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IMPROVED PROPAGATION TECHNIQUE FOR *NOTHAPODYTES FOETIDA*

Anuradha M[1], Lokesh P[2]*, Pradeep S Kaushik[3] and Balasubramanya S[1]

[1]Rishi foundation,#234,10th C main,1st block Jayanagar, Bangalore-560011, India [2]Padmashree Institute of Management & Sciences, # 149 Komaghatta (V) Sulikere (P), Kengeri Upanagar Bangalore-560060,India

[3]Department of Biotechnology, Acharya Nagarjuna University, Guntur 522510,India

Corresponding Author

connectloki@gmail.com

ABSTRACT

Nothapodytes foetida (Wight) Sleumer, which is having problems with seed germination, is the subject of the present study. Seeds collected from various geographical locations expressed different germinability and Camptothecin content. Successful attempts were made to improve the germination percentage and protocol is derived for maximum production of seedlings by adopting the technique of *in vitro* seed culture. Percent of seedling production by conventional methods and improvised method were compared and found to be highly efficient for mass production of this high value tree species.

Key words: Acclimatization, Camptothecin, Conventional Propagation, *In-vitro* seed germination, Seed Propagation.

INTRODUCTION

Nothapodytes foetida (wight) slemuer belonging to family Icacinaceae is a medium sized tree distributed throughout Western Ghats of India. This tree was identified as a potent source of Camptothecin and its derivatives (Govindachari et al. 1972). Camptothecin and its analogs are the naturally occurring inhibitors of DNA topoisomerases (Giovanella et al., 1989) Cytotoxic activities of CPT and its derivatives was evidenced by Potmesil et al.,(1991 & 1993), Hsaiang et al., (1985 & 1988) and Wani et al., (1966). At present two semi synthetic derivatives of Camptothecin, Topotecan (TPT) and Irinotecan (CPT-11) are widely used to treat ovarian and colorectal cancers (Cunningham et al. 1998, Douillard et al. 2000). CPT-11 has been used in combination chemotherapy along with common chemotherapeutic drugs such as 5-FU and leucovorin for treating colon cancer (Yong Ji et al. 2007). Thus the global demand for CPT has increased as it has been recognized as the starting material for the synthesis of these commercially valuable analogs (Maliepaard et al. 2001).

This valuable medicinally important tree due its commercial importance is being over exploited. The only alternative is to cultivate the tree and avoid wild collections. Cultivation and large scale plantations depend upon the availability of planting stocks. Natural regeneration is through seeds and is curtailed by several factors leading to low percent of germination. Vegetative propagation through rooting the cuttings is also not successful, posing a problem for planting stocks. In the present study various attempts were made to derive a protocol for development of planting stocks.

MATERIALS AND METHODS

Mature fruits were collected from various geographical locations of Western Ghats such as Amboli, Chandgad, Agumbe, Coorg, Chickmaglore, Karad, Kemmangundi, Mahabaleshwar, Sagar, Kodachadri and Khanapur.

Conventional seed propagation

Randomly selected dried fruits were planted in the soil with and without fruit coat.

Seed cultures

Mature fruits with and without coat were treated with detergent (Teepol-0.1%v/v) for 10 min and thoroughly washed under a jet flow of tap water. Later fruits and seeds were subjected to Bavistin (0.1% w/v) treatment for 45min followed by a sterile water rinse. Final surface sterilization procedure was carried under LAF with freshly prepared Mercuric chloride (0.1% w/v) for 7min followed by sterile water wash for five times. Fruits and seeds were soaked in sterile water for 24hours. Disinfected seeds were then placed onto half and full strength MS (Murashige and Skoog, 1962), B5 medium (Gamborg et al., 1968) and WP medium (Lloyd and McCown, 1980**)**.. Media was fortified with 3% sucrose (w/v) and gelled with 0.7% agar. The pH of the medium was adjusted to 5.7 prior to autoclaving at 15psi for 20min. Cultures were incubated at 16hours light and 8 hours dark regimes. The percent frequency of germination was recorded after 25days. Three replicates with 100 seeds each were maintained.

Acclimatization

Germinated seedlings were hardened. The hardening media used was soil: sand: manure (1:1:1) and soil rite. High humidity was maintained for 25days inside poly tunnels under greenhouse conditions.

Extraction and analysis of CPT

Fruit coats and seeds were defatted with petroleum ether and filtered. The residual mass was extracted repeatedly with methanol for five times and evaporated to dryness in a rotavapor and methanolic extract thus obtained was repeatedly extracted with chloroform for 4-5 times. The combined chloroform extract was evaporated to dryness using rotavapor. Quantitative HPLC was carried out on water associate using Nopac C18 column (150mm) and solvent system- KH_2PO_4: CH_3CN: MeOH (7:2:1) was used. The flow rate was adjusted to 1.2 ml min^{-1} and the detector was set at 254nm. The concentration of Camptothecin and 9 methoxy Camptothecin in different samples were measured by standard calibration curves of authentic samples obtained from Rishi Herbal technologies Pvt Ltd, Bangalore.

Statistical analysis:

Data were analyzed using SAS software, ANOVA indicated significant treatment effects based on F-test, the Duncan's multiple range test was used as a method to determine which treatments were statistically different from other treatments.

RESULTS AND DISCUSSION

Seeds collected from various geographical locations were tested for its germinability (Table 1). The percentage of germination varied from one region to another. Maximum percent (48%) was observed from seeds collected from Kodachadri region followed by seeds collected from Amboli region (46.6%). The high degree of variations was attributed to the morphological and phenological differences recorded in this plant. Differences in phenology were obvious and also been reported by earlier workers (Ganeshaiah et al. 2006). The CPT content in fruit coats and seeds were analyzed and found be region dependant. The CPT content was relatively more in fruit coats compared to seeds. Maximum percent of CPT (0.86%) was recorded in fruit coats collected from Kodachadri. Interestingly it was observed that there is an invariable low percent of germination in all the experiments when the fruit coat is intact. Germination percentage improved in all the regions when naked seeds were planted. This indicates the possible inhibitory role of CPT and other germination inhibitors present in the fruit coat. The presence of CPT in fruit coats and seeds can be a way for commercial extractors as a source of raw material for CPT production. To the best of our knowledge there are no existing reports on this type of study.

Among the various media used, half strength B5 basal medium showed a good response with the highest percent of germination of seed without fruit coat (61.6%) and seeds with intact fruit coat (Table 2).The best suitable medium were adopted for developing seed cultures. A comparative account of germinability in all the methods was carried out with seeds collected from the different geographical locations. (Figure 4).

The survival rate of plants obtained from various methods when transferred to field was studied (Table 3). Interestingly it was found that the plants rose through seed cultures obtained by seeds without fruit coat showed the highest survival percent (90.6%). This may be attributed to the fact that the absence of fruit coat would have a preventive effect on embryo as they may produce some inhibitory chemicals along with CPT. The micropropagated plants showed a lower rate of survival indicating the plant's poor response to the method. In the present study it is very clear that seed cultures of *N. foetida* have played a critical role in increased rate of propagation. The removal of fruit coat increased the germinability rate both in conventional seed propagation and seed cultures. These novel observations can be useful in developing an improved protocol for conservation as well as cultivation of this endangered tree.

Table 1*:* Germination of *Nothapodytes foetida* seeds collected from various geographical locations.

Location	CPT content in fruit coat	CPT content in seeds	Percentage of germination with fruit coat	Percentage of germination in seeds without fruit coats.
Amboli.	0.79	0.74	31.67±0.33	46.6
Agumbe.	0.7	0.67	16.67±0.67	28.0
Coorg.	0.52	0.46	9.67±0.33	19.66
Chickmaglore.	0.58	0.54	17.67±0.33	31.6
Karad	0.62	0.55	22.67±0.33	34.33
Kemmangundi.	0.43	0.39	13.67±0.33	25.0
Mahabaleshwar.	0.63	0.6	20.67±0.67	34.3
Sagar	0.4	0.38	20.33±0.33	33.6
Kodachadri.	0.86	0.8	32.67±0.33	48.0
Khanapur	0.64	0.59	28.33±0.33	41.33
Chandgad	0.7	0.68	26.33±0.33	41.33

F (10, 22) = 314 p<0.000001 SS=1618.91 MSe=0.52

Table 2: Effect of media on seed cultures of seeds in presence and absence of fruit coat.

Media	*Percent of germination of seed cultures using seeds with fruit coat	**Percent of germination of seed cultures using seeds without fruit coat
Control	39.67±0.88	37.67±0.33
MS Basal	42.67±0.88	41.33±1.45
Half MS Basal	45.67±0.33	45.00±0.58
B5 Basal	52.33±1.76	50.67±2.19
Half B5 Basal	61.67±1.45	61.00±1.53
WPM	36.67±1.33	34.33±2.33
Half WPM	38.67±1.86	37.67±1.20

*F (6, 14) = 45.7 p<0.000001 SS=1424.00 MSe=5.19

**F (6, 14) = 36.3 p<0.000001 SS=1545.62 MSe=7.10

Table 3: Comparison of survival percent of field transferred plants

Method	Percent of survival
Conventional seed propagation	30.33±0.33
Micropropagated plants	39.33±0.33
Seed cultures using seeds with fruit coat	72.67±0.67
Seed cultures using seeds without fruit coat	90.67±0.58

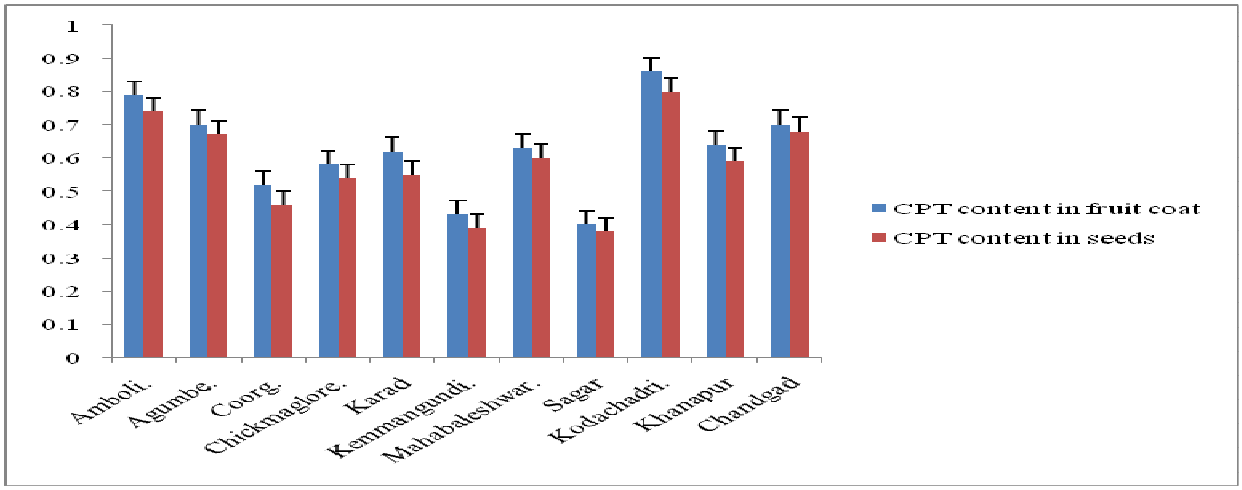
F (3, 8) = 4107 p<0.000001 SS=7187.58 MSe=0.58

Fig 1. *Nothapodytes foetida* Sleumer (Wight)

Fig 2. Seed cultures using seeds with fruit coat

Fig 3. Seed cultures using seeds without fruit coat

Fig: 4 Comparison of CPT content and Percent of germination of seeds collected from different geographical locations using different methods.



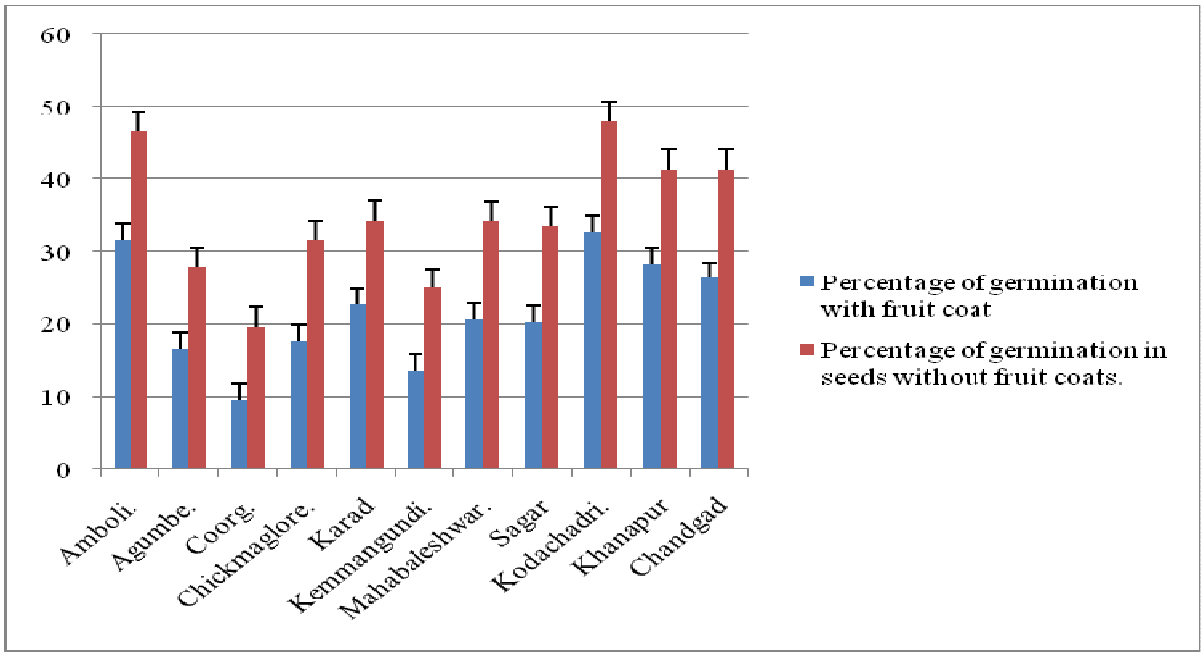


Fig 5. Acclimatization of *Nothapodytes foetida* plants.

CONCLUSION

The major findings of the study include a technology for mass production of *Nothapodytes foetida* a valuable anticancer tree species, screening of the high yielding variety of the tree species, developing protocol for germination of seeds which are inhibited by physical factors and the source of raw material for commercial extractors are reported.

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