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Short Communication

EVALUATION OF DIFFERENT TREATMENTS TO IMPROVE THE SEED GERMINATION AMONG THE POPULATIONS OF *NOTHAPODYTES FOETIDA* (WT.) SLEUMER

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ABSTRACT
Seeds of *Nothapodytes foetida* collected from four different geographical regions of Western ghats of peninsular India were subjected to various treatments. Samples with and without seed coats were appropriately treated to assess the type of dormancy. Less percentage of germination in the samples with seed coats is attributed to the presence of phenolics. The response of seeds to different treatments was not uniform, which depends on the accessions and also the treatments. GA_3 treatments reduce mean germination time, whereas the *in vitro* paper bridge method enhances the percentage of germination and seedling vigour index of both the samples. Seeds of Ooty accession responded positively to all the treatments which have reflected in their better percentage of germination and high seedling vigour. Data thus obtained indicate the presence of seed coat dormancy as well as physiological dormancy.

Key Words: *Nathapodytes foetida, Seed treatments, Germination, Seedling vigor*

INTRODUCTION
Nothapodytes foetida (Wt.) Sleumer belonging to the family *Icacinaceae*, is an Indian source of camptothecin (CPT), an effective anticancer drug. It is a medium sized tree mainly confined to Western ghats of Deccan Peninsula and North-East India. It is believed that CPT is the third most important alkaloid sought after by the pharmaceutical companies around the world (Panneerselvam *et al*. 2004). Because of deforestation and indiscriminate extracts of these trees from their natural habitats over the last 20 years, the taxon has been assigned a vulnerable status at the regional level. Added to this, the seeds lose viability quickly and their poor germination capacity has augmented the threat for its survival in their natural habitats (Sharma *et al*.2000). Hence, the present study aims to determine the germination potential of the seeds collected from the trees growing at different regions of Western ghats by subjecting to various treatments. Study on germination of seeds collected from different localities has become necessity since the content of camptothecin exhibits natural variations among the populations (Suhas *et al*. 2007).

Mature seeds were collected from Mahabaleshwar (Southern Maharashtra), Bisele ghat (Central Karnataka), B.R. Hills (Southern Karnataka) and Ooty (Northern Tamilnadu). Length and volume of the seeds of the above accessions were recorded. Seeds were soaked in sterile distilled water for 24 h and seed coats were removed. Both the samples, with and without seed coats were subjected to appropriate treatments. Since leaching of phenolics was observed when seeds were soaked in water, phenolics content in both the samples were estimated using Folin-Ciocalteau reagent (Bray and Thorpe, 1954). The reaction mixture was comprised of distilled water (5ml), extract samples (1ml), Na_2Co_3 (2ml) and Folin-Ciocalteau reagent (0.5ml). The reaction mixture was boiled for 1min and air cooled. Absorbance was read at 650nm. Gallic acid was used as a standard.

The following treatments were employed to enhance the percentage of germination of seeds with or without seed coats.

a. ***Control:*** Samples with seed coats and without seed coats were washed thoroughly for 30 min in running water and subsequently placed them on wetted filter paper (Whatman No.1) in 9 cm diameter Petri plates under laboratory conditions. In each trial 10 seeds per Petri plate and five replicates were used.

b. ***Mechanical scarification***: It was done by rubbing the samples (with seed coats) kept in-between two pieces of sandpaper and seed coat opposite to hilum was slightly ruptured with a nail cutter.
c. ***Acid scarification***: Seeds with seed coat were soaked in 10mM of H_2SO_4 for 20, 40 and 60 min. After the acid treatment, seeds were washed thoroughly in running water for 30 min and processed for germination as the control**.**
d. ***GA_3 treatment***: Seeds with seed coat and with out seed coats were soaked in GA_3 at the concentration of 0.57 µM for 20, 40 and 60 min. After the treatment both the samples washed thoroughly in running water for 30 min and processed for germination as the control.
e. ***Boiling water treatment***: Seeds with seed coats were placed in muslin cloth bag immersed in hot water maintained at 100˚C for 20, 40 and 60 min. At the end of each treatment, seeds were taken out of the boiling water and washed with running water for 5min before processing for germination as control.
f. ***Desiccators' incubation***: Both decoated and coated seeds were washed in running water for 30 min and placed them on wet Whatman filter paper (No. 1) in desiccator's chamber containing calcium carbonate and sealed the lid with petroleum jelly. The desiccators were kept under laboratory conditions.
g. ***In vitro Paper bridge method***: Both the seeds with or without seed coats were washed in running water for 30 min and treated with saturated chlorine water for 15 and 10 min for seeds with seed coats and without seed coats respectively. They were then treated with mercuric chloride (0.1%) for 1 min and inoculated aseptically onto the autoclaved paper bridges placed in test tubes containing sterile distilled water and plugged with cotton plugs. These tubes were kept in culture room where the temperature was maintained at 25 ± 2° C under 16:8 light and dark regime. The cultures were illuminated with white fluorescent lights with the intensity of 50 µmol m^{-2} s^{-1}
Mean Germination Time (MGT): MGT was calculated following the formula:

$$MGT = \sum (nd) N$$

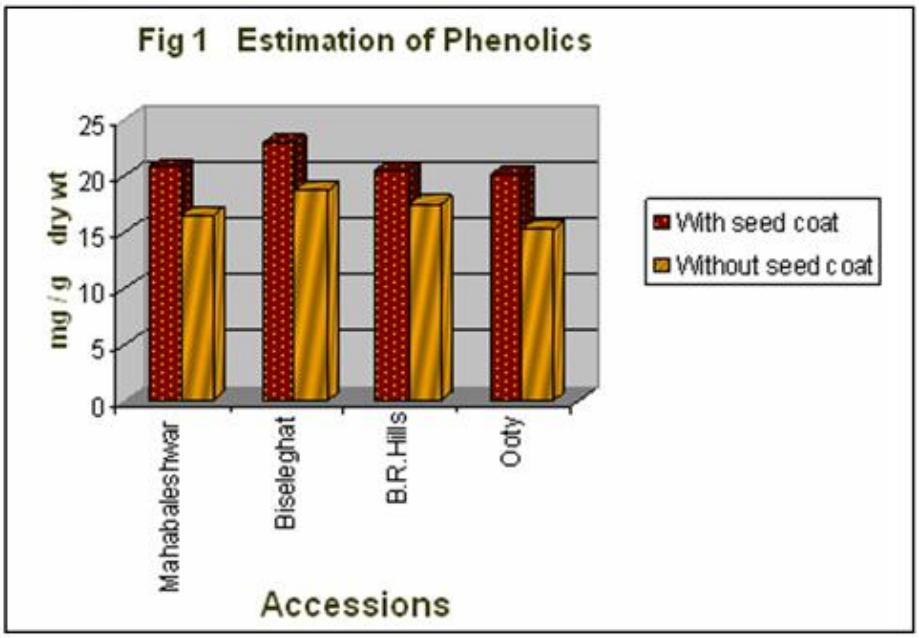
Where n is number of seeds germinated of each incubation period in days d, and N is total number seeds germination at the end of the test (Hartmann *et al.*1990)

Seedling vigor index: It was calculated using the formula

$$VI = (R + S) G$$

Where VI=Vigor index, R=Average root length(cm), S=Average shoot length(cm) and G=Germination percentage (Abdul-Baki and Anderson, 1973).
All the experiments were repeated at least thrice. The data thus obtained was subjected to statistical analysis – one way ANOVA. Significant 'F' ratios between groups means were subjected to least significance difference (LSD).Probability (p) values < 0.05 were considered significant (Snedecor and Cochron, 1994).
Germination studies among different populations provide helpful clues on genetic make-up of the species and its existence in the natural populations. Knowledge of such variation in germination is essential for selection of the elite seeds. In general there are two types of seed dormancy – seed coat dormancy and internal / physiological dormancy. Seeds with seed coat dormancy usually have a very thick coat that is impermeable to oxygen and / or water which are the prerequisite for the initiation of germination. The seed coat dormancy can also be imposed by some inhibiting chemicals such as phenolics and abscisic acid.
Percentage of germination varies between the samples with and without seed coats. Samples without seed coats exhibited higher percentage of germination than the samples with seed coats indicating the presence of seed coat dormancy. Estimation of phenolics in both the samples revealed the presence of more phenolics in the samples with seed coat than the decoated seeds (Fig. 1). Among the accessions studied the phenolic content was found to be less in both the samples of Ooty accessions which may be the reason for high percentage of germination in all the treatments compared to other accessions (Fig. 1). The phenolic content in the seeds of Ooty accessions was 15mg/g DW (without seed coats) and 19mg/g DW

(with seed coats). Phenolics are known to inhibit the enzymes of glycolysis and OPP pathway thereby affecting the respiratory processes of seeds (Muscolo *et al*. 2001).



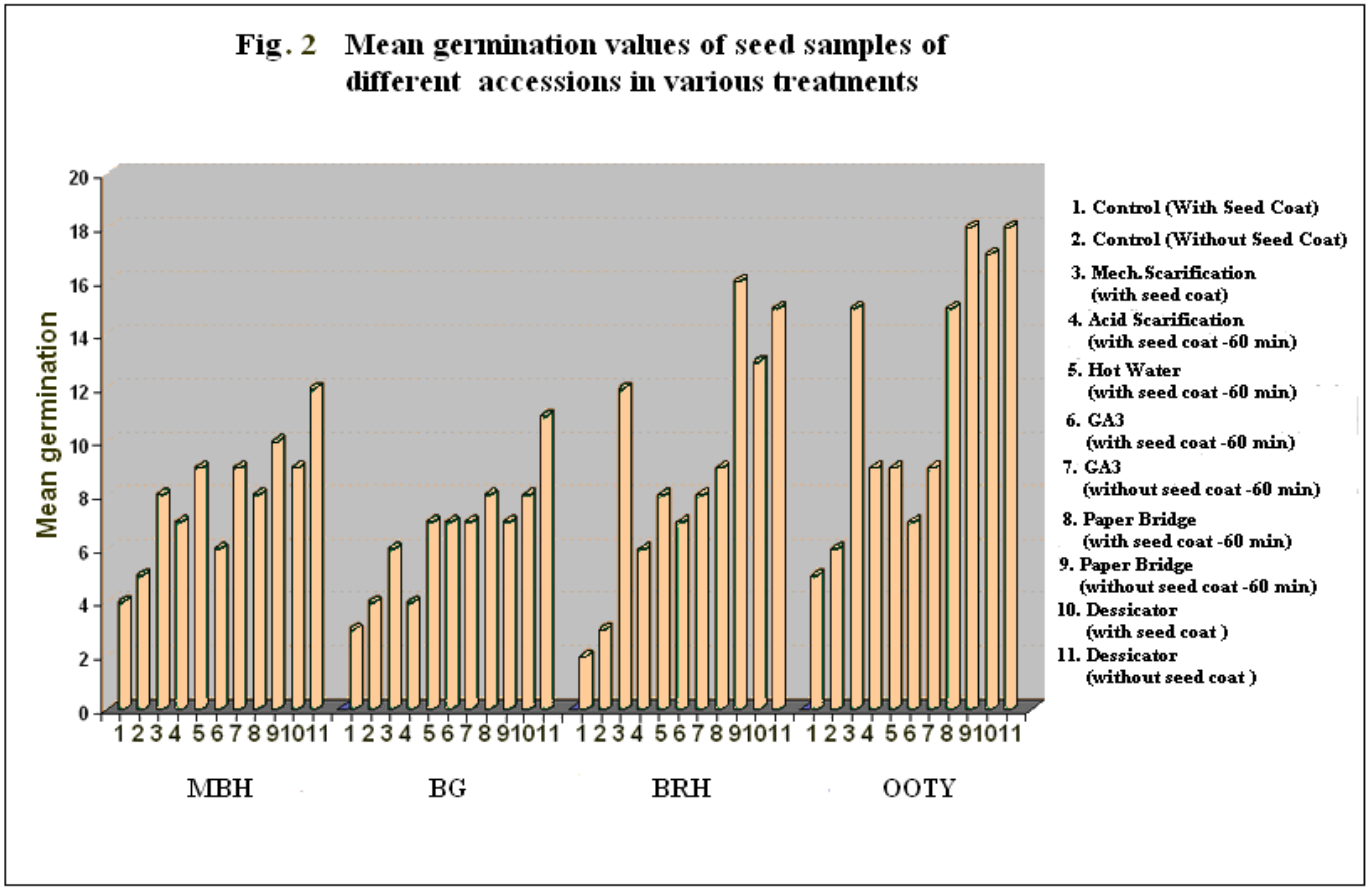
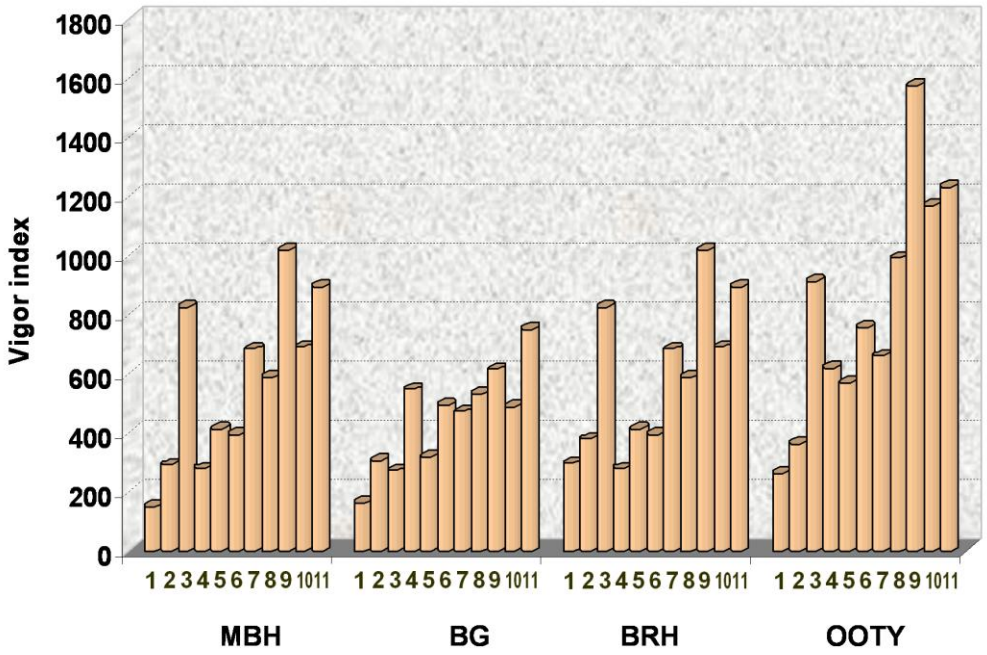


Fig.3 Vigor Index of two months old seedlings of *Nothapodytes foetida.*



1. Control (With seed coat)
2. Control (With seed coat)
3. Mechanical Scarification (With seed coat)
4. Acid Scarification (H_2SO_4-60min)
5. Hot water (With seed coat-60min)
6. GA3 (With seed coat-60min)
7. GA3 (Without seed coat-60min)
8. *In vitro* Paper Bridge (With seed coat)
9. *In vitro* Paper Bridge (Without seed coat)
10. Desiccator Incubation (With seed coat)
11. Desiccator Incubation (Without seed coat)

Many studies have also demonstrated the influences of phenolic compounds on several physiological processes such as cellular expansion, membrane permeability, nutrient intake and water ratio (Leather and Einhellig, 1988).

The response of seeds to different treatments was not uniform which depends on the accessions and the treatments (Fig. 2). In mechanical scarification, the maximum percentage of germination was recorded in Ooty accession with 15.00 ± 0.48 seeds have germination though the MGT was more than the control. The failure of mechanical scarification in reducing the time taken for germination may be due to the presence of phenolics in the seed coats. In acid scarification method, as the treatment was prolonged the percentage of germination also enhances in all the accessions. Beneficial effects of sulphuric acid in breaking seed coat dormancy have been reported in a number of leguminous species (Muhammad and Amuse, 2003). The acid helps in removal of cuticle and softening of testa which facilitate water absorption and gaseous exchange (Govindachari and Viswanathan, 1972). In boiling water treatment the percentage of germination was increased in Mahabaleshwar and Bisele ghat accessions but decreased in B.R. Hills and Ooty accessions as the treatment prolonged. Though MGT was enhanced as the GA_3 period was prolonged, the percentage of germination was significantly improved for both the samples. GA_3 is known to release dormancy of seeds in many plants. Its role in breaking dormancy may be due to the activation of GA synthesis or by reducing the inhibitor levels or both (Sharma *et al*. 2000). However, in *Brassica montana* and *B. oleracea*, GA_3 treatments did not significantly increase germination over that of control (Perez -Garcia, 2005).

In vitro Paper bridge and desiccators incubation methods found to be more suitable for all accessions compared to other treatments in percentage germination and seedling vigour index. Presoaked seeds were placed on wet filter paper in Paper Bridge and desiccator's incubation method. Since, the containers are airtight in both the treatments, high humidity is maintained. This high humidity may be congenial for the germination of seeds in this taxon. However, MGT in these two methods is significantly high compared to other treatments in the samples with/without seed coats. Based on the germination studies in *Brassica japonica*, Takumasu (1970) has concluded that desiccation does not cause dormancy but only maintains it. Once the dormancy has been broken by pre-soaking in water, they will never return to dormant condition even if they are again brought into desiccator's incubation. Hence, the effect of desiccation upon dormancy in pre-soaked seeds of *Nothapodytes foetida* is therefore essentially different from other systems where desiccator's incubation is used to enhance the dormancy period.

Highest vigor index of 1575 was recorded for the seedlings of Ooty accessions raised on Paper Bridge from the decoated samples followed by B.R. Hills and Mahabaleshwar with VI of 1020 (Fig.3). Least was recorded in control samples. The difference in VI between treated and control might be due to altered physiology of embryos and liberation of enzymes by treatments so that developmental processes occur more rapidly after sowing.

The percent of germination and seedling vigor index in Ooty accessions are quite significant among the different populations. Inter-population variability in seed germination may be interpreted as an important survival strategy of species growing under variable and unpredictable environmental conditions (Cruz *et al*. 2003). From the aforesaid data, it can be concluded that the seeds of *Nothapodytes foetida* exhibit both seed coat dormancy as well as physiological dormancy which can be overcome to varying percent by specific seed treatments.

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