Effect of plant growth promoting rhizobacteria and Organixxgro on tissue-cultured bamboo plantlets under nursery conditions

Bamboos are evergreen perennial woody grasses from the Poaceae family distributed in tropical, sub-tropical and temperate regions of the world. As a suitable alternative to timber, bamboo was once considered as the poor man's timber. However, owing to the extensive increase in modern applications with tremendous economic importance, bamboos have rightly been recognized as 'green gold' of the 21st century. In India, 136 species of bamboo belonging to 23 genera are found[1,2]. The North Eastern (NE) states of India alone harbour more than 90 bamboo species, 41 of which are endemic to this region[3,4]. Among the bamboos of NE India, *Bambusa tulda* is the most economically important species prioritized by the National Bamboo Mission for large-scale cultivation. Because of strength, durability and versatility in use, it has great demand in the market[5]. Accordingly demand for quality planting material of *B. tulda* is also very high. To cater to the demand of large-scale bamboo planting material in the country, micropropagation of elite germplasm is practised. Research in the Rain Forest Research Institute (RFRI), Jorhat, has shown that macroproliferation of tissue-cultured propagules in nursery beds is effective and economical in increasing the number of quality planting materials. Generally, soils in the nurseries are low in nutrients and beneficial microbial population due to continuous use for seedling production. Therefore, plant growers are forced to use large quantities of chemical fertilizers to improve plantlet growth. However, increased cost of planting material production and environmental concerns due to the use of chemical fertilizers require alternative methods of large-scale quality planting stock production[6]. The present study was undertaken to assess the efficacy of plant growth promoting rhizobacteria (PGPR) consortium developed by RFRI and Organixxgro (plant supplement) manufactured by Pro Agro Tech, Jabalpur, Madhya Pradesh, India in enhancing the proliferation rate of tissue-cultured propagules.

B. tulda plantlets raised through tissue culture method were transplanted in the nursery beds after primary hardening. Twelve nursery beds were randomized employing on-line statistical software[7] and the experiment was carried out using completely randomized design (CRD). The experiment was performed in three treatments and four replications consisting of control (T1), Organixxgro (T2) and PGPR consortium (T3) for 120 days. The PGPR consortium was prepared using *Bacillus cereus* (accession no. MG196047), *Pseudomonas fluorescens* (MG020682), and *Kosakonia sacchari* KhAn (MG881883) having plant growth promoting traits. The bamboo special Organixxgro (plant supplement) consists amino acid (8%), humic acid (1%), derived from plant extract, elementary macro- and micronutrients (10%) extracted from fruits, vegetables and bioresources (based on manufacturers claim)[8]. PGPR isolates *B. cereus* and *P. fluorescens* were mass multiplied in nutrient broth medium and *K. sacchari*, a free living diazotroph[9] was multiplied in nitrogen-free broth medium. Consortium of these PGPR isolates was prepared by mixing the mass cultures of each isolate in equal proportions and applied at the rate of 50 ml (10^6 cfu ml^{-1}) per plantlet, while 50 ml of Organixxgro was applied at a concentration of 80 ml/l (v/v) following the manufacturer's recommendations. Growth parameters, viz. number of tillers, leaves per tiller, leaf area and chlorophyll content were recorded after 120 days from the date of initiation of the trial. The chlorophyll content of bamboo leaves was estimated using DMSO method[10]. However, root and shoot length as well as dry biomass were recorded after uprooting the plant samples. The data were statistically analysed for calculation of mean values and standard error (SEm) using MS Excel. Initially, the number of tillers, leaves per tiller and average height of plantlets were 2.0 ± 0.12, 5.63 ± 0.34 and 28.57 ± 1.70 (cm) respectively.

The observations revealed that application of both Organixxgro and PGPR consortium was effective to improve plantlet growth and health. Maximum values of chlorophyll *a* (16.15 ± 1.50 mg/g) and total chlorophyll (37.75 ± 1.72 mg/g) were observed in PGPR-treated plantlets followed by Organixxgro. Noticeable improvement was recorded in the number of tillers, leaves per tiller, and shoot and root length of PGPR-treated plantlets compared to Organixxgro. The dry biomass of PGPR-treated plantlets was also higher than the Organixxgro and control (Table 1). Among all the treatments, effect of the PGPR consortium was found to be better than Organixxgro, whereas least values of all the parameters were recorded in control and leaf area of plantlets showed less variation in all the treatments (Table 1). Enhancement in chlorophyll content and dry biomass indicates the positive effect of the PGPR consortium in plantlet health. Increase in the number of tillers, leaves, root and shoot length shows the effectiveness in macroproliferation (Figure 1).

The present study to increase the number of tillers from tissue culture-raised plantlets using plant supplements and PGPR consortium may play a crucial role in large-scale healthy planting stock production. PGPR isolates have long been recognized for their role in soil fertility and crop productivity enhancement across the globe. These organisms have multifarious beneficial properties which are provided to the crops and plants through mutual plant–microbe interactions under a wide range of environmental conditions. Though the readily available plant supplements can serve as a quick source of nutrients and improve plant growth and development within a short period of time, they need to be provided at frequent intervals. On the contrary, PGPRs play a role in nitrogen fixation, phosphate solubilization, biostimulation, biocontrol of soil-borne pathogens and many other functions[11]. Further, they multiply in the soil or in association with the plant to maintain an active population, and generally there will be no need for repeated applications. Therefore, a combination of potent PGPR consortia can improve soil health as well as plant growth and development without the need for repeated applications. Muthukumar and Udaiyan[6] carried out a nursery experiment to determine the effect of *Glomus aggregatum*, *Bacillus polymixa* and *Azospirillum brasilense* on seedling growth promotion of *Dendrocalamus strictus* using Alfisol and Vertisol soil medium with or without fertilizer application. Shoot, rhizome and root length, dry biomass, nutrient concentration and arbuscular mycorrhizal (AM)

Table 1. Effect of Organixxgro and plant growth promoting rhizobacteria (PGPR) consortium on different growth parameters of *Bambusa tulda*

Treatment	Leaf area (cm^2)
T1	247.34 ± 8.56
T2	258.18 ± 7.45
T3	235.40 ± 11.26

Values are mean of 10 leaf samples randomly taken from each replication, ± SEm

	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
T1	9.57 ± 0.47	5.22 ± 0.10	27.89 ± 0.55
T2	12.16 ± 0.73	6.90 ± 0.47	36.86 ± 2.48
T3	16.15 ± 1.50	7.04 ± 0.32	37.75 ± 1.72

Values are mean (mg/g) of randomly pooled leaf samples from each replication, ± SEm

	Number of tillers	Number of leaves per tiller	Shoot length (cm)	Root length (cm)
T1	4.0 ± 0.58	19.83 ± 2.70	65.67 ± 2.29	34.39 ± 1.11
T2	5.5 ± 0.5	36.61 ± 5.80	71.78 ± 3.27	38.0 ± 0.79
T3	7.0 ± 0.70	29.04 ± 4.51	80.65 ± 4.43	36.75 ± 2.78

Values are mean of 10 randomly selected plants from each replication, ± SEm

	Shoot biomass (g)	Root biomass (g)
T1	80.02 ± 8.17	22.03 ± 3.47
T2	105.07 ± 11.91	22.38 ± 2.23
T3	115 ± 6.45	27.14 ± 2.29

Values are mean of 3 sample plants randomly harvested from each replication, ± SEm
T1, Control, T2, Organixxgro and T3, Rain Forest Research Institute-PGPR consortium.

Figure 1. Visible effects of Organixxgro and PGPR consortium on plant growth as compared to the control.

fungi colonized root lengths were determined at harvest. The observations revealed that in treated seedlings (with AM fungi, *B. polymixa* and *A. brasilense*) growth was positively influenced under both soil types. Sachin[12] reported the positive effects of *Azotobacter chroococcum* on the growth of bamboo (*Bambusa bambos*) and maize (*Zea mays*).

In conformity with previous efforts of PGPR application on the growth of bamboo, the present study suggests that the use of bacterial consortium not only improves growth, proliferation rate and health of the planting material, but is also an eco-friendly and cost-effective approach. In addition to its use in the nursery, this PGPR consortium can also be provided

to the bamboo growers and Forest Departments for application in field plantations. Thus this biofertilizer use can be upscaled for quality planting material production of important bamboo species in NE India.

1. Bakshi, M., Sustainable production of bamboo for rural and tribal communities via farmer-friendly technology, 173–180. In Multipurpose forestry. In Proceedings on Managing and Enhancing Ecosystem Services and Production Function of Forests, Woodlands and Trees Outside Forests (eds Negi, S. S. and Dinesh Kumar), FRI, Dehradun, 2010, p. 268.
2. Tewari, D. N., *A Monograph on Bamboo*, International Book Distributors, Dehradun, 1992, p. 498.
3. Hore, D. K., *J. Econ. Taxon. Bot.*, 1998, **22**(1), 173–181.
4. Loushambam, R. S., Singh, N. R., Taloh, A. and Mayanglambam, S., *Indian J. Hill Farm*, 2017, **30**(2), 181–185.
5. Singh, P. K., Devi, S. P., Devi, K. K., Ningombam, D. S. and Athokpam, P., *Not. Sci. Biol*., 2010, **2**(2), 35–40.
6. Muthukumar, T. and Udaiyan, K., *New For.*, 2006, **31**, 469–485.
7. https://iasri.icar.gov.in (accessed on 1 February 2019).
8. www.organixxgro (accessed on 5 August 2019).
9. Giri, K., *J. Soil Sci. Plant. Nutr*., 2019, **19**, 574–579.
10. Hiscox, J. D. and Israelstam, G. F., *Can. J. Bot*., 1979, **57**, 1332–1334.
11. Adesemoye, A. O. and Egamberdieva, D., *Bacteria in Agrobiology: Crop Productivity*, 2013, pp. 45–63.
12. Sachin, D., *Plant Biofront*., 2009, **1**(1), 37–46.

Received 6 August 2019; revised accepted 30 July 2021

KRISHNA GIRI*
SATYAM BORDOLOI
GAURAV MISHRA
R. S. C. JAYARAJ
NAVAJYOTI BORA
RUPJYOTI C. BARUAH
BONDITA BORAH
SAMIRAN KAKOTI

Rain Forest Research Institute,
Jorhat 785 001, India
**For correspondence.*
e-mail: krishna.goswami87@gmail.com