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Effect of Storage Temperature, Storage duration and Supporting Media on in Vitro Seed Germination of Nothapodytes Foetida: An Endangered Medicinal Plant of Western Ghat

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Abstract—*Nothapodytes foetida Grah. (Mabb.) is a medicinal tree species from the Western Ghats which has gained a considerable importance in recent times for its alkaloid Camptothecin. Camptothecin (CPT) is a bio molecule, which has gained attention all over the world because of its role as anticancer drug. Due to the over exploitation of the plant for the extraction of camptothecin, this plant has became endangered. Moreover, seeds also show a viability of about 2-3 months, thus leading to problems in germination. The present study describes in vitro seed germination and storage behavior of Nothapodytes foetida. The results showed that germination percentage can be improved by the use of filter paper bridge as supporting media rather than agar. It is interesting to note that germination on media gelled with agar was only 57 % whereas seed with filter paper bridge as supporting media showed 82% germination. Seeds were tested for two storage conditions over time. Seeds stored at room temperature exhibit viability of less than a year (7-8 months), whereas low-temperature storage enhanced the viability to more than one year. High seed germination, low mean germination time and low rate of fall in seed germination percent of seeds stored at 4ºC was reported. Above 50% seed germination even after 12 months storage at 4ºC was reported which suggest this storage method as an appropriate technique. Cold storage of the seeds of Nothapodytes foetida is recommended for the conservation of germplasm in the seed banks.*

Keywords: *Camptothecin, Nothapodytes foetida, anticancer drug*

1. INTRODUCTION

Nothapodytes foetida is a medicinal plant and endemic to Western Ghats. This species is distributed in patches of the dry and moist deciduous as well as evergreen forests of the Western Ghats in India. It has immense value in treatment of many killer diseases especially cancer. The metabolite–camptothecin (CPT), is known as a potent drug that breaks single-strand DNA in the mammalian systems and tumours[1]. The stem, wood bark, seeds, leaves and various organs of the tree are rich sources of the potent antitumor agents like quinoline alkaloids, camptothecin (CPT) and 9-methoxy-camptothecin (9-OMeCPT).

The content of camptothecin in this tree is very high as compared to *Camptotheca acuminate* [2]. Due to huge global demand of camptothecin, *N. foetida* is being exploited. Perhaps this has led to the large scale exploitation and indiscriminate collection of this species from its wild habitat in the recent years [3]. The species has become endangered due to over-exploitation for medicinal use and habitat destruction in its distribution range area. Therefore, appropriate steps should be taken for its conservation. The present study was undertaken with an aim to test the effect of long-term storage of seeds under different storage conditions on seed germination.

In *N. foetida* seed germination in natural habitat is very less due to the lack of appropriate germination conditions and hard seed-coat. It has been reported that seeds lose their viability after 2-3 months, thus leading to problems in germination [4, 5]. Seed viability in terms of seed germination showed that seeds can be stored up to 60 days with about 30 per cent seed germination [6]. This is due to some sort of seed dormancy. Seed dormancy is caused by the conditions within the seed which prevent germination under ideal conditions. In *Nothapodytes foetida*, dormancy is because of seed coat, physiological conditions and presence of phenolic compounds in the seed coat leading to poor germination [7, 8]. This dormancy is overcome through the application of pre-treatment by many workers [7, 8, 9, 10]. These pre-treatments only help in breaking the seed dormancy and increased the germination, but failed to enhance the viability of *N. foetida* seeds.

Germination of seed is a function of duration of storage, storage temperature and moisture content at storage [11]. Therefore good management of storage temperature and duration may enhance germination. For the large scale production of camptothecin large number of plantation is required. But natural regeneration through seeds is low and vegetative propagation through cuttings is also not successful.

Moreover, no information of seed storage has been reported for this species. There is a need to investigate and characterize specific storage conditions that are optimal for favorable germination. Therefore, in present study, attempt was made to enhance seed viability by storing the seed at low temperature.

2. MATERIAL AND METHODS

2.1Seed collection and storage

Seeds were procured from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (agricultural university) Dapoli, Ratnagiri Maharashtra during the year 2014. The seeds packed in plastic bags with labelling were stored for 1 year at room temperature which was subjected to average temperature of 10-20 ^{o}C during winter and 30-40 ^{o}C during summer. The second lot of the seeds was stored at 4^{0}C in the refrigerator in magenta box sealed with paraffin tape.

2.1 Culture media and growth conditions

The culture medium consisted of MS [12] basal media with 3% (w/v) sucrose and. The pH of the media was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl and 20 ml media gelled with 0.8% (w/v) agar was dispensed in the culture test tubes. For paper bridge method whattman filter paper was cut in M-shape and inserted vertically in the test tube and it was filled with 20 ml distilled water and 20 ml liquid MS medium. The culture tubes were autoclaved at 121^{o} C for 20 minutes. All cultures were maintained at 25±2^{o}C temperature under 16 h photoperiod conditions and light was provided by cool white fluorescent tubes (Philips, India). Each treatment performed using ten replications and experiment was repeated thrice.

2.3 In vitro seed germination

For *in vitro* seed germination seeds were washed with a few drop of liquid detergent for 5 minutes and then in running tap water to remove the superficial dust particle. Prior to inoculation, seeds were sterilized with 0.1% (w/v) HgCl2 for 2-3 minutes and washing in sterilized distilled water for 5 times. This was followed by sterilizing the seeds with 70% ethanol for 30 seconds and washing in sterilized distlled water for 3 times. All steps of surface sterilization of seeds were performed in a laminar flow under aseptic conditions.

Fresh seeds with and without seed coat were inoculated on full strength, half strength Murashige and Skoog (MS) medium gelled with agar and filter paper bridge with sterile distilled water and liquid MS media. Germination under light was exposed to photoperiod (16 h light and 8 dark). Viability of the seeds was assessed by germinating the seeds at an interval of one month. When the seeds kept at room temperature showed zero germination, the refrigerated seeds were germinated at an interval of one month.

3. RESULTS AND DISCUSSION

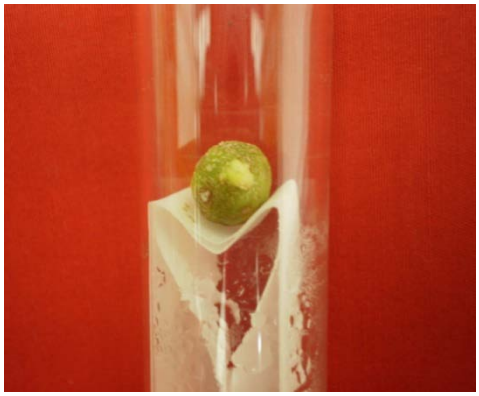
3.1 Effect of media and supporting media

Two supporting medium were employed i.e. agar and paper bridge method. Out of these, paper bridge method gave maximum i.e. 81% germination in seed without seed coat. MS media gelled with agar gave a germination of 57%. The results indicated that in paper bridge method high moisture was maintained inside the test tube. This clearly indicates that seeds of *N. foetida* require high moisture and water content for the process of germination, as shown by other workers also. The containers are airtight in which high humidity is maintained. This high humidity may be congenial for the germination of seeds in this taxon [5].

Seed viability of *Nothapodytes foetida* during different months.

After detailed studies, it was found that the *in vitro* seed germination was 81% of fresh seeds in month of April, 2014. Paper bridge method with distilled water gave maximum germination, so this was used for further study. After 6 month of storage, seed germination was found to be zero in case of seeds with seed coat whereas in case of seeds without seed coat the germination dropped to 7 percent after eight months and after that it dropped to zero.

From the month of November 2014, refrigerated seeds were germinated on Paper Bridge. In the month of November germination was 78% which decreased to 53% in the month of April 2015. Results indicating that percentage germination decreased as age increased of seeds. The storage time and temperature conditions affect the germination rate due to change in physiology of seeds or dormancy of seeds. The changes in temperature and humidity can result in seed vigour, low germination and reduced survival of seedlings [13, 14]. These conditions are believed to affect protein metabolism [13] and cause a reduction of biochemical activity of seed [14, 15].



Seed emergence after 4-5 days

Seed with primary root

Seed with adventitious roots

Full grown seedling after 45 days

Fig. 1: Seed germination in *Nothapodytes foetida*

Previously, it was also reported that seeds of *N. foetida* loose viability after 2-3 months and failed to germinate after sometime [5]. In the present investigation increased seed germination was observed on paper bridge method. Moreover the viability of seeds was further increased by refrigerating at 4°C which showed 50% germination after a year of collection of seeds.

4. CONCLUSIONS

Results show that the seed germination decrease with the increase in storage time. The viability can be increased with low temperature storage of the seeds of *Nothapodytes foetida.* Above 50% seed germination even after 12 months at 4ºC suggest this storage temperature as the most appropriate storage condition for long term storage of seeds. This is the first report on the effect of low temperature storage of seeds of *Nothapodytes foetida.*

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Table 1: Effect of different media strength and supporting media on seed germination

Treatment	With seed coat %Germination	Emergence time	Without seed coat %Germination	Emergence time
MS media gelled with agar				
Full strength	30	19	57	14
Half strength	41	17	61.33	11
Paper bridge				
With dstilled water	52	12	82	4
With liquid MS media	47.33	15	72.66	6
	4.06		4.06	

Table 2: Seed viability of *Nothapodytes foetida*during different months

Months	Duration of initiation	Percent Germination With seed coat	Without seed coat
April	5	56	84.66
May	8	42	73
June	10	33	63.66
July	12	24.6	61.66
August	12	13	45
September	15	6.66	29.33
October	15	-	14.33
November	16	-	7
		3.1	2.6

Table 3: Seed viability of *Nothapodytes foetida*stored at 4^0C during different months

Months	Percent Germination With seed coat	Without seed coat	Duration of initiation
November	-	76.33	5
December	-	72.33	5
January	-	69	6
February	-	64.66	5
March	-	62.66	6
April	-	51.33	5