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Clonal propagation of *Bambusa vulgaris* Schrad. ex Wendl. through adventitious rhizogenesis in mini cuttings

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Abstract: The method describes improvement in adventitious rhizogenesis in *Bambusa vulgaris* var. green. The sturdy mini cuttings collected from one-year-old pollarded culms and grouped in three diameter class, viz. 0-0.5 cm, 0.5-1.0 cm and 1.0-1.5 cm were treated with 100 ppm IBA and control and planted on germination bed of shadehouse in June 2014. Out of different diameter classes, the cuttings in 0.5-1.0 cm diameter class produced a maximum of 83% rhizogenesis with maximum root length (31.63 cm), root number (16.83) and tiller height (20.96 cm). However, the maximum tiller number (1.8) was obtained in 1.0-1.5 cm diameter class. However, the interaction study showed maximum rhizogenesis (100%), root length (32.76 cm), root number (17.33) in diameter class (0.5-1.0 cm) in control, which was significantly higher than the treatment (100 ppm IBA) and in another diameter class viz. 0-0.5 cm and 1.01.5 cm. The findings of the study suggest augmenting adventitious rhizogenesis using mini cuttings (0.5- 1.0 cm diameter) of *Bambusa vulgaris* without any hormone treatment in the month of June. *Key words: Bambusa vulgaris* Schrad. ex J.C. Wendl., mini cuttings, rhizogenesis.

INTRODUCTION

India harbors 10% (125 indigenous and 11 exotic species) (FSI Report, 2017) of the total bamboo species of the world (1250 species) and ranks next to china with 300 species (Hore, 1998; Dhugra, 2013). In India about 50% of the annual production of bamboo is used by many small and big industries like artisans, paper and rayon, scaffolding and bamboo boards (Tewari, 1992). The continuous demand by these industries has put an enormous pressure on bamboo resources and driven by the significant thrust in the bamboo sector. Non-availability of sufficient quantity of quality planting materials remains as a bollteneck. Therefore, efforts are continued to propagate bamboos via different methods. The propagation of bamboo species by seed is most successful and commonly used method but many of them exhibit wide variation with respect to the timing (1–120 years) and nature (sporadic vs. gregarious) of flowering among species (Biswas *et al*., 2016).

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Bambusa vulgaris Schrad. ex J.C. Wendl. commonly known as green bamboo or common bamboo is distributed in north east India and also available in central India as natural forests. This species is commonly used for pulp and paper industries, constructions, scaffoldings, fencing and handicrafts (Salam and Pongen, 2008). Its fiber length (2.33mm) is more and fiber width and flexibility strength is comparable with the parallel species *B.vulgaris* var. striata (Tamolang *et al*., 1980). The species possesses several regeneration constraints. In the literature it is mentioned that in many parts of the world this has not flowered and set fruit due to a cumulative effect of factors such as high pollen sterility, absence of natural pollinators, inhibition of pollen tubes in the stigma tissue etc. (Koshy and Jee, 2001). As seeds and seedlings are not available, such species are being propagated vegetatively (Koshy and Pushpangadan, 1997; Banik, 2000). Several methods of vegetative propagation of *B.vulgaris* Schrad. ex J.C. Wendl. are reported using juvenile branch cutting (Razvi and Nautiyal, 2009), leafy branch cutting (Islam *et al*., 2011) and culm branch cutting (Razvi *et al*., 2011). Now the technique of minicuttings is becoming popular to propagate the propagules vagetatively. Pioneering work done by Xavier and Wendling (1998) reported the use of mini cuttings for propagation of *Eucalyptus*. Mini-cuttings produce better quality root systems with a tendency for development of profuse fibrous root system which provides initially physical strength to the growing culms till the formation of rhizomes in case of bamboos. Apparently, the connection between root and stem tissues in the mini-cuttings is more suitable due to lower lignification of the tissues involved (Assis *et al*., 2004). To meet the demand meant for clonal planting materials of *B.vugaris* Schrad. ex J.C. Wendl., it was imperative to improvise available techniques of Razvi and Nautiyal (2009), Islam *et al*. (2011) and Razvi *et al*. (2011) who routinely used hormones for rooting in juvenile branch cuttings, leafy branch cuttings and culm cutting, respectively. The present communication reports vegetative propagation of *B.vulgaris* Schrad. ex J.C. Wendl. using mini cuttings without any hormonal treatment, which makes this procedure simple, cost effective and feasible.

MATERIALS AND METHODS

The study was carried out in nursery of Genetics and Plant Propagation Division of Tropical Forest Research Institute, Jabalpur, falling in humid subtropical climate. Summer begins in late March lasting until June and temperature ranges from 8^0C to 45^0C.

The single nodded sturdy mini cuttings were collected in the month of June 2014 from hedge garden of one-year-old pollarded culms of *B. vulgaris* Schrad. ex J.C. Wendl. of established in 1993 at silviculture nursery of the institute, receiving average annual rainfall of nearly 1386 mm with maximum and minimum temperature of 45^0C to 8^0C, respectively and found with black cotton soil. The collected mini cuttings were grouped into three diameter classes viz. 0-0.5 cm, 0.5-1.0 cm and 1.0-1.5 cm. Sturdy nodal cuttings of 3-3.5 cm length was prepared and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) aqueous solution for 10 minutes. After surface sterilization, the cuttings were treated with 200 ppm IBA and control (kept in distilled water) for 24 hours. Each treatment consisted of three replicates each of 30 cuttings. After hormonal treatment the cuttings were planted horizontally in sand bed in shadehouse of single layer agro-shade net in natural condition (without misting) and irrigated twice in a week with the help of sprinkler.

After 45 days, the cuttings were scored for rhizogenesis percentage, root length, root length, number and height of culms. The data obtained were subjected to statistical analysis employing analysis of variance (ANOVA), F test for significance at p 0.05 and computing LSD for comparing treatment means.

RESULTS AND DISCUSSION

Rooting parameters

Bambusa vulgaris exhibited adventitious rhizogenesis in the cuttings of all diameter classes of both the treatments (Fig 1 a, b, c), which varied from 83.33% to 51.66% (Table 1). However, better rooting percentage (83.33%) was recorded in the cuttings of 0.5-1.0 cm diameter class, which was significantly higher than other diameter classes. The cuttings without hormonal treatment exhibited 83.33% rooting, which was significantly higher (34%) than the cuttings treated with IBA. However, in the interaction study, the cuttings of 0.5-1.0 cm diameter class without any treatment showed maximum of 100% rooting (Table 1).

Table 1. Effect of diameter class on adventitious rhizogenesis of *Bambusa vulgaris* var. green after 45 days

Diameter class (cm)	Rooting %			Root length (cm)			Root number		
	Control	IBA	Mean	Control	IBA	Mean	Control	IBA	Mean
0-0.5	56.66 (48.85)	46.66 (43.08)	51.66 (45.96)	22.7	24.9	23.80	17.00	11.66	14.33
0.5-1.0	100 (90)	66.66 (54.78)	83.33 (72.39)	32.76	23.23	31.63	17.33	16.33	16.83
1.0-1.5	93.33 (77.71)	50.00 (45)	71.66 (61.35)	20.60	11.2	15.90	14.33	7.33	10.83
Mean	83.33 (72.18)	54.44 (47.62)		25.35	21.98		16.22	11.77	

CD 0.05	CD 0.05	CD 0.05
Treatment 5.98	Treatment 4.80	Treatment 3.42
Diameter 7.33	Diameter 5.88	Diameter 4.19
T*D 8.93	T*D 8.29	T*D 5.90

The use of exogenous application is reported to be prerequisite for adventitious rooting in many bamboo species, including *Bambusa vulgaris* Schrad. ex J.C. Wendl. Working with the same species, Singh *et al*. (2006) reported influence of auxin in single node culm cutting. Islam *et al*. (2011) also reported the nodal leafy cuttings and tip cuttings produced sufficient rooting supplemented with graded doses of IBA. Similarly, Razvi *et al*. (2011) reported maximum of 81% rooting in binodal cuttings treated with 500 ppm IBA. Our results are in contrast to these earlier findings. Control (without hormone) facilitated maximum rooting percentage in *Bambusa vulgaris*. However, the results obtained by Alwis and Ranasinghe (2012) corroborated with the present findings, they reported maximum rooting success in culm cutting of *Bambusa vulgaris* without any hormonal treatment. The rooting in minicutting of *Bambusa vulgaris* is probably because the presence of high level of endogenous auxin in these sturdy cuttings collected in summer. Auxin plays a positive role, needed to direct the expansion of primordia and the differentiation of vasculature (Scarpella et al. 2006). In the month of June high rate of photosynthesis and active growth phase of culms due to high meristematic activity resulted in rapid cell division, initiation of root primordia and root development. The exogenously applied auxin may have raised it to supra optimal levels that were inhibitory and reported to produce considerable lower rooting success as compared to control. In accordance with the finding Titon *et al*. (2003) reported negative effects of hormone on rooting due to the high degree of juvenility of the new shoot of *Eucalyptus grandis*. This is due to the fact that the juvenile minicuttings already have tissues with an endogenous hormonal balance that is favorable for rooting, which then leads to no (Silva *et al*., 2010; Ferreira *et al*., 2010; Wendling *et al*., 2010) or even a negative response to exogenous application of auxin (Xavier *et al*., 2003). In the present study the process of rhizogenesis has been completed in the span of 45 days as compared to the auxin treated branch cuttings which took75 days (Razvi *et al*., 2011). This may be attributed to both higher levels of juvenility and optimal nutritional content of the tissues, which improves the rooting predisposition and speed of root initiation (Assis *et al*., 2004). Our results are in agreement with Nanda and Anand (1970) who have demonstrated the effect of endogenous auxin responsible for rooting in branch cuttings of *Populus nigra* treated without hormone in June. The cuttings without hormone produced significantly higher root length and root number (25.35 cm and 16.22, respectively) which was 15% and 37% higher as compared to IBA treated cuttings. Besides, the interaction study exhibited significantly higher root length (32.76 cm) (Fig 1d) and root number (17.33) (Fig 1e) in 0.5-1.0 cm diameter class without any hormonal treatment. Moreover, the minicuttings produce better quality root systems with higher root number and root length which is apparently more suitable as compared to treated cuttings due to reduced lignifications of the tissues involved (Majada *et al*. 2011).

Fig 1. Propagation of *Bambusa vulgaris* var. green using the mini cuttings; rooting took place in 0.5 cm (a), 0.5-1.0 cm (b) and 1.0-1.5 cm (c) diameter mini cuttings; higher root length and root number in the cuttings of 0.5-1.0 cm diameter class without any hormonal treatment (d,e); clonally propagated plants of
B.vulgaris (f).

Culm number and culm height

In the present study, mini cuttings showed a non-significant effect of treatment and diameter class in culm number. However, the interaction study exhibited a significant variation in culm number and control with 1.0-1.5 cm diameter class produced maximum of 2.20 culms (Table 2). The culm height was found to be non-significant for all diameter class, treatments and their interaction. The mini cuttings were taken from one-year-old branches of pollarded culms and presumed to be good propagules. These have active and vigorous buds that are capable of producing higher culm numbers as compared to cuttings from older culms. The minicuttings of bamboo seem to synthesize food in light and utilize it slowly during development of new shoots. This is in contrast to mini cuttings of difficult to root species like Tectona *grandis* (Paiva and Gomes, 2005) in which the higher reserve level of carbohydate rapidly consumed during the course of root and shoot formation. Similarly, Ruiz and

Montiel (1998) also reported that one-year-old culms, produced more shoots in *Guadua chacoensis* than the three-year-old ones. In the present study, the age of culms of *B.vulgaris* was not a factor of variation, since it sought to control the effect of age by choosing different culm diameter from one year old pollarded culms.

Table 2. Effect of diameter class on number of tiller and tiller height of *Bambusa vulgaris* var. green after 45 days

Diameter class	Number of culm			Culm height (cm) (m)		
	Control	IBA	Mean	Control	IBA	Mean
0-0.5	1.13	1.93	1.53	16.00	25.92	20.96
0.5-1.0	1.57	1.13	1.35	19.07	15.65	17.36
1.0-1.5	2.20	1.40	1.80	22.68	12.66	17.67
Mean	1.63	1.48		19.25	18.08	
$CD_{0.05}$				$CD_{0.05}$		
Treatment NS				Treatment NS		
Diameter NS				Diameter NS		
T*D 0.78				T*D NS		

CONCLUSION

There is no published information available on the use of mini cutting technique in bamboos. The results of this study showed that the mini cuttings of 0.5-1.0 cm diameter without any hormonal treatment produced significantly higher rooting success. This technique is reliable for mass clonal propagation of *B.vulgaris* (Fig 1f) and also being employed successfully at the institute for commercial propagation of other bamboo species like *Bambusa tulda*, *Bambusa nutans* and *Dendrocalamus strictus* available in central India with considerable rooting success . The technique of vegetative propagation of *B.vulgaris* reported herein is cheaper and ease of handling as compared to the traditional technique of culm cutting and tissue culture for stock build up.

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