

## GERMINATION STUDIES ON *ATROPA ACUMINATA* ROYLE EX LINDLEY

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### Abstract

Seeds of *Atropa acuminata* Royle ex Lidley, which were mechanically scarified and then treated with IAA gave 78% germination while seeds subjected to acid scarification and soaking in water gave 50% germination. GA<sub>3</sub> treatment gave 38% germination. Other treatments were ineffective. The delayed and reduced germination was mainly due to the hard impermeable testa and deficiency of germination hormones.

### Introduction

*Atropa acuminata* Royle ex Lidley (Family Solanaceae) is a tall, erect, perennial herb. It is an important medicinal plant containing tropane alkaloids that have anticholinergic, anodyne, antiasthmatic and mydriatic properties. It is an official drug in British and United States Pharmacopoeias (Trease & Evans, 1983). In Pakistan, *Atropa acuminata* grows wild in Azad Jammu & Kashmir, Hazara, Swat, Dir and Murree hills at about 2900 meter height (Zaman & Khan, 1970). It has become a rare species in its habitats perhaps due to exploitation by human beings. It exhibits erratic/reduced and delayed germination of the seeds. The present study was conducted to find out the possible causes of reduced and delayed germination, and to apply various manipulations to enhance its germination.

### Materials and Methods

Seeds of *Atropa acuminata* were collected from Ayubia, District Abbottabad in mid August at a time when the berries were ripe. The seeds were separated from the berries with the help of running water, dried and stored at low temperature (8-10°C) till next January. Preliminary germination studies were carried out at five different temperatures i.e., 15, 20, 25, 30 and 35°C to find a suitable temperature for germination. The seeds were germinated in sterilized Petri dishes with double fold filter papers. For each experiment there were four replicates, each with ten seeds. The preliminary germination tests revealed that the seeds had better germination at 25°C, therefore, rest of the experiments were carried out at 25°C. The Petri dishes were observed daily for 15 days and were kept moist throughout the experiment. The germinated seeds were counted and removed. The following experiments were performed:

**Germination at different temperatures:** The seeds were kept for germination at 15, 20, 25, 30, and 35°C. There was no germination at any of the temperatures even after 15 days and the experiment was stretched up to 25 days. At 25°C only two seeds germinated. This temperature regime was adopted for the subsequent experiments.

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**Effect of mechanical and chemical scarification:** The seeds were gently scarified within the folds of sand papers till the testa ruptured from at least one point. Seeds were then treated with 25, 50% and concentrated Sulphuric acid for 75 seconds to chemically scarify them. They were thoroughly washed with distilled water and kept for germination at 25°C.

**Effect of hot water and soaking by percolating water:** Both unscarified and mechanically scarified seeds were first soaked in water for 20 hrs., at room temperature and then immersed in hot water (60°C) for 5 min. Another batch of seed was washed with running tap water for 48 hrs., and then kept for germination.

**Effect of GA<sub>3</sub> and IAA:** Scarified and unscarified seeds were soaked in 1-ppm solutions of GA<sub>3</sub> and IAA for 20 hrs., and kept for germination.

**Effect of acid scarification coupled with water soaking:** Intact and mechanically scarified seeds were first treated with concentrated Sulphuric acid for 75 sec., and then washed thoroughly with water. These seeds were soaked in water as before and set for germination.

## Results and Discussion

There was no germination of seeds kept at 15, 20, 25, 30 and 35°C for 19 days. However, the first seed germinated on the 20<sup>th</sup> day at 25°C and after 25 days 8% germination was observed. All the experiments were therefore carried out at 25°C. It was seen that the seeds had delayed and erratic germination. Chopra (1958) reported that *Atropa acuminata* seeds take at least 14-21 days at 70°F for germination and while in soil, they take 4-5 weeks for germination. The results of the present study showed that germination was not affected by temperature variation over a wide range. Germination studies made on *Datura innoxia* and *Datura fastuosa* (Hussain *et al.*, 1979), *Hyoscyamus niger* and *Hyoscyamus insanus* (Hussain *et al.*, 1984) and *Withania somnifera* (Hussain & Ilahi, 1988) have revealed various possible causes for delayed and poor germination such as hard seed coat, presence of germination inhibitors, small food reserve and/or immature embryos. A similar situation might be expected for this species also.

To overcome the problem of imbibition of water through the hard testa and seed coat, the seeds were subjected to mechanical and chemical scarifications. Mechanically scarified and acid treated seeds had only 8% germination after 15 days (Table 1). The delayed and reduced germination of *Atropa acuminata* was not just due to hard seed coat as both chemical and mechanical scarifications failed to enhance the germination. These results differed from those of Hussain & Ilahi (1988) who achieved an increase in the germination of *Withania somnifera* by rupturing and splitting the hard testa by either chemical or mechanical means.

Some seeds need hot water stimulation. Keeping this view in mind scarified and nonscarified seeds were treated with hot water. With this treatment there was 23% germination in the scarified seeds as compared to 5% germinations in the nonscarified seeds (Table 1). Water percolation treatment of the unscarified and scarified seeds showed 13 and 28% germinations, respectively (Table 1). The scarified seeds when subjected to water treatment showed 8% germination after 7 days and 50% after 14 days (Table 1). The mechanically scarified seeds did not benefit from acid scarification and water treatment and showed only 13% germination.

**Table 1. Effect of various treatments on the germination percentage of *Atropa acuminata*.**

Treatment	Days			
	13	14	15	
Germination %				
H <sub>2</sub> SO <sub>4</sub>	25 %	--	--	
	50 %	--	--	
	100 %	3.0	5.0	8.0
Mechanical	5.0	8.0	8.0	
Hot Water	unscarified	--	5.0	5.0
	scarified	10.0	15.0	23.0
Water Percolation	unscarified	13.0	13.0	13.0
	scarified	23.0	25.0	28.0
H <sub>2</sub> SO <sub>4</sub> + Water Soaking	48.0	50.0	50.0	
Mech. + Chem. + Water Soaking	13.0	13.0	13.0	
GA <sub>3</sub>	unscarified	10.0	10.0	10.0
	scarified	20.0	23.0	38.0
IAA	unscarified	23.0	22.5	33.0
	scarified	48.0	58.0	78.0

Hot water treatment and water percolation showed some effect on mechanically scarified seeds as it gave 23 and 28% germination respectively but the effect of these two treatments on intact seeds was not so good. It suggested impermeable nature of the testa. It was observed that the testa of *Atropa acuminata* seed was least imbibed by water even after prolonged soaking and was plastic like in nature. Prior to soaking (20 hrs.), hot water treatment and water percolation might have further softened and helped in rupturing the testa of mechanically scarified seeds.

In many seeds hormones and chemical stimulus is needed to trigger germination. This appears to be true in this case as application of GA<sub>3</sub> and IAA promoted the germination. Mechanically scarified seeds, when treated with GA<sub>3</sub>, showed 38% germination compared to 10% in the non-treated seeds. IAA treatment to the unscarified seeds showed 33% germination as compared to 78% germination of scarified seeds treated with IAA. Application of GA<sub>3</sub> and IAA stimulated earlier germination by 8 to 10 days.

The mechanically scarified seeds responded well to the hormonal treatments giving 38 and 78% germination respectively in treatments where GA<sub>3</sub> and IAA were used. The impermeable nature of the testa and deficiency of hormones might be the two main reasons for the delayed and reduced germination of *A. acuminata* seeds. When IAA and GA<sub>3</sub> was applied, the germination started within 8-10 days. Similar results were obtained by Hussain & Ilahi (1988) for *Acacia modesta* and Illahi & Hussain (1995) for *Rhazya*

*stricta*, which gave 90-100% germination respectively within 4 days by the application of GA<sub>3</sub> and IAA over the scarified seeds. The scarification broke the barrier and facilitated the entry of these hormones, which might be deficient in the seeds. Ilahi & Parveen (1986) reported similar results for pine seeds and Akhter (1983) for *Sophora* seeds. Acid scarification and the subsequent water soaking have improved the germination by up to 50% but acid plus mechanical scarification reduced the germination to 13% (Table 1). This might be due to lethal effect of acid to the embryos in the latter case, while in the former case both the chemical scarification and water soaking worked synergistically to soften the testa not injuring the embryo, but the possible deficiency of hormone is evident as only 50% seeds got germinated. These findings are in conformity with the findings of Hussain & Illahi (1988) who obtained 90-100% germination by the combined effect of acid scarification and water soaking on *Accacia modesta* seeds.

A seed must have internal requirements fulfilled and the external factors be optimum for its germination (Karssen, 1982). Vleeshouwers *et al.*, (1995) stated that dormancy is a seed characteristic, the degree of which defines as what conditions should be met to make the seed to germinate. The laboratory results are only an approximation and closely approach the situation that would be expected in nature (Kains & McQuestion, 1960).

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