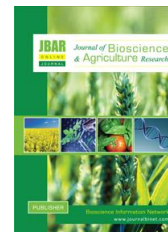


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Germination and initial seedling growth performance of *Vitex peduncularis* Wall. ex Schauer – A threatened native tree species of Bangladesh

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ABSTRACT

Vitex peduncularis Wall. ex Schauer, locally known as Goda, is a threatened native tree species of Bangladesh. In addition to providing many other services this produces, timber of high density, which is traditionally, used for different construction activities. Though seed production is regular but natural germination of seeds is not satisfactory due to the hard seed coat. Effect of pre-sowing methods was studied to find out the best pre-sowing method for higher rate of *V. peduncularis* seeds germination. Randomly selected quality seeds were sown in propagator house with Sylhet sand media, in conventional polybags with 3:1 soil and cow-dung media, and in nursery beds with soil media only. Significantly ($P < 0.05$) highest seed germination (46%) was observed in propagator house. The germination energy (7.9%), plant percent (31.5%) and germination value (147.2) were highest in the seedlings raised in polybags. These seedlings also exhibited better growth in the initial stage at nursery. The records of initial growth of the seedlings indicated highest shoot length (38 cm), root length (34.3 cm), node number (15) and leaf area (18.9 cm × 4.7 cm) for seedlings grown in polybags. Seedlings grown in polybags were more vigor and thrive better than the seedlings germinated in propagator house and nursery bed. This paper will be a supportive material for more advanced studies on seed germination and for establishing plantation with rare but valuable species by raising commercial nurseries.

Key Words: Native tree species, pre-sowing treatments, germination, seedling growth, propagator house and growth media

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I. Introduction

Vitex peduncularis Wall. ex Schauer, locally known as Goda, Arsol, belongs to Verbenaceae family. It is a medium to large sized deciduous tree (Chakraborty *et al.* 2012). The species is restricted in tropical and subtropical regions of Bangladesh, Cambodia, China South-Central, East Himalaya, India, Laos, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam (Kannathasan *et al.* 2007). It is basically growing in mixed forests at elevations from 600 - 1,200 m (Wu *et al.* 1994). In Bangladesh, the species is commonly known as 'Goda or Arsol' distributed in the forests of Chattogram and Chattogram Hill Tracts, Cox's Bazar, Sylhet, Gazipur and Tangail. The tree can grow up to 18 m height.

In addition to timber, the species is used for various medicinal purposes. An infusion of leaves or root bark or young stem bark is used as a cure in several classes of malarial fever, mainly black water fever (Sharma *et al.* 2001; Kirtikar *et al.* 1918; Vaughan, 1921). The plant also exhibits potential inhibitory activity against kala-azar in human (Rudrapaul *et al.* 2014). The decoction of bark is used to cure typhoid fever (Lalfakzuala *et al.* 2015). The bark is also used for making an external application for pains in the chest (Kirtikar *et al.* 1918). Moreover, drinking the extract of boiled bark could cure joint ache (Suksamrarn *et al.* 2002). This plant has anti-inflammatory properties (Nagarsekar *et al.* 2010; Ferreres, 2017) and antioxidant properties (Meena *et al.* 2011) too. Leaves of *V. peduncularis* contain compounds like peduncularaside, iridoid anguside, vitexin, triterpenoids and flavonoids which act as anti-inflammatory properties (Suksamrarn *et al.* 2002; Bheemasankararao and Venkateswara, 1956). Vitexin, isolated from leaves and root bark has both an analgesic and antidepressant-type-effect (Pullaiah, 2006; Borghi *et al.* 2013; Can *et al.* 2013). The plant is also used to improve blood and nutrient flow to the heart muscles and thus cardiovascular health (WHO, 1998). Extract of both leaf and stem has considerable antibacterial and antifungal properties (Panda *et al.* 2012; Kumar *et al.* 2006). The plant species also possess antibacterial potentiality against human pathogenic bacteria (Kannathasan *et al.* 2011). The plant extracts contain antimicrobial properties that can be used in therapeutic treatments (Arora and Kaur, 2007). Besides anti-inflammatory it can also be used as analgesic, antimalarial, antispasmodic and as a remedy of diarrhoea and dysentery (Ambasta, 1986).

In Bangladesh, this plant is harvested from forests for local medicinal use. As a cure of jaundice, extract of bark is taken by Chakma tribe. Leaves and barks are used as a remedy of diabetes and malaria in Khagrachari (Uddin, 2018). The plant is also important for its timber value and can also be used as shade tree. Wood of this plant is heavy hence it is used to prepare house posts, beams and agricultural implements by rural farmers (Ahmed *et al.* 2008). The wood is also used as firewood and charcoal (Lalfakzuala *et al.* 2015). *V. peduncularis* is naturally regenerated from seed but, germination rate of this hard-coated seed is too poor in natural condition. Forests of its natural distribution are deforested heavily and thus degraded due to anthropogenic disturbances (Hasnat *et al.* 2018). In addition to that, over extraction of *V. peduncularis* for timber disturbed its natural restoration capability severely. These issues lead the conservation status of this species to be "Not Evaluated but seems to be rare" (Ahmed *et al.* 2008). The aim of this study was to find out the germination behavior and initial seedling growth performance of *V. peduncularis* in nursery stage with proper care in comparison to the natural condition in forests. This research will help the government organizations and nursery owners to find best regeneration technique for raising seedlings for *in-situ* or *ex-situ* conservation and save this ecologically rare species. It will also open scope for more advanced researches with this rare species.

II. Materials and Methods

Seed collection and handling in nursery

Fruits of *V. peduncularis* were collected from Dulhazara, Bangladesh during August 2012. Phenotypic characteristics of fruits and seeds were then measured in the Seed Research Laboratory of Institute of Forestry and Environmental Sciences, Chittagong University (IFESCU). Fruits selected randomly for the experiment ranges from 0.8-0.9 cm in length and 0.9-1.0 cm width. About 1667-2000 fruits and 6000-6667 seeds were found in one kg. After measurements seeds were extracted manually by rubbing fruits on rough surface. Before rubbing it needed to immersion fruits in water for overnight. Extracted seeds were weighted and then dried in open sun for three days. After that the dried seeds were sown in different sowing media. All the processes were completed within one week of fruit collection.

Experimental design

The experiment was conducted through 1 and half-year period starting from August 2012. It was designed with three sowing media treatment methods with three replications for each treatment. A total of 2800 seeds were sown in all sowing media. Each experimental plot was planned with a complete randomized design. Including control, three treatments were applied: i) Seeds sown in nursery bed¹, ii) Seeds sown in polybags, and iii) Seeds sown in propagator house². Growing media used in the experiment was collected from the forest floor. After collection soil was screened well with <3 mm sized sieve and mixed with decomposed cow-dung in a ratio of 3:1 to prepare soil and cow-dung media. The three types of treatments with growing media were i) Polybags of 15 cm × 10 cm (6" × 4") filled with the soil and cow-dung mixture (3:1) and put in open sun light, ii) Propagator house with *Sylhet* sand³ media and controlled from rain and open sun with special plastic shade, and iii) Nursery bed prepared as an alternative of forest floor and set with soil only. Partial shade with trees was provided in the nursery bed. Seeds were selected randomly and then sown in different media. Seeds were sown in rows in polybags and propagator house, while in nursery bed seeds were scattered as in natural forest floor. Daily weeding and two times watering were done regularly. The experiment was conducted in the normal atmospheric temperature where mean annual temperature was 25.7°C, humidity 78.04% and rainfall 2,794 mm. Only in propagator house temperature and humidity was relatively high as it controlled, and daily sprinkling at two times was provided for required water and moisture.

Data collection and analysis

Germination behaviors were recorded in daily basis at the date of seed sowing and continued up to eight months. Germination percentage measured by counting germinated seeds out of 100 (Kumar, 1999). Cumulative germination percent measured at the end of total seed germination by summed up daily germination. The germination energy assessed by calculating daily germination percentage of its peak time (Dwivedi, 1993). Initial germination was estimated using the Maguire index rate (Maguire, 1962), a time-weighted cumulative germination index that measures the speed of germination (Brown and Mayer, 1988), calculated as the sum of the percentage of seeds germinating on each day divided by the number of days since the germination test began (Schrauf et al. 1995).

Mean time to germination (MGT), also expressed as speed of germination (Soleymani and Shahrajabian, 2018), measures of the rate and time-spread of germination (Bewley et al. 2013). It is calculated as the weighted mean of the germination time. The number of seeds germinated in the intervals of time established for data collection is used as weight (Ranal and Santana, 2006). Rate of germination is the reciprocal of MGT (Mukarati et al. 2013). The coefficient of uniformity of germination measures the variability among seeds in relation to the mean germination time of the sample (Heydecker 1973; Bewley and Black, 1994). Survival percentage was determined by counting total seedlings survived at the end of the experiment. Germination value was found after multiplying peak value of germination (PV) and mean daily germination (MDG). The following formulas were used to find out germination percentage, cumulative germination percentage, rate of germination, plant percent and plant value:

$$\text{Germination \% (GP)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Cumulative germination \% (CGP)} = \frac{\text{Cumulative number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Germination index (GI)} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

$$\text{Mean germination time (MGT)} = \frac{\sum Dn}{\sum n}$$

$$\text{Rate of germination (R)} = \frac{1}{\text{MGT}}$$

¹**Nursery bed:** A nursery bed is a specially prepared portion of land put aside for raising seedlings. This is a temporary home for young plants. The width of a bed is 1 m and length according to the requirement.

²**Propagator house:** Greenhouse is a structure, primarily of glass, in which temperature and humidity can be controlled for the cultivation or protection of plants. Propagator house is one type of greenhouse with a bed consists of *Sylhet* sand with controlled temperature and humidity

³**Sylhet sand:** River sand with higher proportion of silica found in Sylhet region of Bangladesh.

$$\text{Germination uniformity (GU)} = \frac{\Sigma n}{\Sigma (Fn-t)^2 \times n}$$

$$\text{Plant \% (PP)} = \frac{\text{Number of surviving seedlings}}{\text{Number of seeds sown}} \times 100$$

$$\text{Germination value (GV)} = \text{Peak value of germination (PV)} \times \text{Mean daily germination (MDG)}$$

Germination capacity is the percentage of seeds in a sample that actually germinate irrespective of time. The germination capacity was categorized by following Kumar (1999) where - i) 100-90 % - very good, ii) 90-70 % - good, iii) 70-50 % - average, iv) 50-30 % - poor, v) 30-20 % - very poor, and vi) (<) 10 % - extremely poor. After six months of germination started three vigor seedlings from each replication were selected for measuring shoot height, root length, node numbers, leaf length and leaf width. Shoot length was measured from collar region to the tip of the seedlings. Root length measured from collar region to root tip. For calculating average node number both leaf fall scar and leaf present were counted.

Statistical analysis

Statistical analysis of data was done using the computer software package Statistical Package for the Social Sciences (SPSS). The analysis of variance (ANOVA) was studied by applying Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

Germination behavior

Germination behavior significantly ($P < 0.05$) differ among seeds sown in polybag, propagator house and nursery bed. Seed germination revealed first in polybags (11th day) after seed sowing. In propagator house, germination observed even after 210 days and showed highest germination percent (46%). But germination energy (7.9%), germination index (12.3), rate of germination (0.06), germination uniformity (0.71), plant percent (31.5%) and germination value (147.2) were highest in poly-bags. Seeds sown in polybags and propagator house performed poor germination capacity, and in nursery bed it was extremely poor (Table 01).

Table 01. Variation in germination behavior of *V. peduncularis* seeds in different sowing media

Location	Germination start (day)	Germination end (day)	Cumulative germination (%)	Germination energy (%)	Germination energy <small>period (week)</small>	Germination Index (GI)	Mean Germination Time (MGT)	Rate of Germination (R)	Germination Uniformity (GU)	Plant percent (%)	Germination value	Germination capacity
Nursery bed	22	32.33c*	12.8a	4.7a	30c	1.88b	27.54a	0.04b	0.0039a	12.3a	10.1a	Extremely poor
Poly-bag	11	23b*	38.3b	7.9a	16a	12.3c	16.24a	0.06c	0.71b	31.5c	147.2b	Poor
Propagator house	25	Continued	46c	5.3a	25b	0.43a	97.76b	0.01a	0.0001a	23.3b	2.5a	Poor

*Means followed by the same letter (s) are not significantly different at $P < 0.05$, Duncan's Multiple Range Test (DMRT).

Mean cumulative germination percent

Germination revealed in polybags at 11th day of seed sowing and cumulative germination percent was evidently highest up to sixth month. At the seventh month of seeds sown the cumulative germination percentage surpassed the cumulative germination of polybag. Germination ended in polybags at 32nd day and curve of cumulative germination percent remained constant up to seventh month. Seeds sown in nursery bed germinated at 22nd and ended at 31st day. Only in propagator house, germination started at last (25th day) and gradually raised from lowest to highest even after seven months later from seed sown (Figure 01). Moreover, germination found to be continued after one year of seed sown.

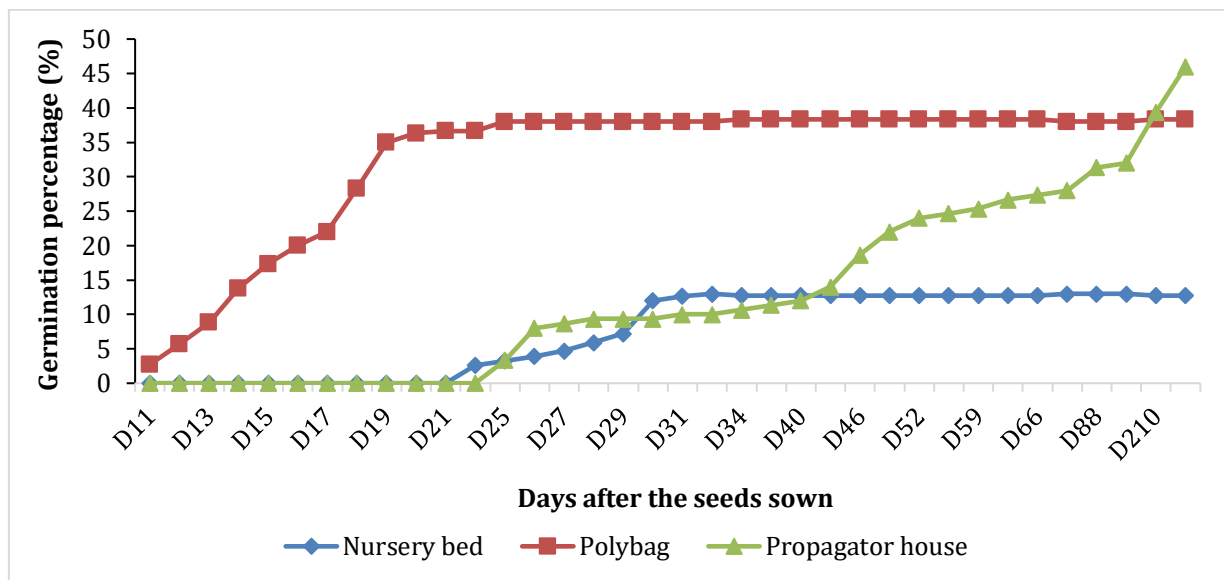


Figure 01. Cumulative germination percent of *V. peduncularis* seeds in different sowing media.

Variation in seedling morphology in poly-bag and propagator house

Though germination behavior in propagator house was significant but morphological behavior of seedlings was very poor. Mean shoot length recorded in polybags was 38 cm while in propagator house it was only 8.6 cm. Mean root length in polybags was 34.3 cm and in propagator house 12.3 cm. In polybags an average of 15 nodes were counted and in propagator house it was 7. Mean leaf size of seedlings was apparently higher in polybags (18.9×4.7 cm²) than those of propagator house (4×1.3 cm²) (Table 02).

Table 02. Initial seedling growth behavior of *V. peduncularis* in polybags and propagator house.

Location	Six months old seedlings after germination					
	Shoot length (cm)	Root length (cm)	Total (cm)	Node number	Leaf length (cm)	Leaf width (cm)
Polybag	38	34.3	72.3	15	18.9	4.7
Propagator house	8.6	12.3	20.9	7	4	1.3

Variation in seedling morphology germinated in nursery bed and propagator house

Seedlings germinated in nursery bed were transplanted into polybags after one month of germination and initial growth was measured at the age of five months (Figure 02). Mean shoot length in transplanted seedlings found 8.4 cm and in propagator house 8 cm. Mean root length of transplanted seedlings was 20.8 cm whereas in the seedlings of propagator house root length was 11.8 cm. In average 10 nodes were observed in transplanted seedlings while 7 in propagator house. Leaves were larger in transplanted seedlings (6.9 cm×2.6 cm) than in propagator house (4.7 cm×1.6 cm) (Table 03).

Table 03. Initial growth performance of *V. peduncularis* between transplanted and propagator house seedlings

Location	Five months old seedlings after germination					
	Shoot length (cm)	Root length (cm)	Total (cm)	Node number	Leaf length (cm)	Leaf width (cm)
Transplanted from bed to polybags	8.4	20.8	29.2	10	6.9	2.6
Propagator house	8	11.8	19.8	7	4.7	1.6

From the results of this study, it is clear that though seeds sown in propagator house showed highest cumulative germination percent but seedling quality was very poor. Like natural forest condition seeds

and seedlings in nursery beds were more prone to insect attack. To save from termite, seedlings in beds were transplanted to polybags after one month of germination.

Cumulative germination percentage of *V. peduncularis* fresh seeds sown in nursery beds found only 12.8%. In propagator house with special care it was found 46% germination, but other germination behaviors observed highest in polybags. Hasnat *et al.* (2014) reported similar observations about a native rare tree species *Schleichera oleosa* where plant percent found better in polybags with soil and cow-dung media (20%) than the Sylhet sand (3%). In another experiment on rare indigenous tree *Canarium resiniferum* (Hasnat *et al.* 2017) revealed similar results that seedlings grow in polybags with soil and cow-dung media provided better plant percent (20%) than seedlings grown in Sylhet sand media (10%). In the present study seeds were sown immediately after collection from forest and sown in all sowing media at a time. Germination in propagator house with Sylhet sand media found even after one year of seed sowing. This revealed that under unfavorable environmental condition seeds remain dormant and viable up to more than one year. But both in soil, and soil and cow-dung media no such dormancy or viability observed. It because after a certain period seeds decay naturally or attacked by pests and loss their viability. Moreover, through observation of the present study it was found that seedlings growth performance was better in soil and cow-dung media in polybags than the propagator house and nursery bed. More vigor and healthy seedlings were produced in polybags.

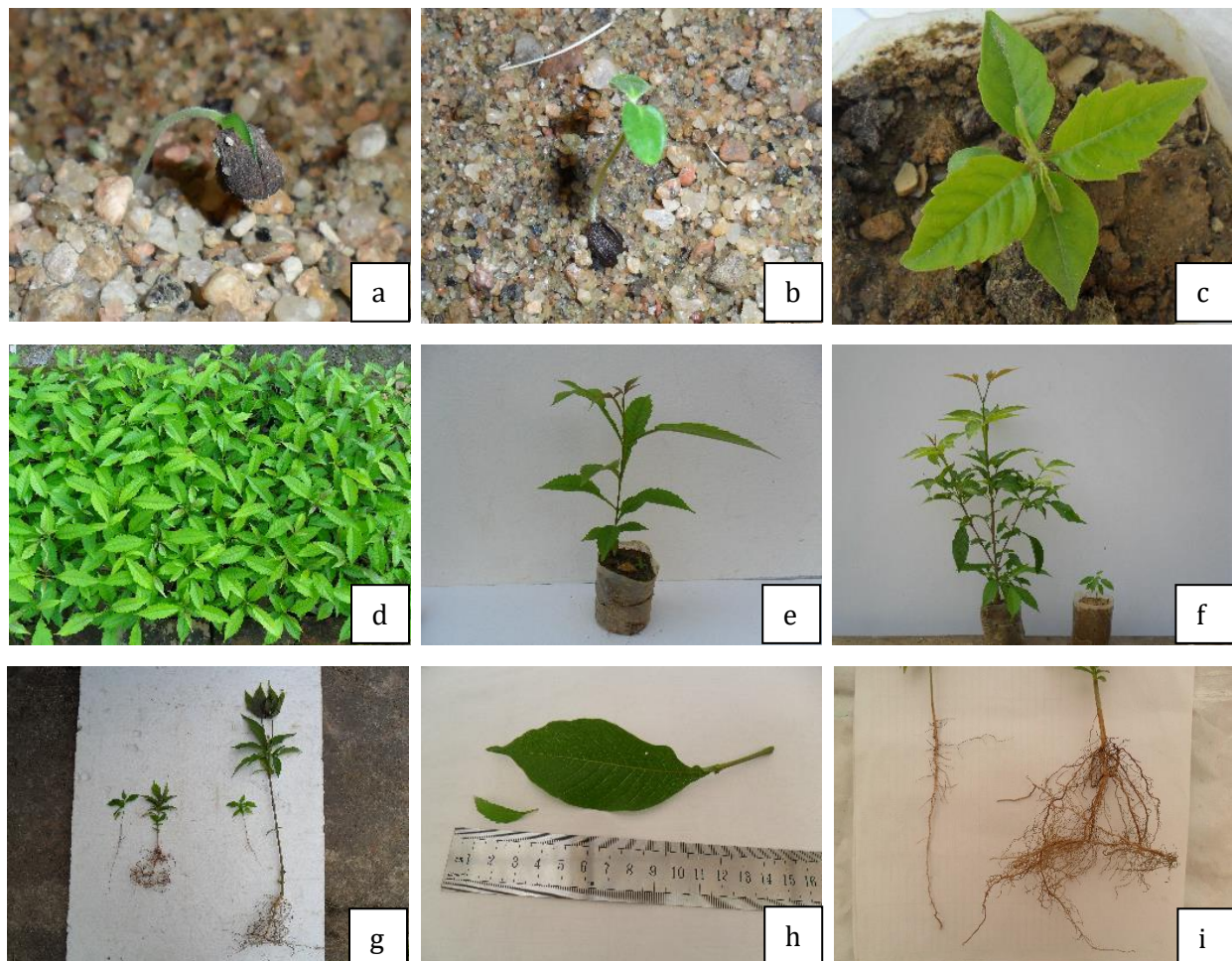


Figure 02. Different growth stages of *V. peduncularis* during the experiment- a) seed germination, b) one day old seedling germinated in propagator house, c) one week old seedling germinated in polybag, d) Six weeks old seedlings in polybag, e) Seven weeks old seedling in polybag, f) Six months old seedling germinated in polybag (left) and propagator house (right), g) five months old seedling germinated in propagator house (left), transplanted from nursery bed (right), g2) five months old seedling germinated in propagator house (left) and Polybag (right), h) leaf of six months old seedling germinated in propagator house (small) and leaf of six months old seedling germinated in polybag (large), i) roots of 5 months old seedlings from propagator house (left) and polybag (right).

IV. Conclusion

This study explored the basic germination characters and initial growth of the seedlings including germination percentage, energy, value, root and shoot growth etc. Based on the findings of this study it may be concluded that seeds of *V. peduncularis* responded differently to the different sowing media. Seeds sown in the propagator house revealed best germination performances (46%) than control (12.8%). Seedlings grown in polybags with soil and cow-dung media showed better morphological behaviors than seedlings grown in other sowing media. From the study, it is suggested to raise seedlings in polybags with soil and cow-dung media for vigor seedlings. Further researches are recommended on seed viability, germination and vigor seedling production.

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