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Full Length Research Paper

Effects of cold stratification pretreatment and pH level on germination of *Centaurea tchihatcheffii* Fisch. et Mey. seeds

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***Centaurea tchihatcheffii* Fisch. Et. Mey. is a critically endangered (CR) plant species due to destruction of habitat, strong anthropogenic pressure, biotic and abiotic factors. It grows in limited areas in Gölbaşi district of Ankara, Turkey and in the world. It is very necessary to conserve it and prevent its extinction. Previous studies emphasise that low germination rate of its seed is primarily due to deep seed dormancy. Therefore, studies was done on *C. tchihatcheffii* seeds, to investigate the effects of cold stratification for different periods (90, 120 and 150 days) and different pH values (pH 6.5, 7.5 and 8.5) on seed germination. The highest germination percentage (58.89 ± 6.39%) was observed in seeds stratified for 150 days at all pH values of growing media, followed by seeds stratified for 120 days (42.78 ± 4.57%). An increase in pH level had negative effects on seed germination, the lowest germination (27.22 ± 4.18 %) was determined at pH 8.5.**

Key words: *Centaure tchihatcheffii*, critically endangered, seed germination, stratification, pH values.

INTRODUCTION

Turkey is one of the leading countries in terms of biological abundance and endemism, with about 33% endemic plants. Natural ecosystems are degrading and declining rapidly, because of increased human populations. The destruction of habitats through human encroachment seems to be the principal cause of the loss of biodiversity (Lopez-Pujol et al., 2006). Due to the rapid population increase in Turkey within the last few decades, many natural habitats have been fragmented and reduced in size or degraded (Kaya and Raynal, 2001). Gölbaşi district of Ankara is important for its complex ecosystem and its habitat is critically endangered (CR) of endemic annual plant species *Centaurea tchihatcheffii* Fisch. Et. Mey. family *Asterace*a per categorization of IUCN (Ekim et al., 2000; Güner et al., 2000; IUCN, 2001; Arif et al., 2004). Previous studies show that the plant show narrow endemism and is susceptible to extinction, with one of the most important reasons being the destruction of habitat primarily due to strong anthropogenic pressure, fast urbanization and seed dormancy (Wagenitz, 1975; Ekim et al., 2000; IUCN, 2001; Arif et al., 2004). The precise evaluation of the conservation status of this species is necessary to prevent its extinction. The plant bears pinkish red flowers, branches from the base, grows to a height of 30 to 40 cm and it is acclaimed as queen of all knapweed species (Figure 1). Although, government regulations prevent trade in critically endangered species, the beautiful flowers of the plant are collected illegally for use in cut flower industry during April-June season each year and its flowers could be easily found as trade commodity in the cut flower markets in Ankara.

Limited literature is available on taxonomy, biology, botanical characteristics and its conservation on *C. tchihatcheffii* (Boşgelmez, 2005 and 2006; Çakaroğullari,

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Figure 1. The appearance of the *C. tchihatcheffii*.

2005; Tan and Vural, 2007). Previous studies showed propagation of *C. tchihatcheffii* through plant tissue culture and cuttings (Özel, 2002; Özel et al., 2006a, b). On the other hand, the *C. tchihatcheffii* is propagated by seed under natural conditions, and its seeds have low germination. This does not make it feasible to be propagated by seed (Çakirlar et al., 2005, 2006). Propagation of this species has remained low in its natural habitat due to deep seed dormancy of embryo and needs a long vernalization to break it (Çakirlar et al., 2005 and 2006; Günöz, 2008; Okay and Günöz 2009). Tipirdamaz et al. (2006), emphasize that better propagation rates could be obtained through development of *in vitro* and *ex vitro* propagation techniques including propagation through seeds. Propagation of this plant by breaking seed dormancy could help in restoration of this ill fated species. Acquaah (2005), suggests that its seeds be kept in a multi stratification environment to meet the resting need of the seeds for improved germination and the pH level in the soil is also quite effective on rate of the seed germination. With the increase of the pH level, the available amount of microelement content decrease, which cause negative impacts on germination of the seeds and development of the plant (Acquaah, 2005).

In view of this, the aim of this study was to find the effects of cold stratification pretreatment and pH levels of the germination media on germination of *C. tchihatcheffii* seeds.

MATERIALS AND METHODS

The mature seeds (achene fruits) of *C. tchihatcheffii* were collected from Gölbaşi district of Ankara during June 2008. They were dried for a week at room temperature and categorized for similar morphological characteristics and seed size.

Stratification treatments

The seeds were put into plastic stratification cups (sized 10.5 x 15 x 3.5 cm) and stratified for 90, 120 and 150 days at 0±1°C with thin sand as stratification medium. The sterilization of the stratification medium was achieved by treating the medium with fungicides (Pilben 50 wp + 50% Benomyl; 4 g/l); and keeping it covered in greenhouse environment for three days followed by drying and spreading it under room temperature. Finally, it was kept at 70°C sterilizer for 24 h. The stratification cups were then surface sterilized with 5% NaOCl solution (Günöz, 2008). The selected seeds were stratified in the aforementioned medium. To keep stratification medium humid, it was sprayed with 150 ml of distilled water at periodic intervals.

The preparation of sowing medium and seed sowing

At the end of stratification periods, the seeds were sown at different pH values on the same day. The peat+perlite mixture (1:1) was used as the sowing medium which was disinfected with fungicides and the pH values were set at 6.5 (light acid), 7.5 (neutral) and 8.5 (alkaline) (Özcan et al., 2005; Günöz, 2008). The non treated surface sterilized seeds kept at room temperature without stratification were also sown as control treatment. The sowing trays werekept in environmental chambers at 25 ± 2°C and 70 to 80% humidity with 16 h light photoperiod following Çakirlar et al. (2005). The humidity of the sowing medium was checked every other day and maintained using distilled water set at 6.5, 7.5 and 8.5 pH. The sowing medium was disinfected by aforementioned fungicides.

The germination process was observed weekly and the germination percentages (%) including days to germination were determined for 90 days. The germination percentage was expressed as number of germinated seeds in percentages (%), while the germination time was evaluated according to Demir and Ellis (1992).

Statistical analysis

The experimental design was a randomized complete block design in factorial arrangement with three replications (100 seeds for each replication). Data from the treatments was analyzed using the Minitab 15.1 statistical software after arc-sinus transformation was applied to germination percentages to meet ANOVA assumptions. The Duncan multiple range test (DMRT) was performed to compare the differences between control and treatments using MSTAT computer software with significance level at (α) set at $p < 0.05$ or $p < 0.01$ (Snedecor and Cochran, 1967).

Table 1. The effects of the stratification applications and periods on the germination percentage (%) of *C. tchihatcheffii* seeds*.

Stratification application	pH 6.5	pH 7.5	pH 8.5	
No stratification (Control)	21.67	18.33	0.00	13.33 ± 4.00^{B}
90 days stratification	26.67	23.33	13.33	$21.11 \pm 2.61^{A,\ C}$
	24.17 ± 3.52^{A}	20.83 ± 2.01^{A}	6.67 ± 3.57^{B}	
No stratification (Control)	23.33	21.67	15.00	20.00 ± 2.20^{B}
120 days stratification	48.33	48.33	31.67	$42.78 \pm 4.57^{A,\ B}$
No stratification (Control)	$26.67 \pm 4.41^{b,\ A}$	$23.33 \pm 4.41^{b,\ A}$	$18.33 \pm 3.33^{b,\ A}$	
150 days stratification [1]	$76.67 \pm 4.41^{a,\ A}$	$63.33 \pm 6.01^{a,\ A}$	$36.67 \pm 4.41^{a,\ B}$	58.89 ± 6.39^{A}
Stratification Periods [2]	50.56 ± 8.01^{A}	45.00 ± 6.29^{A}	27.22 ± 4.18^{B}	

*The differences between the mean averages containing different letters are statistically important; [1]Small letters show the differences between the applications on mediums with the same pH values; capital letters show the differences between the pH values of the mediums in the same application; [2] Capital italic letters show the differences between stratification periods.

RESULTS

Germination percentage (%)

The results showed significant effects ($p < 0.01$) of pH and stratification applications for 90 days; with no interaction. Whereas, stratification period of 120 days showed significant effects of only stratification on seed germination ($p < 0.01$). 150 days stratification application showed significant interaction between pH and stratification application ($p < 0.05$). If we compare, stratification period and pH, we find that both of them affected seed germination separately with no interaction ($p < 0.01$) (Table 1).

The germination percentage of the seeds increased significantly in comparison with the control seeds at all pH values kept in the stratification medium for 90 days (control: 13.33 ± 4.00%, 90 days stratification: 21.11 ± 2.61%). The highest germination percentage was observed in mediums that were set at 6.5 (24.17 ± 3.52%) and pH 7.5 (20.83 ± 2.01%), with significantly lower seed germination (6.67 ± 3.57%) at pH 8.5. Of the seeds that were stratified for 120 days, the germination percentage (42.78 ± 4.57%) was significantly high when compared with the germination of the untreated (control) seeds (20.00 ± 2.20%) sown without stratification. It is important to note that the germination percentages of the seeds that were sown in pH 8.5 level were relatively low. The seeds stratified for 150 days showed a statistically higher germination percentage when compared to those without stratification. However, the germination percentage of seeds that were sown in mediums at pH levels of 6.5 and 7.5 (respectively 76.67 ± 4.41 and 63.33 ± 6.01%) were statistically high when compared to the germination percentage of seeds that were sown to mediums with a pH level of 8.5 (36.67 ± 4.41%) (Table 1). At all pH levels, the germination percentage of stratified seeds remained higher when compared to the germination of the control seeds (Figure 2).

Comparing the stratification periods, the highest germination percentage (58.89 ± 6.39%) was observed in seeds that were stratified for 150 days, followed by seeds stratified for 120 days (42.78 ± 4.57%). In the seeds that were stratified for 90 days, the germination percentage (21.11 ± 2.61%) in all the mediums was the lowest. In terms of medium pH values, the highest germination percentage was 50.56 ± 8.01% at pH 6.5 and 45.00 ± 6.29% at pH 7.5 value. The germination percentage (27.22 ± 4.18%) of the seeds that were sown in a medium with the 8.5 pH value was significantly low (Table 1).

Germination time (days)

If days to seed germination are considered, there was a significant interaction between medium pH values and stratification periods for 90 and 120 days stratification ($p < 0.01$). No significant differences were recorded in germination period when they were left for 150 days before stratification ($p < 0.01$, $p < 0.05$). If all stratification applications are compared, only stratification period seemed to have a positive or negative effect on germination ($p < 0.01$) (Table 2).

The difference between germination time of the seeds that were stratified for 90 days and the control seeds is important statistically, only in comparison to the seeds that were sown in a medium with the pH 8.5. This is due to the fact that there had been no germination in the seeds that were sown at pH 8.5. In terms of medium pH values, the differences between the germination time of seeds that were stratified for 90 days and sown to mediums with pH 6.5 and 8.5 values were important. The germination time of the seeds that were stratified for 120 days and sown to a medium with a pH 6.5 value (55.58 ±3.36 days) were significantly short when compared to the germination time of the control seeds (68.25 ± 4.63 days). In terms of the pH values of the mediums, the control seeds that were sown in a medium with a pH

Figure 2. Seed germination of (a) stratified and (b) non stratified *C. tchihatcheffii*.

Table 2. The effects of the stratification applications and periods on the germination time (days) of *C. tchihatcheffii* seeds*.

Stratification application	pH 6.5	pH 7.5	pH 8.5	Stratification period[2]
No stratification (control)	47.75 ± 3.47 [a, A]	58.53 ± 6.57[a, A]	0.00 ± 0.00[b, B]	
90 days stratification[1]	63.93 ± 0.47 [a, A]	48.22 ± 6.54[a, AB]	42.19 ± 7.65[a, B]	51.45 ± 4.35[B]
No stratification (control)	68.25 ± 4.63[a, A]	56.77 ± 1.70[a, B]	49.78 ± 4.12 [a, B]	
120 days stratification[1]	55.58 ± 3.36[b, A]	63.39 ± 3.83[a, A]	60.70 ± 3.92[a, A]	59.89 ± 2.18[B]
No stratification (control)	73.27	64.55	56.00	
150 days stratification	66.87	68.49	70.87	68.74 ± 2.68[A]

*The differences between the mean averages containing different letters are statistically important.
[1]Small letters show the differences between the applications on mediums with the same pH values; capital letters show the differences between the pH values of the mediums in the same application.
[2]Capital italic letters show the differences between stratification periods.

value of 6.5 and the germination time of 68.25 ± 4.63 days were longer when compared to the seeds that were sown in other mediums (Table 2). When a comparison is made between the stratification times, the earliest germination was observed in seeds that were stratified for 90 days (51.45 ± 4.35 days) and the delayed germination was observed in seeds stratified for 150 days (68.74 ± 2.68 days) (Table 2). This indicated a negative relationship between the germination time and germination percentage. Thus, it was concluded that a low germination percentage means increased germination time.

DISCUSSION

It is advisable to use stratification pretreatment in a humid and cold medium to improve germination (Acquaah, 2005). This is in agreement with this research; where, all pH values of seed sowing mediums and the germination percentages of stratified seeds were statistically higher than control treatments (Table 1). The findings are in agreement with the findings of Özel (2002), Çakirlar et al. (2005, 2006) and Okay and Günöz (2009) who also reported that the *C. tchihatcheffii* seeds need a long resting or dormancy period.

Another point is that, as the pH value of the sowing medium increases, the germination percentage decreases. This is also true for the development of new plantlets. Plantlets that germinated at a high pH value medium did not grow and die in a short time. In all of the stratification periods, the seeds that were sown in a medium with a pH value of 8.5 and the germination percentage remained low (Table 1). One of the most important factors which limit the quantity of plant nutrients that are available in the soil, by decreasing the benefits of the plant nutrients for the plants, is the pH level. As the pH level increases in the soil, the microelement content

decreases; thus causing a negative effect on the germination percentage and plant development. It has been observed that pH values between 5 and 7.5 do not affect the plant root development and that although plant species show various reactions to the adaptation of pH stress, the root development in high (higher that pH 9.0) and low (lower that pH 5.0) pH values is prevented due to toxic effects (Acquaah, 2005). Özel (2002) and Özcan et al. (2005) emphasized that the majority of the soil in Gölbaşi district which is the natural habitat of the *C. tchihatcheffii* plant, has pH values around 7.25 and 8.88.

Seeds that were stratified for 120 days and sown in a medium with pH 6.5 value germinated significantly in shorter time when compared to control (Table 2). When a comparison is made between different stratification periods, the germination rate of the seeds that were stratified for 150 days remained low when compared to the seeds that were stratified for 90 and 120 days (Table 2). In the applications where the highest seed germination percentages were obtained (Table 1), it is interesting to note that the germination time was comparatively long (Table 2). When the daily seed germination activities are taken into consideration, it has been observed that in the applications with a low germination rate, the seeds germinated in the first few days upon sowing, but towards the middle of the germination period, there has not been any new seed germination. In the applications with a high germination percentage, the seed germination started in the first few days upon sowing and continued until the end of the germination period. In other words, in applications which display a high seed germination percentage, seed germination tends to be continuous and takes place in a longer period of time. This is also true for the pH values of the media. In media with a pH value of 6.5 and 7.5, seed germination continues until the end of the germination period, whereas in seeds that were sown into media with 8.5 pH values, new germination was not observed some time after the beginning of germination.

Conclusion

The results are in agreement with Özel (2002), Çakirlar et al. (2005, 2006), and Okay and Günöz (2009), and showed that germination percentage of *C. tchihatcheffii* seeds were quite low. However, it has been observed that by keeping the seeds in stratification for 120 to 150 days prior to sowing, increases germination. In addition to this, it was seen that pH values of 6.5 and 7.5 were optimum for seed germination.

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