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Effect of Cytokinin Concentrations on Shooting ability of plantain Genotypes; 'Efol' and Pita 14.

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ABSTRACT

This study was aimed at determining the effect of different6-Benzylaminopurine (BAP) on shooting abilities of two plantain cultivars ('Efol' and Pita 14). The shoot tips of each genotype were isolated using appropriate protocol for shoot tip isolation under aseptic conditions. After which they were cultured on media supplemented with BAP at four different concentrations being 0.00 mg/l, 0.0025 mg/l, 0.005mg/l and 0.0075 mg/l,and on three growth environments (growth room, bench top and screen house). The results indicated that the number of replication increased at 0.0025 mg/l and 0.005mg/l, with optimum replication observed at 0.005 mg/l for 'Efol' genotype in growth room and screen house and Pita 14 genotypein growth room. Significant difference was determined at $P < 0.05$ and observed for 'Efol' cultured in growth room and screen house and also for Pita 14 cultured in growth room. The research has shown that BAP concentration at 0.005 mg/l is optimum for both genotypes and bench top is not conducive for in vitro multiplication.
Keywords: Cytokinin, concentration, shooting, genotypes and Efol, Pita 14

INTRODUCTION

Nigeria produces plantain in large quantity but despite its prominence, she does not feature among plantain exporting nations because it produces more for local consumption than for export [1]. In recent years, plantain consumption has grown rapidly due to increasing population and urbanization and the demand for easy and convenient food by the populace. Besides being the staple for many people in more humid regions, plantain is a delicacy and favoured snacks for people even in other ecologies. A growing industry, mainly plantain chips, is believed to be responsible for the high demand being experienced now in the country [2]. The establishment of plantain plantation has some economic implications, which directly or indirectly stimulates agricultural and commercial ventures.

For commercialization, it is important that consistent supplies of good quality plantain are produced. This could be achieved through tissue culture technique which provides high rate of multiplication of genetically uniform, pest and disease-free planting materials. Propagation of *Musa spp* through *in vitro* techniques has been reported [3]. This multiplication is achieved with the aid of hormones. Plant hormone such as 6-Benzylaminopurine (BAP) and kinetin are known to reduce the apical meristem dominance and induce both auxiliary and adventitious shoot formation from meristematic explants in plantain. However, however during application, its concentration varies

depending on genotype. As described by [4], concentration of exogenous cytokinin is likely the main factor affecting multiplication. Although, BAP stimulates shoot proliferation, it also has mutagenic effects at high concentration producing off type plantlets [5].

Plantains are known to be favoured under a certain environmental condition, therefore this study seeks to discover the best concentrations of cytokinin that can induce shoot proliferation in vitro on two plantain genotype ('Efol' and PITA 14) and how each respond to different treatments of cytokinin.

MATERIALS AND METHODS

Materials

[6] basal medium, ethanol, autoclave, beakers, hand gloves, laboratory coat, laminar flow cabinet, magnetic stirrer, microwave, pH reader, sterilized foot wears, surgical blade, sodium hypochlorate, sterilized knife, sucrose, agar, BAP and Indole Acetic Acid (IAA).

Method

Preparation growth media

This study was conducted at the Tissue culture laboratory of National Roots Crops Research Institute (NRCRI).The propagules used were excised from the healthy young suckers (0.75 to 1.0 m). The culture medium used for this study was prepared using 4.4g/l of [7] basal medium, stirred with 30 g/l sucrose and gelled with 8 g/l of agar. BAP of different concentration (0.00,0.0025, 0.005 and 0.0075 mg/l) was added to different media. The pH of each medium was adjusted to 5.8 using NaOH or HCl (0.1 or 1N) before autoclaving. The medium was autoclaved at 1.2 KPa and 121ºC for 20 min, and then cooled at room temperature before use.

Preparation of propagule

The pseudostem was trimmed with knife to remove superfluous corm tissue, roots and leaf sheaths. Propagules were washed thoroughly in running tap water for 120 seconds. The leaf sheaths near the bases were again removed from the pseudostem leaving the young leaves around the meristem until the shoot tip became about 2 cm in length. Surface sterilization was done under laminar airflow cabinet which was sterilized with 70% ethanol. The explants were surface sterilized with 8% sodium hypochlorite for 5 minutes. After which, they were rinsed three times with distilled water. Final removal of outer tissue of meristem was done with the aid of a sterile blade. Sterilized explants were collected individually and inoculated on different initiation MS media containing concentrations of BAP for shoot multiplication. The initiated cultures were incubated for 5 weeks aseptically at 25±1 ºC under 16 hour light and 8 hours dark environment of a growth room. The rest were kept on lab bench and screen house. Each concentration was replicated 5 times. After 5 weeks of initiation stage, contamination-free shoots with multiple shoots were separated and shoots that were single were decapitated at 7 to 10 mm height and split longitudinally into two or more parts depending on vigor and thickness. For each genotype, number of shoots per plant per concentration was recorded. After which, they were transferred to rooting medium.

In Vitro Rooting of Shoots

In root development stage, well grown shoots with expanded leaves were separated and transferred singly to fresh rooting culture medium supplemented with0.2 mg L^{-1}ofIAA.

Hardening of Plantlets

After two weeks of culture into rooting media, elongated and rooted plantlets (about 6 cm with 3 to 4 leaves) were taken out from culture vessels and roots were carefully washed thoroughly with running tap water to remove the agar. Individual plantlets were then transferred into small nylon pots filled with mixture substrates (sand to soils) in a 1:3 ratios. Plants were kept inside chambers covered with transparent plastic for a week in the screen house to maintain high humidity (80 to 90%) for hardening. The plastics were gradually perforated and the plantlets exposed gradually to the

environment. After which, the plantlets were fully exposed and ready for field establishment

Data Analysis

The data was analysed with analysis of variance (ANOVA) to obtain their relationship and least significance difference (LSD) used to separate their mean.

RESULTS

Table 1 below shows the effect of different concentration of BAP on 'Efol' genotype. 0.00 mg/l concentration of cytokinin had the least shooting ability, with the mean shoot number 0.80±0.20 at growth room, 0.60±0.24 at bench top and screen house average number of 0.60±0.24. At 0.0025 mg/l, growth room has mean replication value of 2.40±0.60, 0.60±0.24 at bench top and 1.80±0.66 at screen house.0.005 mg/l had the highest replication value of 3.40±0.80 at growth room, 0.80±0.20 at bench top and 5.40±1.67 at screen house. Finally, at 0.0075 mg/l, growth room mean replication value was 1.00±0.32, bench top was 0.40±0.24 while screen house was 1.00±0.45. Significant difference was observed in growth room and screen house at $p < 0.05$ while there was no significant replication in the bench top medium at $p < 0.05$.

Table 1: The mean and standard deviation of shooting ability of 'Efol'

S/N	Concentration (mg/l)	Shoot number in growth room*	Shoot number on bench top	Shoot number in screen house*
1	0.00	0.80±0.20	0.60±0.24	0.60±0.24
2	0.0025	2.40±0.60	0.60±0.24	1.80±0.66
3	0.005	3.40±0.80	0.80±0.20	5.40±1.67
4	0.0075	1.00±0.32	0.40±0.24	1.00±0.45

* = significant difference at P < 0.05

Table 2shows the effect of different concentration of cytokinin (BAP) on Pita 14 genotype. 0.00 mg/l concentration of cytokinin also had the least replication value, with the mean replication of 0.60±0.24 at growth room, 0.60±0.25 at bench top and screen house replication of 0.60±0.25. At 0.0025 mg/l, growth room had mean replication value of 1.60±0.51, 0.80±0.20 at bench top and 0.80±0.20 at screen house. 0.005 mg/l had the highest replication value of 3.40±0.68 at growth room, 1.00±0.00 at bench top and 1.20±0.20 at screen house. Finally, at 0.0075 mg/l, growth room mean replication value was 0.80±0.49, bench top was 0.60±0.25 while screen house was 0.60±0.25. There was a significant difference in replication at growth room at $p < 0.05$.

Table 2: The mean and standard deviation of shooting ability of Pita 14

S/N	Concentration (mg/l)	Shoot number in growth room*	Shoot number on bench top	Shoot number in screen house
1	0.00	0.60±0.24	0.60±0.25	0.60±0.25
2	0.0025	1.60±0.51	0.80±0.20	0.80±0.20
3	0.005	3.40±0.68	1.00±0.00	1.20±0.20
4	0.0075	0.80±0.49	0.60±0.25	0.60±0.25

* = significant difference at P < 0.05

DISCUSSION

The significant difference in replication observed at different culture and growth environments (Table 1 & 2) shows that plantain are influenced by environment. Highest shooting was observed in media supplemented with 0.005 mg/l BAP, this is in contrast with [8] work which explained a high replication response at 2.50 g/dl concentration of cytokinin. But in agreement with the work done by [9] [10] whereby the optimum concentration of cytokinin for most banana cultivars was 5.00g/dl. During the present study, the saturation concentration of BAP for the both genotypes was 0.005 mg/l, beyond which multiplication rate started to decline. [11] also found that the saturation concentration of BAP for 'Basrai' was 6.0 mgL–1, beyond which multiplication rate started to decline. Higher concentrations of BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during in vitro multiplication of 'Nendran' [3].

The replication values reduced at the 0.0075 mg/l concentration in the two genotypes. This could be because the optimum concentration of the cytokinin for replication was increased. [7] observed that the number of shoots regenerated from a single explant increased with increases in BAP concentration in the medium. With BAP at 8.0 mgL^{-1}, the number of shoots regenerated from the single explants decreased, which indicated that BAP had an adverse effect when used at a higher-than optimal concentration. Similar proliferation behavior of BAP in banana was observed by [10] [11] during multiplication of 'Nzizi', 'Kibuzi', and 'Ndiziwemiti'. During in vitro multiplication of 'Ndiziwemiti' and 'Kibuzi', the rate of multiplication increased with increase in BAP concentration up to 16 mM (3.6 mgL^{-1}), beyond which it declined suddenly. Conclusively, Cytokinin (BAP) plays a good role in the replication of the interest genotypes ('Efol' and Pita 14) with optimum concentration of BAP for shoot proliferation being0.005 mg/l.

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