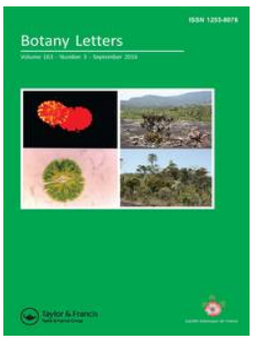

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Treatments for seed germination improvement in *Prunus azorica*, *Frangula azorica* and *Morella faya*, three native species of Azores Islands

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ABSTRACT

The aim of this study was to determine the best treatment to maximize seed germination of three native species of the Azores islands (*Prunus azorica*, *Frangula azorica* and *Morella faya*). In all three species the effect of gibberellic acid (GA_3) application and cold stratification on seed germination under controlled conditions (light and temperature) was investigated. In particular, seeds of all three species were treated with 500 and 1000 mg L^{-1} GA_3 for 24 hours and were subsequently cold stratified at 3–5°C for 0, 1 and 2 months. In addition, seeds from each species were only cold stratified for 0, 1 and 2 months and also seeds with the endocarp (stones) of *P. azorica* only were subjected to outdoor stratification from 1 October 2014 up to 28 February 2015. Untreated seeds of all three species exhibited a very low germination percentage, indicating that the seeds of all three species were dormant. For all three species, the germination of seeds untreated with GA_3 significantly increased as the cold stratification period increased whereas GA_3 application was more effective in improving germination in *P. azorica* and *F. azorica* than *M. faya* seeds. Furthermore, stones of *P. azorica* that were subjected to outdoor stratification and then transferred to germinate under controlled standard conditions exhibited high and rapid germination.

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Keywords; *Frangula azorica*; *Morella faya*; pre-germination treatment; *Prunus azorica*; seed dormancy; seed germination

Introduction

The Azores archipelago is located in the North Atlantic Ocean. It is composed of nine islands that are divided into an eastern group (Sao Miguel and Santa Maria islands), a central group (Terceira, Graciosa, Sao Jorge, Pico and Faial islands), and a western group (Flores and Corvo islands). The Azorian flora is relatively depauperate. Of the total flora (811 species) only 197 species (24%) are considered indigenous (Schafer 2003), of which 70 species are classified as endemic to the archipelago (Schafer 2005). Human activities such as agriculture, clearance of the forest for pasture, as well as the introduction of exotic species (flora and fauna) have resulted in habitat loss and degradation of the Azores islands (Haggar 1988; Silva and Smith 2004). Over the last decades there has been an increased interest in conservation and restoration of these plant communities (Pereira et al. 1998) and reforestation efforts have focused on reintroduction of native forest species and particularly on endemic rare species (Ferreira and Eriksson 2006).

Prunus azorica (Hort. ex Mouillef.) Rivas Mart., Lousa, Fern. Pietro, E. Dias, J.C. Costa & C. AIguiar (Rosaceae), *Frangula azorica* V. Grubow (Rhamnaceae) and *Morella faya* (Aiton) Wilbur (syn. *Myrica faya* Ait.) (Myricaceae) are forest species native to the Azores Islands. *Prunus azorica* and *F. azorica* are endemic tree species to the Azores, listed as endangered and as near threatened, respectively, on the International Union for the Conservation of Nature (IUCN) Red List. *Morella faya* is an evergreen shrub or small tree native to Macaronesia (the Azores, Madeira and the Canary Islands) (Walker 1990). The above species have to be propagated in nurseries and, subsequently, transplanted to restoration sites.

For many species, propagation from seeds is the most common and the cheapest method used in nurseries (MacDonald 2006). As a further benefit, the genetic diversity is promoted by propagation from seeds. However, a major constraint to the sexual propagation of many species is the poor germination of their seeds. This is possibly due to low seed viability or, more frequently, to seed dormancy, which is a physiological state preventing a viable seed from germinating even when the environment is favourable to germination (MacDonald 2006). The causes of seed dormancy can be attributed to exogenous factors (seed coat and other structures prevent germination), or endogenous factors (embryo characteristics that prevent germination), or a

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combination of both (Nikolaeva 1977). Various methods such as scarification (mechanical or chemical), cold moist stratification, gibberellic acid (GA_3) application are used to overcome different types of dormancy, so that the highest percentage of viable seeds could be brought to the point of germination (Baskin and Baskin 1998; MacDonald 2006).

The seeds of *Prunus* species have two different types of dormancy: internal, e.g. embryo dormancy, and external, e.g. endocarp dormancy (Mehanna and Martin 1985; Martinez-Gomez and Dicenta 2001; Grisez, Barbour and Karrfalt 2008; Pipinis et al. 2012b). The presence of a stony endocarp in *Prunus* fruits, that is water permeable (Dirr and Heuser 1987), may restrict seed germination (Young and Young 1992). According to Moreira et al. (2012), the removal of endocarp in *P. azorica* is the most effective treatment in breaking seed dormancy; true seeds germinate at high percentage without cold stratification when they are incubated at 10/5 or 15/10°C. In contrast, the endocarp removal alone is not enough to break dormancy in other *Prunus* species (Garcia-Gusano, Martinez-Gomez and Dicenta 2004; Cetinbas and Koyuncu 2006; Pipinis et al. 2012b). Whereas cold stratification (60 or 90 days) or warm (30 days) followed by cold stratification (60 days) is necessary for germination of *P. azorica* stones at 10/5°C (Moreira et al. 2012). Gibberellin's applications have been reported to improve germination in *Prunus* true seeds (Cetinbas and Koyuncu 2006; Imani et al. 2011; Pipinis et al. 2012b; Moreira et al. 2012).

Germination studies have been conducted in the *Frangula* genus. Keeley (1987) reported that the seeds of *Frangula californica* stratified at 5°C for 1 month germinated readily. The best results in germination of *Frangula purshiana* were achieved using seeds stratified in the dark at 2–5°C for 112 days (Radwan 1976). Apart from cold stratification, dormancy of *F. purshiana* seeds was completely broken by treatment with 500 ppm K-GA_3 for 48 hours in the dark. Furthermore, Dirr (1990) suggested stratification for 2–3 months in moist peat at 5°C for seeds of *Rhamnus cathartica.*

According to Griffin and Blazich (2008), the seeds of *Morella/Myrica* species require pre-germination treatments for optimum germination. In *Myrica pensylvanica* and *Myrica cerifera*, first the wax coating on fruits must be removed and then moist stratification of seeds for 3 months at 5°C is necessary to overcome dormancy (Fordham 1983). The maximum germination in *Myrica gale* is achieved by cold treatment of seeds at 5°C for 6 weeks (Schwintzer and Ostrofsky 1989), whereas in *Myrica rubra* seed germination is achieved by warm stratification at 20/30°C for 8 weeks followed by cold stratification at 4°C for 12 weeks (Chen, Kuo and Chien 2008). However, Walker (1990) refers that mesocarp removal increases germination of *Morella faya* seeds and the scarification of endocarp resulted in the most rapid germination.

The supply of native species seeds is often limited; therefore understanding the requirements for seed dormancy breaking and germination is necessary to maximize propagation. The present study aims to (i) evaluate the effect of cold stratification (CS) and GA_3 treatments (and their combinations) on germination and (ii) to propose effective treatments that maximize germination of *P. azorica*, *F. azorica* and *M. faya* seeds.

Material and methods

Mature fruits of *P. azorica*, *F. azorica* and *M. faya* were collected in the middle of September 2014 from trees growing in their natural habitat at Furnas in Sao Miguel island, Azores. The collected fruits were immediately transported to the laboratory of Silviculture, Department of Forestry and Management of the Environment and Natural Resources, Democritus University of Thrace, Greece. The fruits were pulped by hand and the pulp (exocarp and mesocarp) was removed with water. Then, the clean seeds (with endocarp) that sunk to the bottom of the container were spread out on filter papers in laboratory conditions and left to dry. After drying, the seeds were stored in glass containers in a refrigerator (3–5°C) until used in the experiments.

Seed treatment

Germination experiments were started the following October and conducted in the Department of Forestry and Management of the Environment and Natural Resources, Democritus University of Thrace.

Prunus azorica

The *Prunus* species are characterized by a hard endocarp which surrounds the true seed. The endocarp and seed are usually called the stone (Grisez, Barbour and Karrfalt 2008). For *P. azorica*, stones (true seed plus endocarp) and seeds (true seeds) of which the endocarp was removed by cracking using a vice were used in the experiments. Seeds were soaked in 0 (distilled water, control), 500 or 1000 mg L^{-1} GA_3 (two volumes of GA_3 solution for each volume of seeds) for 24 hours at room conditions. For each treatment with GA_3 there were 240 seeds. Subsequently, the seeds were mixed with moist, sterilized river sand (five volumes of dry sand for each volume of seeds) in plastic containers and cold stratified for 0, 1 and 2 months in a refrigerator at 3–5°C. Three plastic containers corresponded to the three concentrations of GA_3. In total, nine treatments (combinations between GA_3 and CS) were applied. In addition, on 1 October 2014, 100 stones were prepared as described above and then subjected to outdoor stratification up to 28 February 2015. The temperatures during outdoor seed stratification are presented in Table 1. During seed stratification (in refrigerator or

Table 1. Average monthly temperatures during outdoor seed stratification.

Months	Temperature (°C)		
	Mean	Mean max.	Mean min.
October 2014	13.7	19.6	8.4
November 2014	9.0	12.8	5.7
December 2014	6.0	9.3	3.1
January 2015	3.8	8.5	–0.4
February 2015	5.9	10.2	2.0

Source: National Observatory of Athens, meteorological station of Orestiada.

outdoors), sand moisture was checked periodically and distilled water was added whenever necessary to keep it moist.

Frangula azorica and Morella faya

Seeds of *F. azorica* and *M. faya* were treated with GA_3 and stratified in a refrigerator at 3–5°C, as described above for the seeds of *P. azorica*. For both species and for each treatment with GA_3 there were 360 seeds. In the cases of *F. azorica* and *M. faya*, the term "seed" refers to the true seed plus endocarp. As far as *M. faya* is concerned, a "seed" may consist of up to five fused endocarps plus the true seeds (Walker 1990).

Germination test

For each species, at the end of each stratification period, a random sample of seeds was taken from each plastic container. For *F. azorica* and *M. faya* species, for each treatment there were four replications of 30 seeds. Whereas for *P. azorica*, for each treatment there were four replications of 20 seeds, but in the outdoor stratified stones there were four replications of 25. The seeds were randomly placed on sterilized river sand moistened with distilled water in 9-cm plastic Petri dishes. Before their arrangement in Petri dishes, the seeds were dusted with fungicide (Captan) to avoid the development of fungi. The Petri dishes were randomly arranged on the shelves of the growth chamber and were watered with distilled water, as necessary. The temperature in the growth chamber was set at 20°C for a 16-hour dark period and 25°C for an 8-hour light period (provided by cool white fluorescent tubes with an intensity of 75 μmol m^{-2} s^{-1}). The germinated seeds of *F. azorica* and *P. azorica* were counted once a week for a period of 9 weeks, whereas the germinated seeds of *M. faya* were counted once a week for a period of 13 weeks. A seed with a radicle that was at least 2-mm long was considered germinated (ISTA 1999).

The germination percentage (GP) and the mean germination time (MGT) were calculated as the average of the four replications. MGT was calculated according to the following equation:

$$\mathrm{MGT} = \sum(Dn) / \sum n$$

where n is the number of seeds which germinate on day D and D is the number of days counted from the beginning of the test (Ellis and Roberts 1981).

At the end of the germination test, due to low GP, *F. azorica* seeds were subjected to a tetrazolium test.

Statistical analysis

For each species, a completely randomized experimental design was used. The GP data were arc-sine square root transformed before analysis (Snedecor and Cochran 1980). The transformed data as well as the MGT data were checked for normality and homogeneity of variances and then analysed by one-way analysis of variance. Comparisons of the means were made using the Duncan test (Klockars and Sax 1986). All statistical analyses were carried out using SPSS software (SPSS, Inc., Chicago, IL, USA).

Results

Prunus azorica

A CS period > 1 month was not used for the seeds that had been treated with GA_3 solutions (500 and 1000 mg L^{-1}) as, at the end of the first month of CS, germinated seeds appeared.

Untreated seeds exhibited very low GP (15.00%) and high MGT (43.02 days) (Table 2). Stratification of untreated seeds (1 and 2 months) resulted in a significant increase in the GP (77.50 and 88.75%, respectively) and a significant decrease in the MGT (19.08 and 10.68 days, respectively). Non-stratified seeds treated with 500 and 1000 mg L^{-1} GA_3 exhibited higher GPs (72.50 and 76.25%, respectively) and lower MGT (29.60 and 26.90 days, respectively) than control seeds (15% and 43.02 days). After each treatment with GA_3 solution (500 and 1000 mg L^{-1}), a 1-month period of CS significantly improved the GP (from 72.5 and 76.25% to 85 and 87.5%, respectively) and reduced the MGT (from 29.6 and 26.9 days to 10.21 and 8.7 days, respectively) of seeds. Furthermore, stones that were subjected to outdoor stratification exhibited GP (79%) as high as seeds that were only stratified for 1 month or only treated with GA_3 and MGT (11.32 days) as low as seeds that were stratified for 2 months or were treated with GA_3 and then were stratified for 1 month (Table 2).

Table 2. Effects of gibberellic acid (GA_3) combined with cold stratification, and autumn sowing on germination percentage and mean germination time of *Prunus azorica* seeds.

GA_3 (mg L^{-1})	Cold stratification (months)	Germination percentage (mean ± SD)	Mean germination time (days) (mean ± SD)
Control	0	15.00 d ± 4.08	43.02 d ± 4.58
	1	77.50 bc ± 6.45	19.08 b ± 2.34
	2	88.75 a ± 4.79	10.68 a ± 1.00
500	0	72.50 c ± 6.45	29.60 c ± 2.83
	1	85.00 ab ± 5.77	10.21 a ± 0.92
1000	0	76.25 c ± 2.50	26.90 c ± 2.98
	1	87.50 a ± 6.45	8.70 a ± 0.59
Autumn sowing		79.00 bc ± 3.83	11.32 a ± 0.99

The analyses of variance indicated that there were significant differences in germination percentages as well as in mean germination times (a = 0.05) among the treatments that were applied to *P. azorica* seeds ($F_{(7,24)} = 55.67$, $p = 0.000$ and $F_{(7,24)} = 103.90$, $p = 0.000$, respectively). Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using the Duncan test.

Table 3. Effects of gibberellic acid (GA_3) combined with cold stratification on germination percentage and mean germination time of *Frangula azorica* seeds.

GA_3 (mg L^{-1})	Cold stratification (months)	Germination percentage (mean ± SD)	Mean germination time (days) (mean ± SD)
Control	0	22.50 d ± 3.19	33.45 c ± 2.85
	1	40.00 c ± 6.09	24.61 b ± 2.89
	2	51.67 ab ± 6.38	18.54 a ± 1.57
500	0	47.50 bc ± 5.00	20.56 a ± 1.24
	1	50.00 ab ± 4.71	18.46 a ± 1.97
	2	55.83 ab ± 3.19	18.04 a ± 2.03
1000	0	55.83 ab ± 5.69	20.08 a ± 2.05
	1	56.67 a ± 7.20	18.14 a ± 2.00
	2	56.67 a ± 6.09	17.93 a ± 1.27

The analyses of variance indicated that there were significant differences in germination percentages as well as in mean germination times (a = 0.05) among the treatments that were applied to *F. azorica* seeds ($F_{(8,27)} = 17.91$, $p = 0.000$ and $F_{(8,27)} = 24.50$, $p = 0.000$, respectively). Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using the Duncan test.

Table 4. Effects of gibberellic acid (GA_3) combined with cold stratification on germination percentage and mean germination time of *Morella faya* seeds.

GA_3 (mg L^{-1})	Cold stratification (months)	Germination percentage (mean ± SD)	Mean germination time (days) (mean ± SD)
Control	0	1.67 f ± 1.92	*
	1	60.84 c ± 6.31	58.12 c ± 3.78
	2	75.83 ab ± 5.69	20.90 a ± 1.82
500	0	10.84 e ± 5.00	43.75 b ± 3.50
	1	63.34 c ± 6.09	48.11 b ± 4.37
	2	67.50 bc ± 5.00	17.57 a ± 1.26
1000	0	28.33 d ± 6.39	43.66 b ± 4.47
	1	58.34 c ± 5.78	47.24 b ± 4.57
	2	78.33 a ± 6.39	17.90 a ± 0.82

The analyses of variance indicated that there were significant differences in germination percentages as well as in mean germination times (a = 0.05) among the treatments that were applied to *M. faya* seeds ($F_{(8,27)} = 89.80$, $p = 0.000$ and $F_{(7,24)} = 87.57$, $p = 0.000$, respectively). Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using the Duncan test.
*Mean germination time was not calculated because in one of the four replications, no seed germinated.

Frangula azorica

Untreated seeds of *F. azorica* exhibited low germination capacity (GP 22.5% and MGT 33.45 days) (Table 3). The cold stratification period of 1 and 2 months resulted in a significant increase in the GP (to 40.00 and 51.67%, respectively) and a significant decrease in MGT (to 24.61 and 18.54 days, respectively). Cold stratification treatment showed no additive effect with GA_3 pretreatment. In non-stratified seeds as well as in seeds stratified for 1 month, the pretreatment with 500 and 1000 mg L^{-1} GA_3 significantly improved the GP and reduced the MGT of seeds. In all cases, GP and MGT reached about 55% and 18 days, respectively (Table 3).

Morella faya

Untreated seeds of *M. faya* germinated poorly (1.67%) (Table 4). Cold stratification improved strongly the germination of seeds; the increase in the CS period from 1 and 2 months resulted in a strong and significant increase of GP (to 60.84 and 75.83%, respectively) and a reduction of MGT (to 58.12 and 20.9 days, respectively). In non-stratified seeds, the pretreatments with 500 and 1000 mg L^{-1} GA_3 increased the percentage of germinated seeds (to 10.84 and 28.33%, respectively), whereas no statistically significant differences were observed in MGT between seeds treated with 500 mg L^{-1} (43.75 days) and seeds treated with 1000 mg L^{-1} GA_3 (43.66 days). In seeds stratified for 1 month, the pretreatment

with 500 or 1000 mg L^{-1} GA_3 significantly improved only the MGT of seeds. In seeds stratified for 2 months, there were no statistically significant differences in GP as well as in MGT between control seeds and seeds treated with GA_3.

Discussion

Untreated seeds of *P. azorica*, *F. azorica* and *M. faya* exhibited low and slow germination (GPs: 15.00, 22.50, 1.67% and MGTs: 43.02, 33.45 days and indeterminate, respectively). These results indicate that the seeds of all three species were dormant; they required CS for efficient dormancy breaking. Furthermore, the results of our study showed that the pretreatment of seeds with GA_3 had a differential stimulatory effect on germination of the three species.

Moreira et al. (2012) found that mature seeds of *P. azorica* germinated at high percentages (> 80%) when they were placed at alternating temperatures 10/5°C or 15/10°C, whereas the germination percentages significantly decreased (< 40%) at higher alternating temperatures 20/15°C or 25/20°C. The seeds of *P. azorica* may be conditionally dormant and they germinate efficiently over a narrow range of low temperatures (< 15°C). In the present study, a CS period of 1–2 months applied to the seeds untreated with GA_3 significantly increased GPs from 15.00% to 77.50 and 88.75%, respectively. Seeds came out of dormancy in response to CS treatment and germinated at higher alternating temperatures (25/20°C) than those proposed by Moreira et al. (2012). According to Baskin and Baskin (1998), as conditional dormancy loss occurs in seeds, germination can take place over a wide range of thermic conditions. In contrast, in the experiment of Moreira et al. (2012), the CS treatments resulted in a decrease in GPs when the seeds were incubated at 25/20°C. Here we found that a pretreatment of *P. azorica* seeds with 500 or 1000 mg L^{-1} GA_3 strongly stimulated their germination (72.50 and 76.25%, respectively) at 20/25°C. Similar GP (about 75%) was observed by Moreira et al. (2012) in seeds that were treated with 500 mg L^{-1} GA_3 for 24 h and then incubated at 20/25°C. Possibly, the treatment of seeds with GA_3 solutions counteracts the inhibitory effect of high temperatures on germination of the dormant seeds. Cold or warm-plus-cold stratification is needed to overcome the dormancy of stones in most *Prunus* species (Lockley 1980; Dirr and Heuser 1987; Chen et al. 2007; Grisez, Barbour and Karrfalt 2008; Iliev, Petrakieva and Milev 2012). Furthermore, Pipinis et al. (unpublished data) observed that the seeds with the endocarp of some *Prunus* species germinate in high percentages when they are immediately sown outdoors after collection and cleaning. For the above reasons, in the present study, the stones of *P. azorica* were subjected to outdoor stratification. After such a stratification, in many stones, a split in the endocarp was observed and when the stones were incubated at 20/25°C they germinated at high percentage (79%). During outdoor stratification the stones firstly received some exposure to high temperatures (warm stratification) before they were exposed to winter temperatures (see Table 1). The exposure to this sequence of temperatures resulted in overcoming the resistance of the endocarp and breaking the physiological dormancy of seeds. As far as the germination rate is concerned, CS treatment accelerated the germination process. The same effect of CS on germination rate has been observed in a number of species (Pipinis et al. 2009; Pipinis et al. 2012a). Furthermore, in seeds stratified for 1 month, the germination was accelerated by pretreatment with GA_3. This effect of GA_3 was also observed in seeds of *P. azorica* (Moreira et al. 2012) and of *P. mahaleb* (Pipinis et al. 2012b).

In the case of *F. azorica* and *M. faya*, the GP was significantly improved in seeds given CS. In both species, a 2-month period of CS maximized the GP of seeds untreated with GA_3. Similarly, CS treatment has been found to be effective for breaking dormancy in *F. californica* (Keeley 1987) and *F. purshiana* seeds (Radwan 1976). For the first species Keeley (1987) proposes CS of seeds at 5°C for 1 month, whereas for the second species Radwan (1976) proposes CS of seeds in the dark at 2–5°C for 112 days. Concerning *M. faya*, Walker (1990) found that the GP of seeds (with endocarp) sown in a greenhouse (at 24 ± 10°C) reached 82% after 92 weeks. In the present study, non-stratified seeds of *M. faya* exhibited very low germination (1.67%) and an increase in the CS period to 1 and 2 months resulted in significantly increased GPs of 60.84 and 75.83%, respectively. Possibly, the treatment of seeds with CS may reduce the resistance of the endocarp and simultaneously increase the growth potential of the embryo as reported by Carpita et al. (1983) and Rascio et al. (1998) in other species. Griffin and Blazich (2008) mentioned that the seeds of *Morella/Myrica* species require pregermination treatments for optimum germination. According to Fordham (1983), a 3-month CS period at 5°C is necessary to overcome dormancy of *Myrica pensylvanica* and *Myrica cerifera* seeds. Furthermore, Schwintzer and Ostrofsky (1989) suggest a 6-week CS period at 5°C for the maximum germination (66.3%) of *Myrica gale* seeds. Whereas, seeds of *Myrica rubra* require 8 weeks of warm stratification followed by 12 weeks of CS for germination (Chen, Kuo and Chien 2008). In the present study, the response to increasing periods of CS was also a significant reduction of MGT for *F. azorica* and *M. faya* seeds. In practice, apart from high seed germination, uniform and rapid seed germination is also significant to avoid environmental hazards in the nursery.

The results of our study showed that the GA_3 application replaced entirely the requirement for CS in *F. azorica* seeds. The germination of seeds that were treated with 500 or 1000 mg L^{-1} GA_3 was as high and rapid as the germination of seeds that were cold

stratified for 2 months. Similar results have been obtained for *F. purshiana* seeds (Radwan 1976). It is obvious that the endocarp and the other structures that cover the embryo were permeable and GA_3 application may have increased the growth potential of the embryo and germination occurred. Furthermore, exogenous GA_3 applications have been reported to be effective in breaking dormancy and in substituting for the CS requirement in seeds of many species (Baskin and Baskin 1998). Here, the maximum GPs obtained for *F. azorica* seeds were c. 57% (Table 3), when a tetrazolium test indicated that the viability percentage was 55% for the seeds used in this study. Hence, very probably the non-germinated seeds were not viable. In *M. faya*, the most dormant seeds here studied, the GA_3 treatment significantly but weakly improved the germination of non-stratified seeds. Hence, the endocarp and the other structures that cover the embryo could be slightly permeable to GA_3. The high GP obtained after CS (about 78%) showed that the non-germinated control seeds were perfectly viable. In the same way, according to Chen, Kuo, and Chien (2008), in non-stratified seeds (with endocarp) of *Myrica rubra* the GA_3 application was effective in breaking dormancy.

Conclusions

The results of the present study demonstrate that mature seeds of the three Azorean species *P. azorica*, *F. azorica* and *M. faya* exhibited various levels of dormancy. A 2-month period of stratification at 3–5°C was essential for breaking this dormancy and maximizing germination in seeds of all three species. High germination of *P. azorica* and *F. azorica* seeds was also achieved when the seeds were only pretreated with GA_3 solution (500 or 1000 mg L^{-1}) for 24 h. Furthermore, seeds with endocarp of *P. azorica*, which were subjected to outdoor stratification and then incubated in standard conditions, exhibited high and rapid germination. In practice, the removal of endocarp without damaging the embryo is difficult for large amounts of seeds, so for propagation purposes, we suggest that seeds with endocarp of *P. azorica* be immediately sown outdoors after collection and cleaning and the germination will occur the next spring. The results of the present study on seed germination provide useful information to the nursery industry, as they can be applied to improve the method of propagation of the above species with seeds.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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