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In-Vitro Studies on the Germination of *Atropa belladonna* Seeds under Different Conditions

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Abstract: ***Atropa belladonna contains tropane alkaloids and a raw material for pharmaceutics industry. Large scale cultivation of Belladonna is carried both by bulb and seeds. Seeds are usually sown during the first half of March and it take almost 3 months for germination and hence farmers prefer bulbs of belladonna for cultivation. Prolong germination of seed is due to seed Dormancy. Dormancy is a mechanism to prevent germination during unsuitable ecological conditions, when the probability of seedling survival is low. The present investigation was carried out to improve germination percentage of Atropa belladonna seeds by different Scarification methods. Mechanical and Chemical Scarifications were carried on seeds of belladonna. Both mechanical and chemical methods significantly stimulated seed germination in varying percentage. Seeds were germinated on full strength M S media with or without GA3 under invitro condition. Maximum germination was obtained by Acid treatments and also by boiling water treatments. Hard impermeable Testa of the seeds was successfully broken by above Scarification treatment and even insufficient germinating hormones were also supplied for good germination percentage in present investigation.***

Keywords: *Atropa belladonna*, Germination, Scarification, GA3, Testa.

1. Introduction

Atropa belladonna which is commonly known as Deadly nightshade is native to South Africa. The whole plant contains tropane alkaloids and a raw material for pharmaceutics industry. Large scale cultivation of Belladonna is carried both by bulb and seeds. Seeds usually sown during the first half of March and it take almost 3 months for germination and hence farmers prefer bulbs of belladonna for cultivation. Prolong germination of seed is due to seed Dormancy. Dormancy is a mechanism to prevent germination during unsuitable ecological conditions, when the probability of seedling survival is low. Seeds of belladonna are small dark brown in color. Seed cot or Testa of belladonna seeds has both physical and physiological dormancy. Scarification and hormone treatment alters seed coat and may favor germination.

The germination of seeds which have hard testa can be increased by treatment with Gibberlic acid (Nikolaeva, 1982). Significantly different temperature was also used (Elena Genova 1997). Effect of gamma rays and gibberlic acid also proved good for germination (M S Abdel- Hady, 2008).

The aim of this study was to determine different scarification process involved to break the dormancy of Atropa belladonna seeds both in the influence and in absence of Gibberlic acid. Seeds were subjected to both mechanical and chemical treatments to break physical and physiological dormancy of seed and subjected to germination test.

2. Materials and Methods

Seeds of Atropa belladonna were collected from The Jammu Kashmir Medicinal Plant Introduction Centre, Srinagar, Kashmir. 2 years old dormant seeds were subjected to Scarification treatment and detail listed below. All pretreated seeds were transferred to two different combinations of media. One is blank M S media and another is M S media with 0.6 mg/l Gibberlic acid hormonal concentration. Each inoculated petri plates consists of 10 seeds and are incubated at lower temperature of 23±3ºC and also under 200 lux light. Daily reports were collected from the sowing day to one month. All results were recorded purely on percentage basis.

Mechanical Scarification

a) **Sand paper process:** To remove hard seed coat, seed is rubbed against sand paper gently so the seed coat is removed. This process harmed the seeds and damaged the cotyledon and when transferred to media they got mouldy.
b) **Using Needle or Knife:** Seed coat is removed with the help of a needle under the dissection microscope. This is 100% removal of seed coat but limited to lab work, as the seeds are too small to handle and dissect; this cannot be practiced in large quantity.
c) **Boiling water treatment:** Seeds were transferred to boiling water and boiled together for 20 minutes which loosens the seed coat. When those are seeds transferred into media they showed very promising results.
d) **Altered Temperature:** Seeds were transferred in to a water bath whose temperature was maintained at 30ºC and 45ºC for 5 hour. Results were recorded late.
e) **Stratification or cold water treatment:** Seeds were subjected to Cold storage at 4ºC for period of 15 days. That is soaking of seed in water and maintaining the temperature of 4ºC and periodically the water is changed. The germination rate was found to be fast.

Chemical Scarification:

a) **Acid Treatment:** Seeds were pretreated with different concentrated acid solutions for 30 second, 1, 2 and for 5 minutes. Sulphuric acid, Hydrochloric acid, Salicylic acid and Nitric acid were used. Nitric acid results were good compared to other acids.

b) **Alkali Treatment:** Alkali stress may affect the germination of seeds. To study this effect *Atropa belladonna* seeds were treated with 25%, 50% and 100% Sodium hydroxide and Sodium bicarbonate solutions for 1 hour. Before inoculation seeds were washed with sterile water.

c) **Hormonal Treatment:** Seeds were treated with Gibberlic acid and Indole acetic acid of 1ppm for 2 hours, 6 hours and 12 hours and later inoculated on blank M S media. Here M S media with GA3 was not used.

d) **Alcohol Treatment:** Seeds were treated with 5% Ethanol for 10 minutes. Later washed with distilled water and inoculated into suitable media. This experiment did not prove to be feasible.

3. Results and Discussion

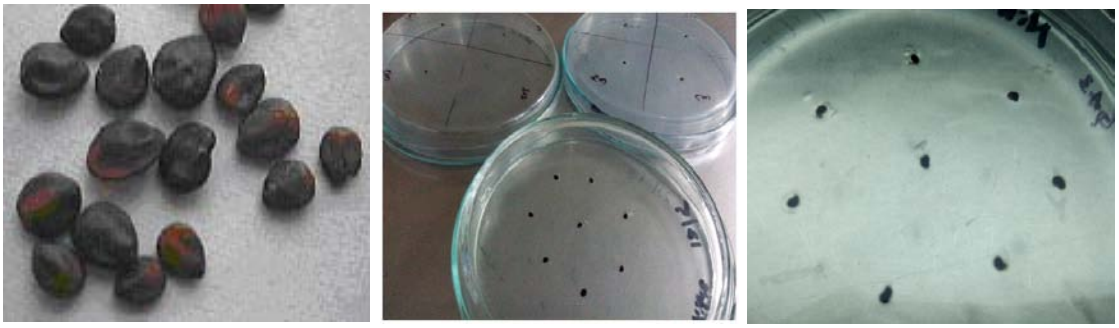

Figure 1: Belladonna Seeds and its inoculation on MS media

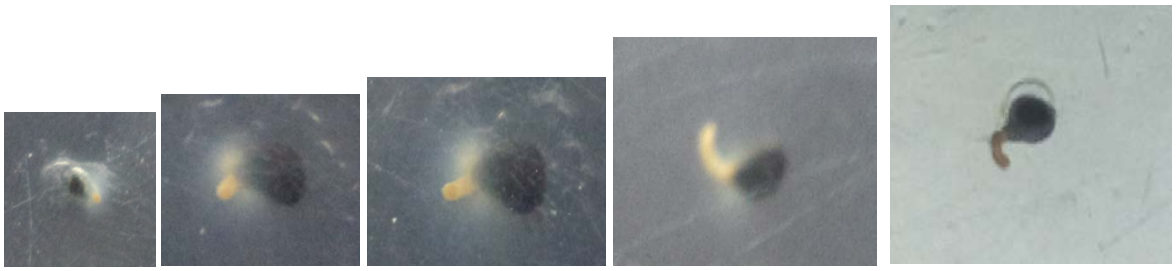

Figure 2: Stages of Germination in Atropa belladonna

The reduced rate of germination of Atropa belladonna is not just due to hard seed coat, but may also be because of chemical insufficiency. Some seed even need hot water stimulation. Water imbibitions inside seed were most important to overcome all this problems. So both mechanical and chemical scarification was carried to break testa so that water imbibes easily and leads to seed germination. Germination results varied as per treatment. There was no germination for 15 days in many of the treatments. Sand paper treatment was not effective and even damaged the seeds. Removing seed coat using a needle was quite effective and germination was recorded in 10 days in both the plates and GA3 growth was high but as the seed size is too small it's very difficult to dissect seed coat from the seed and its time consuming. This method is totally rejected for large scale. Boiling water treatment responded almost 90% on seed and even seed germination started by the next day of the treatment. GA3 plates showed poor growth while the results in M S media was best.

Table 1: Mechanical method treatment in germination

Sl No	*Treatment Carried*	*Days Required For Sprouting*		*% Of Sprouting*	
		Blank MS	*MS+ GA3*	*Blank MS*	*MS+GA3*
01	Sand paper treatment	---		---	
02	Needle or Knife treatment	10 days	10 days	60%	80%
03	Boiling water treatment	2 days	2 days	90%	70%
04	Altered temperature 30°C Treatment 45°C	8 days	10 days	60%	60%
		25 day	20 day	----	20%
05	Cold water treatment	7 days	7days	70%	90%
06	Acid treatment	Results Tabulated Below			
07	Alkali treatment				
08	Hormonal treatment				
09	Alcohol treatment	---	25	---	50%

High percentage germination in seeds of Leucaenia leucocephala and Acacia nilotica with increasing ratio of seed weight to hot water volume was reported (Duguma et al 1988). Temperature is the most important factor in regulating the changes in dormancy (Bouwmeester, 1990). Under influence of different temperature both testa and seed function was affected and germination was achieved. This result was favored by much previous work (Ovcharov, 1976). The germination noticed on 8th day where seed treated at temperature of 30^{0}C for 6 hrs and on the 25th day germination reached 60% and this data was also supported by previous experiments (Dubinskaja, 1949). Meanwhile 45ºC treatment was not that effective but germination was noticed on 25th day and was just 20% and further growth was arrested. Many horticulture departments which grow belladonna practice cold water soaking method of germination including JKMPIC. Chilling treatment allowed seeds to germinate after 7 days and rate of germination was 90% on GA3 plates and 70% on M S blank plates, but

further seedling growth was comparitively slow on both blank M S and GA3 plates Table-1.

Table 2: Acid treatment

SL NO	Acid Used	Duration of treatment	Days Required For Sprouting		% Of Sprouting	
			Blank MS	MS+ GA3	Blank MS	MS+ GA3
01	Nitric acid	30sec	---	---	---	---
		1 min	20 days	18 days	80%	80%
		2 min	10 days	15 days	80%	80%
		3 min	3 days	3 days	95%	95%
02	Sulphuric acid	30sec	---	---	---	---
		1 min	---	---	---	---
		2 min	25 days	20 days	60%	60%
		3 min	20 days	18 days	60%	80%
03	Hydrochloric acid	30sec	---	---	---	---
		1 min	30 days	30 days	20%	30%
		2 min	25 days	20 days	30%	30%
		3 min	20 days	20days	40%	60%
04	Salicylic acid	30sec	10 days	7 days	30%	50%
		1 min	---	---	---	---
		2 min	---	---	---	---
		3 min	---	---	---	---

Chemical Scarification treatment has proved to be more successful than mechanical treatment as it removes the seed coat successfully. Acid scarification was first reported by Bonner *et,al* in the year 1974. Seeds scarified with sulphuric acid for 3 minutes showed better germination but Nitric acid increased the germination percentage to great extent. Soaking seeds in concentrated nitric acid for 3 mins resulted in 95% of germination both on blank M S and GA3 supplemented media within 5 days both on M S and GA3 plates. Same results was noticed in Panicum seeds (Previero 1996) and in tropical grass seeds (Geetha 2001). According to Bewley and Black (1983), KNO3 raises the ambient oxygen levels by making less oxygen available for citric acid cycle. Hydrochloric acid treatment was slow and germination was reported after 20 days. GA3 plates showed germination rate of maximum 60% than MS blank Table-2.

Germination of pre treated seeds with salicylic acid is accompanied by abrupt hormonal changes in the plant due to accumulation of IAA (Hayat & Ahmad, 2007). Thus germination is probably because of accumulation of IAA. Concentrated salicylic acid treatment for 30 second resulted positive only after 7 days but 2 and 3 minutes treatment did not proved good Table-3. Using NaOH solution and breaking seed dormancy was used in seeds of *Zoysia japonica* (Han 1996) and also reported in Chrysanthemum seeds (Chiang & Park 1994). The same process was implemented on belladonna seeds. Germination reached maximum 50% in both 25 and 50% solutions. Further growth rate was totally stopped on both blank and GA3 MS plates may be because of high salinity. Same results were also recorded from Sodium bicarbonate plates. Concentration of 1ppm at an exposition of 12hours affected the germination process Table-4. Both GA3 and IAA recorded maximum 80% of germination and same recorded in earlier work too (Shain 1987). Growth rate of seedlings was slow but seedlings were healthy and germination recorded within 15 days. Ethanol has been reported to have stimulatory effect on the germination of seeds of many plant species (Bewley and Black, 1982). Ethanol may involve in modification of membrane thus facilitating water and oxygen. Pre treated ethanol seeds germinated on the 25th day of inoculation only on GA3 plate and blank M S media did not show any change.

Germination differs from species to species. Species with small seeds tend to require light for germination more than large seeded species (Milberg et al., 2000). Hence all the above experiment were carried out in the presence of light. After germination, germinated seeds were transferred into root inducing M S media and further to shoot inducing M S media. Later obtained plantlets were transferred into sterile, minute saline soli for their growth.

Table 3: Hormonal effect on Seed germination

S. No	Hormones Used	Concentration of Solution	Duration of Treatment	Days Required For Sprouting	% of Sprouting
				Blank MS	Blank MS
01	GA3	1 ppm	2 hours	---	---
			6 hours	20 days	40%
			12 hours	15 days	80%
02	IAA	1 ppm	2 hours	---	---
			6 hours	30 days	50%
			12 hours	15 days	80%

Table 4: Effect of Alkali treatment on seed germination

S. No.	Alkali Used	Concentration of solution	Days Required For Sprouting		% of Sprouting	
			Blank MS	MS+GA3	Blank MS	MS+ GA3
01	Sodium hydroxide	25%	15 days	15 days	40%	20%
		50%	20 days	20days	---	50%
		100%	---	---	---	---
02	Sodium bicarbonate	25%	30 days	30 days	20%	20%
		50%	20 days	15 days	50%	50%
		100%	---	---	---	---

The level of dormancy in seeds is determined by several factors such as maternal environment during maturation, chemical composition, hormone concentration and position of the seeds on the plant (Fenner, 2005). Over all view support that, seeds require proper internal environment and adequate external factors for germination. The laboratory report and analysis are only approximation and close approach of situation expected in nature (Kains & McQueston 1960).

4. Conclusions

Needle treatment for removing seed coat was unsuccessful and cannot be carried for *Atropa belladonna* species due to its minute size and careful handling. Boiling water treatment for 20 minutes on seeds proved immensely best mechanical

treatment and can be carried out by a normal farmer before planting Atropa belladonna. Among chemical treatment to break dormancy, use of Nitric acid proved best among all acids and almost 100% result were obtained within less interval of time. Salicylic acid treated seeds were able to grow even at higher temperature. Hormones also effected the germination proving the physiological dormancy of the seed. Alkali treatment also proved positive for seed germination and also registered that even soil pH also influence germination of seeds of belladonna.

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