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In vitro Regeneration of *Musa* Spp. Plantlet CV. Grand Naine by Plant Tissue Culture Technique

Rahul R. Rodge[1*], Anis Mirza[2], Harjinder Kaur[1], Lakhesing Girase[3] and Khan Jabroot J.[1]

[1]Ph.D. Scholar, Department of Horticulture,
School of Agriculture, Lovely Professional University, Phagwara (Punjab), India.
[2]Associate Professor and Head, Department of Horticulture,
School of Agriculture, Lovely Professional University, Phagwara (Punjab), India.
[3]Ph.D Scholar, Guru Kashi University, Talwandi Sabo (Punjab), India.

(Corresponding author: Rahul R. Rodge)*
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ABSTRACT: Banana (*Musa* spp.) is one of the most consumable fruits and cultivated around the globe. It was majorly propagated through tissue culture technique. It contains high nutritional value as well as the high demand of the market. There are many challenges in banana production long gestation durations, low yields, excessive flood irrigation resulting to high mortality rates, and trouble propagating disease-free uniform suckers were problems that conventional banana farming techniques started to cause for growers. These difficulties may result from several things, including a scarcity of disease-free plants and planting materials or farmers who have not been exposed to new technology we contribute way to produce good quality Banana Tissue culture plant using with PGR and AC. To enhance the tissue culture technique for production of cv. Grand Naine plantlets we use 6-Benzylaminopurinefor shoot proliferation and Indole butyric acid and Activated charcoal for more root proliferation investigate the effect of different cytokinin concentrations, such as BAP (6-benzyl amino purine), on a shoot, leaf, and multiplication, as well as different auxin concentrations, such as IBA (Indole 3-butyric acid) + (AC) Activated charcoal, on root proliferation, to develop a protocol for in vitro plant regeneration from shoot tip explants of the Grand Naine variety of Banana. Different concentrations of BAP (0.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg/l) and IBA (0.5, 1.0, 1.5 mg/l) combination with AC (1.0, 1.5, 2.0 gm/l). The highest number of the shoot after 55 days was observed (6.16) MS medium supplemented with (4.0 mg/1 BAP) the highest length of shoot (5.76) cm that was recorded in MS medium supplemented with (4.0 mg/1 BAP) and highest number of leaves (4.5) found in MS medium supplemented with (4.0 mg/1 BAP) highest length of leaves 4.13 recorded in MS medium supplemented with (3.0 mg/l BAP) and highest number of Root recorded (5.0) in MS supplemented (2.0 gm/l AC+1.5 mg/l IBA) highest length of Root 8.25 recorded in MS supplemented (2.0 gm/l AC+1.5 mg/l IBA) respectively root proliferation recorded in 95 days. In the present study, we found (4.0 mg/l BAP) is best for a shoot, leaf proliferation, and multiplication, and AC + IBA is best for Root.

Keywords: *BAP* cytokinin, *IBA* auxin, *Activated charcoal*, in vitro shoot, leaf, Root proliferation.

INTRODUCTION

In terms of growth and trade, bananas are the world's most valuable crop. The crop's production and trade volumes have increased dramatically in recent years in response to fast population increase in emerging countries as well as growing worldwide import demand. However, because the bulk of banana planting is done informally by smallholder farmers, exact worldwide banana production numbers are difficult to get. Annual global banana production increased from 69 million tonnes in 2000-2002 to 116 million tonnes in 2017-2019, with a value of roughly 31 billion USD, according to current estimates USD (FAO, 2020). The major research gap in this area is that to produce quality planting material for banana propagation, which also increase the yield control soil borne disease we need to incorporate tissue culture techniques. It also reduces the input cost of farmers to control the diseases in orchard at the early stages of plant life. For thousands of years, products derived from natural sources have been used. Bananas are well-known for their traditional, medicinal, and nutritional uses. It is high in carbs (22.84 g/100 g) and energy (about 370 kg/100 g) and is regarded as one of the greatest sources of potassium (358 mg/100 g), accounting for 8% of the daily required requirement. Bananas have exceptional medicinal properties in addition to their particular nutritional composition. One of these is a banana fruit, which may be processed in its whole, including its flesh and skin, to make banana chips, banana flour, banana cookies, and the rest of the time, banana juice is utilized. (Nadeem *et al.,* 2022).

Banana (*Musa* spp.) is a branch of the Musaceae family and ranks fourth among some of the world's most important crops. Indonesia ranks sixth in the world in banana production, with 6.19 million MT, trailing India (in millions MT) (24.86), China (10.55), the Philippines

(9.22), Ecuador (7.01), and Brazil (6.90). (Maps of World, 2014). Banana ranks first in Indonesia, becoming the most significant fruit in terms of production and harvested area. Several studies have reported success of tissue culture in banana crop from different types of genome groups, and it has been well established that the regeneration ability in vitro is highly genotype specific and strongly impacted by growth regulator (Karim *et al.,* 2009; Sipen and Davey 2012; Sazedur *et al.,* 2013; Iqbal *et al.,* 2013).

Tissue culture is also essential for germplasm distribution, conservation, and the safe exchange of internal planting material. For banana improvement, Mass propagation of chosen genotypes, soma clonal variation methods, genetic modification, and other biotechnological applications are all examples of biotechnological uses. based on efficient plant regeneration protocols may be used. Tissue cultured bananas are gaining commercial acceptance among farmers due to their uniform, fast growing appearance, pest and disease-free seedling, and earlier crop maturity than suckers. Tissue culture has shown to be an effective method for producing millions of identical plantlets that are disease-free and true to parental form (Akbar and Roy 2006).

Plants grown in test tubes provide for the safe transport and handling of germplasm within laboratories within and across countries. Higher yields and returns are expected. (Hussein, 2012). Banana has been subjected to various of in vitro techniques, including shoot regeneration from cultured tissue via organogenesis and somatic embryogenesis for micropropagation, embryo rescue, Soma clonal variation, and gene transfer via somatic hybridization and transformation. (Sipen *et al.,* 2011).

MATERIALS AND METHODS

Study area. Tissue culture Laboratory Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani - 431402 (Maharashtra) from October 2020 to January 2021.

Preparation of explants. The explant were selected from farmer field selected sucker carefully removed from mother plant we chose a one-year old Banana orchard suckers 40-100 cm height are most commonly used as explant.

Surface sterilization of explant. It was then washed with running tap water to remove the sticky soil did his first chopping after chopping he was soaked in water containing Bavistin for 7 hours, the fungus and fungus spores remove explants was then taken to the lab and washed with tap water then soak in detergent for 30 Minutes after that they are washed in tap water washed suckers are transferred to a laminar flow chamber for further sterilization. The suckers are sterilized in the chamber using mercuric chloride. The sucker was first sterilized with 0.12 percent $HgCl_2$. The sucker put into the bottle containing $HgCl_2$. Shake the flask well for 2 min. then Again treated with Sodium hypochlorite for 1 min and wash sucker with double distilled water this step repeated for 2 min, 3 min, 5 min, 12 min. After the sterilization layer of sucker remove carefully on Laminar hood.

Experimental treatment and design. In MS Medium preparation (Table 1) for shoot Proliferation Concentration of Growth regulators (BAP: 0.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0) and for Root Proliferation (IBA: 0.5, 1.0, 1.5) + (AC: 1.0, 1.5, 2.0) After initiation callus form in 10 Days. First multiplication done in 10 days second multiplication after 15 days. Reading will taken 45 and 55 days. All cultures were maintained at 22°C with a 16/8 h light/dark cycle. Illumination was provided by white fluorescent light with an intensity of 120 mol m^{-2} s^{-1}. The plantlets are then placed in Rooting Medium. It will take between 75 and 95 days to root reading. The study used a Complete Randomized Design (CRD) with five replications and nine treatments. The data acquired, such as the number of shoots, the length of the shoots, the number of leaves, the length of the leaves, and the number of roots and the length of the roots, were evaluated using statistical significance one factor opstat.

Table 1: Ms composition (Murashige and Skoog 1962).

Components	Concentrations(gm)	Components	Concentrations(gm)
Stock A: Micro Element	For 2 Lit	Stock B: Macro Element	For 2 Lit
Boric acid	1.55 gm	Potassium Nitrate	48 gm
Caboltous Chloride	63 (mg)	Ammonium Nitrate	41.5 gm
Zinc Sulphate	2.15 gm	Calcium chloride	11 gm
Magnesium sulphate	6 gm	Magnesium sulphate	9.5 gm
Molybdenum	63 (mg)	Potassium Dihydrogen	4.5 gm
Copper sulphate	63 (mg)		
Potassium Iodide	2.75 (mg)		
Stock C	For 500 ml	Stock E	For 500 ml
EDTA	1.800 (mg)	Myo-Inositol	50 (mg)
Stock D Vitamin	For 250 ml	Growth regulators	For 500 ml
Pyridoxin HCL	250 (mg)	BAP	1 gm dissolve in 10 ml NaOH
Thiamine HCL	250 (mg)	IBA	1 gm dissolve in 10 ml NaOH

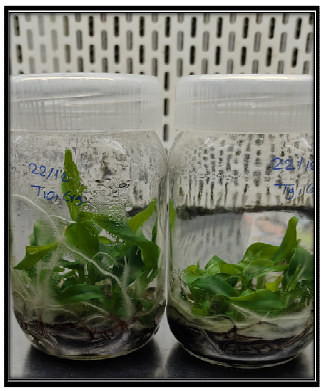

Fig. 1. Highest length, Number of Root in Media containing IBA + Activated Charcoal.

RESULT AND DISCUSSION

A. Shoot Proliferations. Number of shoots

The different concentration and its effect of BAP on banana (cv. *Grand naine*) Number of shoots were recorded at two stage 45 days after 55 days after represented in Table 2.

The highest number of the shoot after 45 days was observed 4.66 that was recorded in T_7 (4.0 mg/1 BAP) which is followed by 3.5 in T_5 (3.0 mg/1 BAP) then third highest 3.16 in T_6 (3.5 mg/1 BAP) also the medium number of shoot recorded was 2.66 in T_9 (5.0 mg/1 BAP) then 2.83 in T_4 (2.5 mg/1 BAP) and the lowest number of shoot was observed 1.0 in T_1 (0.0 mg/1 BAP). While the number of shoot similarly which was recorded after 55 days that was observed the highest number of shoot 6.16 recorded in T_7 (4.0 mg/1 BAP) followed by 5.00 in T_5 (3.0 mg/1 BAP) then third highest was 4.16 in T_6 (3.5 mg/1 BAP) also the medium number of the shoots recorded 3.66 in T_9 (5.0 mg/1 BAP) then 3.33 in T_4 (2.5 mg/1 BAP) and the lowest numbers of shoots was observed 1.5 in T_1 (0.0 mg/1 BAP) (Table 2). Our result was supported and similar result was observed Khatun *et al.* (2017) when good number of shoot in 32 days is (4.60) proliferation was achieved at medium supplemented with (4.0 mg/ L BAP) and also in 48 days observed (6.80) number of proliferation was achieved at medium supplemented (4.0 mg/ L BAP). Sazedur *et al.* (2013) where they observed the most shoots (5.9) for each explant at 4.0 mg/l BAP utilising Banana (Musa sp.) cv. Agnishwar Al-Amin *et at.* (2009). It was discovered that if the BAR1 Banana-1 explant in the culture media is not infected by fungus or bacteria, the explant grows just a single shoot in the long run. The superiority of BAP over other cytokinins in the proliferation of shoot tips has also been observed in other banana cultivars. However, our current results differed from. Rahman *et al.* (2004) who found the highest number of shoot (4.52) at the combination of 1.5 mg/l BAP.

(i) Shoot Length. The different concentration and its effect of BAP on banana (cv. *Grand naine*) shoot length were recorded at two stage at 40 days after and 55 days after represented in (Table 2).

The highest length of shoot 4.56 that was recorded in T_7 (4.0 mg/1 BAP) followed by 4.38 in T_9 (5.0 mg/1 BAP) and then 3.61 recorded in T_5 (3.0 mg/1 BAP) lowest length of shoot was observed 1.161 in T_1 (0.0 mg/1 BAP) which are significant at 45 days after. While 5.76 longest shoot that was observed in T_7 (4.0 mg/1 BAP) followed by 5.35 in T_9 (5.0 mg/1 BAP) and then 3.85 recorded in T_5 (3.0 mg/1 BAP) lowest length of shoot was observed 1.41 in T_1 (0.0 mg/1 BAP) which are significant at 55 DAS. respectively (Table 2). Our result was supported and similar result was observed by Fahima *et al.* (2013) who observed longest shoot length 4.0 and 6.1 was achieved at 4.0 mg/l BAP while the minimum shoot length was observed 1.5 and 2.2 cm at 0.0 mg/l BAP at 40 and 55 DAS respectively. Also found similar result Reddy *et al.* (2014) He observed longest length of shoot 3.95 using (1.5 mg/l BAP) recorded in 45 Days. However, our present result were different from Rahaman *et al.* (2004) BARI Banana-I produced the longest shoots at 5.0 mg/L BAP (3.62 cm), followed by 1.5 mg/l NAA and 4.0 mg/L BAP (3.40 cm). They also found the smallest shoot length (1.05 cm) in the control treatment, which missing growth hormones.

(ii) Number of Leaves. The different concentration and its effects of BAP on banana (cv. *Grand naine*) Number of leaves were recorded at two stage 40 days after and 55 days after represented in (Table 2).

The highest number of leaves 3.66 was recorded in T_7 (4.0 mg/1 BAP) followed by 3.5 in T_5 (3.0 mg/l BAP) and then 3.00 was recorded in T_6 (3.5 mg/l BAP) and lowest number of leaves 1.00 was recorded in T_1 (0.0 mg/1 BAP) which are significant at 45 days after. while 4.5 in T_7 (4.0 mg/1 BAP) followed by 4.16 in T_5 (3.0 mg/l BAP) and then 3.83 observed in T_9 (5.0 mg/l BAP) and lowest number of leaves 1.33 recorded in control T_1 (0.0 mg/l BAP) respectively at 55 days after (Table 2). Our result was supported and similar result was found Fahima *et al.* (2013) Who recorded the highest number of leaves (3.01. 3.29 and 4.24) at 2, 4 and 6 WA. respectively was produced on the medium supplemented with (4.0 mg/l BAP) while the second highest number of leaves (2.6, 3.1 and 3.5) at 2, 4 and 6 WA. medium supplemented with (3.0 mg/l BAP) and also he found lowest number shoot (0.60, 1.0 and 1.6) at 2, 4 and 6 WA. respectively was produced on the medium supplemented with control treatment (0.0 mg/l BAP). According to Chariya *et al.* (2012). In media containing 5.0 mg/L BAP, 2-4 leaves with healthy shoot development were observed, while in media containing 6.0 mg/L BAP, two leaves with slight healthy shoot development were observed in Musa sp. However, our present result were different from, Gebeyehu (2013). The medium supplemented with 5.00 mg/l BAP + 0.50 mg/l NAA produced the most leaves (2.33, 3.00, 3.67 and 4.33 leaves per explant), while the medium supplemented with 5.0 mg/l BAP + 1.0 mg/l NAA produced the second highest number of leaves (2.00, 2.67, 3.3, and 4.00 leaves per explant) at 10, 20, 30, and 60 DAI, respectively.

(iii) Leaves Length: The different concentration and its effect of BAP on banana (cv. *Grand naine*) Leaves length were recorded at two stage 40 days after and 55 days after represented in (Table 2).

The highest length of leave 2.97 was recorded in T_5 (3.0 mg/l BAP) followed by 2.88 length of leaves recorded

in T_7 (4.0 mg/l BAP) and then 2.85 length of leave recorded in T_9 (5.0 mg/l BAP) and lowest length of leaves was recorded 1.1 in T_1 (0.0 mg/l BAP) which are significant at 45 days after. While the highest length recorded 4.13 was recorded in T_5 (3.0 mg/l BAP) followed by 4.00 length of leaves recorded in T_7 (4.0 mg/l BAP) and then 3.98 length of leave recorded in T_9 (5.0 mg/l BAP) and lowest length of leaves was recorded 1.48 in T_1 (0.0 mg/l BAP) which are significant at 55 days respectively (Table 2). Our result was supported and similar result was found Fahima *et al.* (2013) who recorded the highest number of leaves (2.0, 2.7 and 4.2) at 2, 4 and 6 WA. respectively was produced on the medium supplemented with (3.0 mg/l BAP) while who recorded lowest length leaves (0.50, 1.0 and 1.7) at 2, 4 and 6 WA. medium supplemented with (0.0 mg/l BAP). However, our present result were different from Gebeyehu (2015) at 10, 20, 30, and 60 DAI, the explants with the longest leaves (1.4, 2.2, 2.8, and 3.2 cm) were grown with various composition of 5.0 mg/l BAP + 0.5 mg/l NAA. Al-amin *et al.* (2009) obtain longest leaves 0.85. 2.70, and 4.23 cm with 7.5 mg/L BAP + 0.5 mg/L NAA at 10, 20, and 30 DAI using BARI Banana-i, respectively. Variations in findings achieved by different authors might be attributed to differences in genotypes and explants used.

Table 2: Effect of Different concentration of BAP on shoot, leaves, proliferation.

Treatments	BAP	No. of multiple shoots		Length of multiple shoots		No. of multiple Leaves		Length of multiple Leaves	
	Mg/l	45 Days	55 Days	45 Days	55 Days	45 Days	55 Days	45 Days	55 Days
T_1	0.0	1.00	1.5	1.18	1.41	1.00	1.33	1.1	1.48
T_2	1.5	2.16	2.66	2.65	3.23	2.16	2.83	1.82	1.87
T_3	2.0	1.66	2.16	2.00	2.2	1.16	1.83	1.42	1.97
T_4	2.5	2.83	3.33	1.68	1.65	2.83	3.33	2.82	3.33
T_5	3.0	3.5	5.00	3.61	3.85	3.5	4.16	2.97	4.13
T_6	3.5	3.16	4.16	3.5	4.1	3.00	3.66	2.77	3.23
T_7	4.0	4.66	6.16	4.56	5.76	3.66	4.5	2.88	4.08
T_8	4.5	2.16	2.66	2.11	2.16	2.00	2.66	1.92	2.27
T_9	5.0	2.66	3.66	4.38	5.35	2.5	3.83	2.85	3.98
	C.D	1.61	2.08	1.63	1.94	1.72	2.04	1.39	1.68
	SE (m)	0.56	0.72	0.57	0.68	0.60	0.71	0.48	0.58
	SE (d)	0.8	1.03	0.81	0.96	0.85	1.01	0.69	0.83

B. Root Proliferations

(i) Number of Roots. The different combination and its effect of IBA and AC on banana (cv. *Grand naine*) Number of root were recorded at two stage 75 days after and 95 days after represented in (Table 3).

The highest number of root 4.0 was recorded in T_9 (2.0 g/l AC + 1.5 mg/l IBA) followed by 3.3 recorded in T8 (2.0 g/l AC + 1.0 mg/l IBA) then 3.0 number of root recoded in T_7 (2.0g/l AC + 0.5 IBA) and lowest number of root 1.66 recorded in T_1 (1.0 g/l AC + 0.5 IBA) which were significant at 75 days after. While highest number of root 5.0 was recorded in T_9 (2.0 g/l AC + 1.5 mg/l IBA) followed by 4.66 recorded in T_8 (2.0 g/l AC + 1.0 mg/l IBA) then 4.0 number of root recoded in T_7 (2.0g/l AC + 0.5 IBA) and lowest number of root 2.5 recorded in T_1 (1.0 g/l AC + 0.5 IBA) which were significant at 95 days after (Table 3). Our result supported and similar result reported by Uzaribara *et al.* (2015) who recorded more number of root 4.0 in different concentration of (2.0 g/l: AC + 1.5 mg/l IBA) followed by who record 3.3 number of root in (2.0g/l: AC + 1.0 mg/l IBA) then 3.2 number of root found in (2.0 g/AC + 0.5mg/l IBA) respectively in 60 DAS. while highest number of root 4.9 recorded in (2.0 g/l + 1.5mg/ IBA) followed by 4.7 number of root found in (2.0g/l AC + 1.0mg/l IBA) significant at 75 DAS. However, our present result different from Kelta *et al.* (2018). The largest mean number of roots was generated at 1.5 mg/l IBA (5.12 and 4.69) in poyo and Giant cavendish, respectively. After four weeks of inoculation, the treatment 1.5 mg/l IBA produced 5.12 mean number of root in poyo and 4.69 mean number of root in Giant cavendish.

(i) Root Length. The different combination and its effect of IBA and AC on banana (cv. *Grand naine*) Length of Roots were recorded at two stage 75 days after and 95 days after represented in (Table 3).

The highest length of roots 6.3 was recorded in T_9 (2.0 g/l AC + 1.5 mg/l IBA) followed by 5.31 recorded in T_8 (2.0 g/l AC + 1.0 mg/l IBA) then 4.6 length of root recoded in T_7 (2.0g/l AC + 0.5 IBA) and lowest length of root 1.95 recorded in T_1 (1.0 g/l AC + 0.5 IBA) which were significant at 75 days after. While highest length of root 8.25 was recorded in T_9 (2.0 g/l AC + 1.5 mg/l IBA) followed by 7.35 recorded in T_8 (2.0 g/l AC + 1.0 mg/l IBA) then 7.1 length of root recoded in T7 (2.0g/l AC + 0.5 IBA) and lowest length of root 2.45 recorded in T_1 (1.0 g/l AC + 0.5 IBA) which were significant at 95 days after (Table 3). Our result supported and similar result reported by Uzaribara *et al.* (2015) Who recorded highest length of root 6.4 in concentration of AC+IBA (2.0 g/l :AC + 1.5 mg/l IBA) followed by who recorded 5.5 length of root in (2.0g/l: AC + 1.0 mg/l IBA) and also reported 4.7 length of root (2.0 g/AC + 0.5mg/l IBA) respectively in 60 days They also found highest length of root 8.9 in concentration of AC+IBA (2.0 g/l :AC + 1.5 mg/l IBA) followed by who recorded 7.7 length of root in (2.0g/l: AC + 1.0 mg/l IBA) and also reported 7.3 length of root (2.0 g/AC + 0.5mg/l IBA) respectively in 75 days. However, our present result different from Paulos *et al.* (2015), who record the longest root length 5.7 and 6.7 were measured on media composition supplemented with 0.50 mg/l IAA while the minimum value recorded respectively.

Table: 3: Effect of different growth regulators diff concentration on Root proliferation.

Treatments	AC + IBA	Number of multiple Roots		Length of multiple Roots	
	gm/l + mg/l	75 Days	95 Days	75 Days	95 Days
T_1	1.0 + 0.5	1.66	2.5	1.95	2.45
T_2	1.0+ 1.0	2.16	3.0	3.06	3.6
T_3	1.0 + 1.5	1.83	2.5	2.11	2.58
T_4	1.5 + 0.5	1.5	2.16	3.11	3.3
T_5	1.5 + 1.0	2.83	4.5	3.75	4.81
T_6	1.5 + 1.5	2.66	3.83	3.4	3.6
T_7	2.0 + 0.5	3.0	4.0	4.6	7.1
T_8	2.0 + 1.0	3.3	4.66	5.31	7.35
T_9	2.0 + 1.5	4.4	5.0	6.3	8.25
	C.D.	1.51	1.90	1.67	1.85
	SE(m)	0.53	0.66	0.58	0.65
	SE(d)	0.75	0.94	0.82	0.92

Fig. 2. Steps follow during experiment.

CONCLUSIONS

Application BAP Growth hormone supplemented on MS culture medium at 4.0 mg/l and 5.0 mg/l performs better for new plantlet regeneration capabilities via in vitro shoot-tip culture. BAP concentration of 4.0 mg/l was shown to be the optimum concentration for shoot proliferation, shoot elongation, and leaf proliferation in the Banana cv. Grand Naine variety. For Root Proliferation and Root Elongation, Activated Charcoal (AC) is ideal for the combination with IBA growth regulators This combination gives the highest number

of roots (2.0 g/l AC + 1.5 mg/l IBA) and the longest root length (2.0 g/l AC + 1.5 mg/l IBA) suitable for rooting proliferation alone IBA is not that much effective if we use Activated charcoal combine with IBA is best for Root proliferation early growth in tissue culture Banana on commercial production.

FUTURE SCOPE

Future scope of this study is that to produce Banana planting material using Growth regulators with Activated charcoal for better rooting helps for anchorage of the plant, absorption of water and dissolved minerals and conduction of these to the stem, and storage of reserve foods in banana plant.

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Conflict of Interest. None.

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