Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.1, 2015

IN VITRO Proliferation of Plantain using Different Concentration of Auxin and Cytokinin

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Abstract
This study was carried out in the Tissue Culture Laboratory of Nigerian Agricultural Quarantine Service (NAQS), moor plantation, Ibadan. The explants used were obtained from National Horticultural Research Institute (NIHORT) and the growth parameter studied is the number of proliferated buds. MS (Murashige and Skoog) media supplemented with $0.18mgl^{-1}$NAA + $2.3mgl^{-1}$BAP supported the growth and bud formation in meristem culture of *Musa paradisiaca* cv. Agbagba during the initiation stage. After the first apical buds appeared, the buds were transferred to MS media supplemented with different combinations of Naphthalene acetic acid (NAA) and Benzylaminopurine (BAP) at different concentrations ($0.0mgl^{-1}$NAA + $0.0mgl^{-1}$BAP, $0.1mgl^{-1}$ NAA + $3.5mgl^{-1}$ BAP, $0.18mgl^{-1}$ NAA + $4.5mgl^{-1}$ BAP, $0.26mgl^{-1}$ NAA + $5.5mgl^{-1}$ BAP). The results obtained showed that proliferation media supplemented with $0.18mgl^{-1}$ NAA + $4.5mgl^{-1}$ BAP yielded the highest number of buds. This study however revealed the effect of hormone in the initiation and proliferation of plantain buds as essential and MS medium with NAA ($0.18mgl^{-1}$) and BAP ($4.5mgl^{-1}$) was the optimum concentration required for the proliferation of plantain.
Keywords: Optimum concentration, *In-vitro*, MS medium, proliferated buds

1. Introduction

Plantain (*Musa sp*, AAB genome) is a perennial herbaceous monocot plant belonging to the genus Musa in the family *Musaceae* with species name of *paradisiaca*. It is believed to be one of the earliest plant species to be domesticated. They are cultivated over a wide range of agro ecological zones and produces fruit all year round and contributing to their importance as staple food and valuable export commodity (FAO, 2005; Innocent *et al*., 2011). Most of the cultivated plantain accessions are diploid and triploid and it is believed that they originated from intra and inter-specific hybridization (De Langhe *et al.*, 2010). Nigeria is one of the largest plantain producing countries in the world (FAO, 2006). Though, the gains derivable from plantain are numerous, its level of production in Nigeria has been inconsistent and low (FAO, 1990). Despite its prominence, Nigeria does not feature among plantain exporting nations because it produces more for local consumption than for export (Akinyemi *et al.,* 2010). The consumption of plantain has risen tremendously in Nigeria in recent years because of the rapidly increasing urbanization and the great demand for easy and convenient foods by the non-farming urban populations. 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 (Akinyemi *et al.*, 2010). The establishment of plantain plantation has some economic implications, which directly or indirectly stimulates agricultural and commercial ventures. This leads to accelerated developments crucial to national growth (Innocent *et al.,* 2011). For commercialization, it is important that consistent supplies of good quality plantain are produced. However plantain production is greatly threatened by pests and diseases, which has been increasing during the past 20 years. Most alarming has been the outbreak and spread of more virulent forms of the fungal disease fusarium wilt caused by *Fusarium oxysporum* which greatly reduces the yield of plantain production. To harness the export potential of plantain, the current level of its production must be improved. This could be achieved through clonal planting materials obtained through tissue culture propagation technique. This technique 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 by many workers using different explants sources and methods (Strosse *et al.,* 2006; Venkatachalam *et al.,* 2006, 2007; Resmi and Nair, 2007; Shirani *et al.,* 2009). Farahani *et al.* (2008) and Buah *et al.* (2010) stated that the effectiveness of BAP over other cytokinins and NAA over other auxins in inducing multiplication of shoot tip cultures has been reported in different cultivars of plantain. Shoot proliferation rate and elongation are affected by cytokinin type and their concentration. Although BAP stimulates shoot proliferation, it is also known to possess mutagenic effects at high concentration which leads to the production of off-type plantlets which is of great disadvantage to in vitro multiplication of plantain. Therefore, the objective of this study is to determine the optimum growth regulators (auxin/cytokinin) combinations required to modify Murashige and Skoog (MS) medium for *in vitro* proliferation of plantain.

2.0 Materials and Methods

2.1 Plant materials

Young suckers of *Musa paradisiaca* (plantain) were collected from a healthy true to type mother plants at National Horticultural Research Institute, Ibadan. After removing the leaves and the roots, the suckers were thoroughly washed with tap water to remove adhering soil. The samples were trimmed to a size of 3-4cm and 1-2cm in diameter by removing the layers of the developing leaves. The suckers were then placed under running water for 30mins before the shoot apices were transferred to a laminar flow. The shoot apices were sequentially treated with 70% ethanol for 10mins followed by mixture of 10% sodium hypochlorite (NaOCl) mixed with few drops of Tween 20 for 5minutes. After these treatments, explants were rinsed with sterilized distilled water thrice and dried with filter paper. Subsequently, the explants were trimmed to a size of 1.5-3 cm in length and 1-1.5 cm in diameter.

2.2 Initiation Media

The culture initiation media was prepared by adding 0.1g Inositol, 30g sucrose and $7gl^{-1}$ of Agar powder as solidifying agent to MS (Murashige and Skoog) basal medium and supplemented with $0.18mgl^{-1}$ NAA + $2.3mgl^{-1}$ BAP. Cultures were maintained on the hormone concentration until the first individual bud appeared. Subsequently, the explant was cut into four parts and kept in the culture initiation media for 300days. During the initiation stage cultures were sub-cultured twice to reduce browning due to the presence of phenolic compounds.

2.3 Proliferation media

The initiated buds were subsequently transferred to MS basal medium with the combination of different concentration of low level of auxin (NAA) and higher level of cytokinin (BAP) as shown in table 1, to induce shoot bud formation as shown in table1. Transfers to fresh medium and sub-culturing were carried out every 30 days up to 2 cycles. In the proliferation stage, the number of buds per explant were counted and recorded.

2.4 Culture Conditions

The pH of all culture media was adjusted to 5.7 ± 0.1 prior to the addition of Agar at $7gl^{-1}$ and autoclaving. All cultured media were autoclaved at 121ºC for 20 minutes. Cultures were maintained at 28ºC under 16-h photoperiod with light intensity of $31.4\mu molm^{-2}s^{-1}$, 8hours dark periods and temperature of about 25°C to 28°C was administered and maintained in the growth room.

2.5 Data Analysis

The experiment was laid out in a Completely Randomized Design (CRD). Data taken were subjected to Analysis of Variance (ANOVA) and means with significance differences separated by Duncan Multiple Range Test (DMRT) at 5% confidence level.

Table1. Levels and combinations of auxin and cytokinin supplements of Murashige and Skoog (MS) medium used in shoot buds proliferation of plantain

Treatments	MS medium supplements (mgl^{-1})
P1	No supplement (control)
P2	0.1 NAA + 3.5 BAP
P3	0.18 NAA + 4.5 BAP
P4	0.26 NAA + 5.5 BAP

P1 = MS Medium with no supplements (control)
P2 = MS Medium + 0.1 NAA + 3.5 BAP
P3 = MS Medium + 0.18 NAA + 4.5 BAP
P4 = MS Medium + 0.26 NAA + 5.5 BAP

3.0 Results

3.1 Culture Initiation medium

Murashige and Skoog (MS) medium supplemented with $0.18mgl^{-1}$NAA + $2.3mgl^{-1}$BAP induced bud formation (plate 1). After the first four weeks of culturing, the external leaf primordia of shoot tip turned green which were initially creamy white. The size of the explant also increased, while blackening was observed at the base of the explant. This blackening of the explant may be due to secretion of phenolic compounds.

3.2 Bud Proliferation of Shoot Regenerated Plantain

Meristem (explant) with newly formed buds transferred to media with or without the combination of different concentration of hormones produced rapid shoot growth with bud multiplication. Result indicated that $0.18mgl^{-1}$

NAA + 4.5mgl^{-1} BAP at 6wks induced the highest number of buds (19.4 ± 9.0) followed by 0.1mgl^{-1} NAA + 3.5mgl^{-1} BAP while 0.26mgl^{-1} NAA + 5.5mgl^{-1} BAP produced the least number of proliferated buds (table 2 and figure 1).

Table 2. Effect of MS medium with varied hormone supplements on shoot buds proliferation of plantain

HORMONES	WEEKS					
	4	5	6	7	8	9
P1	1.6±1.5	2.2±1.8	2.4±1.8	2.4±1.8	2.6±1.8	4.0±2.5
P2	4.4±1.9	5.8±2.6	6.4±2.7	6.8±3.0	9.0±4.1	11.2±5.3
P3	3.6±0.5	6.0±2.3	7.4±2.6	8.8±2.9	14.8±7.1	19.4±9.0
P4	1.8±1.5	2.2±1.9	2.4±1.9	2.6±2.1	3.0±2.5	3.6±3.4

Values are the mean ± S.D
P1 = MS Medium with no supplements (control)
P2 = MS Medium + 0.1 NAA + 3.5 BAP
P3 = MS Medium + 0.18 NAA + 4.5 BAP
P4 = MS Medium + 0.26 NAA + 5.5 BAP

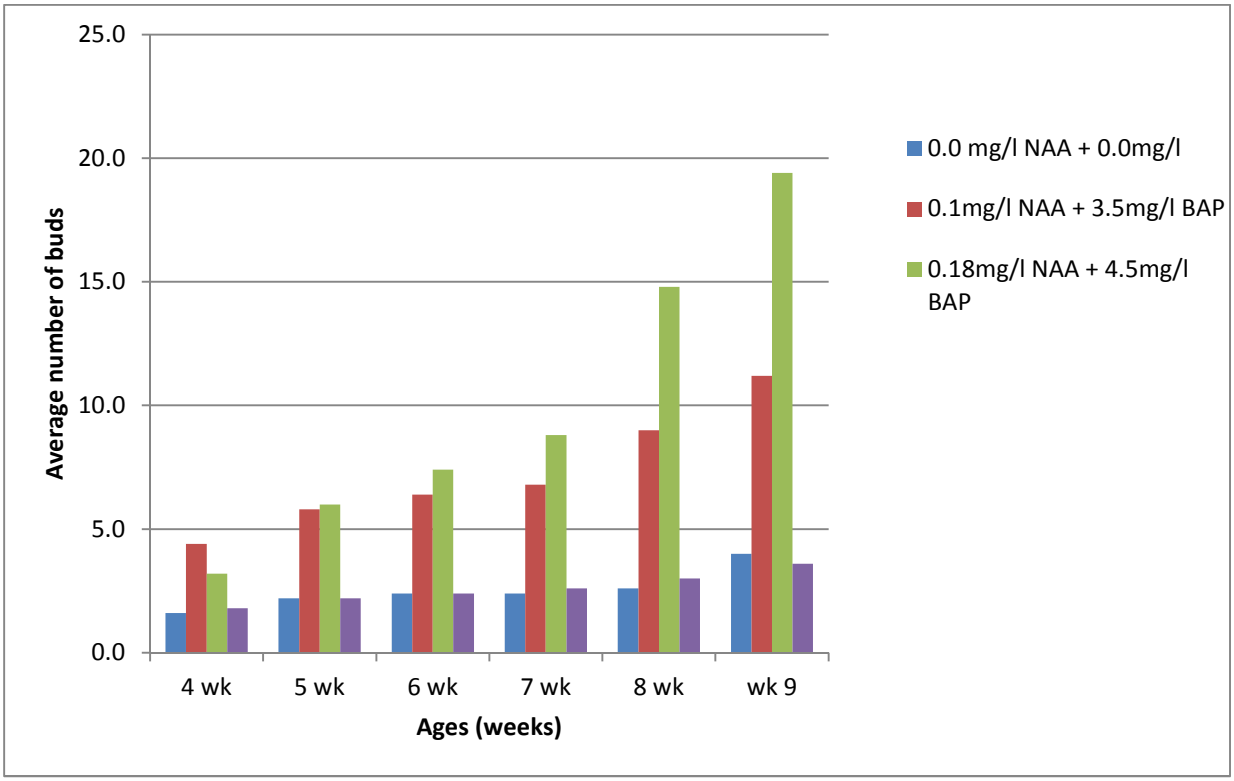

Figure 1: Average number of proliferated buds of plantain on modified Murashige and Skoog (MS) medium at different concentration

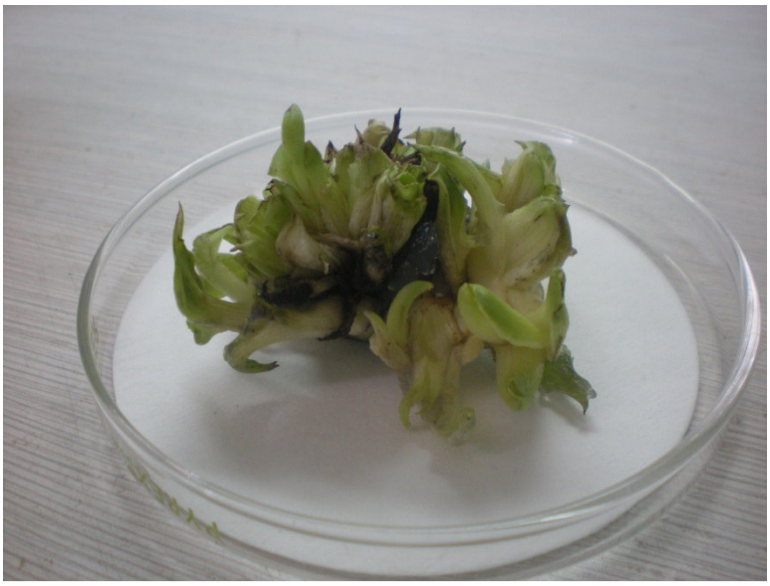

Plate1a: Regenerated plantlets from initiation medium

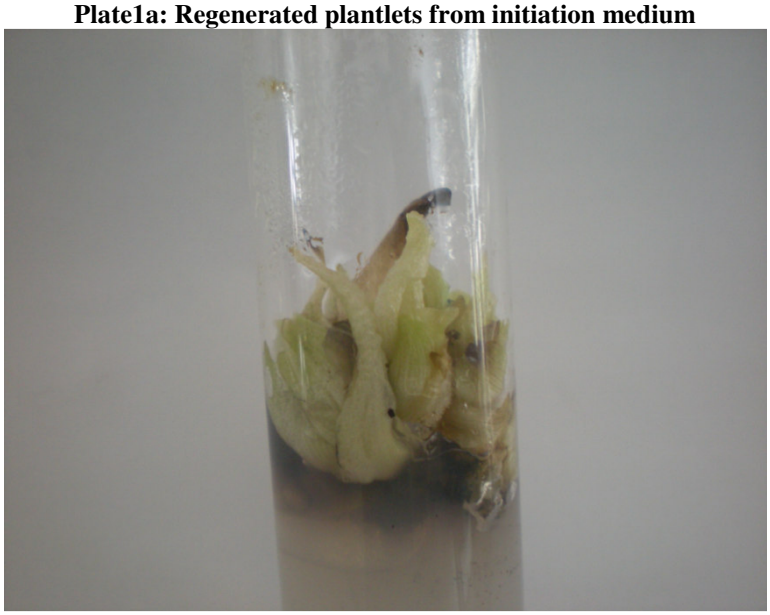

Plate1b: Regenerated plantlets from initiation medium

4. Discussion and Conclusion

This study had shown that the combination of NAA and BAP at the concentrations of $0.18mgl^{-1}$ and $2.3mgl^{-1}$ respectively during the initiation stage enhanced bud formation (plate 1). The important application of high BAP concentration to initiate bud formation from explant was reported by Zaffari *et al.* (2000). However, Arinaitwe *et al.* (2000) reported that *in vitro* bud initiation from banana was cultivar dependent. Upon subculture for shoot proliferation, the vegetative manipulations of plantain explant in this study showed that different concentration using Naphthalene acetic acid (NAA) and 6-Benzyl amino purine (BAP) used at $0.1mgl^{-1}$NAA + $3.5mgl^{-1}$BAP, $0.18mgl^{-1}$NAA + $4.5mgl^{-1}$BAP, $0.26mgl^{-1}$NAA + $5.5mgl^{-1}$BAP and MS medium without supplement for bud proliferation gave comparative results that support proliferation of buds in plantain. This was similar to Farahani *et al.* (2008) who reported that different shoot multiplication was affected by the concentration of BAP.

The results indicated that at 4weeks, the highest number of buds (4.4 ± 1.9) was derived from the medium supplemented with $0.1mgl^{-1}$NAA + $3.5mgl^{-1}$BAP followed by the medium supplemented with $0.18mgl^{-1}$NAA + $4.5mgl^{-1}$BAP while the medium without supplement produced the least number of buds, although rooting was initiated (figure 1 and plate 2a). Boxus *et al.* (1991) reported that the development of very vigorous leaves and roots on banana plants reduces the number of proliferated buds. Aish *et al.* (2004) reported different rate of multiplication among the explant of the same genotype. This different in growth may be due to physiological response of different rhizome. Generally at 6weeks, the result shows that bud multiplication was significantly better in the presence of NAA and BAP. Dhed'a *et al.* (1991) reported that combinations of BAP

with IAA or NAA were effective for *in vitro* multiplication of bananas and plantains. Meanwhile, Resmi and Nair (2007; 2011) reported high bud multiplication but a reduction in the length of the buds in media with a combination of BAP and IAA in triploid cultivar by using inflorescence explants.

MS medium supplemented with 0.18mgl^{-1}NAA + 4.5mgl^{-1}BAP at 8weeks significantly produced the highest number of proliferated buds (19.4 ± 9.0). This was justified by Mendes *et al.* (1996) who observed multiplication of shoot tips of cv. 'Naincao' on MS media supplemented with 4.5mgl^{-1}BAP. The better performance of 0.18mgl^{-1}NAA + 4.5mgl^{-1}BAP compared to 0.26mgl^{-1}NAA + 5.5mgl^{-1}BAP could be due to inhibition at the higher level. This is in agreement with Buah *et al.* (2010) who reported better performance of BAP at 4.5mgl^{-1} when compared to 7.5mgl^{-1}BAP but contrary to Sreeramanan *et al.* (2008) who reported that BAP at 8.0mgl^{-1} gave 10buds/plants where as BAP at 4.5mgl^{-1} gave only 5buds/plant. Venkatachalam *et al.* (2007) also reported reduction in the number of buds that occurred with exposure to high levels of BAP (44.44 µM) in banana. The synergistic effects of plant growth regulators have influenced the cultural response in bud proliferation of plantains.

The development of an *in vitro* regeneration system is an integral part and an essential pre-requisite for studies related to propagation, conservation and genetic improvement. However, micropropagation of many elite plantain cultivars of local importance is still confined to research laboratories. The combination of BAP and NAA generally gave the highest number of proliferated buds in the plantain cultivar used. This was pronounced in 0.18mgl^{-1}NAA + 4.5mgl^{-1}BAP and beyond this, the number of buds declined. Therefore, from the commercial point of view, MS medium with BAP (4.5mgl^{-1}) and NAA (0.18mgl^{-1}) is the optimum concentration required for the proliferation of plantain.

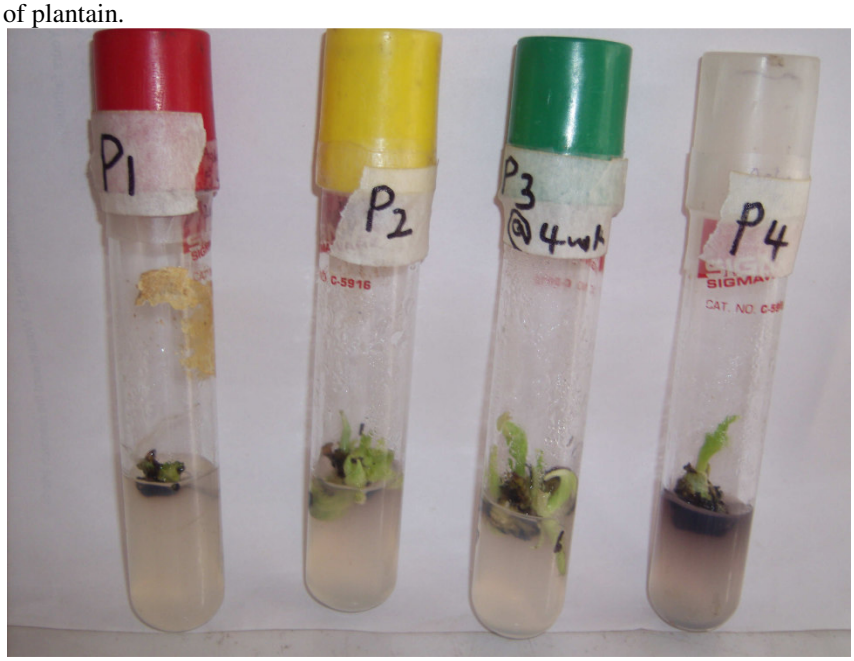

Plate 2: Proliferated buds of plantain at different concentration of NAA and BAP

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