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Macropropagation of banana - Effect of bio-fertilizers and plant hormones

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ABSTRACT

Stimulation of lateral bud development and plantlet production is generally accomplished through decapitation methods in banana. Attempts were made in the present study to enhance the efficacy of decortication in elite cv. Bangladesh Malbhog using additives like bio-fertilizers and plant growth hormones. This trial was carried out with suckers weighing 1.0-1.5 kg and sawdust as substrate. All treatments tested, showed good response in terms of plantlet production and enhanced bud proliferation, growth and better root profiles compared to control. Treatment T_{11} (*Bacillus subtilis* + BAP) produced the maximum number of primary buds (3.77) followed by T_3 (*Trichoderma viride*) and T_6 (AMF + *T. viride*) with 3.50 and 3.47 buds respectively as compared to control (2.03 buds). Secondary bud production was also observed higher in treatment T_{11} with 5.70 buds per sucker followed by treatments T_3 and T_6 with 4.70 and 4.57, respectively. As far as tertiary bud production was compared, T_{11} gave the highest of 7.33 buds followed by T_3 (7.20) and T_6 (6.70) with a least of 3.33 buds in control. Addition of IBA and *Azospirillum* (T_7) were observed to have good response in terms of root formation and enhanced bud regeneration (5.77 tertiary buds). Total number of buds produced was also observed highest in T_{11} (16.80) followed by T_3 (15.40) and T_6 (14.73) suggesting that treatment combinations, *B. subtilis* + BAP and AMF + *T. viride*, were effective for macropropagation of cv. Bangladesh Malbhog.

Key words: Bio-fertilizers, decortication, decapitation, macropropagation.

INTRODUCTION

Natural regeneration in banana is comparatively slow due to hormone-mediated apical dominance exerted by the main plant. Depending on the variety, a plant produces 5-15 side suckers during its life span. Shy suckering is the major constraint in the production of sufficient planting material through conventional approach. Of several propagating units in banana, sword sucker is the best for better crop stand. Micropropagation assures rapid production of healthy, vigorous, and disease-free planting material. However, due to the large capital investments required for tissue culture facility, the plantlets produced are fairly expensive and beyond the reach of resource poor farmers. Thus, tissue culture as a method of generating planting material is not an option for small-scale farmers; hence, there is a need for cheap and simple techniques that increase the sucker multiplication at farm level and warranting minimum technical skill. Macropropagation is one such cost effective technique where repression of apical meristem will stimulate the regeneration of lateral meristem (Uma *et al*., 13). Increased suckering rate can be achieved through complete/ partial decapitation on a field grown plant or detached corm technique (Baiyeri and Aba, 2) and sawdust is the best substrate over others like rice hull, sand etc. with higher water holding capacity (Baiyeri and Aba, 1). In the present study, attempts have been made to enhance the rate of plantlet production through macropropagation by the addition of bio-fertilizers (AMF, *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azospirillum*) and phytohormones (BAP and IBA) to the explant/ substrate.

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MATERIALS AND METHODS

The experiment was conducted at the Crop Improvement Division, National Research Centre for Banana, Trichy, Tamil Nadu. Sword suckers of healthy plants of cv. Bangladesh Malbhog (AAB-Silk) weighing 1.0-1.5 kg were collected from NRCB farm, and washed in running tap water for 15-20 min. The ensheathing leaf bases were removed from the pseudostem and detopped just above the juncture of the corm and aerial shoot.

The remnants of the pseudostem and roots were removed and external layer of the corm was scraped using a sharp knife, to ensure freeness from all nematodes and other root-borne pathogens. The apical meristem was removed to a depth of 2 cm leaving a cavity of 2 cm diameter in the rhizome. The rest of the corm was given 6-8 cross cuts and incised up to 0.25-0.50 cm depending on the sucker size. The corms were washed with 0.3% Bavistin® (to ensure freedom from soil-borne diseases) and air-dried in shade for 3-4 h before planting. The decapitated corms were planted individually in earthen pots (30 cm dia.) filled with sawdust leaving 5 cm from the top. Sawdust was

pre-cooled before starting the experiment facilitating dissipation of heat. Corms were buried 5 cm deep in the substrate and respective treatments were imposed and covered with sawdust up to a height of 2 cm. Commercially available bio-fertilizers and plant growth hormones were used as additives in the present study. Indole butyric acid (IBA) treatment was given by dipping the corm region of decorticated suckers in 0.25% IBA solution for 20 min. prior to planting. The apical meristem was scooped out to a depth of 2 cm near the crown region. Then the corms were given 4-6 transverse incisions to a depth of 2 mm (Fig. 1) and 4 ml of 40 ppm BAP was poured in to the cavity left by the removal of the apical meristem. The same treatment was imposed during the primary and secondary decapitation stages. The suckers were fully covered by sawdust to prevent exposure to direct sunlight.

Five different bio-fertilizers were used alone or in combinations to improve the bud proliferation rate at primary and secondary decapitation stages. Thirty gram of each of the bio-fertilizers was mixed with the substrate before planting. Decorticated suckers without additives served as control. After primary decortication, the emerging shoots were allowed to grow for 25-30 days and when they attain three leaf stage (height 15-20 cm, stem girth 2.5 cm), the secondary decapitation was imposed. The aerial portion of the plantlet was decapitated, juvenile meristem was removed and 4-6 horizontal incisions were given for the young rhizome and covered with sawdust. The same procedure was repeated for secondary and tertiary decapitations. At the end of tertiary bud stage, the corm was removed from the substrate and washed carefully. Each plantlet was

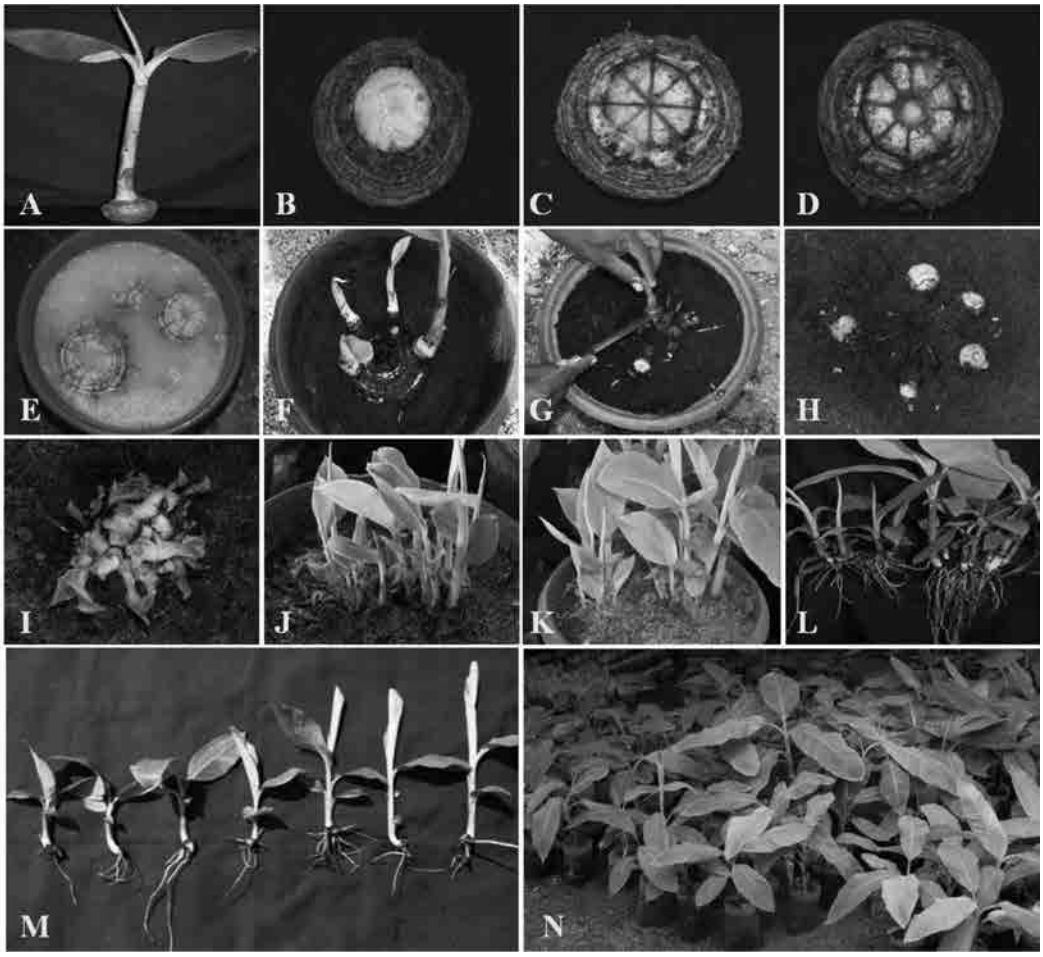

Fig. 1. Stages of macropropagation of banana cv. Bangladesh Malbhog.

A. Sword sucker
B. Decapitated sucker
C. Decapitated sucker with cross cuts
D. Decorticated sucker
E. Bavistin treatment to suckers
F. Primary buds
G. Decapitation of primary buds
H. Decapitated primary buds
I. Secondary buds
J&K. Tertiary buds
L&M. Tertiary buds with roots
N. Hardened plantlets

separated so as to retain at least 2-3 ramified roots and was treated with IBA (0.25%) before hardening. The separated plantlets were hardened in mixture of red soil, sand and farmyard manure (1:1:1) filled in polybags with drainage holes. Plantlets were watered sufficiently and maintained under shade for 45 days. The treatment details are given in the Table 1.

Time taken for bud (primary buds-G1) regeneration from the decorticated suckers was recorded. Total number of primary (G1), secondary (G2) and tertiary (G3) buds formed at the end of 3rd month was recorded. Other parameters measured were plant height, pseudostem girth and number of leaves at hardening stage. Plant height was measured from the base of the stem to the angle made between the youngest and first open leaf. Girth of pseudostem was measured at 1-2 cm above the collar region of the plantlets.

The experiment was laid out in a Completely Randomized Design with eleven treatments and ten replications. The experiment was repeated thrice. The data was subjected to statistical analysis using SPSS (Statistical Package for Social Studies) and the means were compared using DMRT (Duncan's multiple range test) at 5% confidence level.

Table 1. Treatment combinations used in macropropagation of banana.

Symbol	Treatment
T1	Sawdust
T2	AMF
T3	*Trichoderma viride*
T4	BAP
T5	*Azospirillum*
T6	AMF + *T. viride*
T7	IBA + *Azospirillum*
T8	AMF + BAP + *Pseudomonas fluorescens*
T9	*Bacillus subtilis*
T10	*B. subtilis* + *P. fluorescens*
T11	BAP + *B. subtilis*
T12	AMF + BAP + *B. subtilis*

RESULTS AND DISCUSSION

Application of bio-fertilizers exhibited positive impact on plant growth either directly or indirectly. The beneficial effects of microorganisms are usually greater, and the overall results are demonstrated by plant growth promotion and faster germination. In recent years, control of plant pathogens is being achieved through beneficial biocontrol agents. Certain strains of bacteria which belong to Plant Growth Promoting Rhizobacteria (PGPR) act as effective biocontrol agents through induced systemic resistance to fungal, bacterial and viral diseases (Chen *et al*., 4). They suppress the pathogens by various mechanisms namely competition for food, root colonization and antibiosis by producing antibiotics.

All the treatments were found to be statistically significant (Table 2). The number of primary buds (G1-1st generation buds) obtained ranged from 2.03 (T_1-control) to 3.77 (T_{11}) with an average of 3.05 buds/explant. Almost all the treatments except T_4, T_5 and T_7

Table 2. Effect of different treatments on the number of buds at primary, secondary and tertiary stages of decortication of cv. Bangladesh Malbhog.

Treatment	Primary buds (mean)	Secondary buds (mean)	Tertiary buds (mean)
T1 (Saw dust)	2.03 ± 0.88 c	1.23 ± 0.67 c	3.33 ± 0.61 d
T2 (AMF)	3.37 ± 0.88 ab	3.73 ± 0.39 b	5.53 ± 0.57 bc
T3 (*Trichoderma viride*)	3.50 ± 0.34 a	4.70 ± 0.25 ab	7.20 ± 0.35 a
T4 (BAP)	2.97 ± 0.26 ab	3.27 ± 0.39 b	4.50 ± 0.11 cd
T5 (*Azospirillum*)	2.57 ± 0.61 bc	3.80 ± 0.30 b	4.70 ± 0.49 cd
T6 (AMF + *T. viride*)	3.47 ± 0.16 a	4.57 ± 0.18 ab	6.70 ± 0.15 ab
T7 (IBA + *Azospirillum*)	2.57 ± 0.26 bc	3.47 ± 0.26 b	5.77 ± 0.78 abc
T8 (AMF + BAP + *P. fluorescens*)	3.13 ± 0.08 ab	4.07 ± 0.38 b	4.40 ± 0.11 cd
T9 (*Bacillus subtilis*)	3.23 ± 0.03 ab	3.90 ± 0.90 b	5.23 ± 0.74 bc
T10 (*B. subtilis*) + *P. fluorescens*)	3.03 ± 0.12 ab	4.40 ± 0.43 ab	5.27 ± 0.08 bc
T11 (BAP + *B. subtilis*)	3.77 ± 0.37 a	5.70 ± 0.49 a	7.33 ± 0.78 a
T12 (AMF + BAP + *B. subtilis*)	3.00 ± 0.57 ab	4.23 ± 0.32 ab	4.97 ± 0.29 c

*Data pooled across 3 replicated trials

Means within the same column followed by different letters are significantly different (P ≤ 0.05) using DMRT

produced more than 3 primary buds including control. Treatments (T_2, T_3, T_6, and T_{11}) showed good response on primary bud formation per explant suggesting that bio-fertilizers, AMF, *Trichoderma, B. subtilis* and BAP promoted better auxillary bud regeneration. The role of BAP as a shoot promoting hormone is well known and its activity has been reported by Renu and Rashid (11). Association of *B. subtilis* with variety of plants and involvement in promoting plant growth (Cazorla *et al*., 3) by making nutrients more readily available to plants (Nagorska *et al*., 9).

Formation of secondary buds was enhanced in treatments T_{11} (5.70), T_3 (4.70), and T_6 (4.57) suggesting that *B.subtilis* in combination with BAP and AMF alone or in combination with *T. viride* increased the regeneration efficiency of secondary bud in cv. Bangladesh Malbhog. Similar results are also reported in wild bananas *Musa laterita* (Dayarani *et al*., 5). *Bacillus subtilis* in combination with *P. fluorescens* also triggered secondary bud formation producing 4.40 buds per sucker. Co-inoculation of *B. subtilis* and *P. fluorescens* have been reported to stimulate plant growth by virtue of rapid colonization in the rhizosphere and better nutrient uptake (Marcia *et al*., 8).

Formation of tertiary bud was also greatly promoted by the treatments T_{11} (7.33), T_3 (7.20), T_6 (6.70) and T_7 (5.77). Results indicated that AMF alone or in combination with *T. viride*, *Azospirillum* in combination with IBA and *B. subtilis* in combination with BAP were the suitable treatments for better bud regeneration in primary, secondary and tertiary decortication stages. It was also noted that tertiary buds derived from the treatment combination of IBA and *Azospirillum* showed better root system and survived better during the acclimatization stage. These results are in line with the earlier reports indicating that mycorrhizal symbiosis significantly improved banana nutrition even under low fertile soil conditions as the mycorrhizal hyphae are more efficient than roots alone in nutrient uptake and ability to change the root architecture. It is also reported that *Azospirillum* inoculation significantly increased the root growth. *Azospirillum* was also shown to increase the lateral roots and the diameter of turmeric seedlings and stimulated root elongation without affecting the root density.

The total number of buds formed during three months (primary, secondary and tertiary stages) ranged from 6.59 (T_1) to 16.80 (T_{11}). Treatment T_{11} produced the maximum number of buds (16.80) followed by T_3, T_6 and T_{10} with 15.40, 14.73 and 12.70 buds, respectively with an average production of 12.39 buds per sucker (Fig. 2 & 3). Similar results have been reported by Manzur and Macias (7). Under *in situ* conditions where BAP treated sucker produced an average of 4 buds at both G1s and G2s stages and the same technique applied to G3s produced an average of 13 plantlets. Effect of different bio-fertilizers on the time taken for bud initiation was also statistically significant. In the first trial, AMF and *T. viride* (T_6) combination recorded the earliest bud regeneration in a short time span of 28.3 days, followed by BAP + *B. subtilis* (T_{11}) in 29.70 days and AMF (T_2) in 30 days. Same trend was observed for rest of the treatments for the time taken for bud initiation.

Sawdust was used as substrate for all the treatments because of its better water holding capacity as observed by Baiyeri and Aba (2). The higher water holding capacity of sawdust could be attributed to its better water retention ability. The physical composition of the growing medium is reported to have a profound effect on the supply of water and air to the growing plant as well as known to affect anchorage, nutrient and water holding capacity of the medium (Baiyeri and Aba, 2). Macropropagation along with bio-fertilizer treatments showed varying effects on the growth parameters observed during the hardening stage. Treatment with AMF alone and BAP + *B. subtilis* had almost similar effect on plant height. More than 90% of the plantlets regenerated from different treatments had good roots in terms of number and ramification and survived well while hardening. This is in agreement with the findings of Baiyeri and Aba (1) who reported that proportion of plantlets with roots was higher in sawdust irrespective of the genotype. Price (10) reported that the root system is the link between the plant and the soil which is responsible for the absorption of water and nutrients, anchorage, synthesis of some plant hormones and storage. This could be the reason for the better survival of plantlets with good proportion of roots irrespective of weaning media or genotypes. This gives an implication that plantlets with sufficient roots at transplanting survive better during acclimatization and hence hormonal treatments, which enhance rooting are adopted during macropropagation (Baiyeri and Aba, 1). More than 90% of the plantlets survived in hardening medium consisting of red soil: sand: farm yard manure (1:1:1). The stem girth also had an important role in the survival rate at hardening stage. Plantlets with girth ranging from 2.5 to 3.5 cm were found suitable for hardening. The girth of plants at primary and secondary decapitation stage also had a significant role in the production of next generation buds. The plant girth at hardening stage ranged from 2.4 cm in T4 (BAP alone) to 2.78 cm in T_6 (AMF + *T. viride*) followed by 2.73 cm in T_{11} (BAP + *B. subtilis*) and 2.66 in T_9 (*B. subtilis* alone) and showed good survival at acclimatization stage.

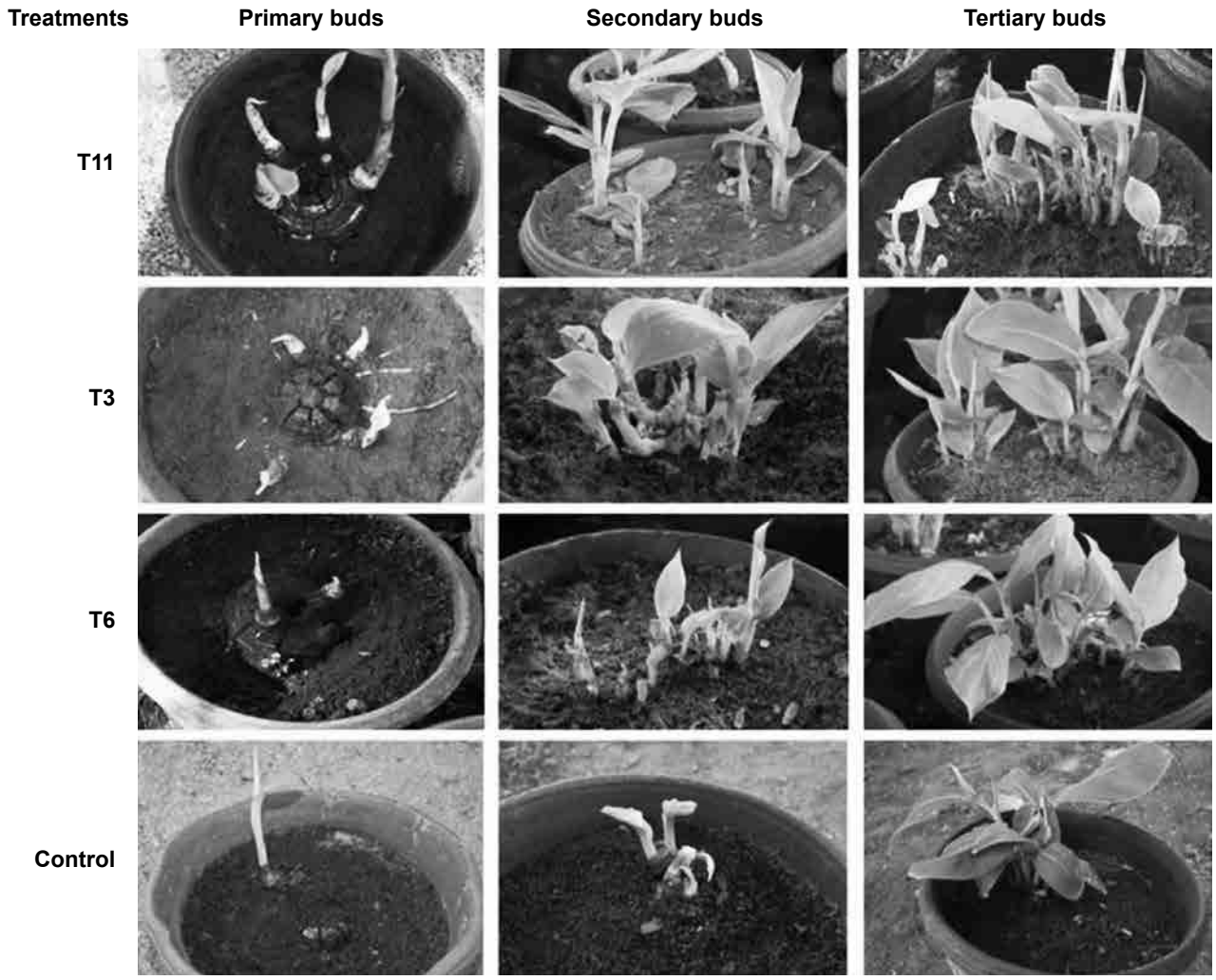

Fig 2. Effect of different bio-fertilizers and plant growth hormones on shoot proliferation of cv. Bangladesh Malbhog.

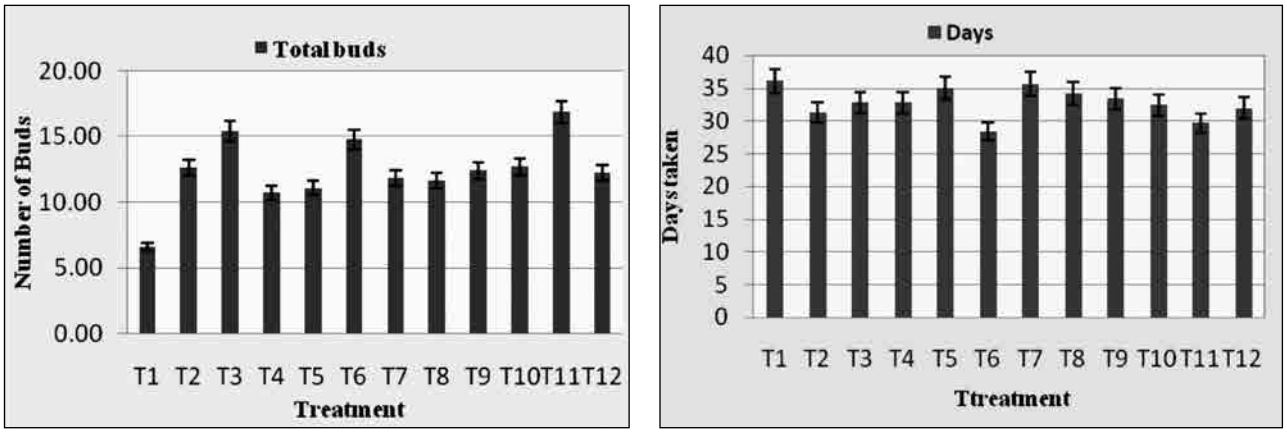

Fig. 3. a. Total number of buds formed in primary, secondary and tertiary stages of decortications. b. Average number of days taken for initiation of primary buds in different treatments.
*Data pooled across 3 replicated trials

Table 3. Economics of macropropagation and cost comparison among the best treatments.

Input	Cost for 100 suckers (Rs.)	Cost for one sucker in best treatments (Rs.)		
		T11	T3	T6
Suckers @ Rs. 4/ sucker	400	4	4	4
Sawdust @ Rs. 4/ kg	280	2.8	2.8	2.8
BAP (growth hormone) @ Rs 0.90/ mg	14	0.14	-	-
AMF @ Rs. 100/ kg	300	-	-	3.0
Bacillus subtilis @ Rs 200/ kg	600	6.0	-	-
Trichoderma viride @ Rs 120/ kg	360	-	3.6	3.6
Hardening media @ Rs 1/ plant	100	1.0	1.0	1.0
Av. No. of plantlets produced		16.80	15.40	14.73
Cost for single plantlet (Rs.)		0.83	0.74	0.97

Note: 40 mg of BAP was used to prepare 1 litre of solution. For hundred suckers, 400 ml of BAP was requires for treatment and cost of BAP is around Rs. 0.9/ mg. 16 mg of BAP was used to prepare 400 ml of solution costing Rs. 14. So, for each sucker the cost of BAP works out to be Rs. 0.14, 30 g of commercially available AMF was used for treatment of one sucker and costs around Rs. 100/ kg and Rs. 3.0 per sucker; 30 g of *Bacillus subtilis* cost around Rs. 200/ kg and works out to be Rs. 6.0 for treating each sucker; *Trichoderma viride* costs around Rs. 120/ kg and the cost for treatment per sucker is Rs. 3.60.

Compared to micropropagated plantlets, macropropagation derived plantlets are more adaptable to the field conditions because they are photosynthetically active as they are regenerated under *in vivo* conditions, while tissue cultured plants are partially photosynthetic and hence are very delicate and do not establish easily under field conditions. This is in conformity with the results of Tenkouano *et al.* (12) who reported that plantlets obtained through detached corm technique are less prone to post establishment stress and field loss.

Treatment combination of *T. viride* (T_3) produced plantlets with a minimum cost of Rs. 0.74 per plantlet followed by BAP + *B. subtilis* (T_{11}) and AMF + *T. viride* (T_6) with Rs. 0.83 and 0.97, respectively (Table 3). The earthen pots were excluded from the total cost as they were reusable and considered as fixed cost. Plantlet production through macropropagation was found economical with the maximum rate being Rs. 0.97 and can be adopted by small and marginal farmers with little access to tissue culture plants due to higher plantlet cost. Higher production cost generally limits the commercial use of tissue culture bananas (Ikram-ul-haq and Dahot, 6) and farmers depend on conventional suckers in spite of increased risk of spread of pests and diseases. These problems could be overcome in macropropagation method by maintaining a disease-free mother block as the source of healthy and high yielding planting materials at a cheaper cost.

Macropropagation offers the cheap alternative with tremendous potential for the production of quality planting material in banana. It is concluded that incorporation of additives like bio-fertilizers and growth hormones to the sawdust substrate has not only enhanced the regeneration of primary, secondary and tertiary buds but also promoted the growth and development, plantlets thereby reducing the post transplanting shock and enhancing the per cent survival in the field. The macropropagation technique optimized in the present study is user-friendly, which requires minimum skill and expertise and suitable for adoption by farmers at the farm level. Further, the cost of per plant production is less than Rs. 1.50 in all the treatments tested, making it a cost effective technique accessible to the small and marginal farmers without compromising on quality.

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