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The effects of different treatments on seed germination and growth of monastery bamboo, *Thyrsostachys siamensis*

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ABSTRACT: Monastery bamboo, *Thyrsostachys siamensis*, is a gregarious flowering species. They can produce large number of seeds but there are few seeds that can germinate and grow in the natural forest areas. This study is aimed at improving the seed germination and growth rate of seedlings in different media and extending seeds storage to increase bamboo seedling production. Monastery bamboo seeds were planted in different media: pure sand, fine sandy loam mixed with bamboo roots and topsoil under the bamboo forest. We found that germination rates were the same in both topsoil under the bamboo forest and fine sandy loam mixed with bamboo roots ($p = 0.134$). However, growth of seedlings was greater in topsoil under the bamboo forest than both fine sandy loam mixed with bamboo roots and pure sand ($p = 0.001$). The treatment of monastery bamboo seeds with potassium nitrate (KNO_3) 0.1%, smoking, gibberellic acid (GA_3) at 250 mg/L, 90% sulfuric acid (H_2SO_4) and untreated control showed that the germination, survival and growth of monastery bamboo was greater in KNO_3 and smoked, but H_2SO_4 killed all seeds after 15 mins. In all cases, a standard temperature of 4 - 5°C was appropriated when focusing on the percentage of germination.

Keywords: Bamboo seed, Propagation, *Thyrsostachys siamensis*, Nursery production.

INTRODUCTION

Monastery bamboo, *Thyrsostachys siamensis*, is a primitive plant which belongs to the same family as grasses (Poaceae) with a hollow stem called culm and caryopsis seeds (Lucas, 2013). Bamboo is a monocarpic plant group that produces several seeds in the flowering season (Wong, 2004). The mass flowering takes long intervals of > 45 - 50 years (Sertse *et al*., 2011). Forest fires are a stimulation factor that favors bamboo flowering (Keeley and Bond, 1999). In nature, bamboo is predominantly an understory species in several Asian forest ecosystems. Bamboo has an economic viability in countries, such as India, China, Japan (Lobovikov *et al.*, 2005) and Thailand due to its rapid growth, high production, and fast maturity (Leksungnoen, 2017).

In Thailand, many bamboo species are established in intermediate moisture sites with relative humidity at 82 – 87% (Leksungnoen, 2017), especially in deciduous forests (Gardner *et al.*, 2000). After flowering, all flowers will fall to the soils and change the soil condition that soil pH, total nitrogen, available nitrogen and exchangeable Ca and Mg content were low in sites where bamboo had flowered and died (Takahashi *et al.*, 2007). The complete destruction of bamboo clumps requires another few years after the first flowering. Bamboo flowering has a considerable effect on the growth of seedlings in the western part of Thailand (Marod *et al.*, 2002). This bamboo flowering and death cycle produced acidic soil conditions with lower concentrations of exchangeable Ca and Mg, leading to lower soil nutrient status (Takahashi *et al*., 2007).

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Vegetative propagation of bamboo is mainly conducted by cuttings and air layering (Ray and Ali, 2016; Sandhu *et al.,* 2018). Cultivation is the proper way to promote bamboo for meeting future demands. The traditional propagation of bamboo consists of cutting branches or rhizomes (Kumar and Pal, 2004; Singh *et al*., 2004; Islam *et al*., 2011). The limitation of this technique is the requirement of the large number of branches and rhizome stocks that may have an effect on cutting the bamboo in the natural forest areas. This technique is also requires at large area of nursery. Tissue culture is another technique used to propagate bamboo seedlings (Agnihotri *et al*., 2009; Devi *et al.*, 2012; Venkatachalam *et al.,* 2015), but this technique is not appropriate for its conservation due to its low genetic diversity. Seed propagation is one of the proper methods to propagate monastery bamboo on a large scale. The only limitation of this technique is the unpredictability of flowering under natural conditions (Janzen, 1976).

The use of seeds is challenging because of the plant' s sporadic flowering nature and long flowering cycles along with seed recalcitrance and consumption by wild animals. The seeds can be collected and propagated in large quantities during one flowering season. With the unpredictability of flowering, the appropriate way to keep seedlings for propagation during non-flowering years is the extension of seed storage period (Balesevic *et al.*, 2010).

In this study, we aim to improve germination, growth rate and seed storage of monastery bamboo. Our work might provide meaningful information for the better propagation of monastery bamboo in terms of conservation and economic values.

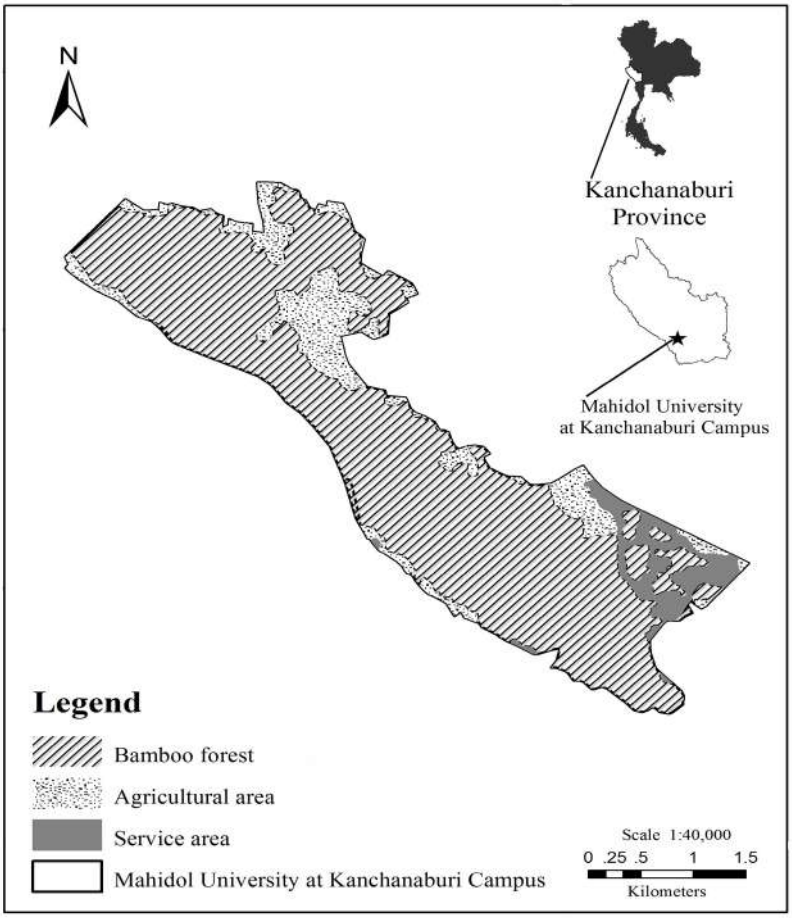

Figure 1. Map of Bamboo forest at Mahidol University, Kanchanaburi Campus, Kanchanaburi, Thailand.

MATERIALS AND METHODS

Study Area

Kanchanaburi province, located in the western part of Thailand, is the largest natural habitat of monastery bamboos in Thailand (about 450,000 ha) (Ueda, 1966). Mahidol University, Kanchanaburi campus is located in Kanchanaburi province covering an area of > 1,000 ha of bamboo forest on a limestone mountain (14°9′54.8″-14°9′54.6″E and 99°6′6.7″- 99°10′0.5″N) (Fig.1). The average precipitation is 1,293 mm yr^{-1} with the highest precipitation being in September and October. The soil in this site contains residuals from the limestone material on upland and hill slope areas. The majority of the forest in this area are mixed deciduous and is dominated by monastery bamboo and other associated trees such as *Anomianthus dulcis, Bauhinia malaburica, B. scandens, Ceasalpinia sappam, Zollingeria dongniaensi, Xylia xylocarpa, and Homalium tomentosum, etc* (Mahidol University, 1998).

Seed Collection

Monastery bamboos produce flowers during October and February and the seeds are ready during March. In Thailand, normally, monastery bamboos are simultaneously producing seeds in large areas (Sungkaew *et al.*, 2011). In 2007, monastery bamboos were flowered and unusually produced seeds in less than 5% of the total study area in Mahidol University, Kanchanaburi Campus. Seeds of the monastery bamboos were collected from the protected areas of Mahidol University, Kanchanaburi Campus. All seeds were dried under the sun for 3 days. Dried seeds were weighed and separated into pure seed, other seeds (crop or weed seeds) and inert matter and seed moisture content was measured in the laboratory of Mahidol University, Kanchanaburi Campus. Seed moisture content (fresh weight basis) was calculated by used of the formulae:

$$\text{Percentage of seed moisture content} = \frac{\text{M2-M3}}{\text{M2-M1}} \times 100$$

Where M1 = Weight of the weighing bottle/container with cover in g
M2 = Weight of the weighing bottle/container with cover and seeds before drying
M3 = Weight of the weighing bottle/container with cover and seeds after drying

Seed Germination

Monastery seeds were separated by hand to test the purity of the seeds. Hundred pure seeds were randomly selected and grown in separate plastic baskets of size 35 cm long, 27.5 cm wide and 10 cm high. Three different media: i) pure sand, ii) fine sandy loam mixed with bamboo roots and iii) topsoil under the bamboo forest were put in the plastic baskets at 3 replications per experiment. The experiment was done in a controlled evaporation greenhouse. The greenhouse temperature was controlled at 25±3°C and the relative humidity at 75±5%. The foggers were continuously spraying for 3 minutes each time. This process was repeated 3 times per day at 7.00 am, 12.00 am and 6.00 pm. The percentage of seed germination was recorded daily for 45 days and seedling survival rates were measured after 6 months. After seed germination, 10 seedlings in each plastic basket were randomly selected and the height measured weekly for 25 weeks.

Seed Germination after Stimulation

This experiment was carried out in a randomized block design (250 pure seeds x 3 replicates for each treatment). Germination was stimulated by soaking seeds in different treatments: 0.1% potassium nitrate (KNO_3), gibberellic acid (GA_3) at 250 mg/L, 90% sulfuric acid (H_2SO_4) (Zare *et al*., 2011) and under

smoke. Smoke was generated in a 130-1 drum using a mixture of dry and fresh plant material gathered in the surrounding vegetation. The smoke was blown, using bellows, through a pipe into plastic tents erected on nine 0.5 × 0.5 m areas containing seed. The system allowed the smoke to cool before it entered the tents (De Lange and Boucher, 1990; Hall *et al*., 2017) for 15 and 60 mins. Percentage of seed germination was measured every day until the last seedling was germinated. Five seedlings in each experiment were randomly selected and planted in plastic container (65 × 55 cm) with fine sandy loam mixed with bamboo roots and kept in the controlled evaporation greenhouse at Mahidol University, Kanchanaburi Campus. The heights of the seedling were measured monthly for 6 months.

Extending Bamboo Seed Life

Extension of the bamboo seed life was tested under pre-chilling (4 - 5°C) and pre-heating (40 - 50°C), compared to room temperature (20 - 25°C as control) for 1, 2, 3, and 4 weeks. After seeds were kept at pre-chilling or pre-heating temperature, the germination in topsoil under the bamboo forest and survival (percentage) were subjected to observation (Ghildiyal *et al.*, 2009). The growth of monastery bamboo seedlings in different media were recorded from 1 to 25 weeks.

Statistical Analysis

The germination data of each treatment were analyzed by using one-way ANOVA. Duncan's multiple range tests at 5% level ($p < 0.05$) were used to separate the difference between means of treatments. All data were analyzed using SPSS statistical package version 18.

RESULTS AND DISCUSSION

Seed Purification

Monastery bamboos produced 664.5 g fresh weight of seeds per clump. These seeds were separated into pure seed (14.1%), other seeds (20.3%) and inert seeds (65.7%) (Table 1). The moisture content of the pure seeds was 25% (n = 25 seeds) (Table 1). Monastery bamboo seeds showed lower purity, moisture content and seed germination rate, which were similar to those in seeds of *Bashania fangiana* in nature (Qin *et al*., 1989) and *Dendrocalamus giganteu*s in vitro (Devi *et al*., 2012). Previous study has reported the seed purity, the moisture content and the germination rate of monastery bamboos seeds were relatively lower than those of *Fargesia qinlingensis* in nature (Wang *et al*., 2007).

Table 1. Percentage of monastery bamboo, Thyrsostachys siamensis, seed purification by weight

Seed type	Weight (g)	Percentage (%)	Moisture content (%)
Pure seed	93.4	14.1	25
Other seed	134.6	20.3	
Inert seed	436.5	65.7	
Total	664.5	100	

Seed Germination

Germination rate of monastery bamboo after sowing in different medias were not significantly different ($p = 0.134$). Even though, in fine sandy loam mixed with bamboo roots, seeds were germinated faster (2 - 3 days) than other media (5 - 6 days). However, survival of seedlings after 6 months was significantly different among different medias; topsoil under the bamboo forest was better than fine sandy loam mixed

with bamboo roots and pure sand ($p = 0.001$), respectively (Table 2). Height of seedlings was also found to be different among different media ($p < 0.001$), while topsoil under the bamboo forest was better than sandy loam mixed with bamboo roots and pure sand (Table 2).

Table 2. Percentage of monastery bamboo, *Thyrsostachys siamensis,* seed germination and survival of seedlings after 6 months, and seedling height during 1 to 25 weeks in different soil types

Experiment	PS	NS	TS	F	P-value
Seed germination (%)	15±1.7	18.7±5.5	11±3.6	2.86	0.134ns
Survival of seedling after 6 months (%)	63.7±0.5	80.2±8.6	96.8±3.7	28.11	0.001**
Seedling height (cm)					
Average	6.7±2.5	15.1±11.9	19.9±13.1	10.47	<0.001**
Range	0.18-8.82	0.59-37.88	0.24-0.45		

PS = Pure sand, NS = Nutrition rich soil for agriculture, and TS = Topsoil under the bamboo forest.

According to other plant species, the germination of *Arundinaria gigantea* varied from 20% to 50% dependent on moisture, nutrition and light intensity (Gagnon and Platt, 2008). The monastery bamboo germinated within a week as found in *Fargesia qinlingensis* (Wang *et al*., 2007). This study suggested that topsoil under the bamboo forest was the most suitable medium such as total nitrogen, available nitrogen and exchangeable Ca and Mg content that were higher than sites where bamboo had flowered and died (Takahashi *et al*., 2007).

For the survival of seedlings when compared to fine sandy loam mixed with bamboo roots and pure sand. Previous observation has revealed that seed survival and germination was reduced on bare mineral soil (Wang *et al*., 2007).

Table 3. Percent germination and survival of bamboo seeds under different stimulated methods at different stimulated times

Time (Min)	Stimulated methodology (%)				
	H_2SO_4	KNO_3	GA_3	Smoke	Unstimulated
Percent germination					
15	3.7±0.5b*-a#	8.6±2.0a-b	4.3±0.5ns-a	5.5±1.1ns-b	7.7±1.6ns-b
60	0.0±0.0a-a	11.5±2.6b-c	6.7±0.9ns-b	6.1±1.3ns-b	10.7±2.2ns-c
Percent survival					
15	62.3±0.6b-a	72.0±3.2ns-b	70.2±7.0b-b	75.0±8.1ns-c	91.3±12.6ns-d
60	0.0±0.0a-a	87.2±11.3ns-c	67.4±6.0a-b	84.7±9.9ns-c	93.6±13.3ns-d

Data with different letter (*) in the same column, or (#) in the same row indicate a significant difference at 5% level according to LSD test, ns = not significant.

Seed Germination, Survival and Growth after Stimulations

Results of seed germination under different stimulation period is given in Table 3. Higher rate of germination was noticed only in KNO_3 pretreatment, but the percentage of germination was not significantly different ($p > 0$ .05). Germination in KNO_3 was increased with period of stimulation ($p > 0.05$). Compared to other treatments, KNO_3 might be the most beneficial method in stimulating germination of seeds and their survival; particularly at 60 mins, and even they were not much different to the unstimu-

lated seeds. Due to the high number of seed production and low germination rate of the monastery bamboo even a low percentage of increase in germination can increase the number of seedling production of this species. With consistency, the stimulation with KNO_3 for 60 mins in monastery bamboo seeds was found to be relatively effective as found in *ex situ* produced seeds of *Swertia chirayita* (Pradhan and Badola, 2010), when compared to stimulation with smoked and unstimulated condition. Treatment with GA_3 has been found to be very effective for breaking seed dormancy and inducing seed germination in many species such as Prosopia koelziana and *Prosopis juliflora* (Zare *et al*., 2011), *Nandina domestica* (Rhie *et al*., 2016).

After stimulation with KNO_3 and smoke, the percentage of survival was not different increased ($p > 0.05$), whereas in case of GA3, it showed elevated survival after 15 mins stimulation but likely decline in the survival at 60 mins stimulation ($p < 0.05$). Under H_2SO_4 treatment, all the seeds were found to be perished after 15 mins. The percentage survival of monastery bamboo showed declined tendency at 60 mins stimulation. In similar, the number of germinated seeds of monastery bamboo was positively correlated with stimulation times with smoked, as found in seeds of pepper (*Capsicum annuum*), salvia (*Salvia sp*.) (Demir *et al*., 2012), and soda apple (*Solanum viarum*) (Kandari *et al.*, 2011), as well as increased seedling emergence of several grasses (Poaceae) species (Ghebrehiwot *et al*., 2012). The survival and seedling vigor were correlated with damaged seeds. In *Quercus suber* acorns, Branco *et al.,* 2002) found that the damaged acorns had a decreasing dry weight, a faster germination, slower growth rate and lower dry mass production and reduced the survival rate of seedling. Treatment with H_2SO_4 even for 15 mins seemed to be a deleterious condition for monastery bamboo seeds, since all seeds perished under this condition. In contrast, H_2SO_4 has been shown to be the most effective germination stimulating agent in several types of vegetation such as *Prosopia koelziana* and *Prosopis juliflora* (Zare *et al*., 2011). Our result also suggested that the germination percentage of monastery bamboo seeds was likely associated to the survival percentage.

The growth of monastery bamboo seeds was assessed by measuring seedling height after 25 weeks growing in topsoil collected from the bamboo forest. As shown in Fig. 2, upon 15 mins (Fig. 2A) stimulation the seedling height was relatively less under stimulating conditions tested, in comparison to untreated condition. Interestingly, the smoke treated seeds for 60 mins (Fig. 2B) showed better seedling height, relative to that of untreated and other pre-treated seedlings ($p < 0.001$). This result indicated that smoke-mediated treatment was practically effective method for inducing growth of monastery bamboo seedlings.

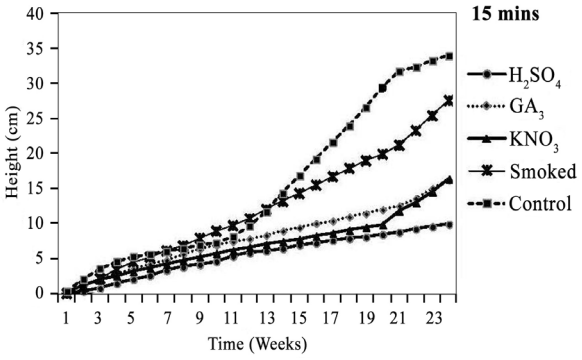

Figure 2A. Effects of different treatments within 15 mins and 60 mins

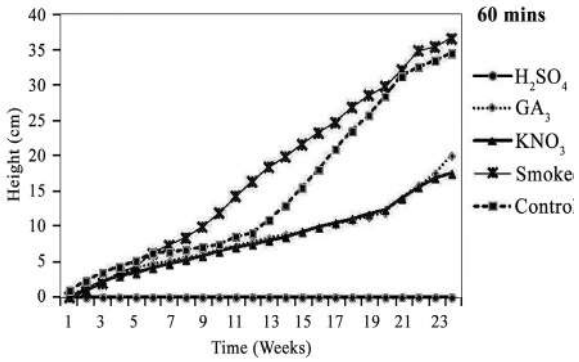

Figure 2B. The growth of monastery bamboo seedlings after cultivated in topsoil for 25 weeks.

A previous study has reported that smoke caused chemical or physical changes in the seed environment that modify germination responses (Pérez-Fernández and Rodríguez-Echeverría, 2003). Previous work has demonstrated that certain native grass species (e.g. *Themeda triandra* and *Tristachya leucothrix)* which frequently dominate in the burnt grasslands of South Africa could respond positively to plant-derived smoke (Ghebrehiwot *et al*., 2009). Recent research has shown that burning positively affected the seed germination of *Pappostipa speciosa* whereas tended to negatively affect the seed germination of *Festuca pallescens* (Franzese and Ghermandi, 2012). It is likely that a short exposure of seeds to high temperatures negatively affected germination percentages of *F. pallescens* while a short exposure to smoke increased *P. speciosa* germination percentages, indicating that chemical constituents in smoke might be considered as plausible stimulating factor for seed germination. It has also recently demonstrated that *Themeda triandra* was regarded as smoke responsive species, since its seed germination and biomass production in burnt mesic grassland sites were found to be increased, in comparison to unburnt sites (Ghebrehiwot *et al.*, 2012).

Extending Bamboo Seed Life

The percentage of germination with pre-chilling temperature was higher than that pre-heating and at room temperature for 1 - 2 weeks ($p < 0.05$), especially the highest germination incidence being in the first week (Table 4). Pre-chilling showed higher germination than pre-heating ($p < 0.001$). Moreover, the bamboo seeds kept under pre-heating condition showed reduced germination but slight effect on the survival, as compared to the control.

Table 4. Germination rate and percent survival of bamboo seeds after extending in different temperatures and times

Methods	Times (weeks)			
	1	2	3	4
Germination rate				
Kept under 4-5°C	15.0±3.2b*-b#	12.7±3.2b-b	8.1±1.4b-a	6.1±1.5b-a
Kept under 20-25°C	7.7±1.6a-b	5.7±1.0a-a	8.5±1.6b-b	6.0±1.1b-a
Kept under 40-50°C	8.7±1.9a-b	6.8±1.4a-b	3.8±1.0a-a	3.7±0.9a-a
Percent survival (%)				
Kept under 4-5°C	89.5±10.1b-a	88.2±9.9ns-a	91.2±10.9b-a	98.3±5.6b-b
Kept under 20-25°C	72.4±7.8a-a	89.2±10.0ns-c	77.6±8.7a-b	73.2±6.3a-a
Kept under 40-50°C	92.3±13.2b-b	89.2±9.9ns-b	89.6±10.0b-b	84.6±8.6b-a

Data with different letter (*) in the same column,or (#) in the same row indicate a significant difference at 5% level according to LSD test, ns = not significant.

Our result represented that pre-chilling at 4 - 5°C and 85 – 90% relative humidity might be a suggestive optimal condition for storing the seeds of monastery bamboo even at 4 weeks since the germinating capability was comparable and enhanced survival (> 98%) was observed, in comparison to the control. Previous studies have been reported that storage under pre-chilling condition could be beneficial to seed of *Dendrocalamus membranaceus* (Rawat and Thapliyal, 2003) and *Bambusa arundinacea* (Warrier *et al*., 2004). In consistent observation of Warrier *et al.* (2004), it has revealed that within 6 month

seeds still had high viability under pre-chilling storage while they rapidly lost viability under ambient conditions, due to seed moisture content lost. Although seed dormancy in many species can be broken by warm and/or cold stratification are unfavorable for germination (Rhie *et al.*, 2016). Unfavorable temperatures, prolonged light or darkness, water stress, and that anoxia can induce secondary dormancy in weed species (Baskin and Baskin, 2014).

CONCLUSION

Although, monastery bamboo produced a large number of seeds in the flowering season, in an unusual year, they will produce lower number of seeds. The optimal condition can stimulate germination and growth of the monastery bamboo seeds as well as for extending the seed life. This observation would be useful for increasing bamboo seedling production in cultivated areas and can reintroduce to the natural habitats. Taken together, our results suggested that topsoil in the bamboo forest was the most suitable medium for seedling survival. Compared to other stimulating agents (GA_3, smoke, and H_2SO_4) at different exposure times, treatment with KNO_3 at 60 mins seemed to be the most optimal condition in stimulating germination and survival of seeds. Nevertheless, smoke might be practically effective for promoting growth of seedlings in topsoil under the bamboo forest since forest fires probably break the seed dormancy. It furthermore revealed that pre-chilling (4 - 5°C) condition within 4 weeks might be the most effective for life extension of the seeds. Our findings would provide useful information for better propagation of monastery bamboo in terms of conservation and economics.

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