

In vitro and in vivo germination of *Cyclamen alpinum* seeds

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Abstract: *Cyclamen trochopteranthum* O.Schwarz has a confusing history, having been identified in the last part of the 19th century and described as *Cyclamen alpinum* Sprenger. It remained known as *C. alpinum* until 1975, when it was described as *C. trochopteranthum* by Otto Schwarz. *Cyclamen trochopteranthum* is a species distributed in the south-western part of Turkey, but the *C. trochopteranthum* described in 1975 is today describe again as *Cyclamen alpinum*. The seeds of this species were collected from red pine forests in the Gökbel-Dalyan area on May 3-7 and stored with and without capsules in incubators at 20 °C. Among the seeds that were sown 5-6 months later (October-November), the germination rate was 35% at 15 °C and 47% at 20 °C in October. Germination increased to 80% at 15 °C and 87% at 20 °C in November. The highest germination rate was observed in December (95%) and these seeds had been stored for 8 months.

Seeds that were stored for 8 months (using different storage methods) were subjected to in vitro and in vivo germination tests at 15 °C and 20 °C. Seed storage method (in capsules and out of capsules) did not have a significant effect on in vitro germination in Murashige-Skoog medium, but storing seeds in capsules had a significant effect on germination on filter paper and in vivo (mixtures of peat, perlite, and sand). In darkness at 15 and 20 °C high germination rates were obtained, both in vitro and in vivo (in vitro at 15 and 20 °C, max. 95%; in vivo at 15 °C, max. 73%; at 20 °C, max. 93%); however, at 15 °C germination was faster (after the first 20 days at 15 °C, max. 95%; at 20 °C, 73%).

The highest germination rate was obtained after 8-9 months of storage (in vitro average, 94%; in vivo average, 83%). A mixture of peat + perlite + sand in equal parts was a suitable in vivo germination medium (93% germination) for *C. alpinum*.

Key words: *Cyclamen alpinum*, *C. trochopteranthum*, seed germination, seed storage

Cyclamen alpinum tohumlarının in vivo ve in vitro çimlenmesi üzerine çalışmalar

Özet: *Cyclamen trochopteranthum* O.Schwarz karışık bir tanımlamaya sahiptir, 19. yüzyılın son yarısında tanımlanmış ve *Cyclamen alpinum* Sprenger olarak belirlenmiştir. 1975 yılında Otto Schwarz tarafından *C. trochopteranthum* olarak tanımlanmaya kadar *C. alpinum* olarak bilinmiştir. Fakat 1975'de tanımlanmış olan *C. trochopteranthum* günümüzde tekrar *C. alpinum* olarak tanımlanmaktadır ve Türkiye'nin güneybatısında yayılış gösterir. Bu türün tohumları Gökbel-Dalyan'dan kızılçam ağaçları altından Mayıs ayı içerisinde toplanmış ve tohumlar kapsül içerisinde ve kapsülden

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çıkılarak olmak üzere iki farklı şekilde 20 °C'de muhafaza edilmiştir. Tohumların toplanmasından 5-6 ay sonra (Ekim-Kasım ayları) 15 °C'de (Ekim ayında % 35) ve 20 °C'de (Ekim ayında % 47) iyi bir çimlenme elde edilmeye başlanmıştır. Çimlenme oranları Kasım ayında 15 °C'de % 80, 20 °C'de % 87'ye yükselmiştir. En yüksek çimlenme (% 95) tohumların sekiz ay muhafazasından sonra Aralık ayında gözlenmiştir.

Farklı şekillerde sekiz ay muhafaza edilen tohumların 15 °C'de ve 20 °C'de in vitro ve in vivo çimlendirme testleri yapılmıştır. Murashige-Skoog (MS) ortamında yapılan in vitro çimlendirme testlerinde tohumların muhafaza edilme şekli önemli bulunmamıştır. Ancak, filtre kağıdı üzerinde ve in vivo çimlendirmede tohumların kapsül içerisinde saklanması etkisi istatistiki olarak önemli bulunmuştur. 15 °C ve 20 °C'de karanlık koşulda hem in vitro'da hem de in vivo'da yüksek çimlenme oranları elde edilmiştir (in vitro'da 15 °C ve 20 °C'de maksimum % 95, in vivo'da 15 °C'de maksimum % 73, 20 °C'de maksimum % 93). Ancak, 15 °C'de çok hızlı çimlenme gözlenmiştir (15 °C'de ilk yirmi gün sonra maksimum % 95, 20 °C'de ilk yirmi gün sonra maksimum %73).

En yüksek çimlenme tohumların 8-9 ay muhafazasından sonra elde edilmiştir (in vitro'da ortalama % 94, in vivo'da ortalama % 83). *C. alpinum*'da en uygun in vivo çimlendirme ortamı olarak eşit miktarda torf + perlit + kum karışımı bulunmuştur (çimlenme % 93).

Anahtar sözcükler: *Cyclamen alpinum*, *C. trochopteranthum*, tohum çimlenmesi, tohum muhafazası

Introduction

More than 500 geophyte species grow naturally in Turkey (Ekim & Koyuncu, 1992) and the bulbs of the majority of these are exported (Ekim et al., 1991). In general, the production of these geophytes is by vegetative means (Atay, 1996); however, in the propagation of the tuberous geophyte *Cyclamen* L., seeds are used; the tuber can be divided, provided each portion has both a growth eye and part of the rooting region. According to the Cyclamen Society, there are inevitable problems in efficiently sealing the cut edges to guard against rot without the tuber desiccating; division of tubers is not a practical method for production. Many studies have been conducted on seed germination and tuber formation in *Cyclamen* species (Neveur et al., 1986; Corbineau et al., 1989). The seeds of *C. coum* Mill., *C. cilicium* Boiss & Heldr., and *C. hederifolium* Aiton were sowed just after seed maturation (Atay, 1996), but no research has been done on the decorative *Cyclamen alpinum* Sprenger (formerly *C. trochopteranthum*), which has attractive pink flowers and dark green leaves that are grey-green and cream coloured in the centre. The Cyclamen Society provides information only on the seed germination rate of *C. trochopteranthum* O.Schwarz on their website. It is a species that grows naturally in the south-western part of Turkey, especially in Antalya, Muğla, Denizli,

Burdur, and Isparta (Davis, 1978; Mathew & Özhatay, 2001). *C. trochopteranthum* flowers in early spring and has a long flowering period. The flowers have propeller-shaped petals that bend backwards. Tubers are broadly ovate and hollow in the centre. The plants flourish in partially shaded moist habitats with soils rich in humus. It is generally found under *Pinus brutia* Ten. and *Liquidambar orientalis* Mill. forests, or under *Laurus nobilis* L. and *Ceratonia siliqua* L. shrubs. This species is reported to grow at 350-1500 m asl in Turkey (Mathew & Özhatay, 2001). We observed populations at a lower elevation (20 m asl) in Dalyan-Muğla and studied these populations.

Seed size and/or weight in wild and cultivated populations, seed burial depth, temperature, light, pre-treatment, seasonal variation, and plant growth regulators affect the germination of seeds in in vitro medium (Quilichini & Debussche, 2000; Tobe et al., 2000; Jurado et al., 2001; Rojas-Aréchiga et al., 2001; Humara et al., 2002; Navarro & Guitián, 2003; Nikolic et al., 2006).

Studies on the factors that affect germination and tuber development in *C. persicum* Mill. have shown that continuous light inhibits germination, at temperatures below 5 °C and over 20 °C seeds do not germinate, and that germinability is high following dry storage between -30 and 20 °C. Moreover, unripe seeds do not germinate, and germinability increases

with seed maturation and in the presence of gibberellic acid, in particular at 20 °C (Neveur et al., 1986; Corbineau et al., 1989). The International Seed Testing Association recommends germinating *Cyclamen* on filter paper and in sand, at temperatures between 15 and 20 °C following pre-treatment in water or KNO₃ for 24 h (ISTA, 1993).

C. alpinum is a valuable species and its production is of great importance. The aim of the present study was to determine the most effective seed storage method and duration, sowing time and temperature, and sowing material (peat, perlite, and sand) for *C. alpinum*.

Materials and methods

The *Cyclamen alpinum* seeds (Compton et al., 2004) used in our germination experiments were collected from populations growing at sea level in the *Pinus brutia* forest at Gökbel-Dalyan. The soil supporting these plants was analysed for pH (Jackson, 1967), organic matter (%) (Reuterberg & Kremkus, 1951), potassium (ppm), calcium (ppm), phosphorus (ppm) (Kaçar, 1995), and salt (EC; $\mu\text{s cm}^{-1}$) (Soil Survey Staff, 1951).

The seeds were collected during the first week of May and were stored, both with and without capsules, in paper bags in incubators preset to 20 °C (seed storage method). These were tested separately for their germination behaviour. In vitro and in vivo seed germination tests were conducted at 2 different temperatures (15 and 20 °C) and in darkness, according to International Seed Testing Association guidelines (ISTA, 1993).

In vitro germination tests

In vitro tests were conducted in petri dishes and in test tubes. The seeds were placed on filter paper (ISTA, 1993) and in test tubes containing Murashige-Skoog (MS) medium (Murashige & Skoog, 1962) prepared according to Bürün and Emiroğlu (1985). MS medium without a plant growth regulator was supplemented with 30 g L⁻¹ of sucrose and 7 g L⁻¹ of agar, and pH was adjusted to 5.7. After 10 min of surface sterilization with commercial bleach (4.5%

sodium hypochlorite) the seeds were rinsed 5-6 times with sterile distilled water and then placed in culture tubes containing 25 mL of MS medium (Wainwright & Harwood, 1985; Gamborg & Phillips, 1995).

In vivo germination tests

For in vivo experiments a mixture of peat (100-300 mg L⁻¹ of N, 100-300 mg L⁻¹ of P₂O₅, 150-400 mg L⁻¹ of K₂O, and EC-350 $\mu\text{s cm}^{-1}$, pH: 5.4-5.9), perlite (super large form), and sand (0.3 mm) was used. Four sets were arranged as peat, peat + perlite, peat + sand, peat + perlite + sand, all in equal parts by volume. Depth of seed burial was 3-4 mm.

In vitro and in vivo germination tests, as described above, were used in experiment I and experiment II.

Experiment I

Mature seeds were collected in May and stored for 8 months with and without capsules in an incubator at 20 °C. Then, the seeds were germinated at 15 °C in petri dishes on filter paper and in MS medium (in vitro germination tests). Moreover, these seeds were germinated at 15 °C in the mixture of peat + perlite + sand (in vivo germination tests). The germination rates after 60 days are shown in the Table.

Experiment II

Mature seeds that were collected in May were stored with capsules at 20 °C in an incubator. The seeds were germinated at 15 and 20 °C in petri dishes on filter paper every month during 1 year of storage (in vitro germination tests). After 120 days the germination rates were determined and are shown in Figures 1 and 2.

After 8 months of storage, mature seeds were germinated at 15 and 20 °C in a peat + perlite + sand mixture (in vivo germination tests). After 120 days, the germination rate (%) was determined and is shown in Figure 3.

Statistical analyses

Experiments were performed as 3 replicates, in accordance with a factorial experimental design. The data were transformed into Arcsine and evaluated using the SAS statistical program.

Results and discussion

The organic matter content of the soil supporting *C. alpinum* was 5.3% (rich) and the pH was 7.6 (moderately alkaline). Potassium, phosphorus, calcium carbonate, and total soluble salt contents were 225 (medium), 21 (medium), and 7400 (very rich) ppm, and 470 $\mu\text{s cm}^{-1}$ (normal) respectively.

In the fruits collected near Gökbel-Dalyan the average seed number per capsule was 18 (range: 13-27) and 1000 seed weight was 5.5 g.

Experiment I

In vitro and in vivo germination of seeds stored with and without capsules was tested at 15 °C. The results of germination after 8 months of seed storage are given in the Table.

In vitro experiments

Germination on filter paper

Among the seeds with and without capsules germinated after 8 of months at 15 °C, the seeds with capsules germinated after 20 days, while those without capsules germinated after 30 days. The difference in germination between the 2 storage methods was statistically significant (5%). These results show that storing seeds with capsules was more beneficial (Table).

Germination in MS medium

In the germination tests carried out in MS medium at 15 °C, the germination rate of seeds with capsules was lower than of those germinated on filter paper, but the seeds germinated faster. Although in vitro culture medium containing nutrient substances is an important rapid germination technique (Bürün & Gürel, 2001), seed sterilization could cause low germination. In the seeds stored without capsules the

germination rate was highest (average 70%) in MS medium (Table). This could have been due to the effect of various elements used in the medium. The correlation between the germination rate and storage method was not statistically significant in MS medium. With in vitro germination use of a nutrient medium containing macro and micro elements, and sucrose (MS medium) has a positive effect on possibly immature seeds (incomplete maturation of seeds) (seeds stored without capsules).

In vivo experiments

In vivo germination tests showed that the germination rate was higher in the seeds stored with capsules than in those stored without capsules (range: 3.3%-30%). In general, in vivo germination rates were lower than in vitro rates (Table); however, as germination continued after 60 days it is possible that the rate could increase. The highest germination rate was in the peat + perlite + sand mixture medium (47%) and results were statistically significant (5%); however, humidity was of great importance in this respect. The results related to the seed storage methods were statistically significant, as in vitro.

In the present study the peat + perlite + sand mixture was the best medium for the germination of *C. alpinum*.

Experiment II

Effects of temperature and storage duration

The seeds collected in May were stored with capsules at 20 °C in incubators. These seeds were germinated every month for 12 months under in vitro conditions (in petri dishes on filter paper) at 15 and 20 °C. Germination behaviour was monitored for 120 days with regard to the duration of storage in order to determine the best germination period.

Table. Germination (%) (in vitro and in vivo) of *C. alpinum* seeds stored in 2 different forms after 60 days (% germination \pm SD).

Seed Storage Method	In Vitro		In Vivo			
	On Filter Paper	MS Medium	Peat	Peat + Perlite	Peat + Sand	Peat + Perlite + Sand
In capsules	91.7 \pm 5.8*	60.0 \pm 0.0	27.0 \pm 11.6*	37 \pm 20.8*	30 \pm 20.0*	46.6 \pm 11.5*
Out of capsules	53.9 \pm 8.4*	70.0 \pm 10.0	10.0 \pm 10.0*	6.7 \pm 9.4*	3.3 \pm 5.8*	30.0 \pm 10.0*

*Mean difference is significant at the P = 0.05 level (seed storage method).

In vitro experiments

In the tests carried out during the months of June, July, August, and September at 15 °C as well as at 20 °C, germination was extremely poor. After the month of October (5-6 months of storage) germination (%) increased at both temperatures (Figure 1). In the month of November germination was first observed on the 20th day at 15 °C and increased to 43% in 20 days, whereas at 20 °C no germination was observed at this stage. After 60 days germination rates at both temperatures were similar (Figure 2a). In January the germination rate after 20 days was 47% at 15 °C (Figure 2c), whereas in December, February, March, and April germination was observed after 20 days at both temperatures, but was higher at 15 °C (Figure 2b, d, e, f). Similar germination rates were observed in November, December, January, and March at both temperatures after 60 days (Figure 2a-e). In February, however, at both temperatures similar germination rates were obtained in 40 days (Figure 2d). The germination rate in April was lower at 20 °C after 120 days than at 15 °C (Figure 2f). At 15 °C the highest germination rate (95%) was obtained after 40 days in December and this rate did not change in 120 days. The same germination rate was recorded at 20 °C after 120 days. In January 93% and 94% germination rates were recorded at 15 and 20 °C, respectively. According to the Cyclamen Society, the seeds of various *Cyclamen* species sown at 9 and 17 °C after being left for 24 h in lukewarm water showed that the number of days required for the first germination was 44 for *C. trochopteranthum*, whereas in the present study the

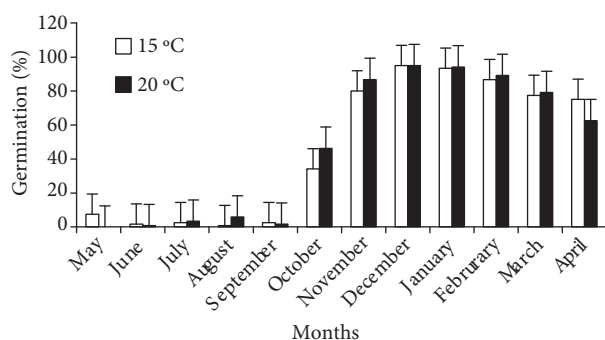


Figure 1. Germination rate (% germination \pm SD) of *Cyclamen alpinum* seeds collected in May after 120 days at 15 °C and 20 °C, according to month.

first germination without any pre-treatment was observed in the first 20 days (the highest in December at 15 °C after 20 days, 66%) (Figure 2b). According to Corbineau et al. (1989), the best germination condition for *C. persicum* is 15 °C in darkness, whereas it is inhibited by white light. Almost all the seeds germinate (max. 99%) at 15 °C after 30 days, but no germination occurs at 5, 25, 30, or 35 °C. It is said that seed size and weight have a positive effect on germination (Rojas-Arechiga et al., 2001; Humara et al., 2002; Navarro & Guitian, 2003). In studies on the germination of different *Cyclamen* species it has been reported by the Cyclamen Society that small seeds do not germinate as well as large seeds. A comparison of the 1000 seed weight (5.5 g) in our species with the commercial F₁ Sierra scarlet 1000 seed weight (7.58 g) shows that seeds of our species were not too small, that germination rates were higher than those given for this species in the literature, and that the number of days for first germination were fewer than that reported in other studies, which might have been due to seed weight differences.

In vivo experiments

After 8 months of storage, the in vivo germination tests in multi-pot trays containing a mixture of equal parts of peat + perlite + sand showed that germination took place at 15 °C and 20 °C in darkness. In the first 60 days a higher rate of germination was observed at 15 °C (63%) than at 20 °C (31%); however, after 120 days the germination rate was higher at 20 °C than at 15 °C (Figure 3).

Successful results were obtained in different *Cyclamen* species in the germination tests performed by the Cyclamen Society, according to the Reading method. In an experiment carried out in autumn at 15 °C 58% germination was obtained in *C. trochopteranthum* and first germination was observed after 49 days (last germination after 122 days). In our in vivo studies, both at 15 and 20 °C, germination was observed after 20 days and after 120 days a 93% (at 20 °C) germination rate was achieved. A very high germination rate was obtained with the germination medium of peat + perlite + sand, as it maintained humidity very well (Figure 3). Müftüoğlu et al. (2006) reported that the best sowing time for *C. hederifolium* seeds collected in May was between 1 and 15 March (respectively, emergence 66.7%-56.7%) and 1 and 15

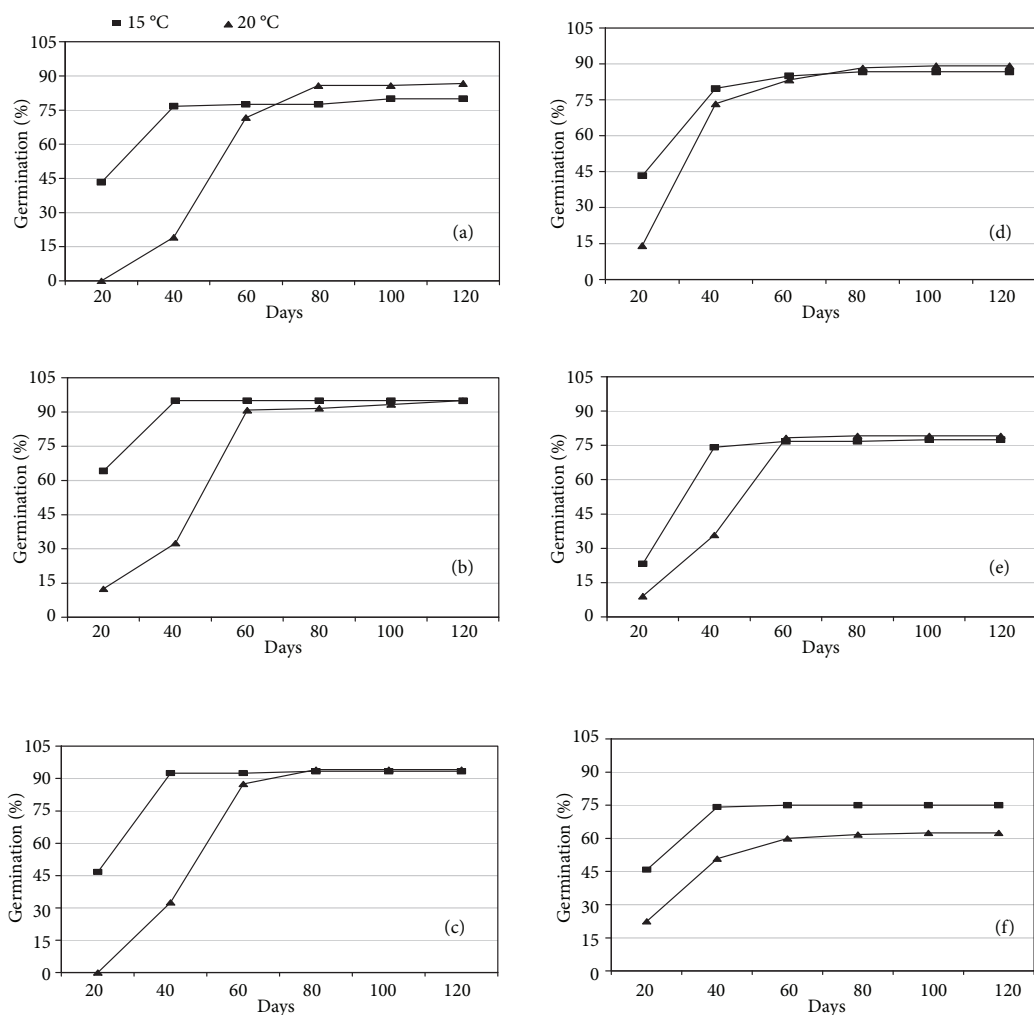


Figure 2. Seed germination rate after 120 days at 15 °C and 20 °C in November (a), December (b), January (c), February (d), March (e) and April (f).

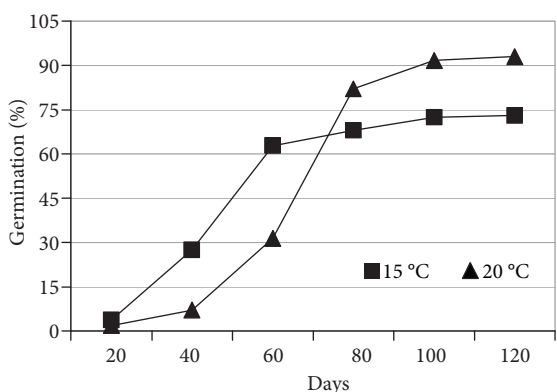


Figure 3. Germination rate of *C. alpinum* seeds at 15 °C and 20 °C in peat + perlite + sand after 8 months of storage.

November (respectively, emergence 58.3%-51.7%). In another study on seed germination conducted by the Cyclamen Society in 1994, which included different *Cyclamen* species, the germination rate in *C. trochopteranthum* seeds sown in October was 40% after 3 months (10 seeds sown, 4 germinated); however, our research on *C. alpinum* shows that much higher germination rates were obtained than those previously reported for seeds sown in October after being collected from Dalyan in the beginning of May. It increased up to 95% during the following months.

In conclusion, *Cyclamen alpinum* seeds that were collected as mature (the first week of May in Dalyan) were appropriate for storage with capsules at 20 °C.

Sowing time of these seeds should be after October (after 5-6 months of storage). The seeds should be sown in a mixture of peat + perlite + sand (equal

volumes) and germinated at 15-20 °C in darkness. For propagation, culture studies on this species must also be conducted.

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