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Seed germination behaviour of *Diplotaxis tenuifolia*

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Abstract
Genus *Diplotaxis* is represented by more than 20 species mainly distributed in the North Mediterranean. Out of these *Diplotaxis tenuifolia* (wild rocket) is used widely in Italian and French cuisine. It has also been used for medicinal purpose for different illnesses throughout the history. In this study the germination behaviour of the seeds of *D. tenuifolia* was investigated. The seeds were very sensitive to pH and salinity stress. The seed germination increased under daylight conditions reaching a level of 80 percent but decreased in dark and continuous light. A remarkable increase was recorded (60%) in the germination of seeds subjected to +4°C shock for a week or two before left for germination. Pre-hydration followed by re-drying increased the germination to some extent (28%). These findings reveal that the storage conditions are important for establishing a seed bank of *D. tenuifolia*, in order to get highest germination.

Keywords: Breaking dormancy, cold stratification, drying-rehydration, germination, stress.

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INTRODUCTION

The genus *Diplotaxis* DC. (Brassicaceae) comprises over 20 species with an essentially Circum-Mediterranean distribution (Perez-Garcia et al. 1995). *Diplotaxis tenuifolia* (L.) DC. is a perennial ruderal plant distributed in many sandy and calcareous areas of Mediterranean Basin. In Turkey the concentration of distribution of this species is especially high in Afyon, Kutahya and Isparta districts and also distributed along west part of Anatolia including European part (Bianco and Boari 1997).

It is economically and therapeutically important plant used as alternative food and some medical purposes. It is not common to consume as a food source around the world. But in Europe, particularly in Italian and French cuisine it is consumed directly as salad, seasoning or by cooking (Bianco 1995, Pignone 1997, Martinez-Laborde 1997). In the Americas, people have introduced with this species through European immigrants, who have brought this crop into their diet, especially with the younger generations searching for natural food (Pignone 1997).

Physiological treatments to improve seed germination and seed emergence under various stress conditions have been intensively investigated in the past three decades (Baskin and Baskin 1998, Zheng et al. 1998). A number of studies have established about seed, molecular and genetic structure and also soil-fertility relations of *D. tenuifolia* (Pignone 1997). However, germination behavior studies of *D. tenuifolia* are not exactly investigated.

The main objectives of this work: (1) to study the germination behaviuor of seeds of *D. tenuifolia* under the controlled light, different pH, salinity stress; (2) to study the germination response of seeds over time after storage; (3) to break down the dormancy of seeds using cold shocking and pre-hydrating and re-drying methods. For this purposes, different conditions for germination of seeds were prepared.

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MATERIAL AND METHODS

Seed supplies

Mature seeds of *Diplotaxis tenuifolia* (L.) DC. were collected from the dry inflorescences of the plants from natural populations from various locations in Turkey (Fig. 1) in October 2002 and subsequently mixed together. The seeds were stored in a hop-pocket, in a ventilated room with natural air and temperatures (22-28°C) with about 48% relative humidity. Seeds were treated using the bleach before the germination process.

Germination determination

Experiments were carried out with four replicates of 25 seeds each, on two filter paper disk, in 7 cm diameter petri dishes at room temperature. Germination was checked every 24 h for 7 d and seeds showing radicle emergence were counted for germination performance. End of the experiments the lengths of roots and shoots were measured.

For determining the effect of pH different acetate buffers were applied to seeds of *D. tenuifolia* at 5, 6, 7, 8 and 9 pH's. Salinity stress experiments were conducted at 0.05, 0.075, 0.1, 0.2, 0.3, 0.4 and 0.5 NaCl solutions. Germination behavior of seeds of *D. tenuifolia* was explored under continuous dark, continuous light and daylight (8 h dark and 16 h light) conditions. Continuous light experiments were carried out under irradiance of 35 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent tube (OSRAM L 58W/20). The seed, collected in November, 2002, were stored at room temperature for a year. Germination was controlled periodically (each month). After 36 weeks (when germination was not seen, the seeds, which was stored at room temperature) was applied to cold shock for a week at +4°C. Second set of seeds was pre-hydrated for 14 h with deionized water at 23°C. The hydrated seeds were briefly blotted with paper towels and then dried back to their original moisture content by leaving them in open dishes at 23°C, 60% r.h. for 16 to 18 h (Zheng 1984).

Statistical analyses were tested by one-way ANOVA at 95% by the Fisher PLSD and Scheffe F-test (Sokal and Rohlf 1995). Results of germination experiments are expressed in percentage (±S.E.).

Fig. 1. Distribution of *Diplotaxis tenuifolia* and sampling points.

Seed germination at different pH

Fig. 2 shows the final germination percentage of seeds of *D. tenuifolia*. There was no germination at pH 5, 6 and 9. At pH 7, germination was 96%. At pH 8, germination decreased to 32%. Data result means are not significantly different (P= 0.34836).

RESULTS AND DISCUSSION

Germination at different concentrations of NaCl solution

After 5 days of incubation, percentages of seed germination at different concentrations of NaCl were summarized (Fig. 3). When comparing the germination of the control of H_2O with germination in NaCl concentrations of 0.05, 0.075, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/L, there were no significant differences, (0.05 (P= 0.2429), 0.075 (P= 0.1635) and 0.1 (P= 0.1572)). Germination at higher concentrations (0.2, 0.3, 0.4 and 0.5 mol/L) were significantly lower than the control (P= 0.04416). The percentages of germination reached at lower initial concentrations (0-0.075 mol/L) were significantly higher than the higher concentrations (0.1-0.5 mol/L) (P= 0.0001) (Fig. 3).

Furthermore, it's also measured the

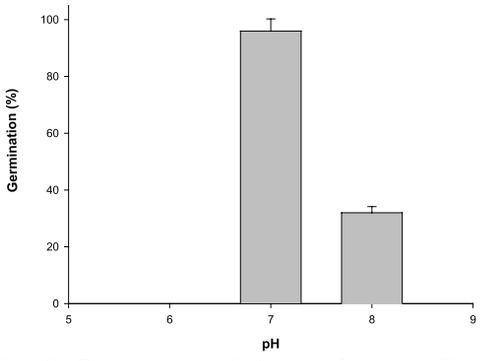

Fig. 2. Germination of *D. tenuifolia* at different pH.

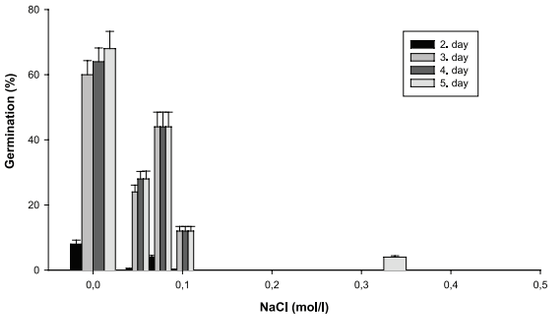

Fig. 3. Germination (% ±S.E.) of *D. tenuifolia* seeds in different concentrations of NaCl solutions (mol/L) after 5 days of wetting.

average length of stem and root of germinated seedlings and showed in Fig. 4. Lengths of root-stem couples are, 27.7-16.5; 22.2-16.2; 13.3-10.3; 7.7-5.8; 5.0-4 for control; 0.05; 0.075; 0.1 and 0.3 mol/L NaCl concentrations, respectively. Average length of stem and root were highly correlated (R^2 = 0.9595) and not significantly different (P= 0.0533) each other. And also have significant reverse correlation with NaCl concentrations (R^2 = 0.6582). Average length of root and stem decreasing, during the increasing of NaCl concentrations and have no significant difference (P= 0.05621).

This is fact that salt stress for seed germination is one of the most sensitive stress conditions (Khatri et al. 1991). Increasing of salt concentration in soil, make difficulties for water absorption by seeds. And of course water is needed for starting enzymatic activation for germination process (Bianco and Boari 1997). Bianco and Boari (1997) shows that the effect of salinity on germination of *D. tenuifolia* at 25°C as seen our experiment they also note that the increasing the salt

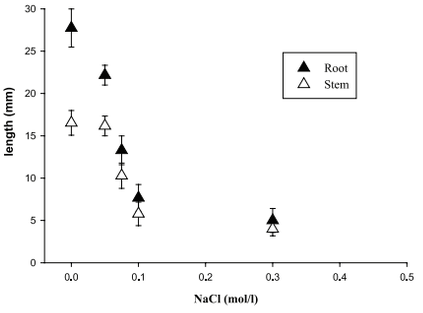

Fig. 4. Average length of stem and root of germinated seedlings in different concentrations of NaCl solutions (mol/L) after 5 d of wetting.

concentration, decrease the germination of seeds. Also salinity causes the marketable yield of wild rocket.

Seed germination in daylight, continuous light and darkness

Seed germinate behavior of *D. tenuifolia* was observed in daylight, continuous light and darkness and showed in Fig. 5. The maximum value of germination was achieved after 5 days of wetting. In daylight, maximum germination (80%) was observed. In darkness germination percentage was 64% and continuous light 56%. Difference was not significant between daylight vs. darkness (P= 0.0705), between daylight vs. continuous light there is also no significantly differences (P= 0.1112). There was significantly difference between daylight vs. continuous light vs. darkness (P= 0.01102).

Some seeds of plants germinate well in dark than in light and vise a versa. Improper light conditions can inhibit the germination (Bannister 1979). Seeds of only a few shrubs, including *Artemisia monosperma* Del., *A. ordosica* Krasch., *A. sphaerocephala* Krasch. (Huang et al. 2003, Huang and Gutterman 1999a, Huang and Gutterman 1999b, Huang and Gutterman 2000) *Capparis decidua* (Forssk.) Edgew. (Qaiser and Qadir 1971), *Pavonia arabica* Hochst. & Steud. ex Boiss. and *Salvia aegyptica* L. (Sen and Chatterji 1968), are highly sensitive to light for germination. Seeds of many shrubs germinate equally well in light and darkness, and those of some species germinate to higher percentages in darkness than in light (Baskin and Baskin 1998).

It shows that under the continuous light, seeds of *D. tenuifolia* expose to light stress

and germination was decreased. The high germination was observed at day light.

Germination in term to time period

Seeds, collected in 09.10.2002 were stored at room temperature, leave to germinate 2 weeks interval. After harvesting, 1st group of seeds left to germinate and 92% of them were germinated. The other seeds were germinated 2 weeks intervals for first mount, 4 weeks interval for rest of time. At that times germinations were 72, 48, 32, 28, 28, 24, 16 and 4% respectively (Fig. 6). After 32nd weeks germination dropped till 4%. At 36th week no germination was seen. There is high reverse correlation ($R^2 = 0.8734$, Fig. 6) between time and germination. Germinations were decreased significantly in term of time period ($P = 0.00342$). After this, seeds were soaked in water for 14 h, dried for 18 h and again soaked in water, finally left to germinate for three days. Germination was increased to 28% after this process.

Furthermore, 36 weeks old seeds expose to cold shock under +4°C for a week. Then these seeds were left to germinate. Although, no germination was seen at control group, 60% of cold shocked seeds were germinated after three days (Fig. 7).

Survival of stored seeds is related to some certain environmental factors as temperature, humidity, oxygen and carbon dioxide concentration. Lewis (1973) proved that, seeds of Brassicaceae member can survive 30 to 50 years under soil. To understand the germination behavior of seeds, several environmental factors as e.g. temperature, rainfall and water availability, nutrient availability, and photoperiod should be taken into account (Alexander and Wulff 1985, Potvin and Charest 1991, Wulff and Bazzaz 1992).

The germination of seeds of *D. tenuifolia* was decreased due to time interval. When seeds expose to different conditions as pre-hydration and re-drying and cold stress, germination rate was increased. Same results were obtained in Canola seeds (*Brassica rapa* L., *B. napus* L.), an other species of Brassicaceae, by Zheng et al. (1998). Cold stratification mostly makes sense about ABA induced dormancy, which cold breaks by ABA induced dormancies (Zhou et al. 2009). Showers also wash up the ABA in dormant seeds and let the seed to germinate.

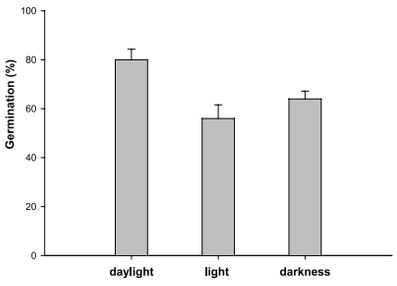

Fig. 5. Germination (% ±S.E.) of *D. tenuifolia* seeds under different light concentrations after 5 d of wetting.

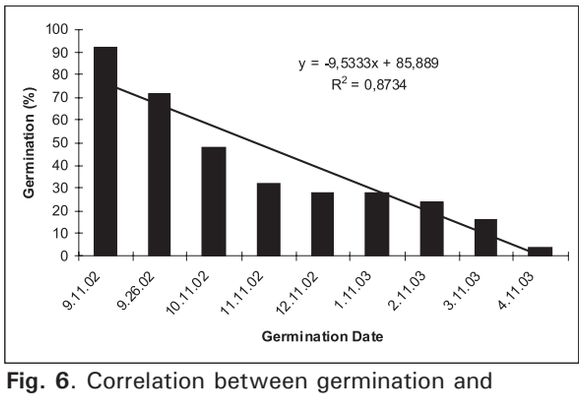

Fig. 6. Correlation between germination and germination date.

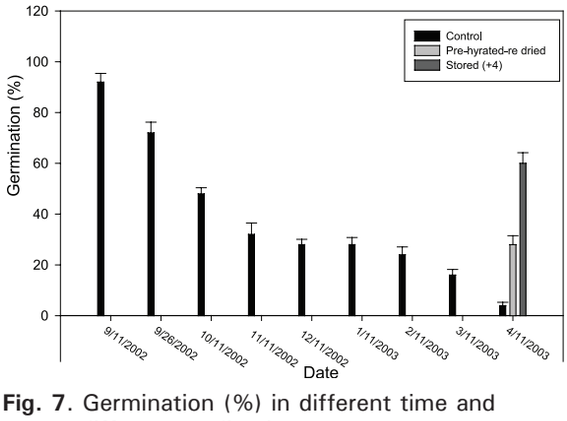

Fig. 7. Germination (%) in different time and different applications.

These findings also can explain the relationship between the distribution strategies of the species. Here the area, which *D. tenuifolia* was most abundant is transitional zone between, Mediterranean and Central West Anatolia plateau. This area is coldest parts of Mediterranean, due to sudden increase of Altitude and geomorphologic structure. Precipitation and temperature regime of area shows transitional properties

(Kocman 1994, Atalay and Mortan 2007). This data also completely overlap with germination behaviour with pre-hydration and re-drying and also with cold stratification.

Breaking dormancy and germination of seed is determined by permeability of testa and/or inhibitors. More over, some environmental factors as pH, salinity, light and climate are also effective on germination (Khatri et al. 1991). Beside of that factors, storage of seeds are very important to sustainability of species.

There was not much studies have been done on seed germination of *Diplotaxis* species (Bianco and Boari 1997). The seeds of *D. tenuifolia*, a perennial shrubby plant, distributed across Mediterranean Basin and some Australia and East part of USA.

Distribution of *D. tenuifolia i*s western part of Turkey. It lies from North to South from Edirne to Isparta. Population of plant is very dense, among Bilecik, Kutahya, Afyon and Isparta, which cities were accepted as transition zone between Mediterranean and Irano-Turanean. That is the temperature sometimes downs less than zero at winter. Further more summer rain regime is interesting. After the shower, sun was shining and gets the temperature warm. These conditions are significantly parallel with result of experiments. Most seeds of *D. tenuifolia*'s dormancy were broken under the harsh condition of winter. Remaining seeds were germinating in summer through the rain fall season.

CONCLUSION

The data, collected from this study are important to understand the germination strategies of *D. tenuifolia*, which have economically and ecologically importance these data may used in further studies to build a seed bank for reproducing and cultivation. For this, it should be known, how to plan the storage conditions for obtaining the most productive germination.

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Diplotaxis tenuifolia'nin Tohum Cimlenme Davranislari

Özet

Ozellikle Kuzey Akdeniz havzasinda yayilis gösteren *Diplotaxis* cinsine ait 20'den fazla tur vardir. *Diplotaxis tenuifolia* (yabani roka) özellikle Italyan ve Fransiz mutfaginda çokça kullanimaktadir. Ayrica tarih boyunca çesitli hastaliklar için tibbi kullanimi da kaydedilmistir. Bu çalismada *D. tenuifolia*'nin çimlenme davranislari incelenmistir. Tohumlar pH ve tuz stresine karsi çok hassastir. Gün isigi kosullarinda tohum çimlenmesi %80'leri asmakla birlikte karanlikta ve sürekli isik altinda çimlendirilen tohumlarda çimlenme verimi düsmektedir. Çimlenmeden bir veya iki hafta sonra +4°C'de korunan tohumlarda farkedilir derecede çimlenmenin arttigi (%60) kaydedilmistir. Islatma ve kurutma islemi yapilan tohumlarda da uzun süre bekletilen tohumlara göre %28'lik bir artis sözkonusudur. Bu bulgular da göstermektedir ki, *D. tenuifolia* için tohum bankasi olusturmada depolama kosullari çok önem tasimaktadir.

Anahtar Sözcükler: Çimlenme, dormansinin kirilmasi, kurutma ve yeniden islatma, soguk, skarifikasyonu, stress.