; p>0.593**			
Survival Time	0	13.067±0.648	c	b	30
	5	11.667±0.969	bc	b	30
	10	10.200±1.143	abc	ab	30
	15	7.800±1.270	ab	a	30
	20	7.267±1.267	a	a	30
	Total	10.000±0.511			150
	ANOVA	**F=5.213; df_1=4; df_2=145; p<0.0001**			

Previous studies also enlighten the negative impact of salt stress on plant growth and development. Kumar (2013) reported that the germination percentage, germination rate and normal seedling percentage decreased in plants under salt stress. Due to the decrease in photosynthesis rate in drought stress, there is a decrease in vegetative growth, stem development and especially leaf development are more sensitive to water deficiency than root development (Çırak and Esendal, 2006). In the first periods of drought conditions, root growth was triggered and stem elongation slowed down in order to reach more water (Öztürk, 2015).

Karakaş et al. (2015) researched the tolerance of seeds of *Salsola soda* L. and *Portulaca oleracea* L., which are salt stress tolerant halophyte plants to increased NaCl doses under *in vitro* germination conditions. At the end of the second week, they found that there was a negative relationship increased salt concentration with the germination percentage, radicle and hypocotyl length, fresh weight, germination and vigor index of germinating seeds.

Şimşek et al. (2018) found that citrus rootstocks continued to survive and reproduce at increasing PEG doses under *in vitro* conditions, but their performance deteriorated. Ertekin et al. (2017) found that with the increasing salt concentration in 4 different common vetch varieties, germination rates, germination indices, root lengths, stem lengths and shoot fresh weights decreased significantly, and average germination times increased. Similar to these researches, radicle length, hypocotyl length, leaf number, plant fresh weight and survival time of *G. pilulifera* were significantly decreased under increasing salt and drought conditions compared to control application.

Germination percentage decreased under salt stress conditions in *G. oblanceolata* Barkoudah, which is an endemic and endangered halophyte species in Turkey. Only

Table 2. Vegetative Growth Values of *G. pilulifera* at Different Salt Concentrations

	Doses (mM)	Mean±SE			N
Radicle Length	0	34.633±1.776	d	d	30
	50	17.900±2.376	c	c	30
	100	6.533±1.500	b	b	30
	150	0.100±0.0557	a	a	30
	200	0,000±0.000	a	a	30
	Total	11.833±1.260			150
	ANOVA	**F=97.618; df_1=4; df_2=145; p<0.0001**			
Hypocotyl Length	0	28.800±1.465	d	d	30
	50	15.300±2.042	c	c	30
	100	5.333±1.224	b	b	30
	150	0.367±0.212	a	a	30
	200	0.000±0.000	a	a	30
	Total	9.960±12.881			150
	ANOVA	**F=94.765; df_1=4; df_2=145; p<0.0001**			
Number of Leaves	0	12.800±0.688	d	d	30
	50	5.400±0.737	c	c	30
	100	2.133±0.498	b	b	30
	150	0.267±0.158	ab	a	30
	200	0.000±0.000	a	a	30
	Total	4.120±0.450			150
	ANOVA	**F=109.154; df_1=4; df_2=145; p<0.0001**			
Fresh Weight	0	0.260±0.015	c	d	30
	50	0.115±0.015	b	c	30
	100	0.041±0.009	a	b	30
	150	0.008±0.004	a	a	30
	200	0.000±0.000	a	a	30
	Total	0.085±0.009			150
	ANOVA	**F=96.772; df_1=4; df_2=145; p<0.0001**			
Germination Time	0	6.067±0.325	b	c	30
	50	5.200±0.737	b	c	30
	10	4.867±0.846	b	c	30
	150	1.800±0.668	a	b	30
	200	0.000±0.000	a	a	30
	Total	3.587±0.326			150
	ANOVA	**F=18.239; df_1=4; df_2=145; p<0.0001**			
Survival Time	0	13.067±0.648	c	c	30
	50	9.333±1.225	b	b	30
	100	7.267±1.266	b	b	30
	150	1.767±0.784	a	a	30
	200	0.000±0.000	a	a	30
	Total	6.287±0.563			150
	ANOVA	**F=35.074; df_1=4; df_2=145; p<0.0001**			

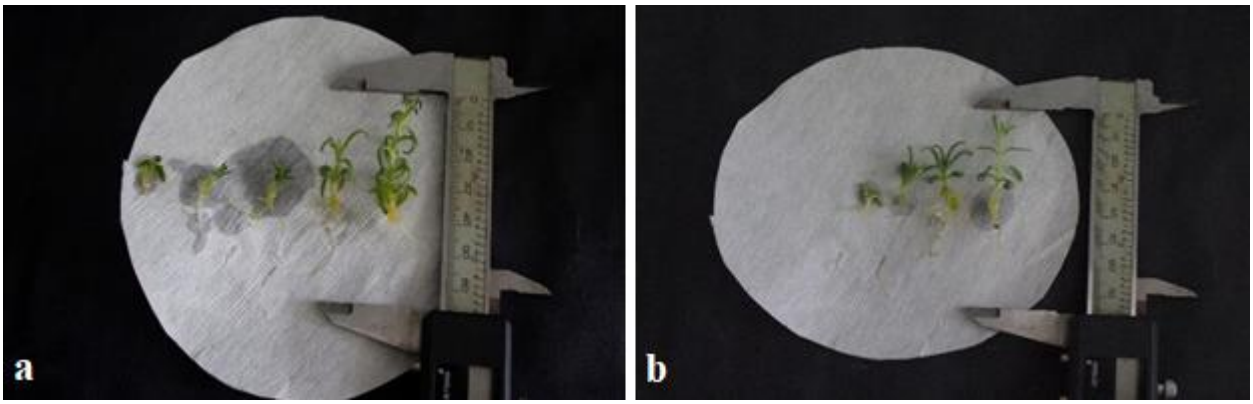

Figure 3. Radicle-hypocotyl length and number of leaves of plants at the end of the third week at different drought and salinity doses (a. Drought: 5%,10%, 15%, 20% PEG 6000 from right to left; b. Salinity: 0, 50, 100, 150 mM NaCl from right to left.)

a few seeds were able to germinate at 100 mM salt concentration (Sekmen et al. 2012). Consistent with this result, *G. pilulifera* was also more sensitive to salt stress than drought stress, and germination rate and vigor index were reduced by half in saline conditions compared to control plants. Even no germination was obtained in the presence of 200 mM NaCl.

While the germination time showed a homogeneous distribution under increasing drought stress, germination

times were also delayed in increasing salinity stress. In addition, at 200 mM NaCl concentration, the vigor index decreased to zero. These results showed that *G. pilulifera* was more sensitive to salinity stress and germination tolerance was higher in drought stress than salinity stress. *G. aucheri* Boiss., a xerophytic plant, was also found to be more tolerant of drought conditions, similar *G. pilulifera* (Esen et al., 2012). The Red List Index, which tracks the average extinction of the species over time, shows that the generations of the endangered groups become more at risk over time (Kurt, 2017). As with many species that have spread in narrow areas and evolved under difficult conditions, the rare compounds found in this species are used in many sectors and their economic importance increases due to these rarity. Crude saponin extract obtained from *G. pilulifera* species, which is in the critically endangered (CR) category and spread in an area open to andropogenic effects, is effective on *Bacillus subtilis* (Özbek Yazıcı and Özmen, 2018). Also, stem extracts and their fractions have free radical scavenging effects (Chima et al., 2014) the species whose roots are very rich in triterpenoid saponins (Arslan et al., 2012) has the potential to be used for both cut flower and landscaping (Kaya et al., 2012) is a very important species in terms of economics. In this study, germination and growth responses of *G. pilulifera* species against drought and salt stress were determined. It has been detected that the species can be grown under increasing drought conditions, but it is important to determine the salinity of the soil, especially by soil analysis. It is thought that the data obtained at the end of this study will contribute to the production and sustainability of the species under salt and drought stress conditions, which are predicted to increase even more in the coming years.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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