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Research Note

Optimization of seed germination in *Prunus* species combining hydrogen peroxide or gibberellic acid pre-treatment with stratification

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Summary

The combined effect of stratification (chilling) with hydrogen peroxide (H_2O_2) or gibberellic acid (GA_3) pre-treatments on seed germination in peach (*Prunus persica*) and three different wild almond species (*P. scoparia*, *P. communis*, and *P. haussknechtii*) was investigated. Mature seeds without endocarp were disinfected with 2% TMTD® fungicide solution for 30 min. Following surface-sterilization, seeds with shells were rinsed three times for 2 minutes each in sterile distilled water and then imbibed for 24 h in either distilled water (control), H_2O_2 (0.5 and 1%, 24 h) or GA_3 (250 and 500 ppm, 30 min). Treated seeds were then stratified at 7°C for 1 to 9 weeks. The number of germinated seeds was recorded weekly for each species. There were significant differences in the percentage and time of seed germination between species and treatments although germination was earlier and more uniform in the treated seeds in comparison with the control in all species. The most effective pre-treatments for breaking dormancy during subsequent stratification were 0.5% H_2O_2 (*P. scoparia*), 500 ppm GA_3 (*P. communis*, *P. haussknechtii*) and 250 ppm GA_3 (*P. persica*).

Experimental and discussion

Seed dormancy, defined as the failure of viable mature seeds to germinate under favorable conditions, is assumed to be an important adaptive trait in nature, enabling seeds to remain quiescent until the conditions for germination and seedling establishment become favorable (Finch-Savage and Leubner-Metzger, 2006). Two main mechanisms of dormancy have been described in *Prunus* species: an external mechanism controlled

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by the endocarp and the testa (maternal tissue), and an internal mechanism controlled by the embryo, which affects later growth of seedlings (Martínez-Gómez and Dicenta, 2001; García-Gusano *et al.*, 2004; 2009). Under natural conditions, release of *Prunus* species dormancy generally occurs during stratification (imbibitions at low temperature), being regulated by a combination of environmental and endogenous signals with both synergistic and competing effects.

A number of different methods have been used to accelerate the breaking of seed dormancy in *Prunus* species. The most common are the application of hormones (Mehanna *et al.*, 1985) or the mechanical removal of endocarp and seed coat (Martínez-Gómez and Dicenta, 2001; García-Gusano *et al.*, 2004). They reported that elimination of the endocarp reduces the period of stratification, probably by the more effective washing of phenolic compounds and alkaloids contained in the seed coat, which inhibit seed germination.

In this study, wild almond species were studied including *P. scoparia* Spach, *P. haussknechtii* C.K. Schneid and *P. communis* L. collected from a single tree in Tehran (north west Iran) and Fars provinces (south Iran) during 2009. Seeds from "Redhaven" peach [*P. persica* (L.) Bastch] cultivar were also included.

Four replications of twenty mature seeds without endocarp of each species were disinfected in a 2% TMTD® (Tetramethylthiuram disulphide) fungicide solution for 30 minutes. They were then soaked in sterile conditions in distilled water (control), hydrogen peroxide (0.5 or 1%), or gibberellic acid (250 or 500 ppm) for 24 hours. The control and pre-treated seeds were placed in sterile conditions in humid vermiculite layers in a re-closable polypropylene Ziplock bag (10 × 5 cm; 1 bag per replicate) and stratified in a cold chamber at 7°C ± 0.5 in darkness, for 1 to 9 weeks. Germination during stratification was considered as the start of visible radicle growth and was observed by removing the Ziplock bags from the stratification conditions and checking the seeds inside. The time of germination was recorded as the time between seed sowing and the beginning of germination. Mean germination time (MGT) was calculated as $\Sigma(nt)/\Sigma n$, where n = number of seed germinating at time t and t = number of days after placing the seeds into the stratification conditions (Ellis and Roberts, 1981). After a multifactor ANOVA for each species and all treatments, MGT values were grouped using Duncan's range test using SAS® statistical software (SAS, 2002).

Results showed significant differences in the percentage and time of seed germination between species and treatments. For all species, germination occurred earlier and was more uniform during the stratification of the treated seeds in comparison with the controls (figure 1), with the final germination at the end of the study (week nine) being 100% for treated seeds and 90% for control. No germination was observed for the control treatments after stratification periods of less than five weeks. The first differences detected as significant were observed in *P. scoparia,* with 51% germination in seeds treated with 0.5% H_2O_2 after 4 weeks of stratification. However, after 4 weeks stratification 500 ppm GA_3 resulted in the earliest germination of *P. communis* and *P. haussknechtii*, and in *P. persica,* 250 ppm GA_3 treatment produced the earliest germination. After 5 weeks stratification, the seed germination percentages observed were 68% in *Prunus scoparia* (0.5% H_2O_2), 60% in *P. communis* (500 ppm GA_3), 42% in *P. haussknechtii* (500 ppm GA_3) and 34% in *P. persica* (250 ppm GA_3) while control seeds failed to germinate (figure 1).

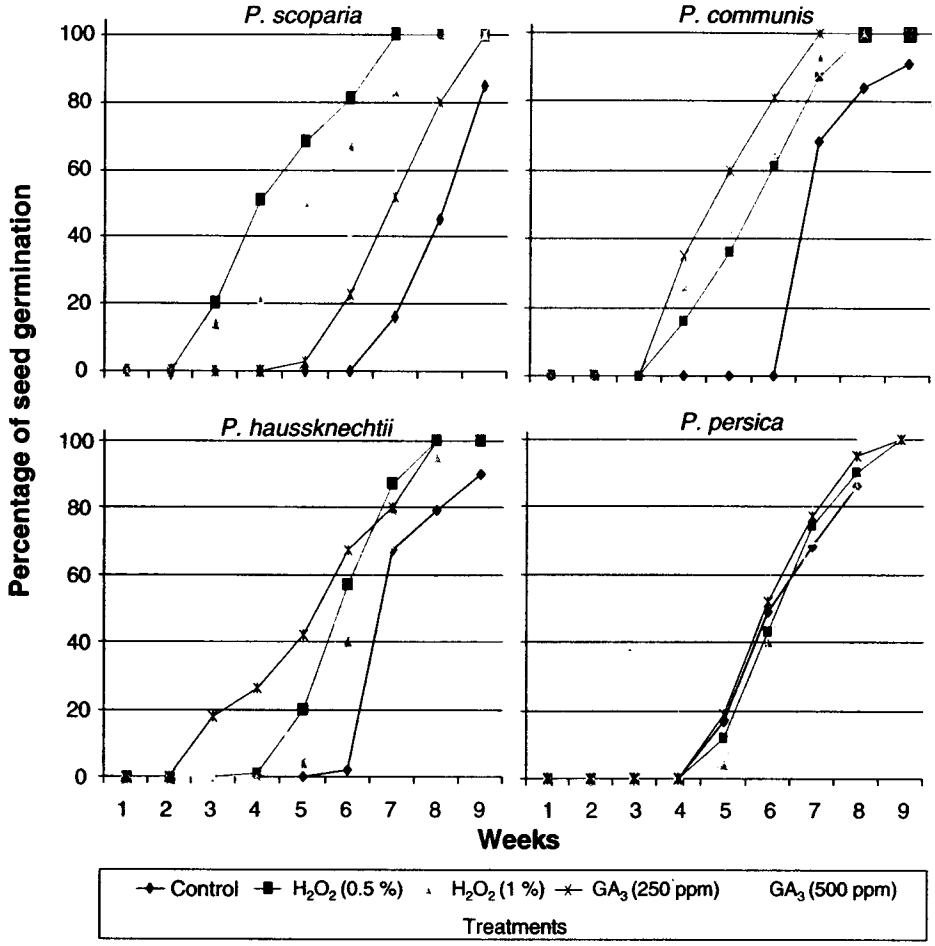

Figure 1. Percentage of germination (%) of three wild almond species and peach seeds stratified at 7°C in dark conditions following 24 h imbibition in different concentrations of either hydrogen peroxide (H_2O_2) or gibberellic acid (GA_3).

Mean germination time (MGT) values indicated that treatment with 0.5% H_2O_2 and distilled water (control) resulted in the most rapid (low MGT) and slowest (high MGT) dormancy breaking in the wild almond species assayed. In the case of peach seeds the treatment with 250 ppm of GA_3 resulted in more rapid dormancy breaking. Wild almond species, *P. scoparia, P. communis* and *P. haussknechtii*, were similar in their behaviour while *P. persica* had the highest rate of germination following treatment with 250 ppm of GA_3 (figure 2).

Germination was more advanced with all the chemical treatments than with control seeds, as shown by Rouhi *et al.* (2006). They obtained significant differences between the control and gibberellic acid treatments, with the best result were recorded with 125 ppm gibberellic acid at 7°C. Probably this hormone acts by replacing the chilling requirement and enhancing endogenous GA_3 levels which is necessary for germination. In addition, H_2O_2 treatments improved the germination of studied species, and these results are in agreement with several reports; exogenous application of H_2O_2 improved seed germination in many plants including almond even in long term stored seeds (Chien and Lin 1994; Zeinalabedini *et al.*, 2009).

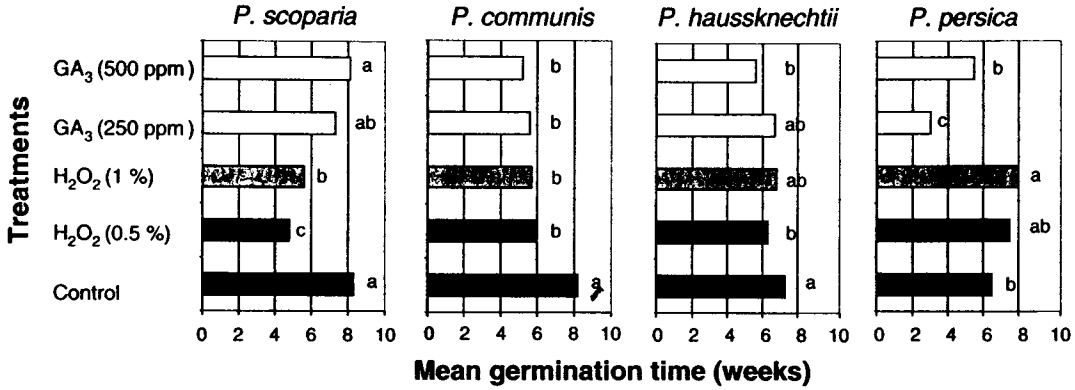

Figure 2. Mean germination time of three wild almond species and peach seeds stratified at 7°C in dark conditions following 24h imbibition in different concentrations of either hydrogen peroxide (H_2O_2) or gibberellic acid (GA_3). Different letters indicate statistical significance according to Duncan test ($P < 0.05$).

In conclusion, our results showed the suitability of the combination of chilling with hydrogen peroxide or gibberellic acid treatments for the optimization of seed germination in *Prunus* species. These findings could have a practical application in nurseries management to increase uniformity and earlier seed germination. The most effective treatments for breaking dormancy during stratification were 0.5% H_2O_2 (*P. scoparia*), 500 ppm GA_3 (*P. communis*, *P. haussknechtii*) and 250 ppm GA_3 (*P. persica*).

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