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Germination study on *Arbutus unedo* L. (Ericaceae) and *Podocytisus caramanicus* Boiss. & Heldr. (Fabaceae)

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Both species *Arbutus unedo* and *Podocytisus caramanicus* that were studied show some difficulty in seed germination. The main factor for the inhibition of germination in *Arbutus unedo* is embryo dormancy and seed coat imposed dormancy in *Podocytisus caramanicus*. These species are commercially valuable for their uses (they are being cultivated as ornamental shrubs and have small requirements in water and nutrients) but they also have a scientific value since few surveys have been conducted concerning dormancy breaking and seed germination. The objective of the present work is to study the effect of various treatments in seed dormancy breaking. After the treatment, the seeds of both species were subjected to germination test under controlled conditions (20˚C/16 h dark period and 25˚C/8 h light period). The maximum percentage of germination for *Arbutus unedo* (85.75%) was observed when the seeds were soaked for 24 h in 500 ppm GA_3 and then stratified (chilled) for a period of 3 months. The maximum percentage of germination for *Podocytisus caramanicus* (63%) was observed when the seeds were immersed in concentrated sulphuric acid (98%) for 20 min.

Key words: *Arbutus unedo, Podocytisus caramanicus,* dormancy breaking, germination.

INTRODUCTION

A seed, which is dormant, will not germinate even when the environment is adequate for germination (Martinez & Dicenta, 2001). The inhibition of germination may originate from "the hardness and impermeability of the seed coat", from "the dormancy of the embryo" or from both. The germination study of several genera of the Leguminosae family, including *Acacia*, *Prosopis*, *Mimosa*, *Calycotome*, *Spartium*, *Medicago* showed that the presence of an impermeable seed coat makes germination difficult (Orozco-Almanza *et al.*, 2003; Pipinis *et al*., 2005). In nature, certain factors such as drought, temperature changes, passage through the animal digestive tracts and bacterial or fungal activity, result in the permeability of the seed coat, thus the uptake of water and oxygen is facilitated. Artificially, this can be achieved by scarifying the seed coat and by treating with concentrated sulfuric acid, or warm water (Baes *et al.*, 2002).

Another type of dormancy is "embryo dormancy". The causes for this type of dormancy are basically hormonal and the phenomenon is mainly due to the disorder of the balance between the substances that cause inhibition and growth of the embryo (Bewley, 1997). The regulation of seed germination is controlled by hormonal interaction (Davies, 1990). Abscisic acid (ABA) has an important role in the inhibition of seed germination (Wang *et al.*, 1995), whereas gibberellins seem to be the most significant endogenous growth substances, which promote seed growth (Davies, 1990). Stratification in appropriate

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temperature is regarded as the most important way to break this type of dormancy. For the dormant embryo, stratification represents an important mechanism to ensure that all processes controlling the balance between the restraining and growth substances take place. According to Allen and Meyer (1990), ''low temperature during stratification is regarded as the most important way to break dormancy in many types of seeds. The use of exogenous gibberellin can partially replace the need for stratification''. Studies concerning a number of species have shown that the combination between GA_3 application and cold stratification gives the best results (Gerçekçioğlu & Cekic, 1999).

Arbutus unedo is a Mediterranean species. It is one of the most important components of the maquis communities, but it grows also in loose oak woods, in open pine forests and sometimes in phrygana. The European altitudinal maximum is in Greece 900 m, while the Asiatic one at about 800 m is in Lebanon (Boratynski *et al.*, 1992). The reproductive cycle is long with the flowers of one year coinciding with the ripening of the fruit of the previous year (Athanasiadis, 1985; Chiarucci *et al.*, 1992). It is a member of the Ericaceae family, which is difficult to propagate by seed due to genetic variation and specific requirements of seed germination (Mereti *et al.*, 2002).

Podocytisus caramanicus is a rather rare species, scattered in northern and central, mainly inland, regions of continental Greece, between elevations of 400 and 1300 m. The highest stations have been found at 1300-1600 m on Mt. Smolikas in Epirus.

The study of seed dormancy and germination of *A. unedo* and *P. caramanicus* has a great commercial and practical interest for nursery owners, because it provides easy proliferation techniques of these species with seeds. They are species that can be cultivated as ornamental bushes with small requirements in water and nutrients and complete studies concerning dormancy breaking and seed germination are limited. For all these reasons, both species were chosen for planting in unfavourable sites of the Egnatia highway banks, in order to restore the heavily disturbed landscape and during the forthcoming years a large number of plants will have to be produced. The aim of the present work was to investigate the effects of the hormone GA_3, of the cold stratification as well as of the combination of GA_3 and cold stratification on dormancy breaking of *Arbutus unedo* seeds and the chemical scarification to improve seed germination of *P. caramanicus* seeds.

MATERIALS AND METHODS

Fruits of *A. unedo* were collected in early December from Ioannina, Western Greece and fruits of *P. caramanicus* were collected in late August from Emathia, Central Macedonia, Greece. The extraction of *A. unedo* seeds started quickly to avoid fermentation. The fruits were pulped manually and for seed cleaning a series of sieves and also running water were used. The pods of *P. caramanicus* were dried first and then were grated by hand in order to break them. Sieving and flotation were used to clean the seeds. Flotation removed trash (parts of pod) and also empty, broken and insect- damaged seeds. In both species, the clean seeds were spread in filter paper and left to dry. After drying, the seeds were stored in glass containers in the refrigerator (2-4˚C) until the beginning of the experiment.

Viability test

In order to determine the viability of *A. unedo* and *P. caramanicus* seeds, the topographical tetrazolium test of viability was used. The solution was triphenyl tetrazolium chloride 1% (TCC). For each species, 100 seeds were used in 4 replicates of 25 seeds (Suszka *et al.*, 1994). The seeds were soaked for 20 h in tap water. Premoistening is necessary because staining is more intense and imbibed seeds are generally less fragile than dry seeds. The seed coat of premoistened seeds was carefully removed and the embryos were immersed in tetrazolium where they remained for 24 h in a dark chamber at room temperature. The embryos with red coloured radicle and cotyledons were considered to be viable.

Seed treatment for Arbutus unedo

Arbutus unedo seeds were soaked in 100, 500, 1000, 2000 and 3000 ppm GA_3 for 24 h and 48 h, at room temperature (Dungey *et al.*, 1980). Seeds were also immersed in warm water (70˚C) for 10 min and then soaked in GA_3 solutions for 24 h and 48 h, respectively. Treatments with GA_3 were followed by cold stratification. Subsequently, seeds were put in petri dishes on filter paper, moistened with distilled water and stratified at 2-4˚C for 1, 2 or 3 months. Also, seeds with no treatments were subjected to cold stratification for 1, 2 or 3 months (control). Afterwards, they were transferred to a growth chamber for the germination test. Each treatment consisted of 4 replicates of 25 seeds.

Seed treatment for Podocytisus caramanicus

The experiment was carried out with the following treatments: seeds were immersed in concentrated sulphuric acid (98%) for 10 and 20 min, seeds were immersed in running water for 24 h and seeds were used as control. After the treatment with sulphuric acid, seeds were washed with running water to remove the acid. They were then transferred to a growth chamber for the germination test. For each treatment 4 replicates of 25 seeds were used.

Germination test

The seeds of both species were placed in plastic petri dishes, 9 cm in diameter, on filter paper moistened with distilled water. The Petri dishes were placed in a growth chamber. The temperature in the growth chamber was set at 20˚C for 16 h dark period and 25˚C for 8 h light period (Young & Young, 1992). Seed germination was defined as the appearance of a radicle, at least 2 mm long, according to the rules of the International Seed Testing Association (1999). Germinated seeds were counted every 4 days for 5 weeks. Each germinated seed was removed in order to avoid counting confusion. The seeds were periodically watered with distilled water to keep them moistened.

Statistical analysis

The experimental design was different for each species. In *A. unedo* a completely randomised factorial design was used, where the GA_3 solution (five concentrations: 100, 500, 1000, 2000 and 3000 ppm), the time of soaking in the GA_3 solution (two times: 24 and 48 h) and the cold stratification period (three periods: 1, 2 and 3 months) were used as factors. The data obtained were statistically treated using GLM analysis. The effect of warm water on germination was not analysed because seeds did not germinate.

In *P. caramanicus*, a completely randomised design was used and the data were subjected to one-way analysis of variance.

In both species, the germination data were statistically analyzed with the program SPSS version 10.0. In order to increase normality, the germination percentage data were transformed to arc-sine square root values, before analysis (Snedecor & Cochran, 1988). The Duncan's test was used to analyse the different treatments, with a 95% degree of confidence (Matis, 1991).

RESULTS

Concerning the viability test, the seeds were rated viable or non-viable according to embryo staining. The embryos which were stained entirely red, or with small unstained areas were considered to be viable. For *A. unedo* seeds the viability was 88%, while for *P. caramanicus* 76%.

Table 1 shows the percentage of germination in the different treatments applied to *Arbutus unedo*. The treatment with warm water was not presented in the table because no seeds germinated. As presented in Table 1, after one month of stratification, the germination percentage in the seeds that had been treated with low concentrations of GA_3 (100, 500 ppm) for 24 h, was either zero or very low (5.75%). On the other hand, the increase in the concentration of GA_3

TABLE 1. Germination percentages in *Arbutus unedo* seeds after 1, 2 and 3 months of stratification (mean ± SD)

Treatments	**Months of stratification**	**100 ppm**	**500 ppm**	**1000 ppm**	**2000 ppm**	**3000 ppm**
24 h GA_3	1	0	5.75 ± 0.975	13.5 ± 3.10	33.75 ± 2.63	45.5 ± 3.10
24 h GA_3	1	1.75 ± 1.70	30 ± 4.08	63 ± 1.24	60.25 ± 2.50	58.5 ± 3.10
Control: 0%						
24 h GA_3	2	48 ± 3.65	30.25 ± 2.53	43.5 ± 3.41	29.25 ± 2.50	43.75 ± 3.59
24 h GA_3	2	42 ± 4.04	39.75 ± 2.50	67.75 ± 2.21	50.5 ± 3.41	46.5 ± 3.69
Control: 26% ± 2.58						
24 h GA_3	3	52.25 ± 4.03	85.75 ± 2.50	65.75 ± 3.30	78.5 ± 2.64	62.75 ± 4.03
24 h GA_3	3	58.5 ± 3.69	82.25 ± 2.98	66.75 ± 3.30	77 ± 2.16	68.25 ± 2.75
Control: 48% ± 2.58						

and the soaking time resulted in higher percentages of germination. The treatment with 3000 ppm GA_3 for 24 h resulted in a germination percentage of 45.5%, while the treatment with 1000 ppm GA_3 for 48 h resulted in the highest percentage of germination (63%). The seeds that were used as control, did not germinate. After two months of stratification, the seeds of *A. unedo* that had been treated with 100 ppm GA_3 for 24 h and 1000 ppm for 48 h, gave the highest percentage of germination, i.e. 48% and 67.75% respectively. The seeds that were used as control, germinated in a percentage of 26%. After three months of stratification, the seeds of *A. unedo* that had been treated with 500 ppm GA_3 for 24 h and for 48 h gave the highest percentage of germination (85.75% and 82.25%, respectively). The seeds that were used as control, germinated in a percentage of 48%. From the statistical analysis it follows that the main effects (CS, ST, GA_3S) and all the interactions were statistically significant (Table 2).

After the comparison of the means of all treatments (30 + 3 controls) with Duncan's test (Table 3), it is concluded that the treatment with 500 ppm GA_3 for 24 h and 3 months cold stratification was the best, although there was no significant difference with the treatment with 500 ppm GA_3 for 48 h and 3 months of cold stratification.

As shown in Table 4, the germination percentage for *P. caramanicus* was 63% when seeds were immersed in H_2SO_4 for 20 min. *Podocytisus caramanicus* seeds that had been immersed for 10 min in H_2SO_4 had a germination percentage of 36%, while

TABLE 2. Analysis of variance in *Arbutus unedo* seeds

Source of Variance	df	Mean square	*F*	*P*
Cold Stratification (CS)	2	16500.94	1727.73	0.00
Time of Soaking (TS)	1	2355.52	246.63	0.00
GA_3 Solution (GA_3S)	4	1706.55	178.68	0.00
CS × TS	2	1438.80	150.65	0.00
CS × GA_3S	8	1526.43	159.82	0.00
TS × GA_3S	4	187.93	19.67	0.00
CS × TS × GA_3S	8	451.14	47.23	0.00
Error	90	9.55		

TABLE 3. Analysis of variance in *Podocytisus caramanicus* seeds

Source	df	Mean square	*F*	*P*
Between groups	3	2625.33	55.46	0.00
Within groups	12	47.33		

TABLE 4. Germination percentages in all four treatments of *Podocytisus caramanicus* seeds (mean ± SD). Percentages followed by the same letter are not significantly different at the 5% level by the Duncan's test

Treatments	Germination (%)
20 min H_2SO_4	63.0 ± 10.32 [a]
10 min H_2SO_4	36.0 ± 6.83 [b]
24 h H_2O	8.0 ± 3.26 [c]
Control	11.0 ± 5.03 [d]

only 8% of the seed germinated when they were immersed in water for 24 h. The germination percentage of the seeds that were used as control was 11%.

After the analysis of variance and the comparison of the means with Duncan's test, it is concluded that among the 4 treatments of *P. caramanicus* seeds exist significant differences (Table 3). The best treatment was 20 min by immersing in H_2SO_4, which had a significant difference from all the other treatments (Table 4). The treatment concerning 24 h immersion in water had not a significant difference from the control seeds.

DISCUSSION

It is well known that hormones are a controlling factor in seed dormancy and germination. Exogenous GA_3 in dormant seeds results in dormancy breaking and germination promotion (Allen & Meyer, 1990; Choudhury & Gupta, 1995). Gibberellins also promote germination in non-dormant seeds. The effect of gibberellins depends on the germination conditions and mainly on temperature, seed coat and balance between the endogenous hormones (Webb & Wareing, 1972).

From the results it is obvious that the combination of GA_3 soaking followed by cold stratification results in satisfactory germination percentages. Full germination of *A. unedo* seeds, which were placed in dark immediately after harvest, occurred at temperatures below 15°C. Prolonged exposure of seeds to temperatures of 25 or 30°C gave no germination due to unfavourable effects of high temperature on membrane stability. The exogenous application of GA_3 to *A. unedo* seeds counteracts to some extent the inhibitory effect of high temperature according to Ricardo & Veloso (1987).

We observed that by increasing the period of stratification, the influence of the time that the seeds were soaked in the solution of the hormone was reduced. For 1 month of stratification, germination was increased in parallel with the increase of the concentration of the hormone solution. An increase in the stratification period resulted in increased germination percentage of *A. unedo* seeds that were treated with low concentration solutions.

Gibberellin can partially replace the need for stratification. For this reason, the stratification period was increased to two and three months, aiming at an increased percentage of the final germination. *Arbutus unedo* seeds that were stratified for two months had an increased germination percentage only at lower concentrations of the hormone, when compared to the previous treatment (one month of stratification). *Arbutus unedo* seeds that were stratified for one month had the highest germination percentage (63%) when they were treated with 1000 ppm GA_3 for 48 h. Seeds that were used as control were not germinated. Seeds that were stratified for two months had the highest germination percentage (67.75%) when they were treated with 1000 ppm GA_3 for 48 h. The seeds that were used as control gave a germination percentage equal to 26%. When the stratification period was increased to three months, the highest germination percentage appeared when seeds were treated with 500 ppm GA_3 for 24 h (85.75%). The seeds that were used as control gave a germination percentage of 48%. According to Karam & Al-Salem (2001), "treatment of seeds of *Arbutus andrachne* with GA_3 was successful in dormancy breaking with 250 or 500 mg l^{-1} resulting in 83-86% germination. This high germination percentage was similar to that of seeds stratified at 4°C for 12 weeks". This implies that treatment of seeds with GA_3 may substitute for cold stratification as was reported for *Prunus persica, Myrica pensylvanica* (Macdonald, 1993) and *Corylus avellana* (Jarvis *et al.*, 1968). Shekatandeh & Sheibani (1987) have reported that "the maximum germination percentage of *Pistacia terebinthus* seeds was observed with GA_3 application". Application of exogenous gibberelin on dormant hazelnut seeds resulted in DNA activation in order to begin the biosynthesis of RNA (Jarvis & Shannon, 1981). Kose (1998) has reported that "*Arbutus unedo* seeds that were immersed in a solution of 400 ppm GA_3 for 24 h at 20°C presented the highest rate of germination (98%) after 30 days". Ak *et al.* (1995) found that "the highest germination percentage (73%) of *Pistacia vera* seeds was observed in 125 ppm GA_3 for 48 h". Khan & Ungar (1995) have reported that "GA_3 decreases the dormancy which is caused by salinity in many halophytic species".

The use of chemical acids, especially H_2SO_4, improves germination in many leguminous species which are characterized by a hard seed coat (cover dormancy), by increasing coat permeability and the exchange of water and oxygen through the membranes of testa (Orozco-Almanza *et al.*, 2003). From the results of our experiments on *P. caramanicus* seeds, the positive effect of sulfuric acid on their germination is obvious. In the 3rd treatment, the seeds

that had not been treated with the acid, gave the lower rate of germination (8%), contrary to the 1st and 2nd treatments, which gave the best results. The stay of seeds for 20 min in the acid gave the best germination rate (63%). This improvement in germination rate is due to the weakening of the seed coat by the acid. We also observed that the seeds used as control had a higher germination rate (11%) compared to those that were treated with water for 24 h (8%). According to Pipinis *et al*. (2005); "in *Calycotome villosa* seeds, the use of concentrated sulphuric acid (98%) for 60 min increased significantly the seed germination in 77%, as well as for the *Spartium junceum* seeds, which gave the maximum germination rate of 87.5%". Also, in *Medicago arborea*, the treatment by immersing the seeds in H_2SO_4 for 10 min gave a very satisfactory percentage (90%). For the same species, immersing in flowing water was less effective (15%) for *Calycotome villosa*, with small percentage (29%) for *Spartium junceum*, but with significant percentage (61%) for *Medicago arborea.* Heit (1977) showed that treatment with H_2SO_4 increases the permeability of the perisperm and facilitates oxygen exchange in seed coats and membranes. According to Khan (1971) "dormancy breaking does not depend on the quantitative changes of one only augmentative substance, but it is the result of the combined effect of augmentative and inhibitory substances contained in the seed".

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