ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 7, Issue 1, pp: (13-20), Month: January - March 2020, Available at: www.paperpublications.org

Optimization of Plant Growth Regulator (PGR) on *invitro* propagation of pineapple (*Ananas comosus (L.)* var. Smooth Cayenne)

Adeoye B.A.[1], Lawyer E.F.[1*], Hassan K.O., Ilesanmi A.O., Richard-Olebe T.C, Oyedeji T.T, Aderemi T.A., Ajongbolo F.B., Adedeji A.A.

National Centre for Genetic Resources and Biotechnology (NACGRAB) moor plantation, Ibadan, Oyo State.

National Biotechnology Development Agency (NABDA) Abuja

National Biotechnology Development Agency (NABDA) University of Ibadan

Abstract: **Lack of planting material is the main factor that reduces pineapple production and this is changing over the years through *invitro* propagation and multiplication of pineapple. The aim of this study was to select the best Plant Growth Regulated medium for initial culture establishment and subsequent shoot multiplication on the *invitro* propagation of pineapple (*Ananas comosus (L.)* var. Smooth cayenne). Disinfected pineapple explants were placed into Murashige and Skoog (MS) medium supplemented with various Plant Growth Regulators (PGR) 6-benzylaminopurine (BAP) or thidiazuron (TDZ) or Kinetin (Kin) in combination with 1-naphthaleneacetic acid (NAA). Results revealed that there was a significant difference (P < 0.05) between different hormone combinations for shoot regeneration and shoot bud formation. Explants placed in 2.0 mg/l BAP + 0.05mg/l NAA produced significantly (P < 0.05) higher rate of regenerated cells after 4 weeks. Transferred regenerated shoot buds from 2 mg L-1 BAP to 5 mg L-1 TDZ after eight weeks showed accelerated production and elongated shoots. The MS medium supplemented with 5.0 mg L-1 BAP and 0.05 mg L-1 NAA produced higher number of normal shoots per explant of pineapple under *invitro* conditions. For root induction, excised individual shoots were transferred into semi-solid basal MS medium Supplemented with different concentration of Plant Growth Regulator (PGR) Naphthalene acetic acid (NAA) or Indole acetic acid (IBA). The best root induction was obtained in 2.0mg/l IBA in combination with 2.0mg/l NAA and they were significant difference among plant growth regulators concentration at (p≤ 0.05).**

Keywords: ***Ananas comosus*****, Smooth cayenne *in vitro* micropropagation, MS medium and Plant growth regulators.**

I. INTRODUCTION

Pineapple (*Ananas comosus L.*) is one of the most economically significant tropical fruit in the world (Duval *et al.*, 2001), and an important plant in the family Bromeliaceae. Pineapple can be cultivated from leave cuttings, lateral shoots, slips, basal suckers or crowns. Pineapple micropropagation can be considered to be no hassle at all, but the multiplication degree is low and it would take years to achieve enough propagules from a mother plant (Almeida *et al.*, 2002). Conventionally, pineapple is vegetatively propagated, where the multiplication rate is low, and ranges from about 11 to 17 plants per five months (Lieu *et al.*, 2004). This breach has necessitated many farmers to purchase suckers from different provenances and also cross border purchase with this planting materials varying in size and weight with packaging putting into consideration, it has given the will and the tendency to increasingly accept smaller sizes and thus longer vegetative cycles with a risk on the quality of product. In the face of its economic role the culture of pineapple especially Smooth Cayenne is faced with some problems which are lack of suckers due to their low rate of multiplication conventionally, good and well identified variety and several diseases challenges like wilt which is induced by Pineapple

Mealybug which is associate with low production of pineapple. Wilt is transmitted by mealybugs (*Dysmicoccus brevipes* and *D. neobrevipes*) and these causes serious loss of production of pineapple (houndedji *et al* 2016).

Due to their low multiplication rate, time and diseased planting materials of this variety Smooth Cayenne which are established and produced with conventional methods hence plant biotechnological methods is therefore a real alternative. Putting to consideration some of Smooth Cayenne short coming necessity to solve some of these problems with the urgency to produce better and clean propagules with improved plantlets multiplication rate which will led to the development of a protocol through tissue culture techniques (Almeda, 1994) is required. Plant tissue culture is about one of the major option to develop an efficient and economical micropropagation protocol for the large scale propagation of pineapples.

Micropropagation is one of the applications of tissue culture, it has the advantage to produce rapidly large number and uniform propagules (firoozabady *et al* 2004) through *in vitro* procedure. *In vitro* regeneration is also influenced by genotype (Jain, 1997.), the realization of an *in vitro* procedure with regards to survival rate and performance of *in vitro* plants depends on several factors in the growth process (Zuraida *et al.*, 2011). *In vitro* shoot proliferation mainly depends on the Phytohormones activities on the plant mainly cytokinins and auxin incorporated in the culture medium (Liao *et al.*, 2004). *In vitro* shoot proliferation mainly depends on plant growth regulators, particularly cytokinins and auxin incorporated in the culture medium. Liao *et al*. (2004) and Debiasi *et al*. (2007) stated the impact of benzyl adenine (BAP), indole-3-acetic acid (IAA), and 1-naphthaleneacetic acid (NAA) on bud initiation.

The objective of this study was to define and optimize an efficient protocol for the micropropagation of pineapple variety Smooth Cayenne using different Plant Growth Regulators (PGR) concentrations in semi-solid culture media to achieve a maximum multiplication rate to make available enough planting materials in a short period of time for farmers to enhance food security and also conservation of the pineapple variety.

II. MATERIAL AND METHOD

This research was carried out at the Tissue Culture Laboratory unit in the Department of Biotechnology of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo state, Nigeria (7°22' N, 3°50'E). The research was conducted from 2017 to 2020.

Plant materials

Pineapple slips of Smooth Cayenne variety were collected from NACGRAB pineapple conservation field in Ibadan, Oyo state. The leaves were removed and the slips were washed under running tap water for 20 minutes to remove soil, dirt, dried and dead leaves matters. Pineapple slips were thoroughly washed with home liquid detergent and later rinsed off using tap water. Pineapple explants were subsequently immersed in 70% ethanol (v/v) for 5 minutes, explants were later disinfected under the Laminar flow hood using Clorox (sodium hypochlorite 6.05%) for 30 minutes with 2 drops of Tween 20 and rinsed three times with sterile distilled water. The meristems were later excised from the sterile explants and inoculated on basal MS medium (Murashige and Skoog, 1962).

Regeneration medium and culture conditions:

Under aseptic conditions, the shoot tip with one to two leaf primordial (approximately 3cm), was inoculated into freshly prepared Murashige and Skoog (MS), (1962) basal medium with vitamins in culture vials. For regeneration, MS medium supplemented with growth regulators: 1, 2, 3 and 4mg/L 6-Benzylaminopurine (BAP), Kinetin (KIN) and Thidiazuron (TDZ) in combination with 0.05mg/L Naphthalene Acetic Acid (NAA) which was constant, 30g/L sucrose, 20mg/L Ascorbic acid and 0.3% phytagel. The cultured explants were incubated in the dark growth room for 7 days at 23^{o}C ±2. Thereafter, they were incubated under florescent lamps with light intensity of 3000 lux at 16 hours photoperiod and 8 hours of darkness for 28 days. The best regeneration medium was used in subsequent studies and the development of shoots regeneration was monitored every week and regenerated plantlets were then recorded after the 4th week.

Shoot proliferation

Initiated shoots obtained from the regenerated plantlets with successive growth on suitable Murashige and Skoog (MS), salt concentrations were sub-cultured twice into media containing different concentrations of Plant Growth Regulators: 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0mg/L for 6-Benzylaminopurine (BAP), Kinetin (KIN) and Thidiazuron (TDZ)) in

combination of 0.05mg/l Naphthalene acetic Acid (NAA) as constant respectively. The number of proliferated plantlets were first recorded after the 4th week and subsequently after the 8th week subculture period. The average plant height (cm), shoot number and leaves number formation were determined in this experiment.

Induction of rooting and acclimatization

For root induction, excised individual shoots were transferred into semi-solid basal MS medium supplemented with different concentrations of Naphthalene acetic acid (NAA) in combination with Indole acetic acid (IBA) (0.5, 1.0, 1.5, 2, 2.5, 3.0 mg/l). All the cultures were incubated at 23±2˚C under white fluorescent lamps at a 16 hours photoperiod. Root number, and root length (cm) were recorded after 4 weeks incubation. Rooted explants were planted in pots containing a sterile soil containing coconut fiber, topsoil and river sand in ratio 5:5:3 respectively and covered with transparent polypropylene bag and kept in the acclimatization chamber for 21days. At the first week, the polypropylene bag was gradually exposed to the environment by perforation of the polypropylene bag until the eventual exposure. At the end of the third week, the plants were transfer into the screen house for more observation and finally to the demonstration field for conservation and also for the uptake by farmers.

Statistical Analysis

All experiments were design with Complete Randomized Design (CRD) in replicates. The results was expressed by the means of the samples with separating mean. Data were statistically analysed using ANOVA with SAS software (SAS Institute, Cary, North Carolina, USA). Mean and analysis of variance and the significant difference between treatments means was estimated by Duncan's multiple range test at 5% significance level.

III. RESULT

TABLE 1: INFLUENCE OF PLANT GROWTH REGULATORS ON SMOOTH CAYENNE PINEAPPLE FOR REGENERATE AFTER 4 WEEKS.

S/N	TREATMENTS	NUMBER OF SHOOT (BUD)	SHOOT LENGTH (CM)
1	BAP1.00mg/l+NAA0.05mg/l	1.00fg	0.70e
2	BAP2.00mg/l+NAA0.05mg/l	4.50a	8.71a
3	BAP3.00mg/l+NAA0.05mg/l	3.70b	5.16b
4	BAP4.00mg/l+NAA0.05mg/l	1.90d	1.10d
5	KIN1.00mg/l+NAA0.05mg/l	2.20c	3.10c
6	KIN2.00mg/l+NAA0.05mg/l	1.24de	0.61de
7	KIN3.00mg/l+NAA0.05mg/l	2.10cd	1.12cd
8	KIN4.00mg/l+NAA0.05mg/l	1.10ef	0.90ef
9	TDZ1.00mg/l+NAA0.05mg/l	0.00g	0.00g
10	TDZ2.00mg/l+NAA0.05mg/l	0.00g	0.00g
11	TDZ3.00mg/l+NAA0.05mg/l	1.00fg	0.70e
12	TDZ4.00mg/l+NAA0.05mg/l	1.00f	0.40f
13	CONTROL	1.20e	0.80e

Values followed by the same letter are not significantly different by DMRT ($P < 0.05$).

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TABLE 2: INFLUENCE OF PLANT GROWTH REGULATORS ON SMOOTH CAYENNE PINEAPPLE SHOOT MULTIPLICATION AFTER 8 WEEKS.

S/N	TREATMENTS	SHOOT NUMBER	SHOOT LENGTH (CM)
1	BAP 1.00mg/l+NAA 0.05mg/l	2.60e	0.94fg
2	BAP 2.00mg/l+NAA 0.05mg/l	2.70de	1.35e
3	BAP 3.00mg/l+NAA 0.05mg/l	1.15hi	0.55ij
4	BAP 4.00mg/l+NAA 0.05mg/l	2.60e	1.14ef
5	BAP 5.00mg/l+NAA 0.05mg/l	7.80a	4.04a
6	BAP 6.00mg/l+NAA 0.05mg/l	2.25f	1.54cd
7	TDZ 1.00mg/l+NAA 0.05mg/l	2.00fg	1.03f
8	TDZ 2.00mg/l+NAA 0.05mg/l	3.00c	1.79c
9	TDZ 3.00mg/l+NAA 0.05mg/l	2.50ef	1.53d
10	TDZ 4.00mg/l+NAA 0.05mg/l	2.10f	0.80g
11	TDZ 5.00mg/l+NAA 0.05mg/l	5.40b	2.50b
12	TDZ 6.00mg/l+NAA 0.05mg/l	1.10i	0.27jk
13	KIN 1.00mg/l+NAA 0.05mg/l	1.00ij	0.67gi
14	KIN2.00mg/l +NAA 0.05mg/l	1.40gh	0.62i
15	KIN 3.00mg/l +NAA 0.05mg/l	1.20h	0.55ij
16	KIN 4.00mg/l+NAA 0.05mg/l	2.95d	1.37de
17	KIN 5.00mg/l+NAA 0.05mg/l	0.90j	0.46j
18	KIN 6.00mg/l+NAA 0.05mg/l	1.80g	0.82g
19	CONTROL	0.60h	0.16k

Values followed by the same letter are not significantly different by DMRT ($P < 0.05$).

TABLE 3: INFLUENCE OF PLANT GROWTH REGULATORS ON SMOOTH CAYENNE PINEAPPLE FOR ROOTING AFTER WEEKS.

S/N	TREATMENT	ROOT NUMBER	ROOT LENGTH (CM)
1	IBA 0.50mg/l + NAA 0.50mg/l	2.10d	1.17d
2	IBA 1.00mg/l + NAA 1.00mg/l	3.05c	1.20c
3	IBA 1.50mg/l + NAA 1.50mg/l	4.15b	1.64b
4	IBA 2.00mg/l + NAA 2.00mg/l	5.30a	2.29a
5	IBA 2.50mg/l + NAA 2.50mg/l	1.25ef	0.36ef
6	IBA 3.00mg/l + NAA 3.00mg/l	1.40e	0.94e
7	CONTROL	0.85f	0.23f

Values followed by the same letter are not significantly different by DMRT ($P < 0.05$).

ISSN 2349-7823

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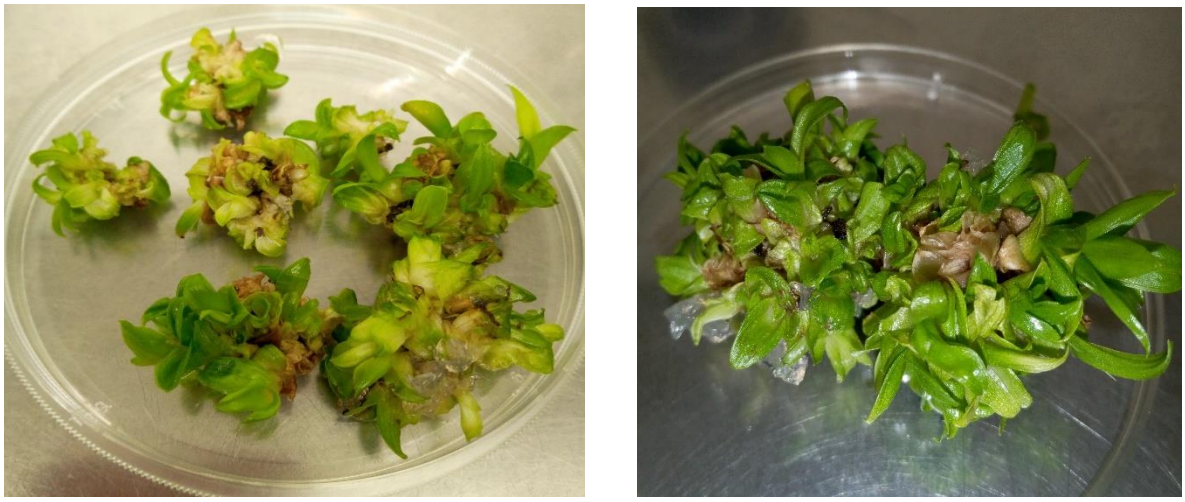

Fig 1. Typical clusters of smooth cayenne proliferating on semi-solid MS medium showing Initial multiplication stage and advanced multiplication stage.

Fig. 2. Root system of Smooth Cayenne plantlet at 3rd subculture and after 8 weeks of subjecting to rooting MS medium of 2mg/L IBA+ 2mg/LNAA.

Fig 3. Well grown plantlets in growth room conditions and already hardened seedling in the screen house.

IV. DISCUSSION

Determination of the best type and concentrations of plant growth regulators as medium constituents is one of the most important aspects of successful micropropagation, among other *in vitro* factors (Shimizu-sato *et al*, 2009). The results showed that all the medium solidification types supplemented with BAP 2.00mg/l in combination with NAA 0.05mg/l concentration promoted regeneration of shoot buds and gave the best regeneration in terms of the average number of shoot buds produced per explant (4.50) and average shoot length (8.71cm) (Table 1).

Shoot multiplication were varied with the different concentration of BAP, TDZ, KIN and constant concentration of NAA with MS medium. Generally, shoot bud number increased with increasing concentration of BAP and TDZ. This is in line with studies by Skoog and Millar (1957) that up to a certain limit, a high cytokinin-auxin ratio favours bud and shoot formation. In this variety, optimal proliferation of highest shoot multiplication occurred at concentration of 5.00mg/l BAP supplemented with 0.05mg/l NAA. However, Duncan's Multiple Range Test ($p<0.05$) showed that the BAP and TDZ concentrations of 5.00mg/l and 2.00mg/l supplemented with and in combination with concentration of 0.05mg/l NAA significantly affected shoot multiplication respectively (Table 2). The use of 5.00mg/l BAP supplemented and in combination with 0.05mg/l NAA during the proliferation stage was the best treatment. An average of (7.80) shoots multiplication per explant was obtained in medium with 5.0mg/l BAP supplemented in combination with 0.05mg/l NAA, as shown in the (Table 2). In sustaining this research work, the effect of BAP levels on the micropropagation of pineapple has been reported (Pescador & Koller, 1992; Kiss *et al*., 1995; Almeida *et al.*, 1997; and Guerra *et al.* 1999) According to Albuquerque *et al.* (2000), the use of BAP in MS medium was essential for the regeneration of plants from shoot apices of pineapple, aiming at plants free of Fusarium. Paiva *et al*. (1998) obtained the best results in the shoot induction of pineapple, var. Skay, with either 1.00 mg/l BAP or 0.1 mg/1 TDZ. Barbosa & Caldas (2001) working with etiolated segments for micropropagation of the pineapple hybrid PE x SC-52, observed that BAP promoted the highest number of plants per shoot and per nodal segment, when compared with KIN, or a combination of BAP and naphthalene acetic acid (NAA). Grattapaglia & Machado (1998) cited BAP as the best cytokinin for the multiplication of aerial plant parts and for the induction of adventitious shoots.

MS basal medium with vitamins supplemented with IBA 2.50 mg/l + 2.50 mg/l NAA gave the lowest average number for root emergence (1.25) while the highest average mean number for root emergence was obtain in MS basal medium with vitamins supplemented with and in combination with IBA 2.00 mg/l and NAA 2.00mg/l gave average number for root emergence of (5.30) roots per shoot. There were significantly difference ($p\leq0.05$) among the plantlets subjected with and in combination with plant growth regulators with different concentration (Table 3). Danso *et al*. (2008) reported that shoots cultured on 2.50 mg/1 BAP and NAA concentrations (7.5 to 15.0 mg/1) did not result in any root formation in MD2 pineapple per shoot. Moreover, Firoozabady and Gutterson (2003) obtained roots from liquid cultures of pineapple cultured on MS medium supplemented with 0.5 mg /1 NAA and 0.5 mg /1 IBA. NAA and IBA are root inducing growth regulators and have been used either alone or in combination for root induction in many cultures (Be and Debergh, 2006), in this study the highest length of root for roots per shoot obtained with medium supplemented with 2.00mg/l IBA and 2.00mg/l (2.29cm) (table 3) is higher than those obtained in medium supplemented with 2.50 mg/l IBA and 2.50mg/l NAA (0.36cm) with the periods of days for induction of root. In contrary to this study, Almeida *et al*. (2002) recorded success in rooting when shoots were transferred to MS medium with half the concentration of salts and no growth regulators for 30 days.

V. CONCLUSION

The results of this study demonstrate the efficient use of semi-solid medium with Moderate concentrations of BAP with low concentration of NAA for *in vitro* regeneration while BAP and TDZ with low concentration of NAA for multiplication of pineapple, as well as the use of MS basal medium with vitamins and moderate growth hormones of IBA supplemented with the same amount of NAA concentration for root induction. The minimal use of materials and Optimization of Plant Growth Regulator (PGR) of pineapple (smooth cayenne) in tissue culture directly translates to low cost of production. This economic approach for multiplication via *in vitro* propagation of this cultivars enhances the availability and affordability of *in vitro* derived pineapple (smooth cayenne) as quality planting materials to meet the ever-increasing demand by farmers and conservation for utilization of this species.

VI. ACKNOWLEDGMENTS

We are very grateful to the Management of The National Centre for Genetic Resources and Biotechnology (NACGRAB), staffs of the Biotechnology unit, The Tissue culture laboratory and National Biotechnology Development Agency Abuja and University of Ibadan who were energetically involved in the study.

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