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STUDIES ON THE GERMINATION OF AN ENDEMIC SPECIES *CENTAUREA TOMENTELLA* HAND.-MAZZ.

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Abstract

Centurea tomentella an endemic species of Turkey is facing a threat of extinction due to its overuse for the decorative purposes. The species is under an abiotic stress of salinity in its natural habitats. Studies on the germination of this species were thus started in order to evaluate the possibilities which would enable us to sustain and cultivate the native populations on a large scale. The germination behaviour of this species was investigated under two different light regimes (8 h light-16 h darkness and 16 h light- 8 h darkness) at a constant temperature of 25 ^{o}C. The results showed that germination was higher (59.50 %) in 16h light- 8 h darkness, but lower in 8h light-16h darkness (52.25 %). Different concentrations of NaCl and KNO_3 were applied to assess if any improvement in germination could be obtained. Both the rate as well as total germination were higher at 0.25 % KNO_3 under 8/16 h light/dark condition as compared to the control. The effect of 0.25 % KNO_3 was significant, but light interactions were non significant.

Introduction

Germination is a crucial stage in the life cycle of plants. Several internal and some external mechanisms regulate the seed germination in their natural habitats and any of these can determine whether a given seed will germinate in a certain place or not (Mayer & Poljakoff-Mayber, 1982). Although ecological conditions prevailing in a given habitat affect germination, microclimatic conditions prevailing in the immediate surroundings of the seed play a dominant role. The regulation of germination of seeds by light would be advantageous in adapting them to their habitat. Many a times, light and temperature interact during this phase and this interaction may be such as to make seeds sensitive to light only at certain temperatures but not at others. The influence of light in such responses is not clear (Gulzar *et al.*, 2001). The importance of light depends upon the size of the seed, its life form (annual or perennial) and the habitat of the species. Addition of exogenous KNO_3 also influences germination during this step. Nitrate-stimulated germination has been associated with gap detection in some species where nitrate levels are lower than that in bare soil (Pons, 1989). Recent physiological models have implicated an interaction of light quality, soil nitrate and phytochrome in light-affected seed germination (Vardar *et al*., 1973; Hilsort *et al*., 1990).

Seed germination in a number of common weed species is stimulated by the presence of nitrate ions in the soil solution (Vardar & Ozturk, 1969). KNO_3 has been previously shown to enhance germination in *Craspedia* sp. (Peishi *et al.* 1998). Positive responses to KNO_3 are linked to phytochrome (Grubisic & Konjevic, 1990).

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The soil may have a salt content which will osmotically delay or prevent germination. Tolerance to salinity during germination is critical for the establishment of plants in an area (Ungar, 1995; Gulzar *et al*., 2001; Khan & Gulzar, 2003a; Ashraf & Foolad, 2005). Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at optimum temperatures.

The genus *Centurea* is represented by 187 taxa in Turkey, out of which 114 are endemics, thus showing an endemism ratio of 60.1%. The gene center of this genus lies in Turkey (Duran & Duman, 2002; Turkoglu *et al.,* 2003; Uzunhisarcıklı *et al.,* 2005; Duman & Aytaç, 2006). One of the important endemic taxa from this group is *Centurea tomentella*. This species is recorded as threatened species in the Red Data Book of Turkish Plants (Ekim *et al.,* 2000). The populations of this endemic species are face to face with a threat of extinction due to its highly attractive flowers. The flowers are collected indiscriminately on a large scale and sold at the markets continuously which is threatening the sustainability of its populations and necessitates its protection and cultivation. As such, studies on the germination of this species were started in order to evaluate the possibilities to start *in-situ* and *ex-situ* conservation studies, which will enable us to sustain and cultivate the native populations on a large scale. This investigation was undertaken to evaluate the effects of light, salinity and KNO_3 in the germination of *Centaurea tomentella*, distributed in the semi-arid environments of Turkey.

Material and Methods

The seeds of *Centaurea tomentella* Hand.-Mazz., were collected from the area between Malatya and Darende (38º21' N 37º 40' E), at an altitude of 1270 m. Germination experiments were carried out in a growth cabinet (MLR-350 Model Sony, Japan) using 9 cm Petri dishes each with a double layer of filter paper. During the experiments, temperature was set at 25ºC ±1ºC and two photoperiods, 8/16 hours light/dark and 16/8 hours light/dark were used. The experimental series were set up using 0.25, 0.5, 0.75, 1 and 1.5%, concentrations of NaCl solution and 0.25, 0.5, 0.75, 1, 1.5 and 2 concentrations of KNO_3 solution. In the control series, only distilled water was used. In each experiment four replicates with 100 seeds per replica were used. The experiments were conducted for 36 days. The emergence of radicle was accepted as the criterion for germination and data were analyzed statistically for constructing ANOVA tables and the means were compared using Duncan Multiple Range test.

The rate of seed germination was calculated according to Djavanshir & Pourbeik (1976), Awodola (1994) and Yucel (2000) as follows:

$DTG=A= X_1 + X_2 + X_3 + X_4$

$DGP=B= A / 4$

$DGS=C= D \text{ (Days) x } B$

$GS= \Sigma \text{ (GP X 100) } / \Sigma \text{ GS}$ where

X_1, X_2, X_3 and X_4 are the numbers of seeds germinated daily, A is the total of these seeds, B is the mean of the daily germinated seeds in four different series, D is the number of days for seed germination, 100 is the constant and C is the daily germination rate.

DGT: Daily total germination; DGP: Daily germination percentage; DGS: Daily rate of germination; GS: Rate of Germination; $X_{1,\ 2,\ 3,\ 4}$ = 1, 2, 3 and 4 sets of Petri dishes. Phyisco-chemical characteristics of the soil collected from the habitats of all three populations of *C. tomentella* are presented in Table 1.

Table 1. Soil analysis of *C. tomentella* populations (0-30 cm).

	Pop.I	Pop.II	Pop. III
pH	7.90	8.10	8.20
$CaCO_3$ %total	6.90	6,82	6,50
Organic Mat. %	5.75	5.40	4.60
P_2O_5%	90	60	50
N%	0.75	0.90	0.70
Ca^{2+}ppm	11750	12500	9950
Mg^{2+}ppm	1450	1300	1250
K^+ ppm	6500	8000	7000
Na^+ ppm	60	75	90

Results and Discussion

Centurea tomentella is one of the important endemic species of Turkey possessing very bright, pink flowers and an attractive capitulum. The seeds are 5.5 – 7.0 mm long, bearing a pappus 7-10 mm in length. Each capitula contains on an average 70-82 seeds. It shows a very narrow distribution in the form of small populations in particular ruderal habitats between 600-1400 m altitudes in the steppes of Adiyaman (Lectotype), Kahramanmaras and Malatya in Turkey (Wagenitz, 1975). The species is tolerant to cold temperatures, but very sensitive to drought, and it grows on moderately alkaline calcareous soils, rich in $CaCO_3$ and organic matter content, but poor in nitrogen (Table 1). This species can be regarded as an indicator species for calcareous habitats. In the distribution areas of *C. tomentella* Mg^{2+} and K^+ ions show normal values. Major associates commonly found with this taxon are *Euphorbia virgata* Waldst.& Kit, *Anthyllis vulneraria* L. ssp. *boissieri* (Lang.) Bornm., *Coronilla varia* L. ssp. *varia*, *Lotus corniculatus* L. var. *corniculatus*, *Melilotus officinalis* (L.) Pers.,*Geranium collinum* Steph. Ex Willd., *Hypericum armenum* Jaub. & Spach., *Anchusa azurea* Mill. var. *azurea*, *Carduus adpressus* C.A. Mey, *Trigonella fischeriana* Ser., *Vicia cracca* L. ssp. *cracca*, *Anthemis cretica* L. ssp. *albida* (Boiss.) Grierson, *Carduus adpressus* C.A.Mey., *Trifolium alpestre* L., and *Centaurea armena* Boiss. All localities are typical natural grazing habitats and basal leaves are consumed by cattle heads deliciously.

Highest germination percentage (61.25 %) was obtained under 8/16 h dark / light condition at 0.25 % KNO_3 in *C. tomentella* (Table 2). The germination percentage in the control group under the same photoperiod was 59.5 %. The rate of germination too was higher in 0.25 % KNO_3 under 8 / 16 h light / dark photoperiod (Table 3). The germination was reduced by 2 % KNO_3, however, an application of 0.25 % KNO_3 improved the germination percentage. This study has shown that nitrate can stimulate germination in conditions which result in enhanced seedling survival.

Seed germination and early seedling growth are critical stages for the establishment of plant populations (Perez *et al.*, 1998; Al-Khateeb, 2006). Germination is most likely to occur at a time when seedlings can survive (Grappin *et al.,* 2000). Dormancy reduces the risk of seedling mortality, when moisture is limited and salinity high (El-Keblawy & Al-Rwai, 2005). Germination is also reduced in several species at unfavourable temperatures (Aiazzi *et al*., 2002). As regards the light, it is abundant only on the soil surface and physiologically active light flux rarely penetrates more than a few mm into soil. As such, it is an important environmental factor that interacts with temperature to regulate seed germination (Baskin & Baskin, 1998). Light requirement for germination may vary with temperature (El-Keblawy & Al-Rwai, 2005). The variations in light conditions play an important role in the seed germination, some seeds being affected adversely by darkness and others not (Redondo *et al.*, 2004). Species that live in highly specific habitats often produce seeds with highly specialised adaptations (Navarro & Guitián, 2003). Light and salinity interact during germination in a number of plants e.g., an increase in NaCl concentration may inhibit seed germination more in the dark than in light (Gul & Weber, 1999). A similar trend has been reported for some shrubs and forbs where inhibition is more substantial in dark than in light (Khan & Ungar, 1997). Also, both darkness and high salinity inhibit germination in *Limonium stocksii* (Zia & Khan, 2002).

Table 2. Effects of different NaCl and KNO_3 concentrations and 8/16 hours (h) dark/light (d-1) conditions on germination rate (GS) and germination percentage (G%) of *C. tomentella* (DW, distilled water, control series)

Concentration		GS	GS ± SD	G %	G % ± SD
NaCl	0.25%	10.76	10.76±0.380.38	41.75	41.75±1.50
	0.5 %	10.78	10.78±0.76	31.25	31.25±2.22
	0.75	9.99 abc	9.66±0.52	23.25	23.25±1.26
	1 %	10 abc	10±1.24	16.75	16.75±2.06
	1,5 %	8.41 a	8.41±0.62	6.75 a	6.75±0.50
KNO_3	0.25%	12.1 e	12.1±0.41	61.25 i	61.25±2.06
	0.5 %	10.6 cd	10.6±0.27	46.75	46.75±1.26
	0.75	10.6 cd	10.6±0.43	35 f	35±1.41
	1 %	10 abc	10±0.77	31.25	31.25±3.40
	1.5 %	9.3 abc	9.3±1.01	19.25	19.25±2.22
	2 %	9.1 ab	9.1±0.67	11 b	11±0.82
Control (8 h l-16 h l)	DW	12 de	12±0.28	59.50 i	59.50±1.29

*Within each column, means with the same letter are not significant; 95 % significant.

Table 3. Effects of different NaCl and KNO_3 concentrations and 16/8 hours (h) dark/light conditions on germination rate and germination percentage of *C. tomentella*.

Concentration		GS	GS ± SD	G %	G % ± SD
NaCl	0.25%	10.4 abc	10.4 ± 0.35	24 g	24 ± 0.82
	0.5 %	10.81 bcd	10.81± 0.7	14.75 f	14.75 ± 0.96
	0.75	10.1 abc	10.1 ± 0.43	11.75 de	11.75 ±0.50
	1 %	9.03 a	9.03 ±1.19	7.25 b	7.25 ±0.96
	1,5 %	9.48 ab	9.48 ±1.55	5 a	5 ± 0.82
KNO_3	0.25%	18.6 cd	18.6 ± 1.47	42.50 i	42.50 ± 1.29
	0.5 %	10.8 bcd	10.8 ± 0.58	31.75 h	31.75 ± 1.71
	0.75	10 abc	10 ± 0,.8	23 g	23 ± 1.83
	1 %	10.2 abc	10.2 ± 0.98	13.50 ef	13.50 ± 1.29
	1.5 %	10.7 bcd	10.7 ± 1.03	10.79 cd	10.79 ± 2.12
	2 %	10.3 abc	10.3 ± 0.64	9.25 c	9.25 ± 0.96
Control(8 h l-16 h d)	DW	11.01 cd	11 ± 0.12	52.25 j	52.25 ± 1.71

*Within each column, means with the same letter are not significant; 95 % significant.

Table 4. Univariate analysis of variance for germination percentage.

Source	df	Mean square	F-Value	Significance
Corrected Model	27	13.1	1.534	0.72
Intercept	1	12737.8	1496.706	.000
Treatments	13	18.8	2.216	.015
Photoperiod	1	16.1	1.888	.173
Treatments- Photoperiod	13	7.0	0.825	0.633

Table 5. Univariate analysis of variance for germination speed.

Source	df	Mean square	F-Value	Significance
Corrected Model	27	1204.6	475.178	.000
Intercept	1	75149.9	29611.05	.000
Treatments	1	2187.6	862.927	.000
Photoperiod	1	2888.9	1139.610	.000
Treatments-Photoperiod	13	92.1	36.139	.000

Soil salinity and moisture content of the habitat are accepted as the most important physical factors in the zonation of plants (Gul & Weber, 1999). However, growth behaviour of majority of the species in particular crop plants is negatively affected by the saline environments due to osmotic impacts, nutritional imbalance, specific ion effect or a combination of these factors depending on the plant species, salt composition and salt concentration (Huang & Redmann,1995; Fanny *et al.*, 2005; Ashraf *et al.,* 2005). Both rate as well as percentage of germination were lower than control series in all NaCl concentrations used by us and the germination was completely inhibited above 1.5 % NaCl concentration. The studies undertaken by several authors support our findings that higher salt concentrations reduce seed germination (Ozturk *et al.*, 1993; Gul & Weber, 1999; Zia & Khan, 2004). This can be attributed to the high osmotic pressure and growth stage of plants as reported by Chi & Huei (1995) and Raccuia *et al.,* (2003). The detrimental effect of NaCl are also attributed to the toxicity of Na^+ that usually causes irreversible damage (Bewley & Black, 1994; Ashraf, 2004). However, the inhibitory effect of salinity sometimes has an ecological significance because it prevents seeds from germinating in salt-affected habitats and consequently avoiding seedling mortality during this period when surface soil salinities are extremely high (Khan & Ungar, 1998). This might be the reason that *C. tomentella* completes its life cycle before the dry hot summer season starts in the steppes of Irano-Turanian plateau.

The germination rates under 8 / 16 h light / dark condition in 0.25, 0.50, 0.75% NaCl and 0.50, 0.75, 1, 1.5 and 2% KNO_3 treatments were near to the control. But germination percentages were much below the control series except 0.25% and 0.50% KNO_3 treatments. Similar findings were recorded under 8 / 16 h dark / light conditions. The germination percentage, in general, was higher under light condition in all treatment groups, but it had no effect on germination rate. According to univariate variance analysis (Tables 4 and 5), the results of germination percentage related to the treatments, photoperiods and interactions of these were significant, but non-significant regarding the rate of germination ($p < 0.05$).

It can be concluded that species growing in the East Anatolia region of Turkey require a number of cues to stimulate germination. These cues are generally associated with winter when continuous moisture is available to ensure seedling establishment.

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