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The Effects of Different Concentrations Cytokinins on the *in vitro* Multiplication of Plantain (*Musa* sp.)

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Abstract: The study was aimed at determining the appropriate type and level of cytokinin required to achieve shooting response in two cultivars of plantain (Oniaba and Apantu pa). Three cytokinin types, Benzylaminopurine (BAP), Kinetin and 2ip at two different concentrations (4.5 and 7.5 mg L^{-1}) were used. The apical meristem of each cultivar was isolated using appropriate protocol for shoot tip isolation under aseptic conditions. Media supplemented with 4.5 mg L^{-1} BAP induced the highest number of shoots after eight weeks of culture. There was also a variation in the ability of the cytokinin types to induce shooting in both cultivars. BAP had the highest shoot induction response in both cultivars, followed by Kinetin and 2ip. Each hormone appeared to have an optimal level of concentration for maximum shooting. Oniaba responded favourably to BAP at 4.5 mg L^{-1} where as Apantu pa was virtually indifferent to the Kinentin types. The degree of efficiency of shooting was therefore found to be dependent on the type of hormone and the plantain cultivar.

Key words: Apical meristem, Benzylaminopurine, Oniaba, Apantu, kinetin

INTRODUCTION

Plantain is one of the staples in most West African countries including Ghana. It is consumed by almost every tribe. Apart from the Northern and Upper regions of Ghana, the crop is grown in almost every region. It is estimated that about 70 million people in West and Central Africa derive more than a quarter of their food energy requirement from plantain (IITA, 1990).

In Ghana plantain contribute 13.1% to the Agricultural Gross Domestic Products (Dankyi *et al*., 2007). It has a per capita annual consumption of 101.8 kg per head (Dankyi *et al*., 2007). Plantain has an export potential because apart from its huge consumption in Ghana, it is also consumed in most parts of Africa.

In spite of its importance in the diet of most Ghanaians and its export potential, the cultivation of the crop has however not caught up with even the domestic demands. In the past, plantain was in abundance in Ghana throughout the year but the present low production situation has arisen due to problems with planting material acquisition disease and pest problems as well as poor agronomic practices. The materials used for conventional propagation include corms, maiden, sword and peeper suckers (Cronauer and Krikorian, 1984).

There have been various innovations in trying to solve the problem of the shortage of planting material; some of these innovations are the split-corm, tissue manipulation and the decapitation techniques. All these have yielded some good results (Dore *et al*., 1983).

The Tissue Culture Technique of growing explants *in vitro*, is also another innovation which has been employed in other countries like the Philippine and Netherlands to produce large amounts of bananas, a plant which is in the same family as plantain. One of the differences between plantain and banana is the high levels of phenolic substances in the plantain, a substance that influences the proliferation of plantain under *in vitro* culture. Plantain is vegetatively propagated because almost all cultivated types are triploid, seedless or produce sterile and non viable seeds. *In vitro* propagation of bananas provides excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability of disease-free material all year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants and faster growth in the early growing stages compared to conventional materials (Daniells and Smith, 1991; Arias, 1992).

Apart from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin type and their concentration (Hamide and Pekmezei, 2004). This study seeks to try different Cytokinins (a substance

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that is used to induce shoot proliferation in tissue culture), at different levels to find out the shoot proliferation rate under the various treatments.

MATERIALS AND METHODS

The experiment was conducted at the tissue culture laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) from Nov. 27, 2008 to March 31, 2009. There were three different types of Cytokinin-based growth hormones: BAP (N^6-benzylaminopurine), Kinetin (N^6-furfurylaminopurine) and 2ip (N^6-isopentenyladenine) at two different levels 4.5 and 7.5 mg L^{-1}. These levels were chosen based on preliminary work which involved two levels of cytokinin at 3.5 and 4.0 mg L^{-1} in which the 4.0 mg L^{-1} gave more shoots compared to the 3.5 mg L^{-1}. Two plantain cultivars Oniaba and Apantu were used as the plant materials.

Murashige and Skoogs (1962) medium (MS medium), was used as the basic medium throughout the experiment. Normal MS medium was initially prepared as the initiation medium and subsequently modified the three cytokinins at two levels used as the shoot proliferation media.

The initiation medium consisted of 4.4 g L^{-1} of full strength of MS basal salts with minimal organics and the following addition of 4.5 mg L^{-1} BAP, 2.5 mg L^{-1} IAA, 30 g L^{-1} sucrose and 0.1 g L^{-1} Myo inisitol (Fisher Scientific UK Ltd, Loughborough, UK). The pH of the solution was adjusted to 5.8. Phytagel was dissolved in a portion of the solution and melted completely in a microwave oven after which it was poured back in the solution.

The medium was then dispensed as 50 mL into labeled 100 mL screw-capped glass jars. (Beatson Clark and Co. Ltd., Rottendam UK). The bottles and their contents were then autoclaved at 121°C for 15 min. Suckers of the 2 cultivars used (Oniaba and Apantu pa) were harvested and air dried under shade for about two weeks. This was necessary to reduce the phenolic content of the material. It also reduced the plant material hypotonic to its growing medium and this created a concentration gradient which helped in the osmotic or passive absorption of nutrients.

The shoot tip isolation protocol (Buah *et al*., 2000), was used to isolate the explants which were then inoculated in the medium.

The materials were then stored in a culture room at a temperature of 28°C with a 16 h photoperiod. The light intensity and humidity were 3000 flux and 60% respectively.

Sub culturing was done at four weeks after the inoculation. At this stage the roots and leaves that had appeared were removed and the materials were vertically divided into two to be placed separately in glass jars. The subculture was done in a proliferation medium as described above. The proliferation medium was an MS medium with 3 different types of hormones as follows: Kinetin, BAP and 2ip all at 2 different concentrations 4.5 and 7.5 mg L^{-1}. There was also a control treatment where no hormone was added to the culture medium. This gave a total of seven treatments and each was replicated four times.

The following data were taken at four week intervals after sub-culture.

- Number of shoots/culture
- Average number of roots/shoot
- Number of leaves/plantlet
- Shoot height/plantlet

Analysis of variance was performed using Genstat version 9 (Hilbe, 2007).

RESULTS

The results of the study showed that the two plantain cultivars responded differently to the hormones used as well as to the different concentrations.

At the end of weeks eight, Oniaba cultured on medium supplemented with 4.5 mg L^{-1} BAP produced an average of 13.25 shoots per explant compared to the 7.5 and 4.5 formed on the same concentrations of kinetin and 2ip (Table 1).

Apantu pa also followed a similar trend in its shoot proliferation ability but the number of shoots formed was fewer than that of Oniaba. It could generally be observed from the results that of the three cytokinin types used, 2ip induced the least number of shoots after the 8th week.

In terms of number of roots, number of leaves and shoot height, BAP did not exhibit superiority in all of these parameters. 2ip at 4.5 mg L^{-1} gave more roots 6.0 per plant in Apanta pa, followed by BAP at 4.5 mg L^{-1} that gave mean root of 5.3 per plant.

The number of roots formed on medium supplemented with BAP at both concentrations was lower, 2.0 and 1.5 per plant than those formed on medium supplemented with 2ip at both concentrations 3.0 and 2.0 mean roots per plant Table 2.

For mean number of leaves Kinetin at 4.5 mg L^{-1} gave the highest value 3.3 per plant for Apantu pa and Oniaba had the highest leave number on BAP 4.5 mg L^{-1}. Mean shoot height was highe,r 39.5 cm, in Apantu pa cultured on 4.5 mg L^{-1} BAP while in Oniaba, the highest shoot height was achieved on medium supplemented with BAP 7.5 mg L^{-1}.

Table 1: Average number of shoots of two plantain cultivars on two different concentrations of cytokinin for eight weeks

	BAP		Kinetin		2ip		
Hormone type (mg L^{-1})	4.5	7.5	4.5	7.5	4.5	7.5	Control
Cultivar Oniaba	13.25±0.98	8.75±0.80	7.25±0.73	10.00±0.54	4.50±0.32	4.45±0.43	5.50±0.29
Apantu pa	11.25±0.68	8.50±0.71	6.25±0.32	6.75±0.56	4.00±0.40	2.75±0.30	4.25±0.18

Control: No hormone added

Table 2: Mean number of roots, leaves formed and shoot height eight weeks after culture on three cytokinin types and concentrations

	Plantain types					
	Mean No. of roots		Mean No. of leaves		Mean shoot height (cm)	
Hormone type (mg L^{-1})	Oniaba	Apantu pa	Oniaba	Apantu pa	Oniaba	Apantu pa
BAP						
4.5	2.0±0.36	5.30±0.64	3.00±0.38	2.80±0.43	13.8±0.55	39.5±0.63
7.5	1.5±0.05	2.50±0.39	2.80±0.34	1.60±0.24	25.0±1.52	29.0±1.70
Kinetin						
4.5	2.0±0.33	1.70±0.09	3.30±0.41	2.30±0.43	20.0±2.10	25.0±1.80
7.5	2.0±0.36	1.70±0.10	1.50±0.12	1.00±0.08	7.5±0.36	21.0±0.93
2ip						
4.5	3.0±0.51	6.00±0.42	1.00±0.31	1.00±0.34	20.0±0.94	15.0±0.83
7.5	2.0±0.44	1.50±0.24	1.30±0.26	2.30±0.46	10.0±0.72	10.2±0.68
Control	2.50±0.36	1.30±0.17	1.42±0.21	1.90±0.30	7.0±0.31	11.7±0.42

Control: No hormone added

This pattern followed through to the end of the experiment. It was observed that as the experiment progressed, the number of roots/plantlet reduced, except Apantu pa on medium supplemented with 4.5 mg L^{-1} BAP. This same pattern was also observed for the number of leaves per plantlet. For plantlet height increased equally among the three cultivars as the experiment progressed with Apantu pa having the highest figures on supplemented with BAP and Kinetin.

DISCUSSION

Even though, cytokinins have been known to induce shoot formation, Buah *et al.* (2000) has been demonstrated from this study that there exist differences in the relative strengths of the different cytokinin types in inducing shoots. This differential ability of the different hormones in inducing shoots *in vitro* could be attributed to factors such as stability, mobility and the rate of conjugation and oxidation of the hormones.

The marked effects of BAP on shoot formation compared to Kinetin and 2ip as observed in this study may be attributed to its high stability in *in vitro* cultures which is in agreement with Rahman *et al.* (2006), who reported that BAP has a superior shoot inducing ability than Kinetin and 2ip. BAP is not easily broken down and therefore persists in the medium. It is also possible that the amount of BAP that got conjugated in the medium was smaller than what happened to the other plant hormones. This would then have larger amount of BAP existing in their free or ionized forms and were readily made available to plant tissues from the medium. This observation agrees with the observation of Klem *et al.* (2004) who reported that BAP is a chemically stable cytokinin in tissue culture whereas most other purine-type cytokinins are considered chemically unstable. Farahani *et al.* (2008), in their work involving different cytokinin hormones realized that shoot multiplication was affected by the concentration of BAP. Similarly, Kadota and Niimi (2003) also observed the important role of BAP in stimulating multiple shoot formation in other species where lack of BAP mostly produced a single shoot, but the addition of 0.25 mg BAP significantly stimulated shoot multiplication. BAP is the most commonly preferred cytokinin as reported by Cronauer and Krikorian (1984) and that, the concentration of exogenous cytokinin appears to be the main factor affecting shoot multiplication. Kalimutha *et al.* (2007) also observed that the best response in bud multiplication was obtained in MS basal medium supplemented with 0.1 mg L^{-1} NAA and 2.0 mg L^{-1} BAP (7-8 shoots/explant). Arinaitwe *et al.* (2000), however, stated that, Thidiazuron (TDZ), a diphenyl urea derivative, rather shows higher cytokinin activity than BAP, Zeatin, 2ip, or Kinetin. Also, the study by Hamide and Pekmezci (2004) showed that shoot proliferation and elongation were significantly greater with TDZ than with BAP in all the three banana types used.

On the contrary, Arildo *et al.* (2003), in their experiment showed that kinetin induced more shoots per explant than the BAP cytokinin which disagrees with the findings of this study.

Each of the hormones tended to have an optimum concentration to achieve maximal shooting response even though BAP generally performed better in this respect. The better performance of 4.5 mg L^{-1} BAP compared to the 7.5 mg L^{-1} BAP could be due to inhibition at the higher level. The better performance of BAP at 4.5 mg L^{-1} in shoot induction as reported in this work is however contrary to Sreeramanan *et al*. (2008) who reported that BAP at 8 mg L^{-1} gave 10 shoot/plant where as BAP at 4 mg L^{-1} gave only 5 shoots/plant. This is further collaborated by Rahman *et al*. (2004), who achieved high shooting of 51.17 shoot/plant at a BAP concentration of 6 mg L^{-1}, which is higher than the best concentration of this study. Teisson and Cote (1985), reported that over exposure to high level of cytokinins may lead to vitrification. Similarly, Okole and Schultz (1996), realized that high concentration of BAP led to poor shoot formation in three Musa cultivars. Though others have achieved shooting on higher concentrations of BAP, there are others who have also used concentrations of hormones lower than what was used to in this study to achieve good results elsewhere. Abbasin *et al*. (2010) in their work with Taxus baccata obtained maximum shoot number, 8 shoots per explant, on medium containing 1 mg L^{-1} BAP but 2 mg L^{-1} BAP significantly reduced shoot number. They also realized that shoot length increased in response to increasing BAP concentration, reaching the highest growth with 0.25 to 1 mg L^{-1}. Similarly, Bashir *et al*. (2007) and Mateille and Foncelle (1988) have all reported favourable shooting in *Musa* spp. With BAP concentrations of 2.25 mg L^{-1} which is lower than the concentrations used in this study. Hamide and Pekmezei (2004), have also reported in their study that BAP below 20 µM or TDZ below 1 µM did not increase shoot proliferation and BAP over 20 µM and TDZ over 2 µM suppressed shoot elongation. They stated that when 11.1 µM BAP induced explants produced an average of 2.4 shoots, while increasing the BAP concentration to 22.2 and 44.4 µM resulted in 2.6 and 4.3 shoots per explant respectively. However, the optimum recommended BAP concentration is 20 µM for banana micropropagation. Kalimutha *et al*. (2007) realized in their study that higher concentrations of cytokinins resulted in profuse callusing and reduction of shoot multiplication. Increasing concentration of BAP at more than 2 mg L^{-1}, TDZ and Zeatin above 0.1 mg L^{-1} in the medium decreased the shoot number with retardation of shoot growth. In this work however, the higher level of kinetin (concentration of 7.5 mg L^{-1}) produced a corresponding higher number of shoots which suggests that kinetin has the capacity to induce more shoots with increased concentrations. Shirani *et al*. (2009), in a similar work reported that BAP resulted in the maximum proliferation response although it was not different from the lower concentrations of TDZ or the highest concentration of Kinetin. As it can be seen from table 2, 7.5 mg L^{-1} Kinetin induced a higher number of shoots than its 4.5 mg L^{-1} counterpart. This suggests that the optimal kinetin level was beyond 4.5 mg L^{-1}. It therefore might have the potential to induce more shoots till the peak level is reached.

Even though shoot formation was low on medium supplemented with kinetin, Oniaba formed more shoots than Apantu pa at both concentrations. Apantu pa was virtually indifferent to the hormonal concentrations. The differences observed might be due to the factors such as the age, genotype. Besides, differences in the genomic constitution or phenolic contents of the cultivars might account for the variation in performance in *in vitro* cultures. This observation is in line with the findings by Arinatwe *et al*. (2000) who stated that shoot proliferation is cultivar dependent. They reported that increasing BAP above 16.8 µM did not significantly increase shoot proliferation in Kibuzior Ndiziwemiti cultivars. However, the cultivar Bware showed significant increases in shoot proliferation rates with increasing BAP concentration from five to eight shoots with an increase from 16.8 and 28.8 µM. Hamide and Pekmezei (2004) and Shirani *et al*. (2009) have all reported that responses among banana and plantain cultivars in influenced by hormone type, concentration and cultivar. It is therefore possible that the differences in our results and the results of other researchers could be due to the cultivar difference. For example, shoot multiplication in Ndiziwemiti progressively increased with increasing TDZ concentrations; but Bwara and Kibuzi decreased with increasing concentrations of TDZ.

2ip though is least in inducing shoots formation, it tended to have some root induction ability compared to BAP and Kinetin as indicated in Table 2, inducing an average of two to 6 roots per plantlet.

CONCLUSION

BAP generally gave the highest number of shoot in the two plantain cultivars used. This was more pronounced with the 4.5 mg L^{-1} concentration and beyond this, the number of shoots declined. However, in terms of shoot height, the BAP at 7.5 mg L^{-1} have the highest figure. Moreover, the two plantain cultivars used, responded differently to the hormones used in terms of shoot numbers and height.

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