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RESPONSE OF INITIATION MEDIA TREATMENT, USE OF PGR ON MACRO-PROPAGATION OF CAVENDISH BANANA CULTIVARS

[1]SUDESHNA BARUAH, [1]UTPAL KOTOKY AND [2]KAUSHIK DAS
[1]Department of Horticulture, [2]Department of Crop Physiology
Assam Agricultural University, Jorhat, Assam
Email: sudeshnabaruah17@gmail.com
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Macro-propagation of banana is done using the principle that when strong apical dominance of banana plant is suppressed by decapitation and decortication (removal of the apical meristem), it gives rise to production of many side suckers. Evaluation of macro-propagation practice using sawdust as initiation media, supplemented with various biofertilizers results in highest number of uniform sized tertiary bud production when the decorticated corm cavity was poured with 40ppm BAP (4ml) and the media is supplemented with *Bacillus subtilis* alone and along with VAM. This treatment also performed better regarding shortest time requirement for production of first primary bud and percent production of sword suckers, and other vegetative growth characters.

Key words: Macro-propagation, Banana, *Bacillus subtilis*, BAP, VAM

Banana belongs to the genus *Musa*, under the family Musaceae and it has been commercialized in the tropics and is the staple food of several African countries. Banana is the second largest produced fruit after citrus that contributes about 16% of the world's total fruit production. Among over 130 banana producing countries, India ranks 1st contributing to 27% of the world's annual banana production. Its cultivation is mostly affected by pests and diseases and scarcity of quality planting material. Farmers prefer naturally regenerated planting material for setting up the new plantations, which may act as vector for most of the banana pests and diseases resulting in reduced productivity and shortened lifespan of new plantations. In this context, high demand of pests and disease free quality planting material of banana is there to set up new plantation or to expand the existing orchards. For producing huge amount of quality planting material, tissue culture technique for mass multiplication of banana was introduced in 1997, but its popularity and adoption is limited because of high cost of micropropagated plantlets, high capital requirement and lack of skill at farmer's level (Njau *et al*. 2011).

To overcome the problem of high cost and skill requirement in micro-propagation technique, macro-propagation had been introduced as an alternative, involving little capital and skill (Njau 2013). The principle behind macro-propagation technique lies in the fact that, when the strong apical dominance of banana is suppressed, it gives rise to many side suckers (Singh *et al*. 2011, Langford *et al*. 2012 and Dayarani *et al*. 2013).

MATERIALS AND METHODS

Cultivars selected for the experiment: *Musa* (AAA) 'Jahaji' (synonyms: 'Dwarf Cavendish', 'Basrai', 'Vamanakeli', 'Pacha Vazhai', 'Saru Jahaji) and *Musa* (AAA) 'Barjahaji' (synonyms: 'Tall Cavendish', 'Giant Governor', 'Grande Naine', 'Nanicao') were selected on the basis of the commercial importance of the available banana cultivars of Assam.

Planting Material: Field extracted healthy, uniform sized corms of both the cultivars, weighing 1.0-1.5kg were selected for the macro-propagation from disease-free mother plant.

Treatments: The seven treatments (T_0, T_1, T_2, T_3, T_4, T_5 and T_6) as mentioned in the Table.1 were given to corms, each replicated three times in completely randomized design under polyhouse-condition

Decapitation and Decortication of corm: The field extracted corms were cleaned by removing roots and detopped just above the

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juncture of the corm and the aerial shoot. The apical meristem was removed to a depth of 2 cm, leaving a cavity of 2 cm diameter in the rhizome and the rest of the corm was given 6-8 cross wise cuts.

Planting: The decapitated and decorticated corms were planted in the sawdust filled pits and irrigated to maintain adequate moisture level in the substrate/initiation media.

Secondary and tertiary decapitation and decortications: The primary buds were decapitated by removing the juvenile meristems and 4-6 horizontal cuts were given for the young rhizome to produce secondary buds. The same procedure was repeated for secondary buds thereby producing tertiary buds.

Separation of individual tertiary suckers and hardening: The tertiary suckers with 3-4 well developed leaves were separated from the mother corm carefully and were planted in polybags containing Soil, Sand and Farmyard manure at a ratio of 1:1:1 and kept under shade net house for hardening. The suckers without well developed roots were replanted in the same initiation media to develop roots. The suckers were hardened for 45 days prior to transplanting in the main field.

OBSERVATIONS

The time taken for the 1st primary bud initiation, average numbers of primary, secondary and tertiary buds formed and percentage production of sword suckers were observed. At the end of the hardening period vegetative growth parameters like plant height, stem girth, phyllochron, sucker weight, number of roots and root length were taken.

RESULTS AND DISCUSSION

Among the treatments combinations, treatment T_5 (Sawdust + BAP + *B. subtilis*) recorded superiority regarding the total number of secondary and tertiary buds formed, and percentage of sword suckers produced (Table.2). T_5 also recorded highest values for plant height, stem girth, sucker weight (Table.3). Addition of VAM along with BAP and *B. subtilis* in treatment T_6 did not show much significant variation with treatment T_5. This treatment was also superior regarding early bud initiation and phyllochron. These treatments were found satisfactory regarding their performance in root growth (Fig.1). Induction of highest number of secondary and tertiary buds in the corms may be attributed to the enhanced callus formation ability of the synthetic cytokinin BAP in addition with the IAA produced by *B. subtilis*. Various stains of *B. subtilis* are capable of producing IAA and therefore, *B. subtilis* can be used in organic nursery, as a supplement to synthetic rooting hormone IBA (Erturk *et al.,* 2010).

In monocotyledonous plants combination of cytokinin/weak auxin increases shoot proliferation rates (George, 1993). Records showed production of maximum primary, secondary and tertiary buds per corm of *Musa laterita* (Bronze banana) when decapitated rhizome were treated with 0.04% BAP (Dayarani *et al*., 2013). Increase in shoot multiplication rate of banana with increased BAP concentration (with or without incorporation of auxin) was also recorded by Jafari *et al*. (2011).

Shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Adenine based cytokinins are used in *in vitro* propagation of several crops. 6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Islam *et al*., 2004; Prathanturarug *et al*., 2003). The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas (Buah *et al*., 2010; Farahani *et al*., 2008; Rahman *et al*., 2006; Resmi and Nair, 2007). BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures.

Plant growth promoting rhizobacteria can exerts a direct effect on plant growth like, production of phytohormones, biological nitrogen fixation, and phosphate solubilization, enhancing the availability of other trace elements, increased iron nutrition through iron-chelating siderophores and volatile compounds that affects the plant signaling pathways; and additionally by antibiosis, competition for space and nutrients, and induction of systemic resistance in plant against a broad-spectrum of root and foliar pathogens (Podile and Kishore, 2006). In both the treatments (T_5 and T_6), addition of *B. subtilis*, which is a plant growth promoting rhizobacteria, might have added to the better root as well as shoot growth. This bacterium produces an endospore that allows it to endure extreme conditions of heat and desiccation in the environment; moreover, *B. subtilis* produces a variety of proteases, alpha-amylase and lipase that enable it to degrade a variety of natural substrates and contribute to nutrient cycling (Claus and Berkeley, 1986). Plant growth may also be stimulated by bacterially produced phytohormones (Okon and Labandra-Gonzalez, 1994; Yanni *et al*., 2001), vitamin-related products (Phillips *et al.,* 1980) or pathogen suppression activities (Haque and Gaffer, 1993). The inoculation of plant growth promoting rhizobacteria could stimulate the root growth and development, which seemed to occur almost in all dimensions. Host plants are benefited by improved root development and subsequent increase in rates of water and mineral nutrient uptake.

Increased plant height and root length of the suckers may also be the result of inoculation of *B. subtilis*. Umesha and Hariprasad (2010) found that seed bacterization with GBO3 strain of *B. subtilis* in tomato increased plant growth under field conditions. The phytohormone produced by *B. subtilis* is recorded to be an auxin (Indole-acetic acid) i.e. responsible for root and shoot elongation in plants (Ajilogba *et al*., 2013). Productions of IAA in plants help to increase root dry weight and thereby increase the plants' ability to take up N, P, K (Etesami *et al*., 2009). It helps to stimulate plant growth and increased the uptake of N, P, K, Ca and Mg and is also responsible for early growth promotion.

The improved nutrient absorption by

Table 1. Treatment details

Treatment code	Treatment combinations	Treatment application
T_0	(Control)	
T_1	Sawdust + VAM	VAM (@30g/corm) mixed with sawdust
T_2	Sawdust + *Trichoderma viride*	*T. viride* (@30g/corm) mixed with sawdust
T_3	Sawdust + VAM + *T. viride*	VAM (@30g/corm) and *T. viride* (@30g/corm) mixed with sawdust
T_4	Sawdust + Indole-3-butyric acid + *Azospirillum*	*Azospirillum* (@30g/corm) mixed with sawdust and corms were dipped in a bucket containing 0.25% IBA solution for a few seconds prior to planting.
T_5	Sawdust + Benzyl Amino Purine + *Bacillus subtilis*	*B. subtilis* (@30g/corm) mixed with sawdust and 40ppm BAP (4ml per corm) poured into the meristematic cavity of the corms.
T_6	Sawdust + VAM + BAP + *B. subtilis*	VAM (@30g/corm) and *B. subtilis* (@30g/corm) mixed with sawdust

Table.2 Performance of Dwarf and Tall Cavendish Banana under various treatments regarding time taken for bud initiation, production of primary, secondary and tertiary buds and production of sword suckers

Treatments	Time taken for initiation of 1st primary bud (days)		Average number of primary buds per corm		Average number of secondary buds per corm		Average number of tertiary buds per corm		Production of sword suckers (%)	
	DC	**TC**	**DC**	**TC**	**DC**	**TC**	**DC**	**TC**	**DC**	**TC**
T_0	15.60	31.10	3.23	3.49	4.70	3.99	13.60	12.43	76.75	79.60
T_1	18.50	17.27	3.28	3.43	6.58	5.43	19.20	18.42	82.12	79.17
T_2	20.77	22.50	3.06	3.61	10.42	7.68	21.25	23.13	85.02	84.47
T_3	18.63	16.27	2.59	3.72	9.26	7.56	24.35	22.52	83.35	82.87
T_4	28.43	24.83	3.02	3.24	6.38	6.35	18.95	17.35	81.88	77.12
T_5	21.50	**11.93**	2.88	3.64	**11.88**	**10.47**	**25.53**	**25.98**	**88.10**	**86.55**
T_6	20.00	13.43	3.63	3.35	9.63	9.18	23.82	21.70	83.15	84.68
CD	**NS**	**4.38**	**NS**	**NS**	**1.13**	**0.87**	**4.70**	**3.83**	**4.95**	**5.30**

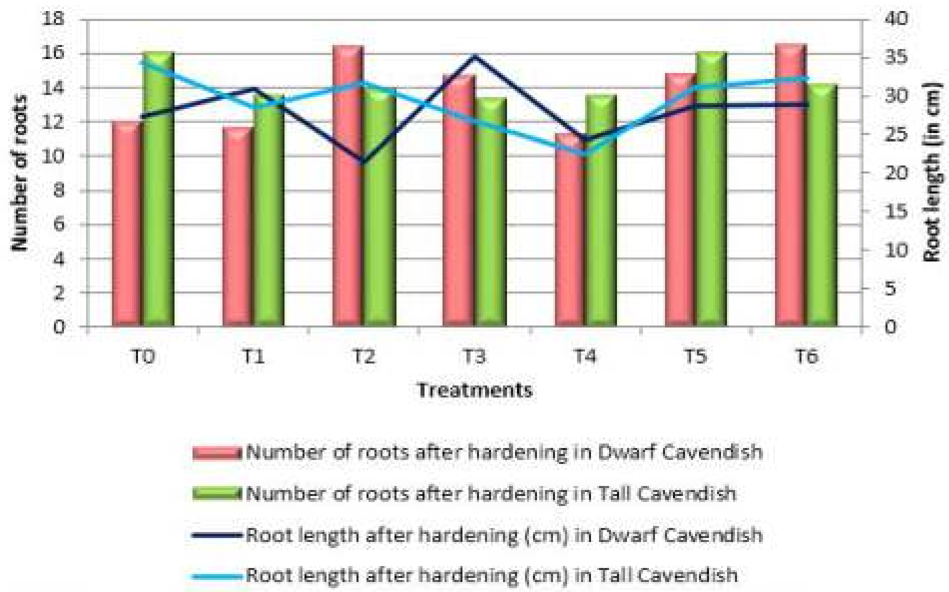
NS: Non-significant, DC: Dwarf Cavendish, TC: Tall Cavendish.

Table.3 Performance of Dwarf and Tall Cavendish Banana under various treatments regarding plant height (cm), stem girth (cm), sucker weight (g) after hardening and phyllochron (days)

Treatments	Plant height after hardening (cm)		Stem girth after hardening (cm)		Sucker weight after hardening (g)		Phyllochron (days)	
	DC	**TC**	**DC**	**TC**	**DC**	**TC**	**DC**	**TC**
T_0	18.03	24.57	7.53	5.56	106.65	146.38	10.47	10.97
T_1	17.27	23.50	6.40	5.98	124.33	122.35	11.56	11.86
T_2	17.62	25.52	6.23	5.87	143.47	141.08	11.36	10.70
T_3	18.43	22.95	7.23	5.43	147.33	113.22	10.78	9.92
T_4	15.95	22.82	6.10	5.78	103.92	106.68	10.39	10.97
T_5	**18.83**	**27.20**	**7.78**	**6.23**	**152.85**	**166.92**	11.90	9.56
T_6	17.90	24.20	7.52	5.83	147.67	165.38	**9.13**	**8.91**
CD	**1.37**	**2.29**	**0.67**	**NS**	**NS**	**32.54**	**NS**	**1.16**

NS: Non-significant, DC: Dwarf Cavendish, TC: Tall Cavendish.

Figure 1: Influence of treatment on number of roots and root length (cm) of Banana cultivars Dwarf Cavendish and Tall Cavandish



Mycorrhiza (VAM), especially phosphorus, had a direct impact on growth parameters. The better growth responses of mycorrhizal plants were attributed to higher nutrients uptake and higher moisture absorption.

CONCLUSION

Macro-propagation technique can help in fast and healthy planting material generation for both the commercial cultivars (Tall Cavendish and Dwarf Cavendish). Adding BAP in the meristematic cavity of banana corm, and mixing of sawdust (initiation media) with *B. subtilis* alone, or in addition with VAM is beneficial for rapid multiplication of Banana through this technique.

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