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Influence of plant growth regulators on macro propagation in different banana genotypes

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ABSTRACT

In vivo macro-propagation of banana has gained its momentum as an alternative source for mass multiplication of quality planting materials following some principles like decapitation, decortication of apical meristem of corm and hardening. 6-Benzylamino purine (BAP) @ 40ppm and 60ppm and Indole-3-butyric acid (IBA) @1000 ppm and 2000 ppm were used for locally grown banana genotypes *viz*. Bharatmani, Chini champa and Bhootmanohar to assess the response of plant growth regulators on shoot and root proliferation. The diseases free uniform corm and sterilized sawdust as uniform rooting media were used in experiment under polyhouse condition. The apical meristem of corm was scooped out with sterilized knife to make a cavity. The growth regulators BAP and IBA) were injected into the cavity of apical meristem 2 days after planting. Proliferation of primary (3.33 and 3.67) and secondary (4.0 and 4.33) buds were noticed significantly by Bharatmani and Chini champa treated with BAP @ 40 ppm and also took less time for bud proliferation.The genotype Chini Champa treated with BAP @ 40 ppm generated maximum number of plantlets per corm (14.67) while the highest number of explants (13.66) per corm after detaching was obtained by Bharatmani using BAP @ 40 ppm.

Figure : 00 References : 16 Tables : 02
KEY WORDS : Banana, BAP, IBA, Macro-propagation, Shoot proliferation

Introduction

Banana (*Musa* spp) is an important perennial herbaceous plant grown in tropical and sub-tropical countries of the world under the family Musaceae. India takes the credit of highest production contributing to 27% share in world. But quality planting materials is still a great challenge in farming community in India. Farmers generate 90 percent planting materials through sword sucker from their own field that is not sufficient for commercial cultivation and mostly infested by diseases and pest in old orchard.Though mass multiplication of tissue culture banana was introduced for rapid production of healthy, vigorous and diseases free planting materials no doubt but the adoption of this method is limited due to high cost of micro propagated plantlets, high investment and lack of knowledge at farmer's level. The *in vitro* technique therefore becomes a very difficult option to adopt for a marginal farmer and moreover tissue culture banana are 4-8 times higher than the natural produced sucker's cost. Hence, an alternative method for rapid multiplication as well as healthy planting material called macro propagation was promoted to increase sucker multiplication at farm levels and to bridge up the gap in supply of healthy planting materials with an affordable cost[5]. Thus, macro-propagation method has been considered as an excellent option, cost effective, easily accessible and affordable for poor farmers and requires little care in banana afflicted areas to overcome the problems for quality planting materials of local genotypes in a short span of time[14]. Macro propagation though depends on its genotype, can produce 8-15 new plants per corm and secondary

scarification of new buds can produce plantlets by a factor of 2-3 times. The principle behind macro-propagation technique is existing fact that many side suckers are induced by suppressing the apical dominance[4,8]. The plantlets obtained by this method are more or less the same with micro propagated plantlets with an advantage of being less prone to post establishment factors in the field[11]. The experiment on macro propagation was undertaken to intensify the standard protocols and technology for mass multiplication of high value local genotypes and make it available easily among the farming community.

Materials and Methods

The experiment was carried out at Instructional cum research farm, Department of Horticulture, SAS, Nagaland University, Medziphema campus during 2019-20.The experimental plot represented sub-humid and sub-tropical climate with moderate temperature prevailing 20ºC to 35ºC during summer and rarely below 8ºC in winter and moderate to high rainfall (2000-2500 mm per annum) with atmospheric humidity varying from 75 to 85%. The experiment was laid out in complete randomized design (CRD) with fifteen treatments and three replications. Three varieties *viz.* Bharatmani, Chini Champa, Bhootmanohar were taken under this experiment on the basis of commercial importance of locally available banana in Nagaland. Healthy diseases free uniform sword suckers of about three months old were collected from well-maintained farm. The treatments were comprised of: T_1: Bharatmani(without growth regulator), T_2: Bharatmani+BAP @ 40ppm, T_3: Bharatmani+BAP @ 60ppm, T_4: Bharatmani+IBA @ 1000ppm T_5: Bharatmani+IBA @2 000ppm T_6: Chini champa(without growth regulator), T_7: Chini champa+BAP @ 40ppm T_8: Chini champa+BAP @ 60ppm T_9: Chini champa+IBA @ 1000 ppm T_{10}: Chini champa+IBA @ 2000ppm T_{11}: Bhoot Manohar (without growth regulator), T_{12}: Bhootmanohar+BAP @ 40ppm T_{13}: Bhootmanohar+BAP @ 60ppm, T_{14}: Bhootmanohar+IBA @ 1000ppm, T_{15}: Bhootmanohar+IBA @ 2000ppm**.** The cleaned and decapitated corms were kept under shade for 2-3 days. The apical meristem was scooped out making a cavity of 2 cm diameter and crosswise incision was given to induce lateral bud stimulation.Then the meristematic cavity of corms were injected with 4 ml of BAP and IBA solution in prescribed concentration before drying up of cavity and the same was again repeated after decapitation of primary shoot to induce secondary bud. The treated corms were air-dried under shade for a day before planting in rooting media. The corms were planted in holes measuring 45×45 cm (fully buried) in the sterilized saw dust with following corm spacing at 15cm×15 cm apart under poly house condition. The plots were sprinkled with water after planting to maintain sufficient moisture level in the substrate under poly house condition.After 3-5 weeks of planting, primary buds appeared. These buds were decapitated when they reached a height of 10-17 cm by destroying the young meristems and 3-4 horizontal cuts were given to induce secondary buds. The secondary buds were harvested when it attained 2-3 well developed leaves and were detached carefully with attached roots. The secondary plantlets were detached from the corm with a sterilized knife. Plantlets with roots were directly planted in polybags filled with prepared rooting media.

Results and Discussion

The equal and uniform size of corm was selected to reduce the experimental error, since the reserved food materials inside the corm play a significant role for induction of primary and secondary buds. The weight of the individual corms under different genotypes varied from 1.62 to 1.77 g (1.68±0.04 g).

Induction of primary and secondary shoots

There was a significant difference for induction of primary and secondary bud emergence using different concentration of growth regulators in different genotypes after decapitation (Table-1). It took around 27.73±1.33 days for primary and 31.93 ± 2.05 days for secondary bud initiation. The minimum duration was noticed in Bharatmani genotype treated with BAP @ 40ppm both in primary (18 days) and secondary (27.33) bud emergence. The genotype Bhootmanohar had taken up maximum duration for primary (38.0 days) and secondary bud (35.0 days) initiation. Rapid multiplication of cell might be the reason for early bud emergence and unfurling of leaves in sucker treated with BAP. The scientist[16] also reported the effectiveness and significant influence in banana bud emergence by application of BAP @ 40 ppm. Induction of primary (2.76±0.58 per corm) and secondary shoot (3.11±0.16 per primary bud) was achieved by using BAP and IBA solution by decortication of apical meristem of corm and primary bud at 2-3 leaf stage. The proliferation of primary bud from a mother corm varied from 1.67 to 3.33 at 27.73±1.33 days after planting the corm in sawdust. The induction of secondary bud was induced from primary bud by injecting 4 ml IBA and BAP solution in different concentrations. The induction of secondary bud varied from 2.33 to 4.33 after decapitation of a primary shoot. The maximum induction of secondary shoot (4.33) from a primary bud was obtained in Bharatmani treated with BAP @ 40 ppm. The genotype Bharatmani, Chini champa and Bhootmanohar treated with BAP @ 40 ppm

TABLE-1: Response of different genotypes of banana with growth regulators on bud and shoot proliferation *in vivo* macro propagation

Treatment	Corm weight (kg)	Days taken for bud emergence		Number of bud emergence		Shoot height (cm)	
		Primary bud	Secondary bud	Primary	Secondary	Primary	Secondary
T_1: Bharatmani (without growth regulator)	1.62	30.67	32.00	3.00	2.33	10.03	7.30
T_2: Bharatmani+BAP @ 40ppm	1.68	18.00	27.33	3.33	4.33	16.80	11.40
T_3: Bharatmani+BAP @ 60ppm	1.71	25.67	30.00	3.33	2.33	15.10	12.10
T_4: Bharatmani+IBA @ 1000ppm	1.67	24.67	31.00	2.67	3.67	13.73	11.90
T_5: Bharatmani+IBA @ 2000ppm	1.71	26.00	31.33	2.67	3.00	13.83	11.86
T_6: Chini Champa (without growth regulator)	1.70	29.33	34.33	2.00	3.00	11.23	10.40
T_7: Chini Champa+BAP @ 40ppm	1.63	20.33	30.67	3.67	4.00	15.60	13.53
T_8: Chini Champa+BAP @ 60ppm	1.74	21.33	29.33	3.33	3.33	15.70	12.43
T_9: Chini Champa+IBA @ 1000 ppm	1.65	29.00	33.67	2.67	3.00	15.27	11.13
T_{10}: Chini Champa+IBA @ 2000ppm	1.71	30.00	33.00	3.00	3.67	12.70	11.83

Treatment	Corm weight (kg)	Days taken for bud emergence		Number of bud emergence		Shoot height (cm)	
		Primary bud	Secondary bud	Primary	Secondary	Primary	Secondary
T_{11}: Bhoot Manohar (without growth regulator)	1.63	38.00	35.00	1.67	2.67	12.60	10.90
T_{12}: Bhootmanohar+BAP @ 40ppm	1.77	31.33	32.33	3.00	3.33	14.27	10.80
T_{13}: Bhootmanohar+BAP @ 60ppm	1.63	31.67	32.33	2.33	2.33	13.03	12.10
T_{14}: Bhootmanohar+IBA @ 1000ppm	1.67	30.67	34.00	2.00	2.67	15.30	11.56
T_{15}: Bhootmanohar+IBA @ 2000ppm	1.65	29.33	32.67	2.33	3.00	13.40	12.66
Mean ±(s)	1.68±0.04	27.73±1.33	31.93±2.05	2.76±0.58	3.11±0.16	13.91±0.47	11.46±0.36
CD @ 5%	NS	6.43	3.89	0.89	1.15	3.41	2.64

showed a quite good response in proliferation and stimulation of primary and secondary buds. The genotypes treated with BAP and IBA solution resulted in higher bud emergence as compared to individual effect of different genotypes. Removal of apical dominance and application of BAP showed a significant response for lateral bud initiation that was fully supported by the scientists[13].

Shoot proliferation

The height of primary shoot during decapitation irrespective of growth regulators application was 10.03 cm, 11.23 cm and 12.60 cm in Bharatmani, Chini champa and Bhootmonohar, respectively while the genotypes treated with growth regulators, the average shoot height of primary bud increased to 14.86±1.43 cm, 14.81±1.42 cm and 14.0±1.01 cm, respectively in Table 1. Primary shoot length varied from 10.03 cm to 16.80 cm at the time of decapitation while the secondary shoot length varied from 7.30 cm to 13.53 cm at the time of separation from the mother corm. Bharatmani and Chini champa treated with BAP and IBA showed a significant difference in shoot length while there was no significant difference in Bhootmanohar groups even after using growth regulators. Similar trend was also reflected in secondary shoot growth in different genotypes using different growth regulators. The enhancement of shoot growth and height of shootlet was influenced by BAP application that might be due to role of cytokine which promoted cell division, rapid multiplication and other metabolic process. Similar findings had been observed by a group of scientists[2,7] who used BAP in plant body and saw dust and *B. subtilis* as rooting media.

The average girth size was noticed high in primary shoot (5.31±0.10 cm) over the secondary shoot (4.87±0.28 cm). Bharatmani treated with BAP @ 40ppm showed the highest girth size both in primary (6.0 cm) and secondary bud (5.36 cm). The scientists[3] also noticed a significant response on shoot proliferation and the highest shoot height using BAP @ 7.5 mgL^{-1} *in vitro* propagation of banana.

Leaf proliferation

The leaf proliferation both in primary and secondary shoots varied significantly among different genotypes using growth regulators (Table-2). The number of leaves varied between 1.94 to 2.72 in primary shoot and 1.94 to 2.67 in secondary shoot at 45 days after decapitation of primary shoot. It was assumed that the variation in leaves might be due to genetic make up and heredity of the variety interacted with environment as well as growth regulators. The highest leaf length in primary bud was found in Bharatmani (16.15 cm) and Chini champa (16.14 cm) treated with BAP @ 40 ppm. Length of leaves in secondary shoots was found to vary in between 6.78 to 12.42 cm (mean value 10.35±0.41 cm) in different genotypes using different growth regulators. Maximum length (12.42 cm) of secondary leaves was recorded in Bharat Mani + BAP @40ppm followed by Chini champa + BAP @ 40ppm (11.60 cm) while the minimum length was recorded in Bharat Mani (6.78 cm) and Bhootmanohar (7.65 cm). The corm treated with BAP @ 40ppm recorded the maximum number of leaves proliferation among all the genotypes compared to individual genotypes. The scientist found that the corms treated with Thidiazuron (TDZ), a urea based cytokinin @ 2.0 mgL^{-1} produced the largest number of leaves per sucker[15]. The result was also quite agreed with a group of scientists[4,15,16] who found a significant response in leaf proliferation *in vivo* macro propagation technique in banana.

Plantlets raised from corm

In vivo macro propagation of different genotypes treated with BAP and IBA showed a significant variation in total number of explants collected from a corm. Maximum number of plantlets (14.67) per corm was obtained in Chini champa treated with BAP @40ppm followed by Bharat Mani + BAP @40ppm (14.00). Among the different genotypes, maximum plantlets (7.33) per corm was obtained by Bharatmani irrespective of application of growth regulators while minimum plantlets were recorded in Bhootmanohar (4.67). The results clearly showed that injection in meristematic cavity with different concentration of growth regulators have significant influence to grow up the explants in corm. The genotypes treated with BAP @40ppm recorded the highest number of explants per corm over the individual genotypes. Earlier scientists[2] treated the corms using BAP @ 40 ppm that resulted in production of 25–27 numbers of uniform tertiary suckers.

Roots induction

The average number of roots in secondary shootlet was 10.87±1.70 after 15 days of separation from corm and planted in polybags. The induction of roots was found to vary from 7.50 to 13.94 in different treatments in Table 2. The maximum number of roots (13.94) per shootlet was recorded in Chini Champa + IBA @ 1000ppm followed by Bharat Mani + IBA @2000 ppm (13.50/plantlet) while minimum was recorded in Bharat Mani (7.50/plantlet) followed by Bhootmanohar (8.78/plantlet). The worker found that corms treated with IBA @ 0.25% prior to planting in saw dust and *azospirillum* induced a greater number of root formation in banana plantlet[12]. Induction and promotion of root elongation in banana was due to analogue of auxin hormone[1,6]. Investigators found better response to IBA

TABLE-2: Influence of physiological growth in different genotypes of banana with growth regulators *in vivo* macro-propagated explants

Treatments	Girth size (cm)		No. of leaves per explants		Length of leaves (cm)		Total no. of plantlets per corm	Plantable explants per corm	No. of roots per shootlet
	Primary	Secondary	Primary	Secondary	Primary	Secondary			
T_1: Bharatmani (without growth regulator)	4.50	4.30	2.31	1.94	10.16	6.78	7.33	6.00	7.50
T_2: Bharatmani+BAP @ 40ppm	6.00	5.36	2.72	2.00	16.15	12.42	14.00	13.66	10.30
T_3: Bharatmani+BAP @ 60ppm	5.77	5.30	2.58	1.94	15.55	11.39	7.67	7.00	11.67
T_4: Bharatmani+IBA @ 1000ppm	5.20	4.86	2.61	2.11	15.57	11.23	10.00	9.66	12.38
T_5: Bharatmani+IBA @ 2000ppm	5.10	4.76	2.52	2.50	13.36	9.36	8.00	7.00	13.50
T_6: Chini Champa (without growth regulator)	4.86	4.73	2.27	2.36	11.91	11.60	6.00	5.66	9.68
T_7: Chini Champa+BAP @ 40ppm	5.36	5.23	2.55	2.67	16.14	11.54	14.67	13.33	10.17
T_8: Chini Champa+BAP @ 60ppm	5.43	4.90	2.38	2.19	15.67	11.42	11.33	9.67	10.83
T_9: Chini Champa+IBA @ 1000 ppm	5.33	4.86	2.60	2.10	13.75	10.75	8.00	6.33	13.94
T_{10}: Chini Champa+IBA @ 2000ppm	5.60	4.60	2.55	2.22	13.16	11.36	11.00	9.67	10.00

Treatments	Girth size (cm)		No. of leaves per explants		Length of leaves (cm)		Total no. of plantlets per corm	Plantable explants per corm	No. of roots per shootlet
	Primary	Secondary	Primary	Secondary	Primary	Secondary			
T_{11}: Bhoot Manohar (without growth regulator)	5.03	4.63	2.16	2.44	11.58	7.65	4.67	4.00	8.78
T_{12}: Bhootmanohar+BAP @ 40ppm	5.53	4.90	2.55	2.30	12.06	10.58	10.00	9.67	10.23
T_{13}: Bhootmanohar+BAP @ 60ppm	5.10	4.76	2.55	2.00	13.17	10.60	5.33	5.00	11.17
T_{14}: Bhootmanohar+IBA @ 1000ppm	5.16	4.93	1.94	2.11	12.85	10.05	5.33	4.66	10.72
T_{15}: Bhootmanohar+IBA @ 2000ppm	5.70	4.96	2.00	2.55	13.73	8.57	7.00	6.33	12.25
Mean ±(s)	5.31±0.10	4.87±0.28	2.42±0.23	2.23±0.23	13.65±0.47	10.35±0.41	8.81±3.15	7.84±0.77	10.87±1.70
CD @ 5%	0.67	0.49	0.39	0.43	0.98	0.89	3.98	2.55	2.77

@ 5ppm for rooting of plantlets in different banana varieties[13].

Conclusion

The findings provided that using of BAP @ 40ppm accelerated more number of suckers with a short span of time from a mother corm to get quality and genuine planting materials. The genotype Bharatmani responded the better performance in shoot proliferation and other growth characters.

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