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EFFECTS OF CITRIC ACID PRESOAKING AND STRATIFICATION ON GERMINATION BEHAVIOR OF *PRUNUS AVIUM* L. SEED

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Abstract

Wild cherry (*Prunus avium* L.) is a fast-growing broadleaved tree of Turkey with great ecological and economic values. Deep and variable dormancy inhibits germination of PA seed. Presoaking seed with a 0.1% citric-acid (CA) enhances pretreatment efficacy for some tree species. Long, constant high temperatures during stratification and germination tests induce secondary seed dormancy in European PA seed sources. This study assesses the effects of 0.1% CA or deionized water (DW) soaking in combination with various pretreatments on the germination of PA seeds from northern Turkey, using alternating temperatures during germination tests. Following presoaking, seeds were put through eight different pretreatments, including complex cold or warm + cold periods ranging from 60 to 135 days. Cumulative germination percentages and the course of germination were determined at the end of the trial. Presoaking seeds in DW for two days increased germination more than presoaking in 0.1% CA solution. Pretreatments affected seed germination significantly differently, whether they were presoaked in CA or DW. For both presoaking treatments, a 15-day warm period followed by a four-month cold period, and then recurrent warm + cold periods followed by a three-month cold period, were the best pretreatments. Germination course results conformed to the cumulative germination results. In conclusion, presoaking seeds in water for two days followed by recurrent warm + cold periods with long cold periods lasting at least four months in total are recommended for adequate germination of *Prunus avium*. Variable and delayed germination may offer PA an ecological adaptation, improving survival in a wide array of environments.

Introduction

Wild cherry (*Prunus avium* L.) is a 'noble hardwood' tree species of both Europe and Turkey with great ecological and economic significance (Eriksson, 2001; Esen *et al*., 2005, 2006). Deep and variable embryo dormancy is the most significant germination-inhibiting factor for PA, which brings about difficulties during seedling production (Suszka, 1967; Grisez, 1974; Finch-Savage, 2001). PA seed germinates after a long complex pretreatment regime with recurrent moist warm and cold periods, imitating end-of-summer or fall sowing in nature (Suszka, 1967; Grisez, 1974; Suszka, 1990; Finch-Savage, 2001). A recent study in Turkey found that consecutive periods of complex warm and cold pretreatments longer than four months with a constant germination temperature of 20°C have partially broken seed dormancy of PA (Eşen *et al*., 2006). However, an alternating temperature regime (high and low) rather than constant high temperatures during germination trials has enhanced seed germination percentage for PA in Europe (Suszka, 1962; Suska, 2005, 2006 pers. comm.). Whether Turkish PA seeds behave similarly to European seed sources under an alternating temperature regime during germination tests is unknown.

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Also, in the Turkish experiment, soaking seeds in 0.1% CA solution for 48 hrs prior to a three-month cold period significantly improved the cumulative germination rate (27%) of PA seed when compared to the cold period alone (14%, Eşen *et al*., 2006). This study, however, provided limited information about the interactive effects of CA presoaking with conventional stratification methods on PA seed germination. Whether CA presoaking is synergistic or antagonistic to the germination behavior of PA seed when used in combination with pretreatments differing in complexity (i.e., single or multiple warm periods during stratification) is not known.

Several researchers studied the dormancy and germination behavior of PA seed in central and northern Europe during the 1960s and 1970s (Suszka, 1962, 1967, 1976; Suszka *et al.*, 1996). However, the climate, growing conditions in Turkey and precipitation regime are much different from those in central and eastern Europe. Therefore, studying germination behavior of PA seed from Turkey is of great interest. Furthermore, the phenology of many European tree species, including PA, has varied due to changes in global climate (Menzel *et al.*, 2006). The dormancy and germination behavior of PA seed may also have changed, suggesting that confirmation of what is known of PA by new studies is a necessity. The present study aims to evaluate the effects of various pretreatments, including deionized water (DW) or 0.1% CA soaking for 48 hrs, followed by different combinations of warm and cold moist periods with alternating temperatures, on the germination behavior of PA seed from the western Black Sea Region of Turkey. This study thereby intends to answer the question of whether or not presoaking seeds in 0.1% CA solution or DW shortens the total period for PA seed stratification. This will help the nursery men to improve the efficiency of generative propagation of PA for commercial and noncommercial (e.g., conservation, biodiversity) purposes.

Materials and methods

Mature fruits of four different PA seed sources (SSs) from the western Black Sea Region of Turkey (Caybuku [Duzce], Ihsangazi and Inebolu [Kastamonu], and Kdz. Ereglisi [Zonguldak]) were collected from open-pollinated single mature trees (nearly 30-40 yrs old) between July and August 2003. Fruits were cleaned of all pulp and juice using sand in water upon collection. Extracted stones (hereafter called seed unless otherwise stated) were floated in deionized water to separate sound seeds. Seeds were later dried in an aerated place at room temperature for two days. Seeds were then stored in a refrigerator in zipped plastic bags at 3°C until treatments began (Eşen *et al*., 2006). The seeds of the four SSs were mixed in equal quantities and homogenized prior to pretreatments. Mean seed moisture content of the seeds was calculated before the beginning of the pretreatments (Anon., 1999). The viability of the seed was also determined using the tetrazolium test (Anon., 1999).

Half of the seeds were soaked in 0.1% CA solution for 48 hrs (Jones, 1963; Esen *et al.,* 2006), whereas the other half were soaked in deionized water (DW) for 48 hrs as the control. Seeds were later mixed in 4:1 proportion with a moist peat moss medium in large plastic containers (Grisez, 1974) for adequate aeration. All seeds were treated with eight different pretreatments, including periods of cold or warm + cold stratifications ranging from 60 to 135 days in total length (Table 1). The seed containers were stored at 3^{o}C (±2^{o}C) in a refrigerator for cold periods (CP). For warm periods (WP), seeds were stored in a growth chamber (Nüve ID 501®, Nüve, Inc., Ankara, Turkey) at 20^{o}C (±0.5^{o}C) (Esen *et al.,* 2006). At the end of the pretreatments, four replicates of 100 seeds each were

Table 1. Effects of different pretreatments on the mean cumulative germination rate of *Prunus avium* seeds pre-soaked in deionized water and 0.1% citric acid solution for 48 hours.

Pretreatment[1]	Germination (%) & Standard Errors (±)	
	Deionized water	Citric acid
15 D WP + 120 D CP	39.0 a[2,3] (±2.3)	21.2 a[2,3] (±3.6)
15 D WP + 15 D CP + 15 D WP + 90 D CP	34.6 a (±5.1)	20.8 a (±3.2)
15 D WP + 15 D CP + 15 D WP + 60 D CP	19.1 a (±2.3)	7.4 ab (±2.1)
60 D CP	5.6 b (±1.4)	1.9 b (±0.7)
120 D CP	4.1 b (±0.4)	4.8 b (±0.9)
15 D WP + 90 D CP	1.8 bc (±1.1)	6.3 ab (±1.6)
90 D CP	0.8 c (±0.4)	4.0 b (±2.1)
15D WP + 60 D CP	0.4 c (±0.4)	6.0 b (±2.5)

[1]Pretreatment and presoaking main and interaction effects were significant according to ANOVA ($p \leq 0.05$) for both deionized water and citric acid treatments.
[2]Transformed data (logged) was used for mean separations.
[3]Means with different letters in each column are significantly different ($p \leq 0.05$).

placed in a moist sand medium in 18-cm glass Petri dishes. The dishes were then placed in the chamber under alternating temperatures (3°C and 20°C for 16 and 8 hrs, respectively) (Suszka, 1967, 1976; Suszka *et al.,* 1996; Suszka, 2005, 2006, pers. comm.) and watered during the germination test as needed. Germination was monitored on days 4, 7, 10, 21, and 28. The seeds with 5-mm-long radicles were considered germinated (Anon., 1999). Cumulative germination percentage was calculated as the proportion of germinants on day 28 to the total number of viable seeds used, multiplied by 100. The experimental design was a randomized complete block design with four replications. The two-way analysis of variance (ANOVA) was used to test for main effects (presoaking or scarification and pretreatment) and interaction effects. The means were separated using the Tukey Mean Comparison Test, with p values ≤ 0.05 considered significant. Logged transformations were made on all cumulative germination percentage data before analysis for normality. The untransformed data were tabulated.

Results

Mean moisture content and viability of the seeds were 10% and 82%, respectively. Generally, germination rates were not high (<40%). Pretreatment effects on seed germination varied substantially by presoaking treatment i.e., significant presoaking x pretreatment effect. Generally, presoaking seeds in DW for two days resulted in greater germination than presoaking them in 0.1% CA for the same period (Table 1). The 15D WP + 120D CP and 15D WP + 15D CP + 15D WP + 90D CP pretreatments resulted in significantly greater mean cumulative germination rates and germination speeds for both the DW and CA presoaking treatments (Table 1, Fig. 1). The two pretreatments did not differ from each other substantially in mean germination rate (Table 1), yet the latter demonstrated a slightly greater speed of germination than the former during the first phase of the germination test (Fig. 1). For the DW-presoaking treatment, treating seeds with a two-week warm period prior to a four-month cold period enhanced mean germination rate by nearly tenfold over treating seeds with a four-month cold period alone. Although its mean germination rate was much lower, the 15D WP + 15D CP + 15D WP + 60D CP pretreatment rate was not significantly different than those of the two best pretreatments, indicating a large variability in the data set. The other pretreatments had very low seed germination rates (Table 1).

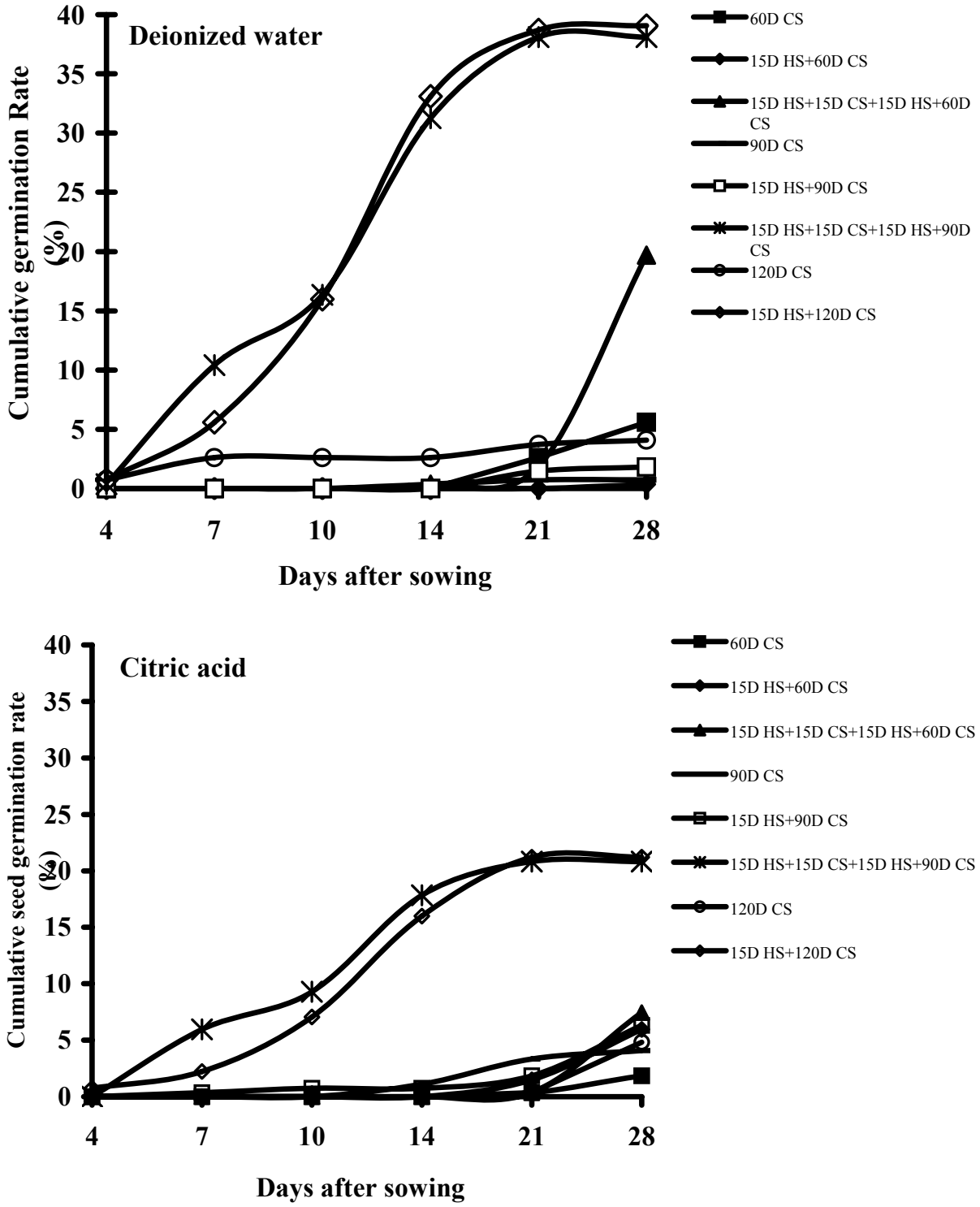

Fig. 1. Germination course of *Prunus avium* seeds presoaked in deionized water (above) and 0.1% citric acid (below) for 48 hours with different pretreatments.

Discussion

The present experiment added another contradiction to the literature with regard to the germination response of the cherry seed to CA presoaking (Jones, 1983; Huntzinger, 1968): The recent Turkish study reported that 48-hr CA soaking prior to a 90-day cold period enhanced mean germination rate for PA when compared to the cold period alone (Eşen *et al.,* 2006). However, in the present experiment, the 48-hr CA presoaking

depressed mean germination rates when compared to DW soaking, suggesting possible embryo damage by CA (Table 1). Great differences exist in many seed traits within a seed source and even a mother tree, most of which are "random" (Grisez, 1974). The physical properties of endocarp may show some similar variations, which might have contributed to the variable results of PA seed in response to the CA presoaking.

The mean seed germination rates of PA in the present study were substantially greater when compared to those of Eşen *et al.* (2006), in which PA seeds were presoaked in DW for 24 hrs prior to similar complex pretreatments. Cherries do not have a hard endocarp and are water-permeable (Grisez 1974, Grisez *et al.,* unpubl). The results of the present and previous experiments on seed germination suggest that presoaking seeds for a sufficient amount of time (at least 48 hrs) softens the endocarp and/or fulfills the imbibition prerequisite for this species (Bonner *et al.,* 1974). Water soaking has also been reported to improve the "viability" of forest tree seeds, especially those from old seed lots (Prescher & Prescher, 2003).

It is well known that the cold period is necessary to break deep embryo dormancy for PA seed (Suszka, 1967; Grisez, 1974; Suszka, 2005, 2006, pers. comm.). However, cold periods alone do not suffice for successful seed germination in this species. Recurrent short, warm periods embedded in pretreatments are required for embryo development (Suszka, 1967; Grisez, 1974; Finch-Savage 2001). This result has previously been reported for Polish PA seed sources (Suszka, 1967) and was also confirmed for Turkish PA seed by the present study. The positive effect of complex stratification with recurrent warm and cold periods has also been documented for black cherry (*P. serotina* Ehrh., Grisez, 1974; Esen *et al.,* 2007). However, the direction of the effect of warm period on PA seed germination depends on the length of warm period and physiological status of seeds in the lot during germination trial: A long, warm period applied to PA seeds that are physiologically ready for germination but have not yet initiated cell growth in the root tip may induce secondary dormancy in these seeds, reducing total germination capacity in a single year (Suszka, 1967; Suszka, 2005, 2006, pers. comm.). This type of behavior in germination increases the percentage of seeds that are physiologically ready for germination in subsequent years and creates a viable seed bank in the soil (Suszka, 1967; Suszka, 2005, 2006, pers. comm.). Once cells in the root tip start growing, the applications of variable temperatures (3^{o}C and 20^{o}C for 16 and 8 hrs, respectively) during the active germination phase circumvent secondary dormancy and improve germination rates in a single year (Suszka, 1967; Suszka, 2005, 2006, pers. comm.). Although the present study did not have a constant temperature regime for comparison, alternating temperatures during active germination tests probably prevented seeds from experiencing a "secondary dormancy" caused by long periods of high temperatures (Suszka, 1967; Suszka, 2005, 2006, pers. comm.), in contrast to Eşen *et al.* (2006). PA is an extremely light-demanding tree species (Savill, 1991). Cherry species preserve a strong viable seed bank in the forest soil (Smith & Linnartz, 1980). The seed bank responds to favorable light conditions after openings of at least one tree height diameter in the stand canopy (Merrit, 1980). The complexity in seed dormancy and germination behavior probably furnishes cherry with an ecological adaptation, increasing survival over a wide array of environments (Grisez, 1974; Radosevich *et al.,* 1997; Eşen *et al.,* 2006), although this strategy often reduces the species' germination in a single year (Swanton, 2003). In conclusion, the present study demonstrated two essential requirements for the increased germination of PA seed: presoaking seeds in water for two days prior to traditional pretreatments, and the application of short, warm period(s) in combination with cold periods for at least a four-month stratification period in total.

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