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Effect of potting media and bio stimulants on growth of banana plantlets cv. grand naine under *ex vitro* conditions

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Abstract
An experiment was conducted on the acclimatization of tissue culture banana plantlets cv. Grand Naine. The different potting media used for the present study were Coco peat, Sawdust, Sand, Red earth, and FYM. In which different propagation media of Cocopeat + Red earth + FYM (1:2:1), Sawdust + Red earth + FYM (1:2:1), Sand + Red earth + FYM (1:2:1) were used for hardening. Different Bio stimulants are used for VAM, KSB, Humic acid, Chitosan and 19:19:19. Which were used in different combinations to study the effect of hardening potting media bio stimulants on vegetative growth performances of banana under *ex-vitro* conditions. The *in-vitro* rooted plantlets were hardened and acclimatized by using different treatments. Plantlets were transplanted from hardening after 60 days of hardening gave maximum survival and growth under *ex vitro* conditions. The maximum survival and growth during hardening (100%) were observed in a shade net with maintained relative humidity and light intensity. Various potting media and bio stimulants were tried, containing (Cocopeat + Red earth + FYM and AMF + KSB) gave maximum growth and development including plant height (cm), pseudo stem girth (cm) leaf length, leaf width, leaf area and growth of tissue culture banana plantlets performances under *ex vitro* conditions.

Keywords: Hardening, *ex vitro*, grand naine, potting media, bio stimulants

Introduction
Banana is one among the twenty-three main crops that form the base of world agriculture. It belongs to the family *Musaceae*. It is the most important fruit crop in the world and is commercially cultivated in tropics and subtropics. Banana is popular among the people as it is the cheapest fruit with abundant nutrients and is rich in carbohydrates, phenols and minerals like potassium and calcium. It is also a good source of vitamin A and a fair amount of vitamins C and B6. By virtue of its nutritional value, availability and price it is relished as "Poor man's apple".

In India, banana is appropriately referred to as 'Kalapatharu' a plant of all virtues. Each and every part of the plant is used for specific purposes. Apart from its use as a dessert fruit and for culinary purposes, the banana plants have multifaceted uses: the leaf is commonly used as a hygienic dining plate; the male flower is a favourite vegetable; the inner core of the pseudo stem is a popular vegetable with many therapeutic uses. It also has several medicinal values and is useful in curing intestinal disorders by converting harmful bacilli into beneficial ones, fighting ulcers by neutralizing acidic secretions and also against gout and arthritis. Its rich iron content makes it a nutritious food for anemic patients.

From backyard cultivation, it emerged as a commercial crop witnessing a great leap in area, production and productivity. India leads the world in banana production with an annual output of about 77.90 million tonnes from an area of 383.77 thousand ha. Andhra Pradesh produces 5003.07 MT over an area of 88.96 thousand hectares and ranks 1st position in India (NHB, 2018) [24]. In Andhra Pradesh, major banana-growing districts are East Godavari, YSR Kadapa, Ananthapur, West Godavari, Vizianagaram, Kurnool, Guntur, Krishna and Prakasham.

Micro propagation of banana has been considered to be the best tool for eliminating the virus from the planting materials apart from other advantages like high multiplication rate and faster growth in early stages compared to the conventional suckers. The success of tissue culture depends on the survival percentage of plantlets. *In vitro* propagated banana plantlets, are transferred to suitable growing media and kept in a greenhouse for hardening purpose before

transferred to the main field by providing light, shade and water under semi-controlled conditions. The physiological and anatomical characteristics of micro-propagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field (Hazarika, 2003) [12]. Therefore, acclimatization is the most crucial step during micro-propagation as the *in vitro* raised plantlets upon transfer to *ex-vitro* conditions are exposed to abiotic stress (altered temperature, light intensity and humidity) and biotic stress conditions, like soil microflora (Deb and Imchen, 2010) [8]. These challenges could be overcome to some extent by using optimum growth media and bio stimulants during acclimatization.

Material and Methods

The present investigation was undertaken in the Department of Fruit science, College of Horticulture, during the year 2018-2022 at Horticultural Research Station, Kovvur, Dr. YSRHU, and West Godavari District of Andhra Pradesh.

The experiment was laid out in Factorial Completely Randomised Design with three replications. The first factor consists of three levels of Potting media viz., Coco Peat + Red Earth + FYM (1:2:1), Saw Dust + Red Earth + FYM (1:2:1), Sand + Red Earth + FYM (1:2:1) and the second factor consists of five levels of Bio stimulants viz., AMF @1 ml/plantlet + Potassium Solubilizing Bacteria @1 ml/plantlet, Humic acid @40 ppm, Chitosan @100 ppm, Conventional method (19:19:19@2 g/L),Water. The experiment included 15 treatment combinations comprising of three levels of potting media and 5 levels of Bio stimulants.

Results and Discussion

Vegetative growth parameters

Under shade net conditions all vegetative growth parameters had a significant effect on plant pseudo stem height at 60 days (cm), pseudo stem girth (cm), number of leaves, leaf length(cm), leaf width (cm) and leaf area (cm^2) among different media and stimulants, and the mean values are represented in Table 1.

Pseudo stem height (cm)

In tissue culture banana, the different potting media combination of bio stimulants, and their interaction significantly influenced the pseudo stem height in the hardening period at 8th week after hardening. (Table 1.)

There was significant influence of potting media on pseudo stem height of tissue culture banana plantlets cv. Grand Naine. Pseudo stem height at 8th week after hardening was significantly highest (8.76 cm) in coco peat, red earth and FYM (1:2:1) media which was lowest (5.69 cm) in saw dust, red earth and FYM (1:2:1).Among the Bio stimulants used Significantly, highest pseudo stem height (7.72 cm) at 8th week under *ex vitro* hardening was recorded in AMF and KSB each applied at 1ml per plantlet, respectively while the lowest (7.31 cm) was found in control (Water). Among the interaction of potting media and bio stimulants, At 8th week after hardening, pseudo stem height (9.53 cm) was highest in coco peat, red earth and FYM (1:2:1) media applied with humic acid @40 ppm, while the lowest (5.25 cm) was noticed in saw dust, red earth and FYM (1:2:1).

This might be due to the fact that coco peat has ability to absorb and retain not only water but also air, due primarily to their microporous nature (Fornes *et al*., 2003) [11] allowing internal retention of both water and air. Enhanced height of plants due to coco peat was reported by Ameri *et al*. (2011) [3] in strawberry, Uzaribara *et al*., (2015) [27] in banana.

During hardening at all-time intervals pseudo stem height was maximum when AMF and KSB were applied. AMF increases the uptake of phosphorus in the plants, permits plant to obtain additional moisture and nutrients .whereas KSB convert insoluble K to soluble forms of K which will be available for plant growth. Similar results were reported by Vasane and Kothari (2008), Mwashasha *et al.* (2011), Patel *et al*. (2015) and Yasin *et al*. (2016) [29, 22, 25, 30] in banana.

Pseudo stem girth (cm)

In tissue culture banana, the different potting media combination of bio stimulants, and their interaction significantly influenced the pseudo stem girth in the hardening period at 8th week after hardening. (Table 2.)

There was significant influence of potting media on pseudo stem girth of tissue culture banana plantlets cv. Grand Naine. At 8th week after hardening, highest (3.66 cm) pseudo stem girth was recorded in coco peat, red earth and FYM (1:2:1) media and lowest (3.12 cm) was recorded in saw dust, red earth and FYM (1:2:1) media. Among the bio stimulants used at At 8th week after hardening, highest (3.56 cm) pseudo stem girth was found in AMF and KSB each applied at 1ml per plantlet, while lowest (3.27 cm) pseudo stem girth was recorded in control (Water).Among the interactions At 8th week after hardening highest (3.89 cm) pseudo stem girth recorded in M_1S_1, while the lowest (3.01 cm) pseudo stem girth found in $M2S_0$.

The enhanced pseudo stem girth in potting media containing cocopeat might be due to good physical properties like high total pore space, high water content, similar reports were given by Ali *et al*., (2011) [2] that coco peat was found significantly best growing media for good vegetative growth of plants. The combined potting media (cocopeat + red soil + FYM) mixed in 1:2:1 proportion might have provided better anchorage to the good plant growth and increased the plant girth along with ample supply of water, nutrients, and aeration (Uzaribara *et al*., 2015) [27] in banana.

The use of AMF and KSB in the potting media proved its superiority as a hardening medium for the better growth and establishment of banana plantlets. AMF enhances nutrient uptake there by increases the plant growth in terms of pseudostem girth in *in vitro* cultured banana plantlets during hardening. Similar findings were reported by Modgil *et al*. (2009) in apple, Khatik *et al.* (2019) in strawberry, Koffi *et al*. (2015), Kumari *et al.* (2017), Esakkimuthu *et al*. (2017), Biswas *et al.* (2018) [21, 14, 16, 10, 6] in *in vitro* raised banana plantlets. KSB solubilises unavailable K in the soil and made available to the plants there by enhances the uptake of K which might have improved the girth of plantlets compared to uninoculated plantlets.

Number of leaves/plantlet

In tissue culture banana plantlets influenced to the different potting media and bio stimulants and their combinations showed significant influence on number of leaves/plants at hardening period of 8th week (Table 3)

There was significant influence of potting media on Number of leaves per plantlet of tissue culture banana plantlets cv. Grand Naine. At 8th week after hardening also. Maximum (7.25) number of leaves were recorded in coco peat, red earth and FYM media while, minimum (6.07) number of leaves were noticed in media with saw dust, red earth and FYM

(1:2:1). Among the Bio stimulants used, At 8th week after hardening, maximum (7.02) number of leaves were recorded in conventional method (19:19:19 applied @2g/L), whereas, minimum (6.54) number of leaves were found in control (Water), Among the interactions, At 8th week after hardening, maximum (7.73) number of leaves were recorded in coco peat, red earth and FYM (1:2:1) media supplied with 19:19:19 @2g/L, while minimum (5.70) number of leaves were registered in in sawdust, red earth and FYM (1:2:1) media applied without any bio stimulant.

The integrated application of inorganic fertilizers along with organic manures, attributed to the increase in nutrient levels of NPK, especially nitrogen, enhanced the vegetative growth like the number of leaves, encourages plant to synthesize more metabolites, exhibiting a high photosynthetic rate during the period of growth and development, proved to be the superior combination for vigorous growth. Similar results were reported by Al-Harthi *et al*. (2009) Balamohan *et al*. (2015), Suhasini *et al*. (2018) and Navgare *et al*. (2021) [1, 4, 26, 23] in banana.

Hence the combination of coco peat containing media and 19:19:19 inorganic fertiliser resulted in the production new leaves at faster rate as it contains readily available nitrogen. These findings are in accordance with the results obtained by Dagnew *et al*. (2012), Uzaribara *et al*. (2015), Maharana *et al*. (2017), Bharati *et al*. (2018) [7, 27, 18, 5] in banana.

Leaf length (cm)

In tissue culture banana, the different potting media combination of bio stimulants, and their interaction significantly influenced leaf length in the hardening period at 8th week after hardening. (Table 4.)

There was significant influence of potting media on leaf length at 8th week after hardening, maximum leaf length (8.82 cm, 11.82 cm and 12.91 cm respectively) was recorded in coco peat, red earth and FYM (1:2:1) media while minimum leaf length (7.30 cm, 8.86 cm and 9.26 cm respectively) was observed in media with saw dust, red earth and FYM (1:2:1).. Among the Bio stimulants used, at 8th week after hardening, maximum leaf length (12.02 cm) was observed when AMF and KSB each applied at 1ml per plantlet while, minimum leaf length (11.31 cm) was recorded in control (Water). Among the treatment combinations At 8th week after hardening, highest leaf length (13.15 cm) was observed in coco peat, red earth and FYM (1:2:1) media with AMF and KSB each applied at 1ml per plantlet, lowest leaf length (8.40 cm) was observed in saw dust, red earth and FYM (1:2:1) media without application of bio stimulants.

In the present study, maximum leaf length recorded in the media with coco peat is due to its porous nature with highest cation exchange capacity enabled roots to spread out easily and thus, gives more breathing space for aeration, and consequently better growth is achieved. The results of the present experiment were agreed with the finding of Bharati *et al.* (2018) [5] in banana, reported that AM related pathways stimulate plant growth and physiology in nutrient independent ways which might be the reason for enhanced growth of leaf in terms of length. These findings are in corroboration with the results obtained by Mathewsel *et al*. (2003), Joolka *et al*. (2004), Vasane and Kothari (2006) and Vasane and Kothari (2008) [28, 13, 29] in micro-propagated banana plantlets. Along with AMF, KSB also plays an important role in K nutrition.

Leaf width (cm)

In tissue culture banana, the different potting media combination of bio stimulants, and their interaction significantly influenced the leaf width in the hardening period at 8th week after hardening. (Table 5.)

There was significant influence of potting media on leaf width 8th week after hardening, significantly highest leaf width (1.64 cm) was recorded in coco peat, red earth and FYM (1:2:1) media while, lowest (1.46 cm) leaf width was recorded in media with saw dust, red earth and FYM (1:2:1).Among the Bio stimulants used, 8th week after hardening significantly maximum (5.44 cm) leaf width was recorded in S_1 (AMF @ 1ml/plantlet + Potassium solubilizing bacteria @ 1ml/plantlet) whereas, the minimum (5.12 cm) leaf width was recorded in treatment S_0 (water). Among the treatment combinations, at 8th week after hardening the maximum (6.54 cm) leaf width was recorded M_1S_1 while, the minimum (3.14 cm) leaf width was found in treatment combination M_2S_0.

Coco peat containing media enhance leaf width compared to other media. It improved aeration, drainage for better growth of roots. Also prevented nutrient leaching by retaining nutrients, released slowly to the plants and thus helped in better nutrient uptake which in turn enhanced growth. Similar results were observed by Bharati *et al*. (2018), Mahendra *et al*. (2020) [5] in banana and Lakshmikanth *et al*. (2020) [17] in strawberry. Mycorrhizal association was found to be beneficial to the plants in terms of better nutrient uptake and water potential which led the plants to become healthier and more productive than the non-mycorrhizal plants as reported by Khaliq *et al*. (2001) in peppermint. AMF play an important role in soil nutrient dynamics by improving soil physical, chemical and biological properties. The present findings are supported by Mathews *et al*. (2003), Vasane and Kothari (2006), Mwashasha *et al*. (2011), Emara *et al*. (2018) in banana and Kumari *et al*. (2017) [28, 22, 16] in strawberry.

Table 1: Effect of media and bio stimulants on pseudo stem height (cm) at 8 the week after hardening under *ex vitro* hardening in banana cv. Grand Naine

Potting media (M)	Bio stimulants (S)					
	Pseudo stem height (cm)					
	S_1	S_2	S_3	S_4	S_0	Mean
M_1	8.93	9.53	8.66	8.63	8.04	8.76
M_2	6.13	5.41	5.76	5.91	5.25	5.69
M_3	8.09	8.10	8.42	8.25	8.18	8.21
Mean	7.72	7.68	7.61	7.60	7.31	
Factor	SE(m)			CD(P=0.05)		
M	0.04			0.12		
S	0.06			0.16		
M x S	0.10			0.28		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Table 2: Effect of media and bio stimulants on pseudo stem height (cm) at 8 the week after hardening under *ex vitro* hardening in banana cv. Grand Naine

Potting media (M)	Bio stimulants (S)					
	Pseudo stem girth (cm)					
	S_1	**S_2**	**S_3**	**S_4**	**S_0**	**Mean**
M_1	3.89	3.57	3.73	3.76	3.35	3.66
M_2	3.16	3.02	3.30	3.13	3.01	3.12
M_3	3.64	3.63	3.60	3.65	3.45	3.60
Mean	3.56	3.41	3.54	3.51	3.27	
Factor	SE(m)±			CD(P=0.05)		
M	0.01			0.04		
S	0.02			0.05		
M x S	0.03			0.09		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Table 3: Effect of media and bio stimulants on Number of leaves at 8th week after under *ex vitro* hardening of tissue culture banana cv. Grand Naine

Potting media (M)	Bio stimulants (S)					
	Number of leaves (No)					
	S_1	**S_2**	**S_3**	**S_4**	**S_0**	**Mean**
M_1	7.17	7.23	7.30	7.73	6.83	7.25
M_2	6.67	5.77	6.10	6.07	5.70	6.06
M_3	6.97	6.93	7.03	7.27	7.10	7.06
Mean	6.93	6.64	6.81	7.02	6.54	
Factor	SE(m)			CD(P=0.05)		
M	0.04			0.13		
S	0.06			0.16		
M x S	0.10			0.29		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Table 4: Effect of media and bio stimulants on leaf length at 8th week after under *ex vitro* Hardening of tissue culture banana cv. Grand Naine

Potting media (M)	Bio stimulants (S)					
	Leaf length (cm)					
	S_1	**S_2**	**S_3**	**S_4**	**S_0**	**Mean**
M_1	13.15	13.04	12.82	12.80	12.75	12.91
M_2	10.24	9.05	9.52	9.11	8.40	9.26
M_3	12.66	12.89	12.61	12.82	12.77	12.75
Mean	12.02	11.66	11.65	11.58	11.31	
Factor	SE(m)±			CD(P=0.05)		
M	0.03			0.07		
S	0.03			0.09		
MxS	0.06			0.16		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Table 5: Effect of media and bio stimulants on leaf width at 8th week after under *ex vitro* hardening of tissue culture banana cv. Grand naine

Potting media (M)	Bio stimulants (S)					
	Leaf width (cm)					
	S_1	**S_2**	**S_3**	**S_4**	**S_0**	**Mean**
M_1	6.54	6.23	6.03	6.14	6.11	6.21
M_2	3.93	3.39	3.67	3.50	3.14	3.53
M_3	5.85	5.99	6.11	5.85	6.11	5.98
Mean	5.44	5.20	5.27	5.16	5.12	
Factor	SE(m)±			CD(P=0.05)		
M	0.01			0.04		
S	0.02			0.06		
MxS	0.03			0.10		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Table 6: Effect of media and bio stimulants on leaf area (cm^2) at 8th week after under *ex vitro* hardening of tissue culture banana cv. Grand naine

Potting media (M)	Bio stimulants (S)					
	Leaf area (cm^2)					
	S_1	S_2	S_3	S_4	S_0	Mean
M_1	72.45	68.13	65.47	64.73	62.08	66.57
M_2	32.21	22.19	27.61	26.49	21.17	25.93
M_3	57.18	61.09	64.34	61.95	59.67	60.84
Mean	52.95	50.47	52.47	51.06	47.64	
Factor	SE(m)±			CD(P=0.05)		
M	0.27			0.79		
S	0.35			1.01		
MxS	0.61			1.76		

M_1- Coco Peat + Red Earth + FYM (1:2:1); M_2- Saw Dust + Red Earth + FYM (1:2:1); M_3- Sand + Red Earth + FYM (1:2:1); S_1- AMF @1ml/plantlet+ Potassium solubilizing bacteria@1ml/plantlet; S_2- Humic acid 40ppm; S_3- Chitosan 100ppm; S_4- Conventional method (19:19:19@2g/L); S_0- water

Leaf area (cm^2)

In tissue culture banana, the different potting media combination of bio stimulants, and their interaction significantly influenced the pseudo stem girth in the hardening period at 8th week after hardening. (Table 6.)

There was significant influence of potting media on leaf width At 8th week after hardening, maximum leaf area (65.57 cm^2) was recorded in coco peat, red earth and FYM (1:2:1) media, while minimum leaf area (25.93 cm^2) was observed in saw dust, red earth and FYM (1:2:1) media. Among the Bio stimulants used, At 8th week after hardening, maximum leaf area (52.51 cm^2) was observed when AMF and KSB each applied at 1ml per plantlet, while minimum leaf area (47.64 cm^2) was recorded in control (Water)..Among the interactions at 8th week after hardening, maximum (72.45 cm^2) leaf area was recorded in treatment combination M_1S_2 whereas, the minimum (27.17 cm^2) leaf area was found in treatment combination M_2S_0

Leaf area parameter is used to predict the photosynthetic primary compound production, evapotranspiration, and also as a reference tool for crop growth. Leaf area plays an essential role in theoretical production ecology (Lakshmikanth *et al.*, 2020) [17]. Cocopeat and AMF help to release the nutrients and provide a better microclimate and enhance greater assimilation of food material by the plant which resulted in greater meristematic activities of cells and consequently the number of leaves, length, and width of the leaf of the plants. AMF mycelia are capable of extending the region of absorption reaching away from the phosphorus depletion zone which forms around the absorbing roots of plantlet (Vasane and Kothari 2006) [28] which plays an important role in promoting the mechanism of cell division and differentiation. There by promote plant growth and effects leaf development. Similar findings were also reported by Mathewsel *et al*. (2003), Uzaribara *et al*. (2015) and Bharati *et al.* (2018) [27, 5] in banana.

Conclusion

The banana plants grown in potting media and bio stimulants containing Cocopeat + Red earth + FYM(1:2:1) along with AMF@1ml/plantlet + Potassium solubilizing bacteria@1ml/plantlet) was plant pseudo stem height, pseudo stem girth, Number of leaves, Leaf length, leaf width, leaf area with respectively in best growth in banana plantlets cv. Grand naine under *ex vitro* conditions.

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