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The effects of different pre-sowing seed treatments on breaking the dormancy of mahaleb cherries, *Prunus mahaleb* L. seeds

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Summary

Seeds of the mahaleb cherry, *Prunus mahaleb* L., used as a rootstock in cherry production, germinate and emerge poorly due to seed dormancy. A greenhouse experiment was done to determine the effects of several treatments to overcome this dormancy, i.e. prolonged (30, 60 and 90 days) stratification followed by hot water treatment at 90°C (for 0, 10, 20 and 30 minutes) or sulfuric acid scarification (0, 10, 20 and 30 minutes) or gibberellic acid, GA_3 (0, 500, 1000 and 2000 ppm). The results showed that stratification for 60 days or more increased germination percentage and decreased mean germination time. Scarification with either hot water or sulfuric acid only improved germination percentage and speed if followed by 60 days of stratification. Treating the seeds with GA_3 resulted in a significantly higher germination rate. The highest germination percentages were attained by treating seeds which had been stratified for 60 and 90 day-periods with GA_3 at 1000 ppm. It is recommended that GA_3 should be used in addition to cold stratification for improving germination percentage and speed of mahaleb cherry seeds.

Introduction

The Mahaleb cherry (*Prunus mahaleb* L.), a member of the family *Rosaceae*, has been identified in Jordan as one of the most important rootstocks commonly used worldwide for sour and sweet cherries (Hrotko and Magyar, 2004). It is used as a preferred rootstock for calcareous soils and arid areas (Buman, 1977; Giorgio and Standardi, 1993). *Prunus mahaleb* is highly drought-resistant and produces well-anchored, sturdy trees. It is preferred for use as a rootstock because of its resistance to pests, early yield and high crop load (Cochran *et al.,* 1961).

The most common methods of cherry propagation are grafting and budding, where mahaleb or mazard cherries are the usual rootstocks used. However, seed propagation is difficult because of low germination percentages resulting from seed dormancy. Seed primary dormancy is an intrinsic inhibition of early germination in freshly harvested mature seeds. It can rely on morphological, physical or physiological factors (Baskin and Baskin, 2004). Seed germination is influenced by internal factors controlling dormancy, including seed coat factors and phytohormones (Hartman, 1997). Therefore, promoting the germination of mahaleb cherry seeds is important in propagation, breeding programs and the use of germplasms. Various methods are used to overcome seed dormancy

depending on the plant species and dormancy type. Although acid scarification was considered an important procedure for overcoming the physical dormancy of some plants, chemical scarification techniques to overcome dormancy in mahaleb cherry seeds have proven ineffective (Gercekcioglu and Cekic, 1999). Physiological barriers to germination in embryos have been overcome with cold stratification (moist-prechilling) in a number of *Prunus* species (Martinez-Gomez and Dicenta, 2001; Garcia-Gusano *et al*., 2004). Stratification plays a significant role as a stimulator that helps to break mahaleb cherry seed dormancy. In order to achieve germination percentages greater than 90% Seeley and Damavandy (1985) found that mahaleb cherry seeds need 100 days of chilling before germination.

Gibberellins are a class of plant growth substances which are most directly implicated in the control and promotion of seed germination (Ritchie and Gilroy, 1998). Studies evaluating the exogenous application of gibberellic acid have been shown to relieve certain types of dormancy, including physiological dormancy, thermodormancy and photodormancy, and to replace partially or fully the necessary cold treatments in different plants (Hartmann *et al*., 1997). A combination of cold stratification and GA_3 treatment was found to improve mahaleb cherry seed germination (Gercekcioglu and Cekic, 1999).

There have been some studies on the germination of different species of Prunus (Finch-Savage, 2002; Jensen and Eriksen, 2001), but apparently little work has been done on mahaleb cherry seeds. The objectives of this study were to determine the effects of chemical scarification, hot water scarification and gibberellic acid treatment preceded by stratification on the germination of *P. mahaleb* seeds and to devise effective methods for overcoming seed dormancy.

Materials and methods

The research trial was conducted in 2004 at the greenhouse of the Faculty of Agriculture, University of Mutah in Karak, Jordan to enhance the germination of *Prunus mahaleb* L. seeds.

Seed materials

The seeds were obtained from naturally grown trees from Al-Hassan Agricultural Station (Tafila) owned by the Ministry of Agriculture, Jordan. The altitude of the collection site is 900m above sea level. This area is dominated by a Mediterranean microclimate. Mahaleb cherry seeds were collected in October 2003 from five individual trees after they were fully ripe.

Seed treatments

The seeds were soaked in a 20% sodium chloride solution (floater test) to separate empty endocarps from filled ones. The viability of seeds was determined by using a standard tetrazolium test. The seeds were divided into 48 groups (150 seeds for each). Each group was divided into three replicates and subjected to different treatments.

Cold moist stratification (moist-prechilling)
Seeds were immersed in tap water for about 2 hours at 25°C to allow water imbibition. The seeds were stratified in plastic bags filled with moist peat moss:perlite (1:1). Methyl 1-(butylcarbamoyl) -2- benzimidazole carbamite (Benomyle) solution (1g L^{-1}) was incorporated into the moss. The bags assigned to moist-chilling treatments of 30, 60 and 90 days with three replicates were incubated in a refrigerator at 4-5°C. The media were checked routinely every week for moisture availability. A group of seeds was kept nonstratified as a control treatment. Following stratification, the seeds were treated with hot water or concentrated H_2SO_4 or gibberellic acid.

Hot water scarification
Stratified and nonstratified seeds were wrapped in white cloth and soaked in hot water at 90°C for 0, 10, 20 and 30 minutes. After immersion, they were removed from the hot water and left to cool for about 10 minutes.

Acid scarification
Stratified and nonstratified seeds were scarified by soaking them in 98% concentrated H_2SO_4 (specific gravity of 1.84 g cm^{-3}) for 0, 10, 20 and 30 minutes with agitation. After scarification, the acid was decanted and the seeds were washed with distilled water several times.

Gibberellic acid pretreatment
Stratified and nonstratified seeds were pretreated with GA_3 at 0 (control, untreated), 500, 1000 and 2000 ppm. Ethanol was used to dissolve GA_3.

Germination
The seeds were placed in peat moss:perlite (2:1) on seedling trays on 26 February 2004 and were left to germinate under greenhouse conditions at an average temperature of 24°C. The three replicates of each treatment were sowed on three separate trays. Seeds were irrigated twice a week with potable water prior to transplanting. The seedlings were irrigated with Benomyle (0.5 g/L) as a protective measure against fungi.

Three weeks after sowing, the degree of germination was calculated weekly until the ninth week (20 May 2004). At the close of the investigation, final seed germination percentages and germination rates (in days) were determined. Seeds were considered to have germinated when the radicle was ≥ 2mm long (Kaye and Kuykendall, 2001). Germination percentage was calculated as the average of three replicates of 50 seeds and the mean time to complete germination (MGT) was calculated according to the equation described by Isfendiyaroglu and Zeker (2002) as follows:

$MGT = \sum(t \times n) / \sum n$; where t is the time in days starting from day zero, and n is the number of seeds which had completely germinated on day (t).

Statistical analysis
The study involved three experiments of 4 × 4 factorial models arranged in a randomized complete block design. For each experiment, there were three replicates per treatment and

50 seeds for each replication. The percent of germination was transformed using arcsine-square root transformation. Data for each experiment were subjected to an analysis of variance (ANOVA) using MSTATC statistical package (Michigan State Univ., East Lansing, MI). The significance of mean differences was determined by using the Least Significant Difference method at P = 0.05 for all statistical analysis.

Results

Germination percentage

Without seed stratification, low germination (3.3 - 5.0%) was obtained for mahaleb cherry seeds which had been kept untreated or soaked in hot water for 10, 20 or 30 minutes (figure 1a). Soaking in hot water had no significant effect on seed germination; however, increasing the duration of stratification from 0 to 90 days resulted in a considerable increase in germination. Furthermore, soaking seeds which had been moist-chilled for 30 days in hot water did not significantly affect germination. Germination of seeds, with or without 30 days of stratification, was the same for all soaking periods. By contrast, when seeds were subjected only to moist stratification for 60 or 90 days, the germination percentage rose to an average of 33.3% and 56.7%, respectively. The highest germination percentage was attained for seeds which had been moist-chilled for 90 days regardless of hot water treatment.

Figure 1b shows that the germination percentage in nonstratified seeds that had not been subjected to scarification with sulfuric acid was very low (3.3-5.0%). Furthermore, scarification of nonstratified seeds with sulfuric acid for different durations had no significant influence on germination rates. On the other hand, treating seeds which had been moist-chilled for 30 days with sulfuric acid led to a significant increase in germination percentages compared to the control seeds. A further increase in germination (40.0-48.3%) of nonscarified or scarified seeds was attained by increasing stratification to duration of 60 or 90 days, thus indicating that *P. mahaleb* seeds lack seed coat dormancy.

Pretreatment with GA_3 failed to affect the germination of seeds which were nonstratified (control) and those that had been moist-chilled for 30 days (figure 1c). However, GA_3 treatment obviously enhanced the germination of seeds that had been moist-chilled for 60 and 90-day periods. The response was dependent on the concentration of applied GA_3. Gibberellic acid treatment at 1000 ppm applied to seeds stratified for 90 days yielded the highest germination percentage (65.0%); the same result was obtained at 2000 ppm. Germination percentages for seeds moist-chilled for 60 and 90-day periods at control or low GA_3 concentration (500 ppm) were significantly higher than those obtained with seeds treated with hot water or concentrated H_2SO_4.

Germination rate

Mean germination time (MGT) varied statistically depending on the stratification duration and subsequent treatment with hot water or H_2SO_4 or GA_3 (figure 2a, b, c). Without application of hot water or H_2SO_4 or GA_3 treatments, the positive effect of stratification for 30 and 60 days on MGT could not be determined. Increasing the duration of stratification from 0 to 90 days hastened seed germination when accompanied by treatments.

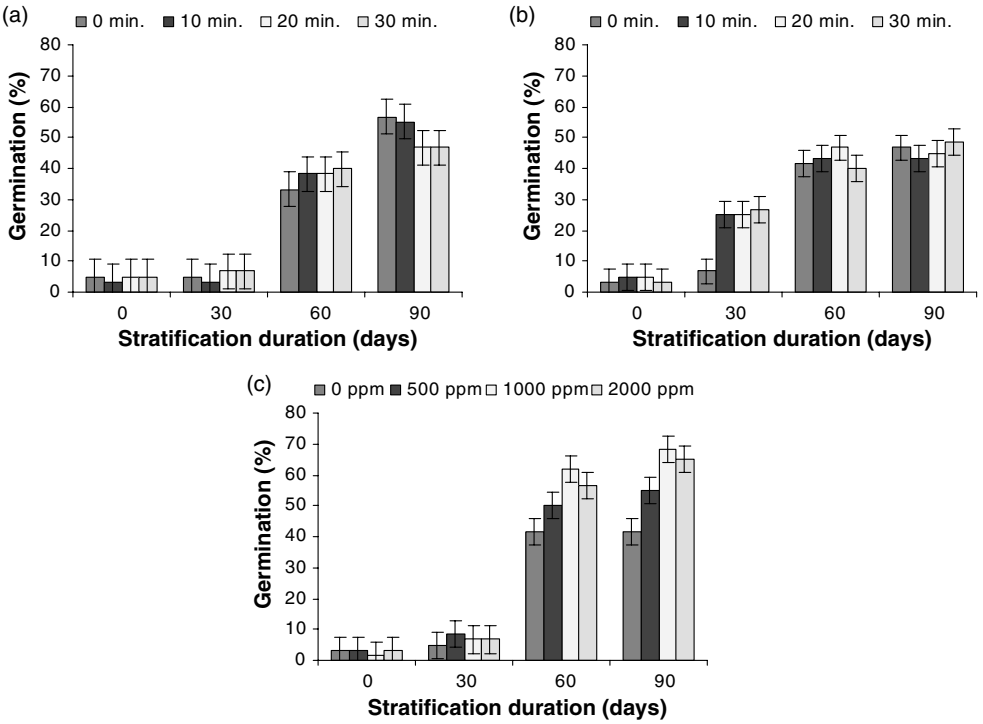

Figure 1. Germination of mahaleb cherry (*Prunus mahaleb*) seeds stratified for 0, 30, 60 or 90 days at 4-5°C treated with hot water (a) or sulfuric acid (b) or GA_3 (c).

The results indicated that the MGT for nonstratified seeds or those moist-chilled for 30 days was not significantly influenced by hot water treatment figure 2a). However, soaking in hot water for 20-30 minutes reduced the MGT for seeds that had been moist-chilled for 60 and 90 days.

Soaking nonchilled seeds in H_2SO_4 for different durations did not change germination rate significantly (figure 2b). A similar trend in MGT was observed for seeds that had been moist-chilled for 30 days. On the other hand, when seeds moist-chilled for 60 days were subjected to acid scarification for 20 to 30 minutes, the MGT declined significantly to 16.8 and 17.4 days, respectively. Stratification of nonscarified seeds for up to 60 days did not give a considerable change in MGT (19.9 to 21.3 days). 90 days of moist chilling did not change the MGT, even for acid-soaked seeds.

The effect of GA_3 alone or in combination with stratification on the germination rate of mahaleb seeds is shown in figure 2c. When GA_3 was used alone without stratification, it was ineffective in hastening germination rates at any of the various concentrations tested. The results also revealed that stratification for 30 and 60 days had no effect on the rate of seed germination. With stratification, the increase in concentration of GA_3 reduced the mean time to complete germination. Specifically, stratification for 90 days plus the use of GA_3 at 1000 or 2000 ppm reduced the mean time to complete germination significantly to 11.07 and 12.10 days, respectively.

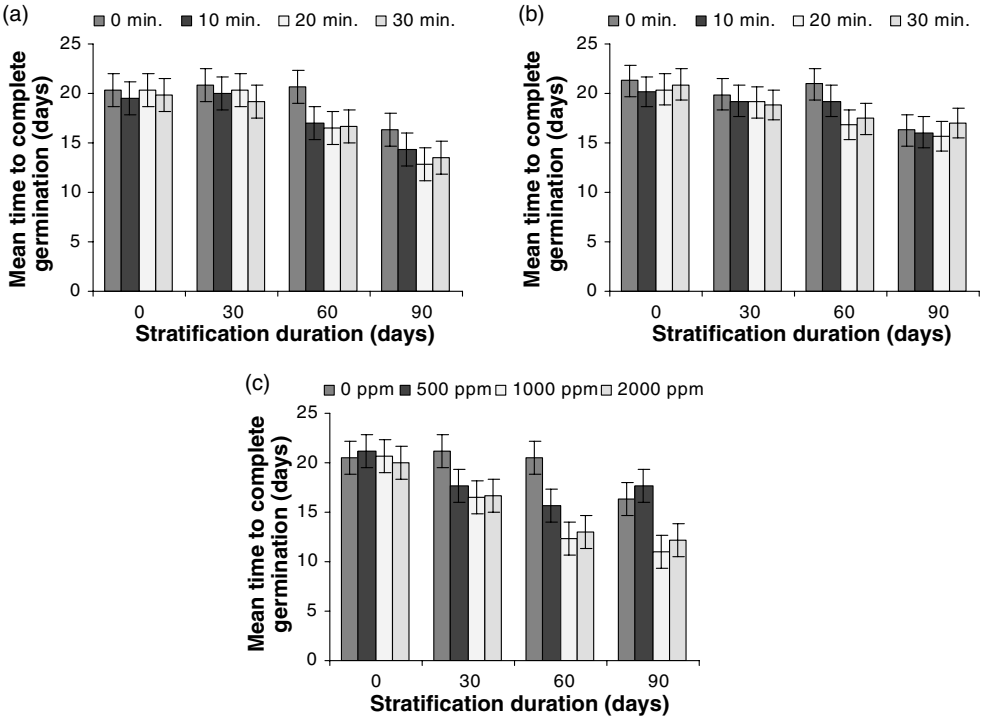

Figure 2. Germination rate of mahaleb cherry (*Prunus mahaleb*) seeds stratified for 0, 30, 60 or 90 days at 4-5°C treated with hot water (a) or sulfuric acid (b) or GA_3 (c).

Discussion

Mahaleb cherry seeds sown immediately after extraction from ripe fruits failed to germinate. The percentage of seeds that germinated without pretreatment was very low (1.7 – 5.0%). This extremely low percentage indicates that seeds of this species are dormant, since 99% of the seeds were viable. Hot water scarification was not successful in enhancing seed germination; only about 5% of the seeds germinated after being soaked in water at 90°C for 20 to 30 minutes. Germination rate for this treatment was still not acceptable in terms of satisfying the primary objective of this study.

The ability of hot water to separate the columnar macrosclereid cells through thermal expansion (Teketay, 1998) was ineffective in permitting water to penetrate mahaleb seeds. This result agrees with that obtained by Ren and Tao (2004).

Although sulfuric acid has been used as an effective scarification agent to overcome seed covering-induced dormancy in different plant species (Ren and Tao, 2004; Smiris *et al*., 2006; Heidari *et al.,* 2008), mahaleb cherry seeds that had been chemically scarified without stratification failed to germinate well, suggesting that dormancy in this species is not a result of a physical barrier or the seed coat. This result confirms the finding of Yang *et al.* (2007). However, it increased the germination percentage for seeds that had

been moist-chilled for 30 days. The use of acids, especially H_2SO_4, improves germination in many Prunus species which are characterized by a hard seed coat by increasing coat permeability and increasing the exchange of water and oxygen through testa membranes (Orozco-Almanza *et al.,* 2003).

Gibberellic acid has been used in attempts to promote the germination of seeds with morphophysiological dormancy (Karam and Al-Salem, 2001; Cetinbas and Koyuncu, 2006), and its effects vary depending on the species and type of dormancy. Without seed stratification, pretreatment with GA_3 had no effect on the germination percentage of seeds, since few seeds germinated with GA_3 application alone, indicating that GA_3 was unsuccessfully substituted for cold stratification. On the other hand, the apparent response to GA_3 pretreatment when stratification was combined with it, suggests a synergistic effect for both. The application of GA_3 after 30 days of stratification increased the germination rate but had no effect on final germination percentages. The lack of GA_3 effectiveness in stimulating the germination of seeds that had been moist-chilled for 30 days might be attributed to the negative effect of GA_3 on levels of pyruvate kinase and malate dehydrogenase and/or production of a protenaceous germination inhibitor (El-Dengawy, 1997). Both germination percentage and rate increased with higher GA_3 concentration for seeds stratified for 60 and 90 days. Previously, it has been reported that an increase in germination resulting from the exogenous application of GA_3 was due to the effect of inhibitors and increasing endogenous gibberellin-like substances (Bewley and Black, 1994). Combinations of GA_3 and stratification for two months or more increased germination percentages above levels attained by stratification without GA_3. Furthermore, germination rate decreased with combination of GA_3 and stratification. The response to the interaction of stratification and GA_3 is in accordance with the results reported by Fang *et al.* (2006).

The stratification period had a significant influence on seed germination, thus showing a positive correlation between germination percentages and the length of the period of stratification. Germination percentages failed to increase with 30 days of stratification. However, further prolongation of stratification periods caused a significant increase in germination percentages. The optimum duration of stratification required for breaking dormancy (60-90 days), observed in the current study, was close to the range (70-112 days) described by Gercekcioglu and Cekic (1999) and less than the range (100-120 days) given by Seeley and Damavandy (1985) for mahaleb cherries. Similarly, it was greater than the 2-8 weeks described for different species of *Prunus* (Martinez-Gomez and Dicenta, 2001; Garcia-Gusano *et al.,* 2004).

The results of this study indicate that endogenous dormancy has a clear inhibiting effect on seed germination. This dormancy was overcome by stratification for 60 days or more. When the seeds were insufficiently stratified (i.e., for 30 days only), low germination percentages were observed. However, periods of stratification ranging between 60 and 90 days were suitable for overcoming embryo dormancy and obtaining the best germination rates. The positive response to stratification indicates that either fall or winter planting, or laboratory stratification prior to spring planting, is necessary to overcome dormancy.

Conclusion

Based on the present study, it may be concluded that scarification with hot water or H_2SO_4 fails to stimulate the germination of *Prunus mahaleb*. However, stratification alone for long periods or in combination with sulfuric acid or GA_3 pretreatment has a greater effect than these treatments applied alone. The effect of stratification was duration-dependent. The most effective and fastest method for overcoming dormancy in mahaleb cherry seeds was to treat seeds stratified for 60 and 90 day-periods with GA_3 at 1000 ppm. It is therefore recommended that GA_3 be included in addition to cold stratification for mahaleb cherry seeds.

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