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Standardization of Vegetative Propagation Through Rhizome Cuttings in *Phyllostachys pubescens*

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Abstract: *Phyllostachys pubescens* (Moso bamboo) is a large monopodial bamboo species, naturally distributed in China and cultivated in neighboring countries. Seed availability of the *P. pubescens* is limited due to its long gregarious flowering cycle. Hence, developing nursery techniques from rhizome cuttings is necessary to fulfill the demand for Moso bamboo plantlets. A randomized block design nursery experiment was conducted with rhizomes which were 2-3 years old, healthy, fresh, not damaged, and have roots. Logistic regression model was used to compare the effect of various treatments and rhizomes lengths. Out of 720 rhizomes, 178 were sprouted (24%). Sprouting probability of rhizome bud was high in treatment T2 (50% rice husk + 50% straw dust) followed by treatment T4 (50% cocopeat + 50% wood dust). The treatment T3 (25% cocopeat + 25% wood dust + 25% rice husk + 25% straw dust) had more negative effects than control treatment. Similarly, sprouting probability was higher when rhizomes with 15 nodes were used for propagation followed those with 10 nodes and 5 nodes. The overall accuracy of the model was 83.9 percent. Results of the study revealed that rhizome cuttings can be used effectively for the production of *P. pubescens* plantlets in nurseries when there is scarcity of seed production.

Keywords: Alternative treatments, Nepal, nursery technique, *Phyllostachys pubescens*, rhizome length.

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Introduction

Bamboo is a large woody perennial grass belonging to the family Poaceae which comprises more than 1,600 species of 75-107 genera (Yuen *et al.,* 2017; Huy *et al.,* 2019). P. pubescens (commonly known as Moso bamboo) is a monopodial bamboo, naturally distributed in China and has also been planted in neighboring countries such as Nepal, Japan, and India (Larcher *et al.,* 2017; Gautam *et al.,* 2018; Isagi *et al.,* 1997; Bhatt, 2015). Moso bamboo can withstand a wide range of temperature varying from -18°C to 29.9°C (Zhou *et al.,* 2011) and precipitation of 800 - 1,800 mm (Fu, 2001). The suitable elevation for the species is between 500 - 800 m in yellowish-brown loam soil (60 cm deep fertile loam soil) with pH 4.5 - 7 (Yang *et al.,* 2016). However, it can also be grown at 10 - 1,700 m towards southern aspect (Lai, 1997; Fu, 2001). It is one of the most viable species providing timber (culms) and tender shoots (Lin *et al.,* 2014). Both parts of the species are easy to process for multiple bamboo products such as medicine, pulp, paper, housing, crafts, panels, boards, veneer, flooring, fabrics, roofing, oil, gas, and charcoal (Huy *et al.,* 2019). Cultivation of Moso bamboo is beneficial due to its characteristic rapid growth. Firstly, it matures within five years with an average culm height 20 m and 6-18 cm DBH (Diameter at Breast Height) (Fu, 2000). Secondly, it is one of the superior species for carbon storage (Yen, 2015; Yen, 2016). Thirdly, this species can be grown on steep slopes. As Nepal is mostly covered with fragile areas often susceptible to landslides (Meena *et al.,* 2019), Moso bamboo could be a suitable species to rehabilitate the land that was degraded by an earthquake-induced landslide and soil liquefaction (Gautam *et al.,* 2018). These features of Moso bamboo along with strong adaptability to various environmental conditions are advantageous over other plant species (Chen *et al.,* 2015; Chen *et al.,* 2016).

Moso bamboo naturally reproduces vegetatively in which it grows from a fragment of a mother plant (Swingle, 1940). As the flowering cycle of the species is 67 years, seeds are sparsely available (Isagi *et al.*, 2004) and also the germination rate from seed ranged from 7 - 60% only (Uchimura, 1990). Therefore, propagation through rhizome cutting could be important for creating a continuous supply of plantlets for Moso bamboo expansion without waiting for its long flowering cycle. The seed of Moso bamboo was first introduced in Nepal in 2006 in the degraded land of Kavrepalanchowk district (Gautam *et al.*, 2018). To test the best practices for sprouting the rhizome cuttings, experimental trials were carried out using rhizomes of different lengths and treatments. The Specific objectives of the study were; 1) To assess the effect of alternative treatments on sprouting of rhizomes, 2) To assess the effect of rhizome length (based on node numbers) on sprouting of rhizomes. It is hypothesized that there will not be a significant effect of alternative treatments and rhizome length on sprouting of rhizome on Moso bamboo.

Materials and Methods

Study site

The experimental site lies in the Kavrepalanchok district of the Bagmati province, Nepal. The nursery lies in Dhaneshwor Baikiwa Community Forest of the district. The nursery is located at an altitude of 1,551m asl with a northeast aspect (27°37'3" N, 85°30'57" E). It has an average annual temperature of 17.2°C and average annual precipitation of 1745 mm. In 2019, the maximum temperature recorded in this region was 30°C and the minimum temperature was 7°C (DHM, 2019). The soil type within the nursery is brown clay and sandy loam with pH 7.14. The natural vegetation in this region is *Schima-Castanopsis* and *Pinus roxburghi* forest with frequent shrubby patches.

A completely randomized block nursery experiment was conducted during February-July 2019. The nursery area was divided into 4 replication blocks; R1, R2, R3, and R4. In each block, four beds of size 1 x 10 m were made. The nursery beds were made up of 3:1 soil and sand as sand maintains good drainage, enables rooted plants to penetrate the surface easily, and establishes roughly uniform temperature (Banik, 1995). Locally easily available materials were used as standard substrates and treatments. Each nursery bed soil was mixed with 2.5 kg of fungus (*Trichoderma viride*) inoculum, 2.5 kg of di-ammonium

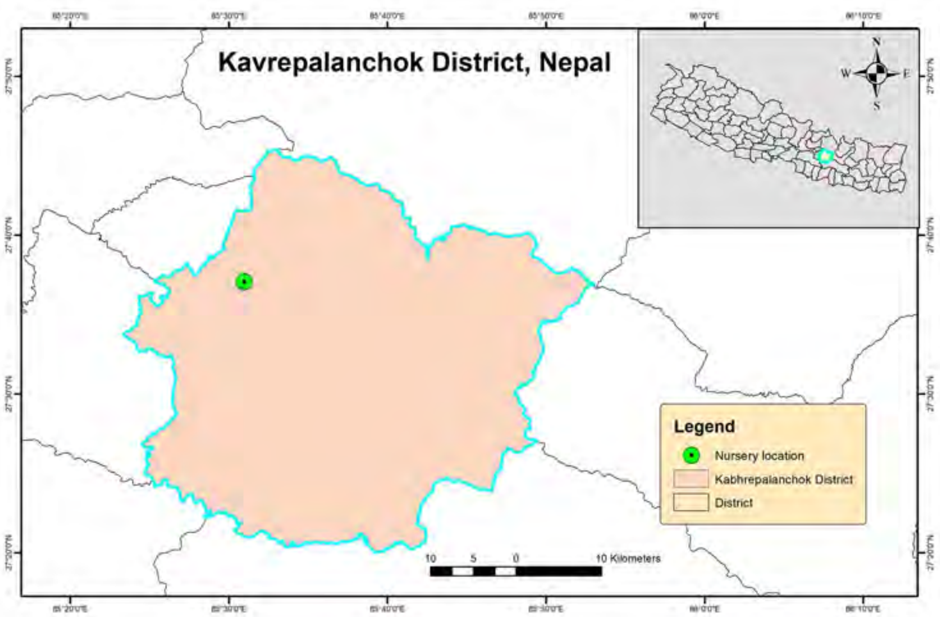


Fig 1. The Study area

phosphate fertilizer, 6 kg of bio-compost (mixture of cow/buffalo dung and dry leaves), and 0.0025 gl^{-1} of calcium carbonate as standard substrate to increase the physiology of rhizomes.

Furthermore, four treatments viz. T1, T2, T3, and T4 were designed to assess the effect on the sprouting of bamboo. T1 was the control as the soil was without any treatment. In T2, 50% rice husk + 50% straw dust was added to the soil. Similarly in T3, 25% cocopeat + 25% wood dust + 25% rice husk + 25% straw dust were added and in T4, 50% cocopeat + 50% wood dust were added to the soil. The treatments were assigned randomly to each block.

Healthy rhizomes which were 2-3 years old, fresh in color, not damaged, and having roots were chosen from a Moso bamboo plantations. Rhizomes older than 3 years produce buds with reduced vigor and growth (Dai *et al.,* 2016). The rhizomes were further grouped into three categories; rhizomes with 5 nodes, 10 nodes, and 15 nodes. The rhizomes with different lengths (based on node numbers) were planted randomly in each block while keeping each bed within the blocks homogenous. For the rhizomes with 5 nodes and 10 nodes, 60 rhizomes were planted with 25 cm spacing homogeneously in one of the beds each. Whereas, for the rhizome with 15 nodes, the remaining two beds were used for plantation (30 rhizomes in each bed) as it was difficult to accommodate all plants within a bed keeping a 25 cm margin. While planting, the bud of rhizomes was pointed upward and buried 10 cm deep in the soil. Overall, 720 rhizomes were planted into four randomized blocks with 180 rhizomes for each treatment, 240 rhizomes of each rhizome length.

Data collection and analysis

Sprouting of each rhizome subjected to different treatments was recorded. Binary values were assigned for each of them. The rhizomes that sprouted were scored as 1, and those that did not sprout as 0. The observation was continued till the final sprouting up to July 2019. The data were analyzed using R version 4.0.1 (R Core Team, 2020).

The logit model

To test and compare the effectiveness of alternative treatments and rhizome lengths on sprouting of the rhizome of Moso bamboo, binary logistic regressions (Peng *et al.*, 2001) were applied using logistic regression equation;

$$\ln(p/1-p) = b0 + b1 \times node + b2 \times treatment \text{ --- eq (1)}$$

(p/1-p) is an odd ratio of sprouting for rhizomes. Sprouting is a dichotomous variable with a value equal to 1 if the sprouting from the rhizome occurred and 0 if the sprouting from the rhizome did not occur. b0 is the intercept. $b_1$and b_2 are coefficients of categorical variables; rhizome lengths and treatments respectively. Rhizome lengths and treatment were control variables.

Akaike Information Criterion (AIC) was used for the selection of the best logistic regression model (Aho *et al.*, 2014), and the model with the lowest AIC was chosen as the best model. The Maximum Likelihood Estimation (MLE) technique was used

The vertical column represents frequency sprouting of rhizome in alternative treatments. The horizontal divides represent the frequency of sprouting of the rhizome of different node length.

Fig 2. A mosaic display for the contingency table data for sprouting of *P. pubescens*

Table 1. Linear estimates of coefficients of alternative treatments and node lengths on sprouting of rhizomes

S.N.	Term	Estimate	Standard error	Z-statistics	p-value
1	Intercept	0.042	0.317	-10.00	1.18E-23
2	node 10	3.95	0.280	4.91	9.30E-07
3	node15	8.01	0.275	7.57	3.73E-14
4	Treatment T2	4.60	0.276	5.54	3.10E-08
5	Treatment T3	0.82	0.320	-0.64	5.24E-01
6	Treatment T4	3.12	0.278	4.09	4.33E-05

for model fit. Likelihood Ratio test (often termed as LR test) based on Maximum Likelihood Estimation is a goodness of fit test used to compare between two models; the null model and the final model. Probability plots were used to examine the change in sprouting potential concerning independent variables.

The Marginal Effect

Marginal Effect computation was used to examine sprouting potential keeping another independent variable constant. Marginal effects can be described as the change in outcome as a function of the change in the treatment (or independent variable of interest) holding all other variables in the model constant (Leeper, 2017). For model evaluation, the whole data set into a training and test/validation dataset in 8:2 ratios. Training data was used to build models and a validation data set was used to evaluate the best model. We assessed the accuracy of the model at the appropriate cutoff value. There was an imbalanced dataset as the dataset had more 0s than 1s. And therefore, Precision, Recall, and F1-score were used to measure the quality of model predictions from a classification algorithm (Wardhani *et al.*, 2019).

Results and Discussion

Frequency of sprouting

Plant vegetative growth is a complex phenomenon that is a joint result of the plant's genetics and various environmental factors (Beveridge *et al.,* 2007). The development of the rhizome lateral bud into the stem depends on the environment, the parent shoot, and the rhizome apex (Bateman *et al.,* 1998). In the present study, out of 720 rhizomes planted only 178 sprouted (24.72% of the total sample taken) which is comparatively low as compared to the vegetative growth of lateral buds from a rhizome in a bamboo forest. The low sprouting capacity may be due to the absence of parent shoots, slightly high pH (7.14) of the nursery bed, and relatively high elevation of the plot.

The frequency of sprouting of the rhizome of 15 nodes was higher (n = 35) in treatment T2 followed by the control T1 (n = 31), T4 (n = 16), and treatment T3 (n = 13). Similarly, the frequency of sprouting of rhizomes with 10 nodes was higher in treatment T2 (n = 28), followed by T1 (n = 21), T3 (n =7), and T4 (n = 6), while results for rhizomes with 5 nodes comprised of T2 (n = 7), T1 (n = 7), T4 (n = 3), and T3 (n = 2). Additionally, the frequency of sprouting of Moso bamboo was higher in rhizomes with 15 nodes (n = 95) followed by 10 nodes (n = 62) and 5 nodes (n = 21) in all categories of treatment applied (Fig.2).

Logit model analysis

Three logit models were tested to assess the effect of each category of control variables on the sprouting of the rhizome. Three logit models were tested for the purpose. The first model was performed to test the effect of treatment only and the second to test effect of rhizome length and model 3 tested the effect of both rhizome lengths and treatments. Based on the lowest AIC value, model 3 was selected as the best fit to interpret the results (model 3: 691.04, model 2: 744.87, and model 1: 758.9).

Logit model analysis result took rhizomes with 5 nodes and Treatment T1 as reference (intercept). It also showed that rhizome with 10 nodes, and 15 nodes, had a significant positive influence ($p < 0.05$), while T3 is insignificant in determining Moso bamboo sprouting (Table 2). For easy un-

Table 2. Estimates of the Average Marginal Effect of alternative treatments and node lengths on sprouting of rhizomes

Factor	Average Marginal Effect	Standard error	z-statistics	p-value	Lower	Upper
Node 10	0.1708	0.0323	5.2814	0.0000	0.1074	0.2342
Node 15	0.3083	0.0347	8.8964	0.0000	0.2404	0.3763
Treatment T2	0.2556	0.0423	6.0363	0.0000	0.1726	0.3385
Treatment T3	-0.0222	0.0348	-0.6386	0.5231	-0.0904	0.0460
Treatment T4	0.1778	0.0414	4.2955	0.0000	0.0967	0.2589

derstanding, we interpreted the linear comparison of coefficients of control variables for 95% confidence intervals. Linear coefficients are exponents of log odd ratios of coefficients of control variables. Binary logistic regression estimated that Moso bamboo sprouting in node 15 is 8 times than node 5 and that in node 10 is 3.95 times than node 5.

Besides, Moso bamboo sprouting in the treatment T2 is 4.59 times higher than T1 that in the treatment T4 level is 3.11 times than T1, whereas, with the treatment T3, it was 0.81 times than T1.

The Likelihood Ratio test revealed that the Log-Likelihood difference between the intercept-only model (null model) and the model with all independent variables did differ ($p<0.05$) which made the model a good fit for the interpretation of the results.

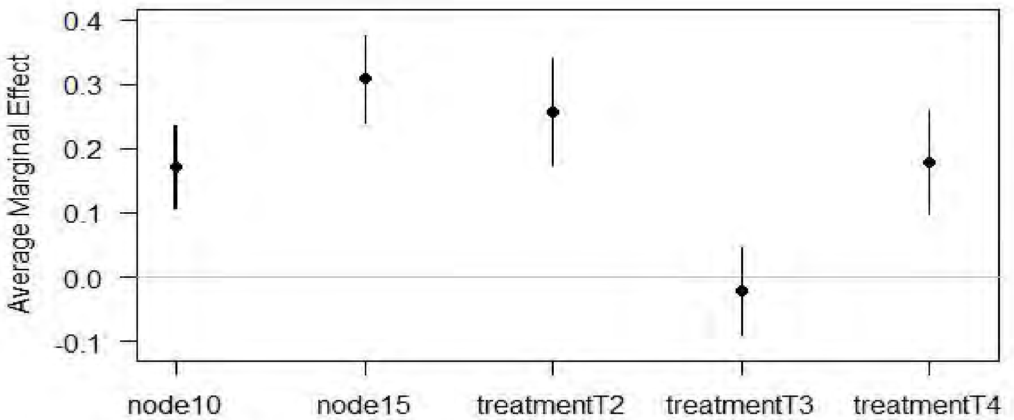
The Marginal Effect of control variables

Average Marginal Effect analysis result indicated that the probability of Moso bamboo sprouting is 25.56% higher in Treatment T2 than in treatment T1, 17.78% higher in Treatment T4 than in control, while lower than control in T3 (2.22% lower). The probability of Moso bamboo sprouting was 30.83% higher for rhizome with 15 nodes than those with 5 nodes. The probability of Moso bamboo sprouting is 17.08 % higher for rhizome with 10 nodes as compared to those with 5 nodes. Overall, rhizomes with 15 nodes have the highest average marginal effect on sprouting of rhizome followed by treatment T2, treatment T3 and rhizome with 10 nodes. The result showed that the treatment T3 harms the sprouting of rhizome (Fig. 3). The result from marginal effect analysis is important because farmers may not have a variety of treatments and a variety of rhizome lengths. In this scenario, farmers can choose the best alternatives for the growth of rhizomes in nursery conditions.

Model evaluation

The model evaluation metrics revealed that the precision and recall values are 0.797 and 0.887 respectively. The precision indicates that 79.7 % rhizome actually sprouted out of labeled as sprouted by the model. However, recall shows that 88.7% rhizomes were labeled as sprouted out of truly sprouted. And the F1 score combines the both precision and recall for final model evaluation. And the F1 score is 0.839, which indicates that the trained model has classification strength of 83.9%.

The present study showed that sprouting from lateral buds of rhizomes can be enhanced by alternative treatments and rhizome lengths. We found that a combination of treatments (rice husk and straw dust) along with the use of rhizomes with a length of 15 nodes were most successful for the conversion of rhizome lateral buds into shoots of Moso bamboo. According to Larcher *et al.*,(2017), a combination of alternative treatments are key for the sustainable cultivation of *P. pubescens*. It also suggests that rice husk is useful for the growth of Moso bamboo in nursery conditions when sprouting percentage is low and it supports the finding of the current study. Kigomo (2007) stated that the secondary roots attached with rhizomes are im-



The horizontal line represents number of sprouting and the vertical line along points shows a range of average marginal estimates

Fig 3. Estimates of the average marginal effect of control variables (node 10, node 15, treatment T2, treatmentT3, and treatment T4) on sprouting of rhizomes of Moso bamboo

portant for the conversion of the lateral bud into bamboo stem because it helps to uptake nutrition from the soil and treatment media. This means the longer the length of the rhizome, the greater the density of secondary roots on rhizomes which could be helpful for rhizome lateral bud proliferation, and this supports the results of our study.

Conclusion and Recommendations

Overall, it can be concluded that that Moso bamboo can be propagated with a moderate success through sprouting rhizome offset. Binary logistic regression suggested that Moso bamboo sprouting in node 15 is 8 times than node 5 and that in node 10 is 3.95 times than node 5. Besides, Moso bamboo sprouting in the treatment T2 is 4.59 times higher than T1 that in the treatment T4 level is 3.11 times than T1, whereas, with the treatment T3, it was 0.81 times than T1. In addition, the marginal effect of control variables showed that the rhizomes having 15 nodes had 30.83% higher sprouting than 5 nodes , while specific soil treatment in nursery beds - 50% rice husk + 50% straw dust (treatment T2), in addition to the standard substrates influenced higher sprouting of rhizomes (25.56%more than that of Treatment T1). Findings of the study could be utilized as a baseline data for planting stock production of *P. pubescens*, and also for other advanced research in propagation methods of *P. pubescens* in Nepal.

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