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Evaluating the Efficacy of Macropropagation Techniques for Red Banana (*Musa acuminata*) Using Biocontrol Agents

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The corm propagation is an simple, alternative and cheap technique for Banana multiplication. The study on the effect of biocontrol agents on macropropagation of Banana cv.Red Banana was carried out at SRM College of Agricultural Sciences, Chengalpattu. The decapitated and decorticated corms were exposed to various treatments with combination of sawdust and cocopeat media supplemented with different biofertilizers and biocontrol agents comprising 13 treatments in 3

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replication adopting Completely Randomized Design and the data recorded underwent analysis of variance (ANOVA). Among the 13 treatments adopted, Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60g/corm) showed significantly best result in terms of number of days taken for primary bud emergence (22.7 days), days taken for secondary bud emergence (46.34 days), days taken for tertiary bud emergence (67.35 days), number of primary buds per corm (1.53), number of secondary buds per corm (3.67), number of tertiary buds per corm (4.33), total number of plantlets per corm (9.54), plant height (73.56 cm) and pseudostem girth (12.34 cm). The next better performance was observed in the treatment Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm) which recorded early emergence of buds and production of more buds. The cormpropagation approach refined in this work is accessible, necessitating less skill and knowledge, making it appropriate for implementation by farmers at the farm level.

Keywords *Corm propagation; suckers; red banana; growing media; VAM; Bacillus subtilis; Pochonia chlamydosporia.*

1. INTRODUCTION

Red Banana (AAA) is a choice dessert cultivar of Tamil Nadu, Kerala, Karnataka and Andhra Pradesh. Its commercial cultivation is prominent in Kanyakumari and Tirunelveli districts of Tamil Nadu. Red Banana plants are traditionally propagated through vegetative means using suckers [1]. However, plants produced through suckers have their own limitations as it leads to disease transmission, low productivity, and poor preservation of original plant genetic material [2] and sucker production is very slow as a single plant will produce only 5 to 15 suckers during its entire life time [3]. Moreover, there is a huge demand for quality planting materials to narrow the gap between demand and supply. In this scenario, micropropagation techniques have been used in many parts of the world to produce healthy, disease-free Banana plants throughout the year that perform better under field conditions [4]. But this cannot be adopted by small traditional farmers as it requires more sophisticated techniques and are more expensive (4-8 times) than traditional suckers. Macro-propagation or corm propagation has been advocated for as an effective alternative method which requires less capital and skills to produce large numbers of better-quality Banana seedlings. In the past many studies were undertaken for macropropagation of banana using different rhizome/corm manipulation techniques and found effective especially for small and rural stakeholders [5,6,7]. Depending on the variety, one corm can yield an average of 10 seedlings, which can be increased by a factor of 3–4 through scarification (*i.e.,* removal of the apical meristem of emerging lateral buds) [8] using this method. Macropropagation is a farmer friendly technology complementing field sucker production. With the aim of utilizing the plant multiplication potential of soilless substrates and biopesticide or bio nematicide potential of VAM, *Bacillus subtilis* and *Pochonia chlamydosporia* in producing a disease free plantlets, the present investigation is experimented to study the effect of growing media and biocontrol agents on corm propagation of Banana cv. Red Banana".

2. MATERIALS AND METHODS

The sword suckers of Red Banana were procured from farmers' field in Theni district of Tamil Nadu. Healthy, disease free sword sucker, weighing 1.0-1.5 kg was used as the planting material. The experiment was laid out in Completely Randomized Design (CRD) with 13 treatments and 3 replications using different growing media (sawdust and cocopeat), biofertilizer (VAM) and biocontrol agents (*Bacillus subtilis, Pochonia chlamydosporia*) [9].

Preparation and planting of corms: Red Banana corms, weighing 1.0-1.5 kg washed in tap water for a duration of 15 to 20 minutes. The leaf bases that were covering the pseudostem were cut off and the top part of the corm together with the above-ground sprout was also removed. In order to eliminate nematodes and other diseases that are transmitted through the roots and soil, the pseudostem and roots were removed, and the outer layer of the corm was scraped off using a sharp knife. The procedure standardised by ICAR- National Research Centre for Banana, Trichy for the preparation of corm is followed. The decapitated and decorticated corms were planted in polybags filled with sawdust or cocopeat or a mixture cocopeat and sawdust (50:50) media supplemented with VAM, *Bacillus stubtilis* and *Pochonia chlamydosporia* in

Chart 1. Treatment details

T_1	Cocopeat + *Bacillus subtilis* (30 g/corm)
T_2	Cocopeat + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_3	Cocopeat + VAM (30 g/corm)
T_4	Cocopeat + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_5	Saw dust + *Bacillus subtilis* (30 g/corm)
T_6	Saw dust + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_7	Saw dust + VAM (30 g/corm)
T_8	Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_9	Cocopeat + Sawdust (1:1) + *Bacillus subtilis* (30 g/corm)
T_{10}	Cocopeat + Sawdust (1:1) + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_{11}	Cocopeat + Sawdust (1:1) + VAM (30 g/corm)
T_{12}	Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)
T_{13}	Control (Sawdust)

required quantity as per the treatment details. The specimens were interred at a depth of 15 cm with treatments administered in accordance with the above prescribed protocol. The planted bags were kept in shadenet (50 %) and watered regularly. The observations were recorded on days taken for primary bud emergence (Days), days taken for secondary bud emergence (Days), days taken for tertiary bud emergence (Days), number of primary buds, number of secondary buds, number of tertiary buds, total number of plantlets per corm, plant height (cm), pseudostem girth (cm).

As the study was conducted entirely in a protected structure (shadenet), the experiments were designed using a Completely Randomised Design (CRD). The data underwent analysis of variance (ANOVA) following the method proposed by Panse and Sukhatme (1967). Critical difference values were computed at a significance level of five percent, specifically when the 'F' test yielded a significant result.

3. RESULTS

Days taken for primary bud emergence (Days): The data pertaining to effect of growing media and biocontrol agent on days taken for primary, secondary and tertiary bud emergence are presented in Table 1.

Significantly the lesser number of days (22.77 days) taken for first bud emergence was observed in corms planted in T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] followed by T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)] which recorded 24.11 days. Among the treatments, maximum number of days for primary bud emergence (28.33 days) was observed in T_{13} (Control).

Days taken for secondary bud emergence (Days): The lesser number of days for emergence of secondary bud (46.34 days) was observed in T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] followed by T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)], which recorded (50.47 days). The control (T_{13}) recorded maximum number of days for secondary bud emergence (66.48 days).

Days taken for tertiary bud emergence (Days): The lesser number of days for emergence of tertiary bud (67.35 days) was observed in T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] followed by T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], which recorded (70.53 days). The control (T_{13}) recorded maximum number of days for tertiary bud emergence (92.46 days).

Number of primary buds per corm: The data pertaining to effect of growing media and biocontrol agent on number of of primary, secondary and tertiary buds per corm are presented in Table 2.

Maximum number of primary buds per corm (1.53) was obtained in the treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] and T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)], followed by T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], T_2 [Cocopeat + *Bacillus subtilis* (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] and T_1 [Cocopeat + *Bacillus subtilis* (30 g/corm)] which

recorded 1.43. The least number of primary buds per corm was recorded (1.00) in T_{13} [Control].

Number of secondary buds per corm: Maximum number of secondary buds per corm (3.67) was obtained in the treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], followed by T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] and T_4 [Cocopeat + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], which recorded 3.33. The least number of secondary buds per corm was recorded (1.67) in T_{13} [Control].

Table 1. Effect of growing media and biocontrol agents on days taken for primary,secondary and tertiary bud emergence in corm propagation of Banana cv. Red Banana (Days).

Treatments	No of days taken for Primary bud emergence	No of days taken for secondary bud emergence	No of days taken for tertiary bud emergence
T_1	25.22	61.63	88.56
T_2	25.55	53.66	79.86
T_3	25.00	59.78	86.32
T_4	25.11	55.46	76.54
T_5	24.77	60.64	81.32
T_6	24.77	62.31	83.47
T_7	24.66	57.34	80.21
T_8	26.22	49.54	70.53
T_9	25.88	58.67	82.78
T_{10}	24.66	55.78	73.12
T_{11}	24.11	50.47	75.56
T_{12}	22.77	46.34	67.35
T_{13}	28.33	66.48	92.46
S.E(d)	**0.77**	**4.54**	**2.36**
S.E(m)	**0.54**	**3.21**	**1.67**
CV %	**3.74**	**9.81**	**3.63**
CD at 5%	**1.58**	**9.35**	**4.86**

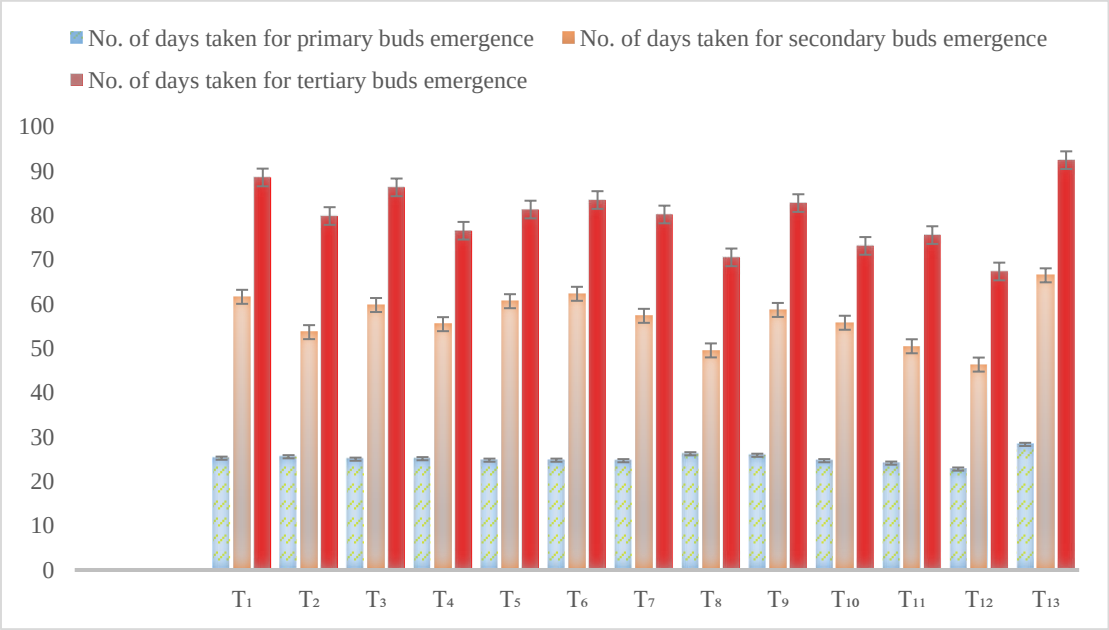

Fig. 1. Effect of growing media and biocontrol agents on days taken for primary, secondary and tertiary bud emergence in corm propagation of Banana cv. Red Banana (Days)

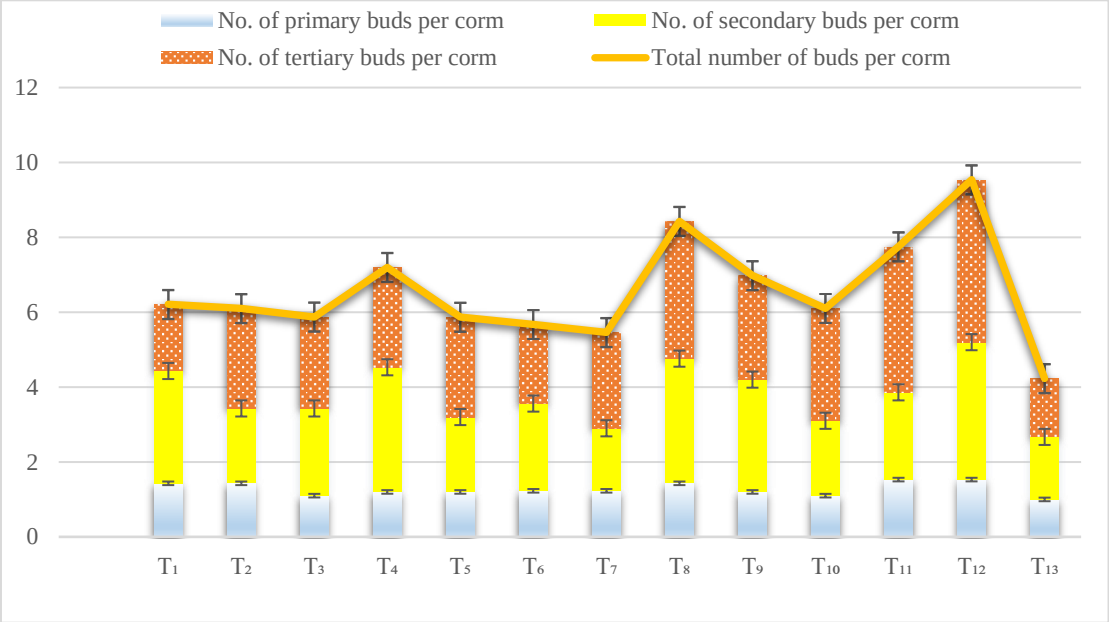

Fig. 2. Effect of growing media and biocontrol agents on number of primary, secondary and tertiary bud developed during corm propagation of Banana cv. Red Banana

Table 2. Effect of growing media and biocontrol agents on number of primary, secondary and tertiary bud developed during corm propagation of Banana cv. Red Banana

Treatments	No. of primary buds per corm	No. of secondary buds per corm	No. of tertiary buds per corm	Total no. of buds per corm
T_1	1.43	3.00	1.77	6.21
T_2	1.43	2.00	2.66	6.10
T_3	1.10	2.33	2.44	5.87
T_4	1.20	3.33	2.66	7.20
T_5	1.20	2.00	2.66	5.87
T_6	1.23	2.33	2.11	5.67
T_7	1.23	1.67	2.55	5.46
T_8	1.43	3.33	3.66	8.43
T_9	1.20	3.00	2.77	6.98
T_{10}	1.10	2.00	3.00	6.10
T_{11}	1.53	2.33	3.88	7.75
T_{12}	1.53	3.67	4.33	9.54
T_{13}	1.00	1.67	1.55	4.23
S.E(d)	**0.10**	**0.19**	**0.40**	**0.50**
S.E(m)	**0.07**	**0.13**	**0.28**	**0.35**
CV %	**9.69**	**9.50**	**17.40**	**9.26**
CD at 5%	**0.20**	**0.40**	**0.81**	**1.02**

Number of tertiary buds per corm: Maximum number of tertiary buds per corm (4.33) was obtained in the treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], followed by T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)], which recorded 3.88. The least number of tertiary buds per corm was recorded (1.55) in T_{13} [Control].

Total number of buds per corm: The maximum number of buds per corm (9.54) was obtained in the treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)], followed by T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] and T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)], which recorded 8.43 and 7.75 respectively. The least

number of buds per corm was recorded (4.23) in T_{13} [Control].

Plant height (cm): The data corresponding to plant height (cm) indicated significant difference among the treatments (Table 3). The treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] recorded maximum plant height (73.56 cm), followed by T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)] and T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] which recorded 70.56 cm and 69.18 cm respectively. The treatment T_{13} [Control] recorded minimum plant height (37.89 cm).

Table 3. Effect of growing media and biocontrol agents on plant height (cm) pseudostem girth (cm) during corm propagation of Banana cv. Red Banana (days)

Treatments	Plant height (cm)	Pseudostem girth (cm)
T_1	57.00	7.16
T_2	59.65	6.42
T_3	43.39	7.31
T_4	63.17	8.34
T_5	45.48	6.89
T_6	58.07	8.11
T_7	56.33	6.15
T_8	69.18	10.36
T_9	49.65	8.46
T_{10}	67.13	7.45
T_{11}	70.46	11.45
T_{12}	73.56	12.34
T_{13}	37.89	5.59
S.E(d)	**1.35**	**0.18**
S.E(m)	**0.96**	**0.13**
CV %	**2.88**	**2.76**
CD at 5%	**2.79**	**0.37**

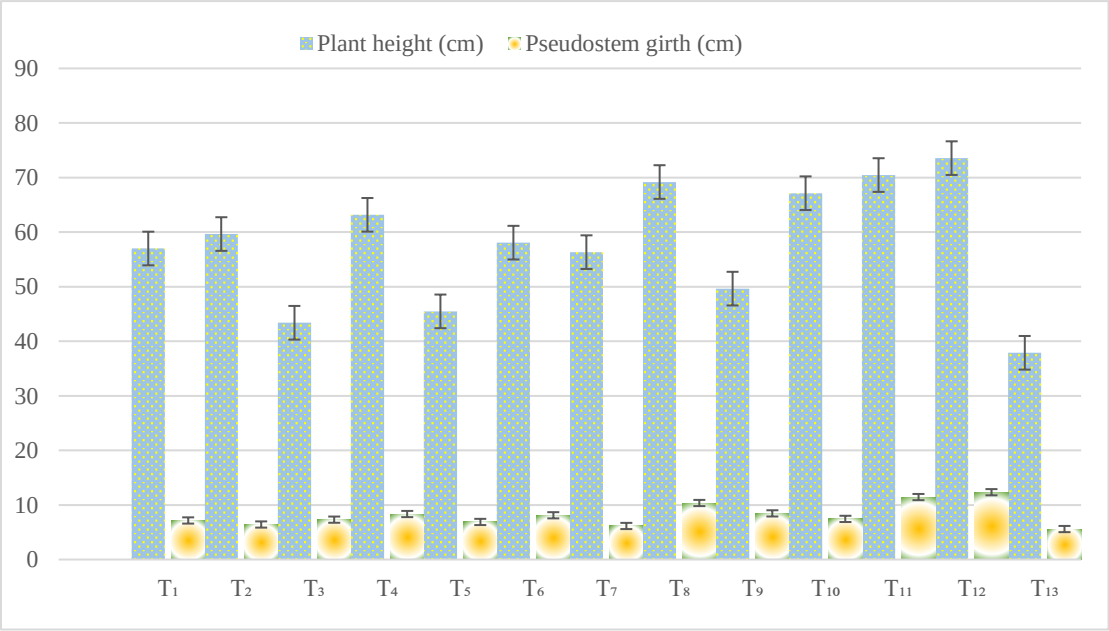

Fig. 3. Effect of growing media and biocontrol agents on plant height (cm) pseudostem girth (cm) during corm propagation of Banana cv. Red Banana (days)

Pseudostem girth (cm): The data corresponding to pseudostem girth (cm) indicated significant difference among the treatments (Table 3). The treatment T_{12} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] recorded maximum pseudostem girth (12.34 cm), followed by T_{11} [Cocopeat + Sawdust (1:1) + VAM (30 g/corm)] and T_8 [Saw dust + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm)] which recorded 11.45 cm and 10.36 cm respectively. The treatment T_{13} [Control] recorded minimum pseudostem girth of 5.59 cm.

4. DISCUSSION

Growing media is a complex mixture of different solid, liquid and gaseous materials [10]. The physical composition of the growth media has a significant impact on supply of water and air for successful plant growth [11] as well as it improves anchorage, nutrient and water holding capacity of the medium [12]. The beneficial microorganisms like antagonistic bacteria (e.g., *Bacillus subtilis*) and fungi (AMF) compete with plant pathogens for nutrients and space, by producing antibiotics, by parasitizing pathogens, or by inducing resistance in the host plants, these microbes have been used for biocontrol of pathogens [13].

In the present experiment, the time taken for primary, secondary and tertiary bud initiation as influenced by different treatments were recorded and revealed that there were significant differences in days required to bud initiation in suckers.

The growing media containing cocopeat and sawdust in equal proportion supplemented with VAM (30 g/corm) and *Pochonia chlamydosporia* (60 g/corm) has shown earliest emergence of primary (22.77 days), secondary (46.34 days) and tertiary buds (67.35 days) (Fig. 1). The earliness may be due to the inherent starch reserve of the mother corm [14]. This may also be due to the use of soilless media for propagation as it reduces incidence of soil borne diseases and pests which leads to a reduction in use of soil fumigant, it improves water use efficiency and fertilizer use due to its high water-holding and cation exchange capacity [15,16]. This findings are in line with Oselebe et al*.* [17] who stated that soil less media be the fastest means of plantlet generation for Musa species at the farm level. In the present investigation, it is found that bud emerged in all the treatment within a month duration from the date of planting. Similar findings were reported by Sannigrahi et al. [18], where the induction of primary shoots took 19.75 days in Grand Naine and 28.25 days in Bagda variety. Sudeshna et al. [19], Baiyeri and Aba [20], Oselebe et al*.* [21], Mensh et al. [22] and Deepa et al*.* [23] reported sawdust as best initiation media for macropropagation of Banana while Pujar et al., [24], Sangey et al. [25], Thungon et al*.* [26] reported cocopeat as the optimal growing medium for macro propagation of 'Malbhog' Banana.

In the present study, corms planted only in sawdust (Control) took more number of days for primary, secondary and tertiary bud emergence (28.33 days, 66.48 days and 92.46 days respectively). It is because even though sawdust having good water holding capacity, it is poor in nutrients and growth chemicals which might have delayed the emergence of buds and as a consequence it took more number of days for bud emergence [27]. Similar results were also reported by Baiyeri and Aba (2005) who reported 40.5 days for emergence of buds in sawdust. The contrast report was given by Manju and Pushpalatha,2023 which states that, plantain variety nendran took the shortest time for primary (20.33 days) and secondary bud regeneration (14.33 days) with sawdust as media.

Number of buds per corm: Growing media are considered major factors in controlling the physiological pattern as well as the morphological traits of many plants. In the present investigation, all the treatments showed significant difference for number of primary, secondary and tertiary buds per corm and total number of plantlets/corm due to the use different growing media supplemented with biocontrol agents. Among all the treatments, T_{12} - Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm) has produced more number of primary (1.53), secondary (3.67) and tertiary buds per corm (4.33). The same treatment recorded maximum total number of buds /corm (9.54) (Fig. 2). This study proves that, when the apical dominance was arrested, it led to the development of the miniature buds immediately as sprouts and then as quality suckers. This is evident in our studies as it is irrespective of treatments all the physical activation technique like decapitaiton and decortication leads to the development of suckers. The earliness in bud emergence and number of Plantlet production from corms under macro-propagation mainly depends on the

corm's reserves, as the continuous harvest of plantlets prevents influx of additional photosynthesis products [28].

The regeneration of more number of buds may also be due to the presence of beneficial microorganisms and essential nutrients in growing substrate which are easily available for plant growth thus helps in producing more number of plantlets per corm. The number of plants produced per corm was found to be high in all the treatments enriched with VAM. This is due to their mutualistic association with most of the vascular plants and for helping in the absorption and assimilation of elements that are less soluble and non available to the plants, i.e. P, Zn, Cu, etc., from the rhizosphere, thereby increasing the growth and productivity of the plants [29].

Similar results were also obtained by Rajera and Sharma [30] in LA lily and Moghadam et al*.* [31] in Asiatic lily hybrid. Kiran [32] reported that macropropagtion of Red Banana in Saw dust + Cocopeat + potting media produced 9.80 plantlets per corm. This report corroborates the findings of our study. Sajith et al. [33] reported maximum number of primary buds with treatment of *Bacillus subtilis*, VAM and BAP. Similar results were also reported in Banana by Singh et al. [34]. In the present study, highest number of buds from the corms in the *B. subtilis* treated media may be attributed to the enhanced callus formation ability of the synthetic cytokinin BAP in addition with the IAA produced by *B. subtilis* [35,36,37]. This finding is in the line of report by Baruah et al*.* [38] in Banana.

Plant morphological characters in growing /initiation media: The morphological characters *viz.,* plant height (73.56 cm) and pseudostem girth (12.34 cm) was maximum in T_{12}, Cocopeat + Sawdust (1:1) + VAM (30 g/corm) + *Pochonia chlamydosporia* (60 g/corm) (Fig. 3). In Banana, the pseudostem is made up of leaf sheaths which is most pronounced at collar and this reflect on pseudostem girth, number of leaves as well the plant vigour [39]. The treatments with VAM showed significant difference in morphological characteristics as the AMF fungi infect and spread inside the root system. They possess special structures known as vesicles and arbuscules. The arbuscules help in the transfer of nutrients from soil to the root system, and the vesicles, which are sac like structures, store P as phospholipids.

AM fungi colonize the root cortex of plants and develop an extrametrical hyphal network that can absorb nutrients from the soil. Enhanced plant growth due to arbuscular mycorhizae (AM) association was well documented by Bagyaraj [40]. In addition, he reported that improved plant growth is attributed to increased nutrient uptake, especially of phosphorus, tolerance to water stress, root pathogens and adverse soil environments and production of growth-promoting substances. The association with the host plant increases the uptake of water and most essential mineral nutrients for their host plant, such as phosphate and nitrogen [41]. But probably also micro-elements such as zinc and in return, AM fungi receives photosynthetic carbon from their host [42].

The combined application of *Pochonia chlamydosporia* with VAM and in the growing media showed significant growth parameter at hardening stage in the present study. This is because of the root endophytic behaviour of *Pochonia chlamydosporia* which improves the growth of a range of host plant species and sustaining their defense reaction to different pathogens [43,44]. Its growth promoting benefits in monocot and dicot crops are reported in barley, wheat, lettuce, pistachio and tomato [45,46,47,48,49,50].

In the present study, the growing media enriched with *Bacillus subtilis* also showed improved plant height and pseudostem girth. This may be due to the improved nutrient uptake, root growth, and the proliferation of plants by *Bacillus subtilis*. It also stimulates seed germination and supports the general health and vigor of the plant. *B. subtilis* has been regarded as biofertilizers, phytostimulators, and biopesticides [51,52]. Association of *B. subtilis* with variety of plants and involvement in promoting plant growth [53] by making nutrients more readily available to plants [42]. This is in accordance with the findings of Baiyeri and Aba [54], Uma et al. [55] and Sajith et al*.* [45] during the macropropagation of Banana.

5. CONCLUSION

In conclusion, the findings of this study indicate that a growing medium composed of Cocopeat and Sawdust in a 1:1 ratio is effective for the corm propagation of Red Banana, yielding a greater number of suckers from a single corm due to its high water retention capacity and porosity. The augmentation of growing media

with biocontrol agents such as *Bacillus subtilis*, VAM, and *Pochonia chlamydosporia* has significantly improved the regeneration of primary, secondary, and tertiary buds in a brief period, while also fostering the growth and development of plantlets, thereby mitigating post-transplant shock and yielding disease-free planting material. The corm propagation approach refined in this work is accessible, necessitating less skill and knowledge, making it appropriate for implementation by farmers at the farm level. This approach offers a method to enhance banana production by boosting the availability of seedlings to small-scale growers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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