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Study of growth hormones and biofertilizers on macropropagation in banana cv. Poovan

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Abstract
The present investigation entitled "Study of growth hormones and biofertilizers on macropropagation in banana cv. Poovan" was carried out during 2019-2020 at the Department of Fruit Science, Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkote, Karnataka. The experiment was laid out in Completely Randomised Design (CRD) with eleven treatments and three replications. Among different growth hormones and biofertilizers used, the corms treated with BAP took less number of days for first bud emergence (25.20 days), secondary decapitation of first bud emerged (51.46 days) whereas, treatment combination of VAM + BAP + P. *fluorescens* + T. *harzianum* (T_{10}) recorded maximum number of primary buds per corm (3.73), total number of plantlets per corm (15.40), shoot girth (23.94 mm), fresh weight of shoot (74.96 g), dry weight of shoot (6.74 g), number of leaves (4.43) and leaf area (470.94 cm^2 /plant).

Keywords: Macropropagation, Poovan, decapitation, growth hormones, biofertilizers

Introduction
Banana (*Musa* spp.) is one of the most important fruit crops of the tropical and sub-tropical regions of the world as it provides food security, nutrition and income for many small farmers (INIBAP 1997) [11]. It is one of the cheapest fruits and commonly known as "Adam´s fig", "Apple of paradise", "Tree of paradise" and "Kalpatharu" (Kumar *et al.*, 2012) [17]. They are the fourth important global food commodity after rice, wheat and milk in terms of gross value of production (INIBAP, 1997) [11]. It is the staple food of Uganda, Bukoba and Tanzania. Year-round availability, affordability, taste, varietal wealth, medicinal and nutritive value makes it one of the favorite fruits among different classes of people across the globe (Pujar *et al.*, 2017) [26].

Different banana varieties are grown in different parts of India as well as in Karnataka. Banana cv. Poovan (AAB) is grown throughout the country with location specific ecotypes and Tamil Nadu is the leading producer of this cultivar. A common limiting aspect for large scale expansion of banana plantations is availability of healthy planting materials. This is due to poor suckering ability (Tenzens du Moncel, 1985) [34] and slow natural regeneration in banana due to the hormone induced apical dominance exerted by the main stem (Esakkimuthu and Shakila 2017) [7] and naturally produced suckers are more likely to carry pest and diseases.

Rapid production of propagating material could be achieved through vegetative method like *in-vitro* micropropagation. But this cannot be adopted by small traditional farmers as it requires more sophisticated techniques and also expensive for them. Therefore, macropropagation is the technique which is relatively easier and require minimum investment to set up and is an excellent technique for producing low cost and quality planting material and the plantlets obtained have the uniformity like tissue culture plantlets (Baiyeri and Aba, 2007) [3]. Keeping all these factors in consideration the present experiment was undertaken to know the effect of different growth hormones and biofertilizers on plantlets regeneration through macropropagation in banana cv. Poovan.

Material and Methods
The experiment was carried out at the Department of Fruit Science, Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkote, Karnataka, India. The planting material of Poovan cultivar were collected from farmers field in Gangavathi, Koppal district of Karnataka, India. The procedure consisted of collecting Healthy

and disease-free sword sucker from mother plant which were pared to detach the older roots and superficial layers; those suckers were cut transversely 2 cm above the collar region, then apical meristem was removed leaving a cavity of 2 cm diameter and 3 cm depth and then corm was given criss-cross incisions to a depth of 1-1.5 cm which will be ending down to the rhizome collar. Later these corms were surface sterilized by dipping in 1 per cent Carbendazim (Bavistin) solution for about 5 minutes to eradicate surface pathogens and shade dried for 20 minutes. These corms were imposed with treatments and were planted in the polybag (50X45 cm) filled with standardized media (from earlier studies) *i.e.,* Potting media [Soil + FYM (2: 1)].

As the complete studies was done in the shade net under normal temperature and light conditions, Completely Randomised Design (CRD) was applied for the experiments. The data recorded was subjected to analysis of variance (ANOVA) as suggested by Panse and Sukhatme (1967) [24]. Critical difference values were calculated at five per cent probability where 'F' test was significant.

The experimental material comprises of eleven treatments, three replications and five plants per replication. The treatment details are given below.

T_1 - VAM (10g / sucker)
T_2 - *Trichoderma harzianum* (30g / sucker)
T_3 - *Psuedomonas fluorescens* (30g / sucker)
T_4 - *Azospirulum brasiliense* (1kg in 50 litres of water)
T_5 - BAP (40 ppm)
T_6 - IBA (Dipping in 0.25% solution)
T_7 - VAM (10g / sucker) + *Trichoderma harzianum* (30g / sucker)
T_8 - IBA (Dipping in 0.25% solution) + *Azospirillum brasiliense* (1kg in 50 litres of water)
T_9 - BAP (40ppm) + *Psuedomonas fluorescens* (30g / sucker)
T_{10} - VAM (10g / sucker) + BAP (40ppm) + *Psuedomonas fluorescens* (30g / sucker) + *Trichoderma harzianum* (30g / sucker)
T_{11} - control (Untreated suckers)

Results and Discussion

Number of days taken for first bud emergence

The data on the effect of growth hormones and biofertilizers on number of days taken for first bud emergence has showed significant differences among the treatments (Table 1) and significantly the lesser number of days taken for first bud emergence (25.20 days) was observed in corms which were planted in Potting media treated with BAP (T_5), followed by in VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (26.73 days) (T_{10}). While, the maximum number of days taken for first bud emergence was observed in control *i.e.* untreated sucker (34.93 days) (T_{11}). This could be due to application of BAP (Adenine based Cytokinin) which reduce the apical dominance and enhance rapid proliferation of auxiliary and adventitious buds in banana (Osei *et al.,* 2005; Kalimutha *et al.,* 2009; Shagufta *et al.,* 2011; Thiemele *et al.,* 2015) [30, 35].

Number of primary buds per corm

The data with corresponding to the effect of growth hormones and biofertilizers on number of primary buds per corm has showed significant differences among the treatments and significantly the maximum number of primary buds (3.73) was recorded in corms which were planted in potting media treated with BAP (T_5) followed by VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (3.53) (T_{10}). While, the minimum number of primary buds were recorded in control *i.e.* untreated sucker (2.33) (T_{11}). The results of present study are close conformity to Macias (2001) [18] whose study reported that application of BAP in FHIA-20 banana enhanced the regeneration of lateral buds under field decapitation technique. Gilmar *et al.,* (2000) [8] and Khalid *et al.,* (2011) [14] recorded maximum shoot development in BAP treated plants. Swennen (1990) [32] obtained similar results in Plantain.

Number of days taken for secondary decapitation of first bud emerged

The data with respect to the effect of growth hormones and biofertilizers on number of days taken for secondary decapitation of first bud emerged (Table 1) indicated that there is a significant difference among the treatments. The minimum number of days (51.46 days) were taken in corms which were planted in potting media treated with BAP (T_5) and is on par with VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (52.46 days) (T_{10}). While, the maximum number of days were recorded in control (untreated sucker) (T_{11}) (66.66 days). This could be due to application of BAP (Adenine based Cytokinin) which reduce the apical dominance and enhance rapid proliferation of auxiliary and adventitious buds in banana (Osei *et al.,* 2005; Shagufta *et al.,* 2011; Thiemele *et al.,* 2015) [30, 35]. Ravani and Patel (2013) [27] observed the effect of BAP on early shoot initiation in banana cv. Grand Naine and Kelta *et al.,* (2018) [13] observed similar results in Poyo and Giant Cavendish banana.

Total number of plantlets per corm

The data pertaining to the effect of growth hormones and biofertilizers on total number of plantlets per corm (Table 1) showed that there is a significant difference among the treatments due to use of different growth hormones, biofertilizers and their combinations. Significantly the maximum number of plantlets (15.40) were recorded in VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) which is statistically on par with BAP (T_5) (14.46). While, the minimum number of plantlets per corm (9.46) were recorded in untreated (T_{11} - control) followed by treatment of corm with *Azospirillum brasiliense* (T_4) (11.13). This might be due to application of different biofertilizers like VAM + *Pseudomonas fluorescens* + *Trichoderma harzianum* which helps in rapid regeneration of lateral buds (Sajith *et al.,* 2014) [29], along with it BAP acts as a better shoot promoting hormone (Renu and Rashid. 2002) [28]. *Trichoderma sps.* will increase the regeneration efficiency of primary and secondary buds in Bangladesh Malbhog. Also, similar results were obtained by Dayarani *et al.,* (2005) in *Musa laterita*. Application of *Pseudomonas fluorescens* helps in stimulating plant growth through rapid colonization in the rhizosphere which helps in better nutrient uptake (Marcia *et al.,* 2008) [20]. Mycorrhizal symbiosis through application of VAM helps in better nutrient uptake even under less fertile soil condition, as the mycorrhizal hyphae favors better nutrient uptake than root alone (Sajith *et al.,* 2014) [29]. Talengera *et al.,* (1994) [33] and Maerere *et al.,* (2003) [19] also observed enhanced shoot proliferation in Plantain and bananas by application of BAP, respectively. Singh *et al.,* (2011) [31] also observed that BAP application enhances sprouting of auxiliary buds in Cavendish banana.

Plant height (cm)

The data corresponding to plant height indicated significant difference among the treatments (Table 2) due to use of different growth hormones and biofertilizers. Significantly highest plant height (34.75 cm) was recorded in the corms planted in potting media treated with VAM + *Trichoderma harzianum* (T_7) which was statistically on par with IBA (T_6) (33.96 cm) and in VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) (33.62 cm). However, the minimum plant height (30.25 cm) was recorded in untreated sucker (T_{11}). This could be due to mycorrhizal symbiosis which improved nutrition level in banana, as the mycorrhizal hyphae helps in better nutrient uptake than root alone helping in faster growth (Sajith *et al.*, 2014) [29]. Harman *et al.,* (2004) reported that application of *Trichoderma* promotes growth and development, improve crop production and nutrient availability. Similar results were also reported by Priyarani *et al.*, (1999) [25] in *Acacia nilotica* plants.

Shoot girth (mm)

The data pertaining to the effect of growth hormones and biofertilizers on shoot girth of the plantlets at the time of secondary decapitation has shown significant difference among the treatments (Table 2) and significantly maximum shoot girth (23.94 mm) was recorded in the treatment VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) which is statistically on par (23.69 mm) with BAP (T_5). While the lowest shoot girth (18.96 mm) was recorded in corms planted in potting media (T_{11}) without treating any growth hormones and biofertilizers. Inoculation of VAM into growing media enhances microbial and enzymatic activities, increases mobilization and absorption of nutrients (Edwards *et al.,* 2004) [6]. *Trichoderma* application promotes plant growth and development, enhance crop production, nutrient availability (Harman *et al.*, 2004) and thus increases shoot girth. Application of *Pseudomonas* enhances the ability of the plants to produce secondary metabolites, phytohormones and enzymes (Nagarajkumar *et al.,* 2004) [21] which helps in increasing shoot girth. Singh *et al.,* (2011) [31] observed that BAP application improves shoot girth in Cavendish banana.

Fresh weight of shoot (g)

The data on shoot fresh weight indicated significant difference among the treatments (Table 2) due to use of different growth hormones, biofertilizers and their combinations. Significantly maximum shoot fresh weight (74.96 g) were recorded in the corms planted in potting media treated with VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) followed by (71.95 g) in VAM + *Trichoderma harzianum* (T_7). The minimum shoot fresh weight (54.94 g) was recorded in the treatment with *Azospirillum brasiliense* (T_4) followed by untreated corms (T_{11} - control) (57.07 g). Khanizadeh *et al.,* (1995) [15] observed that application of VAM increased shoot fresh in Strawberry. Application of BAP helps in enhancing shoot fresh weight in banana micropropagation (Ngoumuo *et al.*, 2013). *Trichoderma* application promotes plant growth and development, enhance crop production, nutrient availability (Harman *et al.,* 2004) and increases shoot fresh (Priyarani *et al.,* 1999) [25]. Application of *Pseudomonas* enhances the ability of the plants to produce secondary metabolites, phytohormones and enzymes (Nagarajkumar *et al.,* 2004) [21] and thus helps in increasing shoot fresh (Gravel *et al.,* 2007) [9]. This shows that application of different microorganism combination improves shoot girth, fresh and dry weight of shoots.

Dry weight of shoot (g)

The data with respect to the effect of growth hormones and biofertilizers on dry weight of the shoot of plantlets removed after secondary decapitation has shown significant difference among the treatments (Table 2). Shoots raised from the corms planted in potting media treated with VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) has recorded significantly highest dry weight (6.74 g), followed by VAM + *Trichoderma harzianum* (T_7) (6.45 g). However, corms treated with *Azospirillum brasiliense* (T_4) has recorded least dry weight (4.54 g) followed by untreated corms (T_{11} - control) (4.64 g). This is because application of VAM increased shoot fresh and dry weight in Strawberry (Khanizadeh *et al.,* 1995) [15]. BAP helps in enhancing shoot fresh weight in banana micropropagation and thus also improves shoot dry weight (Ngoumuo *et al.,* 2013). *Trichoderma* application promotes plant growth and development, enhance crop production, nutrient availability (Harman *et al.,* 2004) and increases shoot dry weight (Priyarani *et al.,* 1999) [25]. Application of *Pseudomonas* enhances the ability of the plants to produce secondary metabolites, phytohormones and enzymes (Nagarajkumar *et al.,* 2004) [21] and thus helps in increasing shoot fresh and dry weight (Gravel *et al.,* 2007) [9]. This shows that application of different microorganism combination improves shoot girth, fresh and dry weight of shoots.

Number of leaves

The data with respect to effect of growth hormones and biofertilizers on number of leaves at the time of secondary decapitation has shown significant difference among the treatments and results shows (Table 3) that highest number of leaves (4.43) was observed in plantlets grown in potting media treated with VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) which is statistically on par with treatment BAP (T_5) (4.30). However, minimum number of leaves (3.43) was observed in treatment *Azospirillum brasiliense* (T_4). Inoculation of VAM to the growing media has a positive impact on synthesis of various hormones like auxins, gibberellins and cytokinin which leads to increased cell division and multiplication (Damar *et al.,* 2014) [4], also it aids in better nutrient absorption especially Phosphorous and increases photosynthetic rate thus aids in production of more number of leaves (Usha *et al.,* 2004) [36]. Ravani and Patel (2013) [27] reported that application of BAP helps in enhancing leaf number. Similar results were also obtained by Kumar *et al.,* (2020) [16] in rough lemon, Aman *et al.,* (2018) [1] and Babu (2019) [2] in Banana. Inoculation of *Trichoderma* improves plant growth, nutrient uptake and improve crop production (Harman *et al.,* 2004) as well as it acts as an antagonistic against many plant pathogenic fungi and control diseases (Vinale *et al.*, 2008) [27]. Inoculation of *Pseudomonas fluorescens* favors synthesis of many secondary metabolites, phytohormones and enzymes. Thus, combined application of all these growth hormones and bio-agents aids in improving number of leaves.

Leaf area (cm^2/plant)

The data on leaf area has shown significant difference among the treatments (Table 3) due to use of different growth hormones and biofertilizers. Significantly highest leaf area

(470.94 cm^2 /plant) was measured in corms which were planted in potting media treated with VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum* (T_{10}) which is statistically on par with (464.96 cm^2/plant) BAP + *Pseudomonas fluorescens* (T_9). While lowest leaf area (407.94 cm^2/plant) was measured in corms treated with *Azospirillum brasiliense* (T_4). Inoculation of *Trichoderma* improves plant growth, nutrient uptake and improve crop production (Harman *et al.*, 2004) as well as it acts as an antagonistic against many plant pathogenic fungi and control diseases (Vinale *et al.*, 2008) [37]. Inoculation of *Pseudomonas fluorescens* favors synthesis of many secondary metabolites, phytohormones and enzymes. Inoculation of VAM to the growing media has a positive impact on synthesis of various hormones like auxins, gibberellins and cytokinin which leads to increased cell division and multiplication (Damar *et al.*, 2014) [4], also it aids in better nutrient absorption especially Phosphorous and increases photosynthetic rate thus enhances growth (Usha *et al.*, 2004) [36]. Ravani and Patel (2013) [27] reported that application of BAP helps in improving leaf area. Similar results were also obtained by Kumar *et al.*, (2020) [16] in rough lemon, Aman *et al.*, (2018) [1] and Babu (2019) [2] in Banana.

B: C ratio

Study on the cost economics of macropropagation in banana cv. Poovan revealed that among eleven treatments used (Table 4) the corms which were treated with BAP (40 ppm) (T_5) has recorded the highest net return (Rs. 1190) and B: C ratio (2.78) when compared to other treatments. This is because of application of BAP which enhanced the quick proliferation of auxiliary shoots in banana (Shagufta *et al.*, 2011; Thiemele *et al.*, 2015) [30, 35] leading to development of more number of plantlets $corm^{-1}$. While the minimum net returns (Rs. 471) and B: C ratio (0.64) was reported in treatment T_6 – IBA (0.25%), because of lesser number of plantlets production per corm in the treatment as well as more cost of IBA.

Table 1: Influence of growth hormones and biofertilizers on plantlet regeneration through macropropagation in banana cv. Poovan

Treatments	Number of			
	Days for first bud emergence	Primary buds per corm	Days taken for secondary decapitation of first bud emerged	Total number of plantlets per corm
T_1	32.40	2.93	60.20	12.33
T_2	32.06	2.86	62.33	12.06
T_3	30.40	3.13	55.13	13.06
T_4	32.73	2.53	63.80	11.13
T_5	25.20	3.73	51.46	14.46
T_6	32.93	2.53	65.06	11.40
T_7	30.80	3.13	56.93	13.40
T_8	32.20	2.93	57.86	11.93
T_9	29.20	3.33	54.53	13.13
T_{10}	26.73	3.53	52.46	15.40
T_{11}	34.93	2.33	66.66	9.46
S.Em±	0.51	0.07	0.96	0.37
CD at 5%	1.50	0.20	2.82	1.10
CV	2.85 (S)	3.85 (S)	2.82 (S)	5.13 (S)

Treatment

T_1: VAM T_2: *Trichoderma harzianum* T_3: *Pseudomonas fluorescens* T_4: *Azospirillum brasiliense*
T_5: BAP T_6: IBA T_7: VAM + *Trichoderma harzianum* T_8: IBA + *Azospirillum brasiliense*
T_9: BAP + *Pseudomonas fluorescens* T_{10}: VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum*
T_{11}: Control (Untreated suckers)

Table 2: Influence of growth hormones and biofertilizers on shoot parameters of plantlets regenerated through macropropagation in banana cv. Poovan.

Treatments	Girth (mm)	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
T_1	21.45	32.95	64.92	5.45
T_2	21.94	32.94	61.96	5.24
T_3	22.45	32.75	66.96	5.04
T_4	19.95	30.79	54.94	4.54
T_5	23.69	33.33	69.97	6.15
T_6	20.96	33.96	63.94	5.45
T_7	22.76	34.75	71.95	6.45
T_8	22.63	31.70	64.95	5.65
T_9	22.89	33.35	69.97	5.95
T_{10}	23.94	33.62	74.96	6.74
T_{11}	18.96	30.25	57.07	4.64
S.Em±	0.32	0.42	1.00	0.09
CD at 5%	0.96	1.23	2.95	0.26
CV	2.56 (S)	2.21 (S)	2.64 (S)	2.70 (S)

Treatment

T_1: VAM T_2: *Trichoderma harzianum* T_3: *Pseudomonas fluorescens* T_4: *Azospirillum brasiliense*
T_5: BAP T_6: IBA T_7: VAM + *Trichoderma harzianum* T_8: IBA + *Azospirillum brasiliense*
T_9: BAP + *Pseudomonas fluorescens* T_{10}: VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum*
T_{11}: Control (Untreated suckers)

Table 3: Influence of growth hormones and biofertilizers on leaf parameters of plantlets regenerated through macropropagation in banana cv. Poovan

Treatments	Number of leaves	Leaf area (cm^2/plant)
T_1	4.03	459.94
T_2	4.03	454.95
T_3	3.83	449.94
T_4	3.43	407.94
T_5	4.30	459.94
T_6	3.63	409.78
T_7	3.70	418.95
T_8	3.60	414.95
T_9	4.13	464.96
T_{10}	4.43	470.94
T_{11}	3.60	419.96
S.Em±	0.08	6.64
CD at 5%	0.24	19.61
CV	3.58 (S)	2.62 (S)

Treatment

T_1: VAM
T_2: *Trichoderma harzianum*
T_3: *Pseudomonas fluorescens*
T_4: *Azospirillum brasiliense*
T_5: BAP
T_6: IBA
T_7: VAM + *Trichoderma harzianum*
T_8: IBA + *Azospirillum brasiliense*
T_9: BAP + *Pseudomonas fluorescens*
T_{10}: VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum*
T_{11}: Control (Untreated suckers)

Table 4: Economics of macropropagation in banana cv. Poovan using different growth hormones and biofertilizer

Treatments	No. of plantlets produced per treatment	Gross return (Rs.)	Total cost (Rs.)	Net return (Rs.)	B:C ratio
T_1	185.00	1295.0	420.0	875.0	2.08
T_2	181.00	1267.0	495.0	772.0	1.55
T_3	196.00	1372.0	495.0	877.0	1.77
T_4	167.00	1169.0	445.0	724.0	1.62
T_5	231.00	1617.0	427.0	1190.0	2.78
T_6	171.00	1197.0	726.0	471.0	0.64
T_7	201.00	1407.0	510.0	897.0	1.75
T_8	179.00	1253.0	765.0	488.0	0.63
T_9	211.00	1477.0	517.0	960.0	1.85
T_{10}	217.00	1519.0	622.0	897.0	1.44
T_{11}	142.00	994.0	405.0	589.0	1.45

Treatment

T_1: VAM
T_2: *Trichoderma harzianum*
T_3: *Pseudomonas fluorescens*
T_4: *Azospirillum brasiliense*
T_5: BAP
T_6: IBA
T_7: VAM + *Trichoderma harzianum*
T_8: IBA + *Azospirillum brasiliense*
T_9: BAP + *Pseudomonas fluorescens*
T_{10}: VAM + BAP + *Pseudomonas fluorescens* + *Trichoderma harzianum*
T_{11}: Control (Untreated suckers)

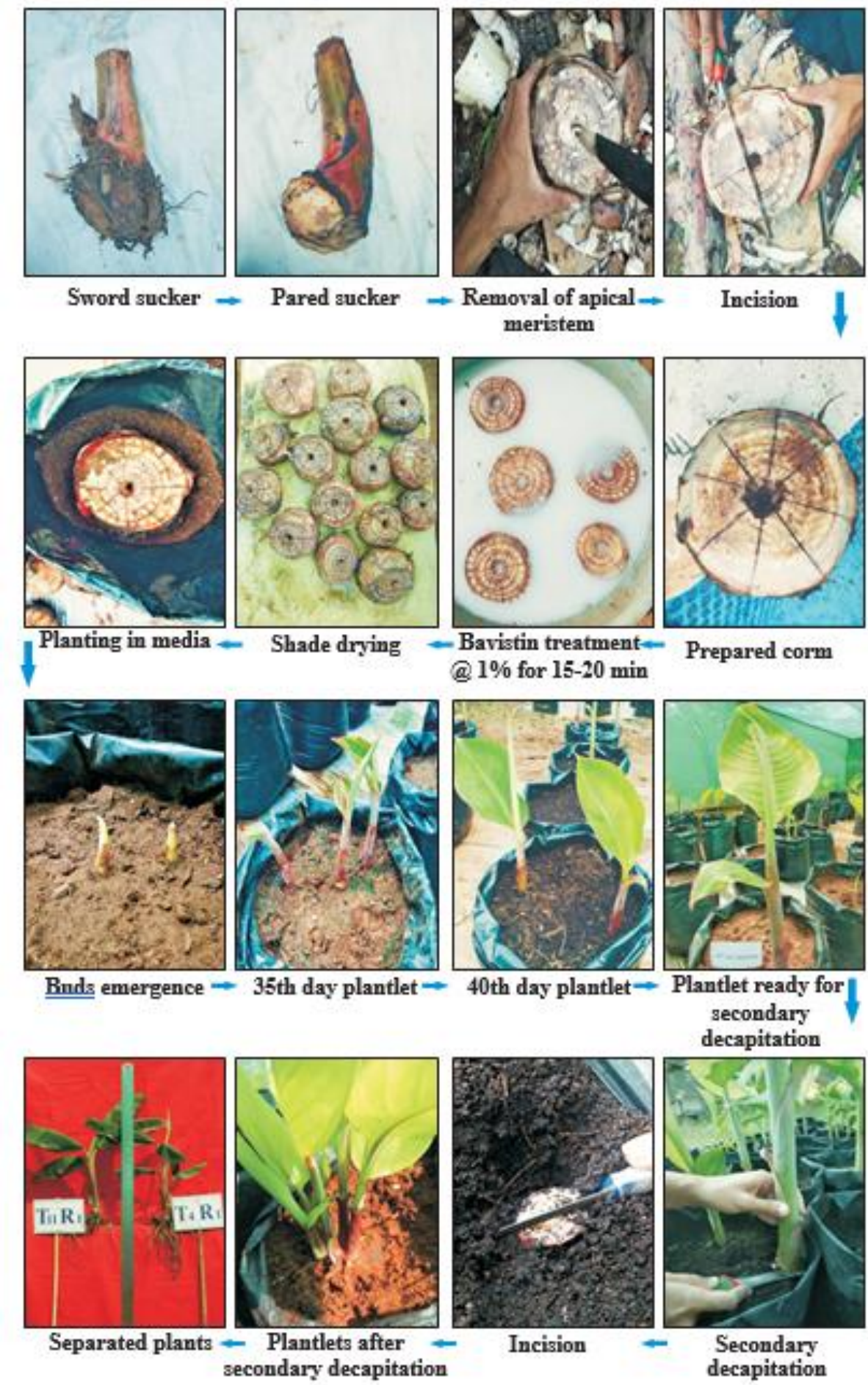

Plate 1: Procedure for macropropagation in banana cv. Poovan

Plate 2: General view of second experimental site of macropropagation in banana cv. Poovan

Number of primary buds per corm

Plantlet ready for secondary decapitation

Number of plantlets after secondary Decapitation

Plantlet of best treatment (Potting media)

Best treatment (T_{10}) – VAM + BAP + *P. fluorescens* + *T. harzianum*

Plate 3: Influence of growth hormones and biofertilizers on plantlet regeneration in macropropagation of cv. Poovan

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