DENDROBIOLOGY

2015, vol. 74, 149–155

http://dx.doi.org/10.12657/denbio.074.015

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Effects of dormancy-breaking treatments on seed germination of *Koelreuteria paniculata* and *Mahonia aquifolium*

Received: 14 December 2014; Accepted: 12 May 2015

Abstract: The present paper examines the germination requirements of the seeds of two woody species (*Koelreuteria paniculata* and *Mahonia aquifolium*). *Koelreuteria paniculata* seeds were subjected to a combination of acid scarification, gibberellic acid (GA_3) and cold stratification treatments. Un-scarified seeds, which were only cold stratified (up to 3 months) as well as the seeds which were only acid-scarified (up to 60 minutes) exhibited low germination percentages. The combination of sulphuric acid scarification and cold stratification treatments significantly improved germination. In acid-scarified seeds, GA_3 application improved germination, but did not fully replace the cold stratification period required to break seed dormancy. *Mahonia aquifolium* seeds were subjected to a combination of GA_3 and cold stratification treatments. Notably, prolonged cold stratification is essential for the germination of *M. aquifolium* seeds since seeds stratified for 1 or even 2 months did not germinate. In almost all cases, the percentage of *M. aquifolium* seeds germinated was higher with GA_3 treatment applied prior to cold stratification than with cold stratification only.

Additional key words: Cold stratification, Gibberellic acid, Sulphuric acid, Scarification

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Introduction

Koelreuteria paniculata Laxm., which is a small deciduous tree native to China, Korea and Japan (Michler and Rudolf 2008), is ornamental, mainly planted in gardens, as its yellow flowers and green leaves are aesthetically appealing (Rehman and Park 2000). *Mahonia aquifolium* (Pursh) Nutt. is an evergreen shrub, native to western North America, which was introduced in Europe as an ornamental species due to its leaves, yellow flowers and blue berries (Kowarik 1992). In addition, *M. aquifolium* is a valued medicinal plant as it is a rich source of phenolics and antioxidants (Gunduz 2013).

The aforementioned species can be propagated with seeds in nurseries, as the specific method is cost-effective (Mcdonald 2006). However, a major constraint to the sexual propagation of many species

is the poor germination of their seeds. This is possibly due to low viability, although it is frequently due to seed dormancy (Mackay et al. 2002), which is a physiological state, during which a viable seed fails to germinate even when the environment is favorable to germination (Mcdonald 2006). The causes of seed dormancy can be attributed to exogenous (seed coat and other structures prevent germination), or endogenous factors (embryo characteristics that prevent germination) and also a combination of both (Nikolaeva 1977). According to Rehman and Park (2000), the reasons of dormancy of *K. paniculata* seeds are found in seed coats and embryos. Furthermore, Michler and Rudolf (2008) state that dormancy is caused by the impermeability of seed coats and possibly by embryos. The presence of a hard, impermeable seed coat (physical dormancy) and embryo (physiological) dormancy in a single seed is called combinational dormancy (Baskin and Baskin 2004). As far as *M. aquifolium* is concerned, in the relevant literature the type of seed dormancy has not been accurately determined. Seeds of some species of *Mahonia* genus exhibit physiological dormancy, whereas an immature or improperly developed embryo is possibly present in seeds of other species (Dirr and Heuser 1987; Baskin et al. 1993).

Various methods are used to overcome different types of dormancy, so that the highest percentage of viable seeds could be brought to the point of germination. Scarification (mechanical or acid) treatment is used to soften the seed coat making seeds permeable to water (Baskin and Baskin 1998), whereas cold moist stratification is widely used for breaking embryo dormancy and enhancing the germination of seeds in numerous species (Macdonald 2006). In case of *K. paniculata*, there are contradictory results regarding the effectiveness of the scarification treatment in dormancy-breaking and germination of seeds. Stilinovic and Grbic (1988) stated that a single mechanical scarification treatment for breaking seed coat dormancy improves the *K. paniculata* seed germination. Furthermore, Dirr and Heuser (1987) stated that if seed coat becomes permeable, some seeds will germinate. In contrast, Rehman and Park (2000) stated that the germination of *K. paniculata* seeds is not induced by a single manual scarification treatment. Thus, the assumption that scarification treatment overcomes dormancy and improves the germination of *K. paniculata* seeds incurs the risk of poor germination. Seed scarification followed by cold stratification is suggested as the best treatment to break dormancy in seeds with combinational dormancy (Baskin and Baskin 1998). For the best results in germination of *K. paniculata* seeds, Rudolf (1974b) recommends scarification with sulphuric acid for 1 hour followed by 90 days cold stratification. Also, Rehman and Park (2000) observed that cold stratification for 60 days increased germination of manually scarified seeds. According to Rudolf (1974a), seeds of *M. aquifolium* require cold stratification for germination, whereas, Dirr and Heuser (1987) state that the embryo of *M. aquifolium* seeds is possibly immature or improperly developed and, for the maximum germination, a warm followed by cold stratification is required. It is also worth noting that growth regulators, such as gibberellic acid, are used to partially or fully replace the period of cold stratification required to break physiological dormancy in the seeds of many plant species (Baskin and Baskin 1998).

The present study aims to evaluate the effect of scarification with sulphuric acid, cold stratification and gibberellic acid (GA_3) treatments (and their combinations) on dormancy breaking of *K. paniculata* and *M. aquifolium* seeds and to enable the understanding of the nature of seed dormancy exhibited by each of the species.

Materials and Methods

Mature fruits of *K. paniculata* and *M. aquifolium* were collected from plants growing in parks in Thessaloniki, northern Greece, during the autumn 2010. After collection, the seeds were extracted manually from the fruits. Subsequently, sieving and flotation were used to clean the seeds and to remove non-filled seeds. The clean filled seeds were dried under laboratory conditions and were stored in sealed containers at 3–5ºC until the beginning of the experiments.

Seed treatment

Germination experiments started in January 2011 and were conducted in the laboratory of Silviculture, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki.

Koelreuteria paniculata

Seeds of *K. paniculata* were immersed in concentrated (95–97%) sulphuric acid and gently stirred periodically (acid scarification, AS). The duration of immersions in acid was 0, 30 and 60 minutes. After treatment the seeds were removed from the acid and were washed thoroughly under running water. The seeds of each treatment with sulphuric acid (0, 30 and 60 minutes) were randomly divided in three groups. Each group of seeds of each treatment with sulphuric acid was soaked in each one of three gibberellic acid (GA_3) concentrations (0, 500 and 1000 mg l^{-1}) for 24 hours, and then placed in plastic containers with moist sterilized river sand and was subjected to cold stratification (CS) for 0, 1, 2 and 3 months. The CS of seeds took place in a refriger-

ator at temperatures fluctuating between 3 and 5°C. There were nine plastic containers corresponding to the nine combinations between duration of AS (0, 30 and 60 minutes) and concentrations of GA_3 (0, 500 and 1000 mg l^{-1}). Totally, 36 treatments (combinations between AS, GA_3 and CS) were applied.

Mahonia aquifolium

Seeds of *M. aquifolium* were soaked in 0, 500, 1000 or 2000 mg l^{-1} GA_3 for 24 hours, and then placed in plastic containers with moist sterilized river sand. Subsequently, they were subjected to CS for 0, 1, 2, 3 or 4 months. The CS of seeds took place in a refrigerator at temperatures fluctuating between 3 and 5°C. There were four plastic containers corresponding to the four concentrations of GA_3. Totally, 20 treatments (combinations between GA_3 and CS) were applied.

Germination test

For *K. paniculata* and *M. aquifolium* species, a random sample of 120 seeds was taken out from each plastic container at the end of each stratification period and randomly placed in 4 plastic petri dishes (90 mm in diameter: 30 seeds per petri dish). In both species, for each treatment there were 4 replications of 30 seeds. Sterilized river sand moistened with distilled water was used as substrate in petri dishes. Prior to the arrangement of seeds in petri dishes, the seeds were dusted with fungicide to prevent fungi development. The petri dishes were randomly arranged on shelves in the growth chamber. 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.

For each species, the germination test lasted 8 weeks and the germinated seeds were counted each week. Seed germination was determined by the appearance of a radicle at least 2 mm long (I.S.T.A. 1999). Finally, for each treatment of each species the germination percentage (GP) were calculated as the average of the 4 replications.

Statistical analysis

For each species a completely randomised experimental design was used. For *K. paniculata*, a CS period longer than 2 months was not used for the seeds which were acid-scarified for 30 minutes as, at the end of the 2-month period of CS, germinated seeds appeared. Similarly, for the seeds scarified for 60 minutes, a CS period longer than one month was not used. It is worth noting that, as regards *M. aquifolium*, treatment combinations (between GA_3 and CS), during which seeds did not germinate were not included in the statistical analysis. The germination percentage data of *K. paniculata* and *M. aquifolium* were arc-sine square root transformed before the analysis (Snedecor and Cochran 1980). The transformed data were checked for normality and homogeneity of variances and then analysed by one-way ANOVA. Comparisons of the means were made using the Duncan test (Klockars and Sax 1986). All statistical analyses were carried out using SPSS (SPSS, Inc., USA).

Results

Koelreuteria paniculata

There were significant differences in GPs (α = 0.05) among the combinations of AS durations, GA_3 concentrations and CS periods [$F_{(26,81)}$ = 122.36, p = 0.000].

In all GA_3 treatments (0, 500 and 1000 mg l^{-1}) of the un-scarified seeds and the seeds scarified for 30 and 60 minutes, the cold-stratified seeds exhibited higher GPs than the seeds which were not stratified (Table 1). In treatments with 500 and 1000 mg l^{-1} GA_3 of unscarified seeds, GP significantly increased ($p < 0.05$) by increasing the CS period. However, in the intact seeds, which were not treated with GA_3, there was not a significant difference ($p > 0.05$) in GP between the seeds stratified for 2 months and those stratified for 3 months. In each treatment with GA_3 (0, 500 and 1000 mg l^{-1}) of seeds scarified for 30 minutes there was not a significant difference ($p > 0.05$) in GP for the seeds stratified for 1 month and those stratified for 2 months. Furthermore, for each CS period (0, 1, 2 and 3 months) there was no difference ($p > 0.05$) in the GPs of the intact seeds among the GA_3 concentrations (0, 500 and 1000 mg l^{-1}). In contrast, concerning the seeds scarified for 30 and 60 minutes, which were not stratified, GA_3 application significantly increased GP ($p < 0.05$). Only the seeds that were not stratified after AS treatment for 60 minutes demonstrated a significant increase in GP ($p < 0.05$) when GA_3 concentration was also increased. As regards the 1-month stratified seeds, in each GA_3 concentration, the increase of AS duration significantly increased ($p < 0.05$) seed germination. It is also worth noting that the highest GPs ($p < 0.05$), regardless of GA_3 application, were yielded in seeds scarified for 60 minutes and then cold stratified for a one-month period. Furthermore, in each GA_3 treatment, the seeds scarified for 30 minutes and then stratified for 2 months exhibited higher GPs ($p < 0.05$) than the unscarified seeds subjected to the same period of CS.

Mahonia aquifolium

There were significant differences in GPs (α = 0.05) among the combinations of GA_3 concentrations and CS periods [$F_{(7,24)}$ = 109.31, p = 0.000].

Table 1. Germination percentages of *K. peniculata* seeds after various combinations of acid scarification, GA_3 and cold stratification treatments

Acid Scarification (minutes)	GA_3 (mg l^{-1})	Cold Stratification (months)	Germination percentage (%, S.E.)
0	0	0	0.83 i[1] 0.83
		1	10.83 h 1.59
		2	34.17 fg 2.84
		3	44.17 def 2.84
	500	0	0.83 i 0.83
		1	14.1 h 3.15
		2	30.00 g 2.72
		3	41.67 ef 2.89
	1000	0	0.83 i 0.83
		1	15.00 h 1.67
		2	36.66 fg 3.04
		3	50.00 de 3.04
30	0	0	0.00 i
		1	75.83 bc 2.84
		2	80.83 b 2.85
		3	–[2]
	500	0	10.00 h 2.36
		1	73.33 bc 3.04
		2	76.67 bc 3.04
		3	–
	1000	0	11.67 h 2.15
		1	67.50 c 3.44
		2	73.33 bc 1.36
		3	–
60	0	0	1.66 i 0.96
		1	92.50 a 2.10
		2	–
		3	–
	500	0	32.50 fg 2.50
		1	94.17 a 2.84
		2	–
		3	–
	1000	0	55.00 d 2.15
		1	95.83 a 2.10
		2	–
		3	–

[1] Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using Duncan test.
[2] For the seeds which were acid-scarified for 30 minutes, a 3-month CS period of seeds was not used as at the end of the 2-month CS period germinated seeds appeared in the refrigerator. Similarly, for the seeds scarified for 60 minutes, a CS period longer than one month was not used.

None of the *M. aquifolium* seeds, regardless of GA_3 application, subjected to CS for 1 and 2 months, germinated. In all GA_3 treatments, an increase in the CS period from 3 to 4 months significantly increased ($p < 0.05$) the germination of seeds (Table 2). Furthermore, in each CS period (3 and 4 months) the seeds treated with GA_3 (500, 1000 and 2000 mg l^{-1}) exhibited higher ($p < 0.05$) GPs than those not treated with GA_3 (0 mg l^{-1}) except for one case. After a 4-month period of CS, untreated and seeds treated with 500 mg l^{-1} of GA_3 exhibited GPs with no significant difference ($p > 0.05$). However, in each period of CS (3 and 4 months) the increase in concentration of GA_3 (500, 1000 and 2000 mg l^{-1}) did not affect ($p > 0.05$) seed germination significantly.

Discussion

As far as *K. paniculata* species is concerned, previous studies have shown that its seeds exhibit exogenous and endogenous dormancy (Rehman and Park 2000; Michler and Rudolf 2008) and need a combination of treatments before sowing in order to germinate (Rudolf 1974b; Rehman and Park 2000). Our results are congruent with the previous statement since seed dormancy was not overcome with a

Table 2. Germination percentages of *M. aquifolium* seeds after various combinations of GA_3 and cold stratification treatments

GA_3 (mg l^{-1})	Cold Stratification (months)	Germination percentage (%, S.E.)
0	0	0.00
	1	0.00
	2	0.00
	3	5.83 d[1] 1.60
	4	57.50 b 2.84
500	0	0.00
	1	0.00
	2	0.00
	3	10.83 c 2.10
	4	65.00 ab 3.19
1000	0	0.00
	1	0.00
	2	0.00
	3	14.17 c 1.60
	4	68.33 a 3.47
2000	0	0.00
	1	0.00
	2	0.00
	3	11.67 c 2.15
	4	70.84 a 2.85

[1] Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using Duncan test.

single AS treatment (up to 60 minutes). The specific result provides sound evidence that *K. paniculata* seeds exhibit physiological dormancy. Although AS degraded seed coat, the seeds did not germinate due to embryo dormancy, which corroborates the conclusions drawn by Rehman and Park (2000), who stated that seeds subjected only to manual scarification (the seeds were pierced by a needle at the end of cotyledon) are unable to germinate. In contrast, as reported by Stilinovic and Grbic (1988), a single mechanical scarification treatment improved the *K. paniculata* seed germination (61%), although germination was significantly increased by acid or mechanical scarification followed by cold moist stratification treatment. Concerning the effect of scarification treatment on seed germination, the results of the research made by Rehman and Park (2000) and also of the present study contradict Stilinovic and Grbic's (1988) research possibly due to the conditions of seed storage. In Rehman and Park's (2000) research and also in the present study, *K. paniculata* seeds were dry stored in a sealed container at 3–5°C until the beginning of the experiments, whereas storage length and seed conditions are unknown in Stilinovic and Grbic's (1988) research, in which the possibility of dry storage at room temperature may explain the different results. According to Baskin and Baskin (1998), a period of dry storage at room temperature replaces or reduces the length of the CS period required to break dormancy in seeds with nondeep and intermediate physiological dormancy. Furthermore, in the present study, the intact seeds, which were only cold stratified (up to 3 months) exhibited low germination percentages (0.83–44.17%, Table 1). Despite the fact that embryo dormancy was broken by CS treatment and growth potential of embryo increased, the embryo force did not overcome the inhibiting effects of the seed coat. These results confirm that, apart from physiological dormancy, the seed coat also inhibits the germination of *K. paniculata* seeds. The results of the present research demonstrated that the combination of AS and CS treatments was necessary to break dormancy and maximize the germination of *K. paniculata* seeds. In 1-month stratified seeds, a 60-minute AS treatment prior to CS (in all GA_3 treatments – 0, 500 and 1000 mg l^{-1}) yielded the highest GPs, which implies that a 60-minute AS duration of seeds overcame entirely seed coat dormancy and an 1-month period of CS was enough to break embryo dormancy. Potentially, in a 30-minute duration of AS, the embryo dormancy was overcome after an 1-month period of CS, but the seed coat was still hard and provided resistance to radicle emergence. Furthermore, for the same AS duration, the penetration resistance of seed coat was not reduced by increasing the CS period from 1 to 2 months as germination was not significantly improved by increasing the CS period. According to Stilinovic and Grbic (1988), a 2-month CS period is needed to maximize germination (88.50%) of seeds scarified with sulfuric acid for 60 minutes. Similarly, Ertekin (2011) found that a 2-month CS period of seeds that have been firstly soaked for 5 days in distilled water at 40°C is needed to maximize germination (90%) of *K. paniculata* seeds. The fact that the germination of intact seeds treated with GA_3 and then cold stratified (for 0, 1, 2 or 3 months) was as high as the germination of intact seeds that were only cold stratified for the same period, whereas the scarified seeds (for 30 or 60 minutes) which were treated with GA_3 prior to CS exhibited significantly higher germination than un-scarified seeds treated with GA_3 and then cold stratified for the same period, which implies that the *K. paniculata* seeds may have an impermeable seed coat and AS treatment erode seed coat making it permeable to GA_3. GA_3 application improved only the germination of scarified seeds which were not stratified. Exogenous GA_3 application may increase the growth potential of embryo; however, it did not manage to break entirely seed coat resistance of an adequate percentage of seeds. According to Baskin and Baskin (1971) and Rascio et al. (1998), pretreatment of *Ruellia humilis* and *Cercis siliquastrum* seeds with GA_3 results in an increase in the growth potential of the embryo. Rehman and Park (2000) found that application of 100, 200 and 300 mg l^{-1} GA_3, increased germination of

manually scarified seeds of *K. paniculata* from 0 (control) to 17, 18 and 15% respectively.

Prolonged cold stratification is essential for the germination of *M. aquifolium* seeds since seeds stratified for 1 or even 2 months did not germinate at all, while 4 months of cold stratification alone yielded germination percentages equal to 57.5%. A CS period longer than 4 months was not used, because towards the end of the 4-month CS period germinated seeds appeared. Previous studies on seeds of *Mahonia* species suggest that they exhibit dormancy and that stratification is required to overcome it. According to Baskin et al. (1993), a 10-week period of CS is required to break completely the physiological dormancy in *M. fremontii* seeds. Similarly, seeds of *M. bealei* and *M. japonica* germinate well when they are firstly subjected to CS for 1 to 2 months (Dirr and Heuser 1987). In contrast, *M. aquifolium* seeds may have immature or improperly developed embryo and 4 months warm stratification followed by 4 months of CS are required for maximum germination (Dirr and Heuser 1987). Furthermore, Neugebauer (1980) reported that *M. aquifolium* seeds require stratification in order to germinate and maximum germination is obtained after a 4-month period of seeds after-ripening in a moist atmosphere at 20°C followed by 4 months of chilling at 2–5°C. It is possibly, if seeds in the present study were subjected to warm stratification prior to CS, germination would have been even higher. In almost all cases, the percentage of *M. aquifolium* seeds germinated was higher with GA_3 treatment prior to CS than with CS only. GA_3 treatment may substitute the warm stratification requirement for germination in seeds. Hidayati et al (2000) found that as regards the seeds of *Sambucus canadensis* and of *Sambucus pubens* which require warm stratification followed by CS for germination, GA_3 substitutes warm stratification rather than CS. Furthermore, in *Carpinus betulus* seeds, GA_3 application appeared to replace entirely the requirements for the warm stratification, to shorten the period of CS requirement and to promote germination (Pipinis et al. 2012).

Conclusions

In conclusion, the dormancy of *K. paniculata* seeds is successfully overcome with sulphuric acid scarification followed by cold stratification. In the case of *M. aquifolium*, prolonged cold stratification is essential for the seed germination and GA_3 (1000 or 2000 mg l^{-1}) application followed by cold stratification is more effective at promoting germination than cold stratification only. Thus, a 60-minute scarification with sulfuric acid followed by an 1-month CS period is recommended as an effective treatment which maximizes germination of *K. paniculata* seeds, whereas GA_3 (1000 or 2000 mg l^{-1}) treatment for 24 hours followed by a 4-month CS period enhances germination of *M. aquifolium* seeds. The results of the present study on seed dormancy and germination are of practical interest in the nursery industry as they can be applied to improve the method of propagation of the above species with seeds.

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