

Seed germination of *Cistus creticus* L. and *Cistus laurifolius* L. as influenced by dry-heat, soaking in distilled water and gibberellic acid

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(Received: December 22, 2006 ; Revised received: March 05, 2007 ; Re-revised received: May 08, 2007 ; Accepted: June 22, 2007)

Abstract: The effects of dry-heat and seed soaking in distilled water or in gibberellic acid on germination performance of *Cistus creticus* L. and *C. laurifolius* L. were studied in the present study. Germination percentages of two *Cistus* species were low due to dormancy. Soaking in distilled water for 24 hr resulted in 28% germination in *C. creticus* and 43% in *C. laurifolius*. Gibberellic acid applications (20, 100 and 250 mg l⁻¹) for 24 hr gave 32, 30 and 23% germination, respectively in *Cistus creticus* and 33, 37 and 28% germination, respectively in *C. laurifolius*. Dry-heat pretreatments at 50, 80 and 100 °C for several times (1-60 min) also significantly increased germination percentage in two species. The highest germination in *C. creticus* was obtained with dry-heat at 100 °C for 1 or 5 min (80% and 83%, respectively) and in *C. laurifolius* at 100 °C for 5 min (87%). A significant increase in germination rate was also achieved under different pretreatments. The physiological dormancy caused by an impermeable seed coat can be overcome by dry-heat pretreatments in two *Cistus* species.

Key words: *Cistus*, Pretreatment, Seed dormancy, Seed scarification
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Introduction

Cistus species (*Cistaceae*) is distributed in some enclaves along the Black Sea coast and Mediterranean region of Turkey, in which it is a prominent element in such vegetation (Greuter *et al.*, 1984; Mayer and Aksoy, 1986). *Cistus* occurs on poor soils of dry scrub or open woodland, and fire is necessary for the colonization of *Cistus* on highly degraded areas (Corral *et al.*, 1990). Regeneration of *Cistus* in natural conditions or burned areas is performed exclusively by seeds (Montgomery and Strid, 1976; Arianoutso and Margaris, 1982). Seed coat-imposed dormancy associated with hardness and impermeability to water has been suggested as the most important causes of the primary dormancy present in several species of the genus *Cistus* (Corral *et al.*, 1990; Nadal *et al.*, 2002). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination. Stratification, scarification and gibberellins have a promotive effect on the germination of many species of angiosperms and gymnosperms (Bradbeer, 1988; Bewley and Black, 1994; Leadem, 1996; Tilki, 2004; Tilki and Cicek, 2005; Esen *et al.*, 2007). Nevertheless, these methods vary from one species to the other, accentuating the need for formulating species-specific treatments.

Pretreatments with high temperatures for different durations were found effective in breaking dormancy in various *Cistus* species (Vuillemin and Bulard, 1981; Troumbis and Trabaud, 1986; Thanos and Georghiou, 1988; Corral *et al.*, 1990; Perez-Garcia, 1997). Soaking seeds in distilled water also increased germination in some *Cistus* species (Corral *et al.*, 1990). However, soaking seeds in distilled water or gibberellic acid applications did not significantly increase germination over untreated seeds in various

Cistus species (Nadal *et al.*, 2002). Germination of *Cistus ladanifer* and *Cistus albidus* was also not favored by the addition of polyethylene glycol (PEG) (Perez-Fernandez *et al.*, 2006).

The present investigation aims to evaluate the effects of dry-heat treatments and seed soaking in distilled water and also in gibberellic acid (GA₃) solutions on germination performance of *Cistus creticus* L. and *C. laurifolius* L.

Materials and Methods

Mature seeds of *Cistus* species were collected from wild populations in September 2006 in Turkey. *Cistus creticus* were collected from Artvin and *C. laurifolius* from Izmir cities in Turkey. Collected seeds were cleaned and stored dry at 4°C until used. Seeds were immersed in three concentrations of GA₃ solutions (20, 100 and 250 mg l⁻¹) or soaked in distilled water for 24 hr at room temperature (around 20°C). To test the effects of dry-heat on breaking coat-imposed dormancy seeds were placed in an oven at 50, 80 and 100°C for 1, 5, 10, 30 and 60 min.

The seeds were enclosed in petri dishes on two layers of filter paper moistened with distilled water, and placed in the germination chamber at 15°C in darkness (Thanos and Georghiou, 1988; Corral *et al.*, 1990; Nadal *et al.*, 2002). The seeds were monitored every day and moistened when dry. The criterion for germination was root emergence. Germination percentages for each trial were calculated after 37 days (Nadal *et al.*, 2002) and germination rate was calculated and expressed as peak value (PV), an index of germination speed which is the highest number obtained when germination percentage is divided by the number of elapsed days (Czabator, 1962).



Table - 1: Effect of heat pretreatments on germination percentage of *Cistus creticus* and *Cistus laurifolius*

Species	Temperature (°C)	Germination percentage (%)					
		Control	1 min	5 min	10 min	30 min	60 min
<i>C. creticus</i>	50	15 ^{aA}	18 ^{aA}	24 ^{abA}	35 ^{bA}	48 ^{cB}	51 ^{cC}
	80	15 ^{aA}	60 ^{cdB}	58 ^{cb}	69 ^{dB}	45 ^{bB}	38 ^{bB}
	100	15 ^{bA}	80 ^{dC}	83 ^{dC}	56 ^{cB}	20 ^{bA}	8 ^{aA}
<i>C. laurifolius</i>	50	20 ^{aA}	24 ^{aA}	30 ^{aA}	52 ^{bA}	54 ^{bB}	57 ^{bb}
	80	20 ^{aA}	36 ^{bb}	39 ^{bA}	57 ^{dB}	50 ^{cB}	60 ^{dB}
	100	20 ^{bA}	66 ^{dC}	87 ^{eb}	48 ^{cA}	28 ^{bA}	14 ^{aA}

Means in the row followed by the same lowercase letter are not significantly different at $p < 0.05$

Means in the column followed by the same uppercase letter within species are not significantly different at $p < 0.05$

Table - 2: Effect of seed soaking in distilled water and in gibberellic acid on germination percentage of *C. creticus* and *C. laurifolius*

Species	Germination percentage (%)				
	Control	Water soaking	20 mg l ⁻¹ GA ₃	100 mg l ⁻¹ GA ₃	250 mg l ⁻¹ GA ₃
<i>C. creticus</i>	15 ^a	28 ^c	32 ^c	30 ^c	23 ^b
<i>C. laurifolius</i>	20 ^a	43 ^d	33 ^b	37 ^c	28 ^b

Means in the row followed by the same letter are not significantly different at $p < 0.05$

For each experiment, there were four replicates with 100 seeds each per treatment arranged in a completely randomized design. Data for each of the experiments were subjected to analysis of variance (ANOVA) using SPSS for windows. Mean comparison was performed using Duncan's New Multiple Range Test, and a significance level of 5% was used for all statistical analyses. The percentage data were arcsine transformed before performing ANOVA (Zar, 1984).

Results and Discussion

Germination response varied significantly across the treatments in two *Cistus* species, and germination response for different durations and temperatures of dry-heat pretreatments revealed a significant difference. The interaction between temperature and duration was also significant (Table 1).

Pretreatment of the seeds at three different temperatures (50, 80 and 100°C) for different durations increased germination percentages in two species significantly. In *C. creticus* germination percentages reached to 48% at 50°C for 30 minutes treatment. Germination percentages increased to 69% at 80°C for 10 minutes. But the highest germination percentages in *C. creticus* were reached with dry-heat pretreatment at 100°C for 1 or 5 min (80 and 83%, respectively).

In *C. laurifolius* germination percentages reached to more than 50% at 50°C after 10 min heat treatment. Germination percentages increased to 60% at 80°C for 60 min and the highest germination percentage in *C. laurifolius* was reached at 100°C for 5 min (87%). Increasing the duration of dry-heat pretreatment at 100°C to 10 min or more in *C. creticus* and *C. laurifolius* reduced germination significantly. The lowest germination was observed after 60 min dry heat treatment at 100°C in two species (Table 1).

The effect of soaking in distilled water and different GA₃ concentrations on germination percentages was shown in Table 2. The highest germination in *C. creticus* seeds was achieved after soaking in 20 and 100 mg l⁻¹ GA₃ (approximately 30%) and in *C. laurifolius* seeds after soaking in distilled water (43%). Although soaking in distilled water and GA₃ application produced an increase in germination percentages of two species over untreated seeds, the highest germination percentages were obtained in dry-heat pretreatment at 100°C for 1 or 5 min in *C. creticus* and 5 min in *C. laurifolius*.

A significant increase in germination rate was achieved after different pretreatments in two species, and dry-heat pretreatment for 5 min at 100°C resulted in maximum increase in germination rate compared to water soaking and 20 mg l⁻¹ GA₃ application (Table 3).

Hardness and impermeability to water of the seed coat were reported as the most important causes of dormancy in the genus *Cistus* (Corral *et al.*, 1990; Nadal *et al.*, 2002). Dry heat treatment affects seed germination of various species (Perez-Garcia and Gonzalez-Benito, 2005; Zida *et al.*, 2005; Perez-Fernandez *et al.*, 2006) and is also effective to break dormancy, improve germination ability and reduce the mean germination time in various *Cistus* sp (Thanos and Georgiou, 1988; Corral *et al.*, 1990; Roy and Sonie, 1992; Valbuena *et al.*, 1992; Perez-Garcia, 1997). Probably dry-heat pretreatments crack the seed coats, particularly the internal layer, with strongly lignified cell wall (Corral *et al.*, 1989).

The highest germination percentages in *C. clusii*, *C. monspeliensis* and *C. salvifolius* were obtained with dry-heat pretreatment at 100°C for 5 min (Nadal *et al.*, 2002). The soaking of seeds in distilled water and gibberellic acid applications did not

Table - 3: Effect of best pretreatments on germination rate of *C. creticus* and *C. laurifolius*

Species	Germination rate (PV)			
	T1	T2	T3	T4
<i>C. creticus</i>	1.2 ^a	7.1 ^c	2.1 ^b	2.4 ^b
<i>C. laurifolius</i>	1.5 ^a	6.8 ^c	3.2 ^b	2.3 ^{ab}

Means in the row followed by the same letter are not significantly different at $p < 0.05$. T1 = Control, T2 = Dry-heat treatment for 5 min at 100°C, T3 = Water soaking, T4 = 20 mg l⁻¹ GA₃ soaking

significantly increase germination over untreated seeds in these three species.

Soaking in distilled water of seed for 24 hr increased germination in *C. ladanifer*, *C. albidus* and *C. laurifolius* (Vuillemin and Bulard, 1981; Corral *et al.*, 1990). In these species the promotive effect of wash can be associated to loosening and detachment of small pieces of the external waxy layer of seed coat (Corral *et al.*, 1990). According to Corral *et al.* (1990) GA₃ application did not significantly increase germination performance in *C. laurifolius*, and germination increased with dry-heat pretreatments although not so markedly. Final germination percentages of the untreated seeds were relatively low (5%) and 5, 10 or 30 min dry-heat pretreatment increased germination to approximately 10%. Germination rate decreased gradually with prolongation of treatments over 30 min. However, in the present study, although soaking for 24 hr in distilled water and 20 or 100 mg l⁻¹ GA₃ application produced an increase in germination percentages of *C. laurifolius* over untreated seeds, the highest germination percentage was obtained in dry-heat treatment at 100°C for 5 min (87%).

The present study shows that *C. laurifolius* and *C. creticus* seeds have seed coat dormancy, present in several species of the genus *Cistus* (Corral *et al.*, 1990; Nadal *et al.*, 2002). Hard seed coats behave as a barrier for water uptake which can be overcome by 1 or 5 min dry-heat pretreatment in *C. creticus* and 5 min in *C. laurifolius*, and natural forest fire can be an effective dormancy-breaking treatment for the seed germination of *C. laurifolius* and *C. creticus*.

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