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Influence of Different Doses of Benzyl Adenine with Constant Naphthalin Acetic Acid on Callus Induction and Mass Multiplication of Pineapple (*Ananas comosus* L. Merr) var. Kew

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A B S T R A C T

A study on influence of different doses of Benzyl Adenine (BA) combined with constant level of Naphthalin acetic acid (NAA) on callus induction, shoot multiplication, and rooting of Kew pineapple was conducted. Early callus response was observed in treatment concentrations of 2mg/l BA with 2mg/l NAA and 3mg/l BA with 2mg/l NAA. Treatment 2mg/l BA with 2mg/l NAA was resulted to replicate the maximum number of shoots (22.71 at 90 days) significantly among the treatments. Shoot length was most enhanced by 0.5 mg/l BA with 2 mg/l NAA (8.09 cm at 90 days) dose. Shoots in control condition developed to be shorter in all the culture periods. On sub culturing in fresh and same media of all the treatments, root initiation was observed 30 days after culture. 0.1 mg/l BA with 2 mg/l NAA was found to be significantly efficient with the number (6.33 at 90 days) and length (2.08 cm at 90 days) of roots developed among the treatments. The plantlets were acclimatised at a satisfactory rate.

Keywords

BA, *in vitro*, Pineapple, Proliferation, Kew, Manipur

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Introduction

Pineapple (*Ananas comosus* L. Merr), locally called "Kihom", is an important fruit crop suitably cultivated over the hill slopes in the state of Manipur. Kew (Syn. Smooth Cayenne) is a preferred variety on account of its nutritive as well as commercial feasibility. Kew has shy suckering habit, incapable of producing enough planting material for large scale production. Cost effective and timely production of planting material is the highlighted objective in the state's area of Kew production. Micro propagation serves the best alternative method over the traditional suckers, leading to production of large numbers of disease free, uniform planting materials in a relatively short period of time.

Micro propagation of shoot tip has been successfully carried out in pineapple (Hamad and Taha, 2008a). Many authors reported micro propagation of pineapple through enhanced axillary bud development and organogenesis (Zuraida *et al.*, 2011; Yapo *et al.*, 2011 Danso *et al.*, 2008). Shoot regeneration and development were positively influenced by MS basal medium incoorporated with hormonal combination of BA and NAA in many cases (Usman *et al.*,

2013; Nikumbhe *et al.,* 2014; Al-Saif *et al.,* 2011; Kothawale *et al.,* 2015). Akbar *et al.,* 2003 found an effective mode of *in vitro* pineapple regeneration through callus using supplements of cytokinin and auxin. Also, there have been many records on better rooting of micro-plantlets with auxin:cytokinin combination in different concentrations (Nikumbhe *et al.,* 2014; Waseem *et al.,* 2011).

Pineapple micropropagation is well established and technically applied in many pineapple growing regions. But, Manipur state is solely dependent on the conventional method of propagation and no record of commercialised micropropagation of Kew pineapple is found in the state. So, the present study was focused on finding an applicable *in vitro* protocol by examining the influence of different levels of Benzyl Adenine (BA) combined with constant level of Napthalin acetic acid (NAA) on callus induction, shoot multiplication, and rooting of Kew pineapple.

Materials and Methods

Crowns of Kew pineapple were used as the source of explant. The material was collected from the Horticulture Research Farm, Andro, CAU, Imphal. For explant preparation, the leaves from the crowns were first removed carefully and were washed under running water for 5-10 mins. The terminal growing point of 1.5 cm diameter, was removed from the crown and placed in a beaker after washing thoroughly in distilled water.

Media preparation

One liter Murashige Scoog media (HI media) pack was dissolved in 600 ml double distilled water in a beaker. After maintaining pH at 5.7-5.8, water volume was made upto 1 litre. Thereafter, the media was autoclaved at 121^0C and 1.5 $kgcm^{-2}$ for 20 mins. After cooling down, the hormone supplements were added as required for different treatments viz., Control, 0.1mg/l BA+2mg/l NAA, 0.5mg/l BA + 2mg/lNAA,1mg/l BA + 2mg/lNAA, 1.5mg/l BA + 2mg/l NAA, 2mg/l BA + 2mg/l NAA, 2.5mg/l BA+ 2mg/l NAA and 3mg/l BA + 2mg/l NAA. The media were poured into sterilized culture jars (20ml each).

In vitro explant sterilization, inoculation and culture conditions

The explants were washed thoroughly under running tap water to remove any field dirt. Under laminar airflow chamber, the explants were soaked in Bavistin for 15 mins, then washed thoroughly with water for 3-4times. The explants were then treated with 0.1% $HgCl_2$ (Nikumbhe *et al.,* 2014) for 5 mins followed by rinsing thoroughly in distilled water for 3-4 times. The explants were trimmed down to $5mm^3$ and inoculated into culture jars containing the media. Sub-culturing was done at 15 days interval, on fresh media for every treatment. A total of 240 crowns were inoculated, 30/treatment and 10/replication. The cultures were incubated under a constant temperature of 25^0C, relative humidity of 60-70% and photoperiodic regime of 16 h/day provided by fluorescent light (2800 lux intensity). Data was collected every 30 days interval. Rooted plantlets from each treatment were planted in sterilised river sand soil medium. The potted plantlets were kept first in Greenhouse, then in shadehouse for 15 days each and finally transplanted in the field. Survival rate was checked after 15 days from the final transplant.

Statistical analysis

The experimental data was analyzed under complete randomized block design. The data were subjected to analysis of variance as suggested by Gomez and Gomez (1984). The significance of calculated variance was determined by "F" test.

Results and Discussion

Influence of different doses of BA with constant NAA on callus induction

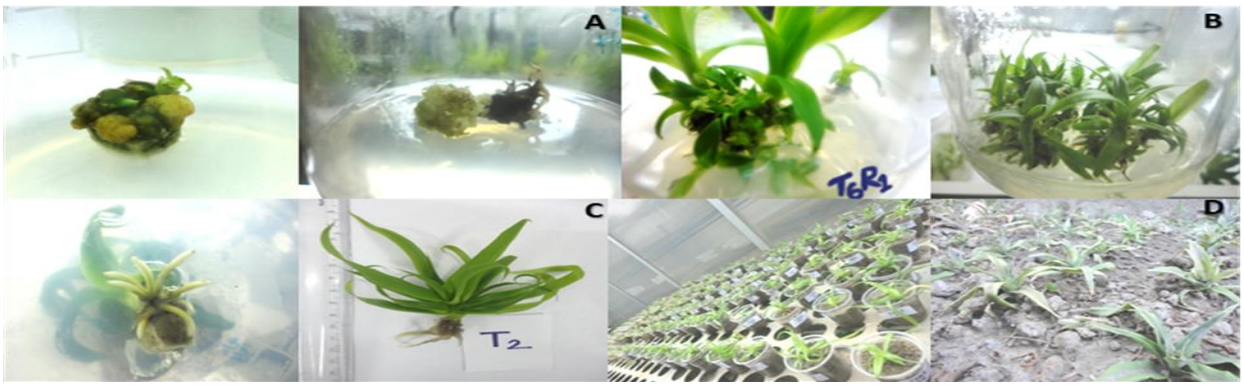
The shortest period for callus initiation (22.68 DAI and 23.53 days) were observed with 2mg/ml BA + 2mg/ml NAA and 3mg/ml BA + 2mg/ml NAA respectively, which were found to be at par (Fig. 1A). Whereas, the longest period (40.57 days) was obtained against 0.5mg/ml BA + 2mg/ml NAA. There were no callus formations on media without growth hormone and with 0.1mg/ml BA + 2mg/ml NAA. The results are in accordance with the findings of (Hussain *et al.,* 2012) and Akbar *et al.,* (2003).

Influence of different doses of BA with constant NAA on shoot multiplication (shoots/explant) and length of shoots (cm)

Treatment 2mg/ml BA + 2mg/ml NAA replicated the maximum number of shoots at all the intervals of observations (Table 1). According to Usman *et al.,* (2013), both cytokinin and auxin influences differentiation pattern in plants. BA and NAA interaction had significant effect on number of plantlets at multiplication. Barboza *et al.,* (2004) showed similar hormonal combination of 2mg/l BAP+ 2mg/l NAA as the best for Smooth Cayenne multiplication. At 60 days, 2mg/ml BA + 2mg/ml NAA at par with 3mg/ml BA + 2mg/ml NAA produced highest number of shoots. The combination of auxins and cytokinins is found to be essential for shoot induction and multiplication, depending on the plant genotype (Zuraida *et al.,* 2011). Be and Deberg (2006) reported production of better axillary shoots at higher cytokinin concentration.

Media incorporated with 0.5 mg/ml BA + 2mg/ml NAA hormonal combination was observed to give better shoot length, although the response was at par with 2mg/ml BA + 2mg/ml NAA in the beginning. However at 60 days, 1.5 mg/ml BA + 2mg/ml NAA and 0.5 mg/ml BA + 2mg/ml NAA led to longest shoots. But, at the end of 90 days, all treatments gave similar shoot length except the untreated and 0.1 mg/ml BA + 2mg/ml NAA, where the shoots appeared shorter. From the investigation, the longer shoots at the initial stages of culture may be due to early growth response and development of the treatments with higher BAP levels. As the culture progressed, there developed an alteration in which lower level of BAP resulted in formation of shoots with longer shoots compared to those with higher BAP concentrations.

Fig.2



A. Callus induction in explants, B. Shoot multiplication, C. Rooting of plantlets, D. Acclimatization of *in vitro* plantlets

Fig.1

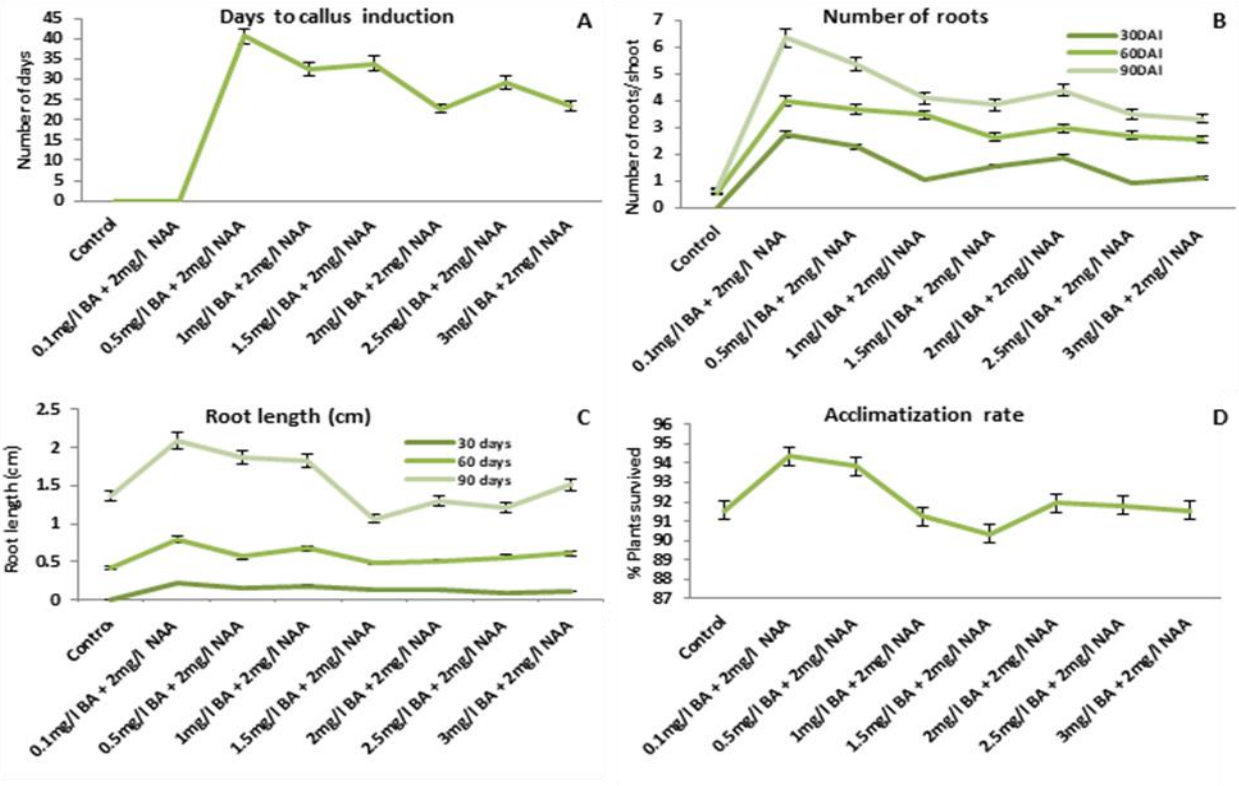


Table.1 Influence of different doses of BA with constant NAA on shoot multiplication (shoots/explant) and length of shoot (cm)

Treatments	30 days after innoculation		60 days after innoculation		90 days after innoculation	
	Shoot multiplication (shoots/explant)	Length of shoot (cm)	Shoot multiplication (shoots/explant)	Length of shoot (cm)	Shoot multiplication (shoots/explant)	Length of shoot (cm)
Control	0.89 (1.18)	0.19 (0.83)	1.63 (1.46)	1.77 (1.51)	2.53 (1.74)	5.42 (2.43)
0.1mg/l BA + 2mg/l NAA	1.32 (1.35)	0.32 (0.90)	2.70 (1.79)	2.20 (1.64)	4.87 (2.32)	5.43 (2.44)
0.5mg/l BA + 2mg/l NAA	2.04 (1.59)	0.64 (1.07)	4.15 (2.16)	4.56 (2.25)	7.30 (2.79)	8.09 (2.93)
1mg/l BA + 2mg/l NAA	2.77 (1.8)	0.96 (1.21)	6.98 (2.74)	4.18 (2.16)	15.45 (3.99)	7.96 (2.91)
1.5mg/l BA + 2mg/l NAA	2.55 (1.74)	1.07 (1.25)	8.14 (2.94)	4.36 (2.20)	15.53 (4.00)	7.55 (2.84)
2mg/l BA + 2mg/l NAA	4.70 (2.28)	1.53 (1.42)	12.51 (3.61)	3.53 (2.01)	22.71 (4.82)	7.96 (2.91)
2.5mg/l BA + 2mg/l NAA	3.61 (2.02)	1.23 (1.32)	10.71 (3.35)	3.96 (2.11)	15.90 (4.05)	7.40 (2.81)
3mg/l BA + 2mg/l NAA	3.53 (2.00)	1.08 (1.26)	12.22 (3.57)	4.16 (2.16)	20.83 (4.62)	7.51 (2.83)
SE (d) ±	0.09	0.05	0.05	0.04	0.05	0.03
$CD_{0.05}$	0.20	0.1	0.12	0.09	0.11	0.07

Note: Number of explants inoculated in each treatments=30. (Square root transformed values in parenthesis).

Increase in proliferation over time could easily mask the treatment effect on shoot length, as according to Hamad and Taha (2008b). Moreover, it might be because higher cytokinin level overcomes apical dominance which releases lateral buds from dormancy (Staden *et al.,* 2008). Hence, treatments enhancing shoot proliferation produced shorter shoots. This is supported by the results of Rao *et al.,* (1993) and Macedo *et al.,* (2003).

Influence of different doses of BA with constant NAA on root growth and development (Number and length of roots/shoot)

Different levels of BA had significant influence on production of micro-roots per shoot among the treatments, led by 0.1mg/ml BA + 2mg/ml NAA at every culture periods (Fig. 1B). Minimum number of roots was obtained in 2.5mg/ml BA + 2mg/ml NAA at 30 days. It can be observed from the present findings that number of micro-roots was observed to be reducing with higher BAP concentration. Higher auxin level enhances better root initiation and development, contrary to the shoot development enhanced by lower auxin and higher cytokinin. Macedo *et al.,* (2003), reported that there was better rooting in media with lower BAP: NAA concentration in the cultures of pineapple cv. Peroira. Moreover, Bhatia and Ashwath (2002) observed that repeated sub-culturing in the same medium resulted in root initiation which could serve as both multiplication and rooting medium. They figured out that the protocol eliminates the extra step required for root initiation**.**

Treatment 0.1mg/ml BA + 2mg/ml NAA developed the maximum root lengths in all the culture periods (Fig. 1C). While, 2.5mg/ml BA + 2mg/ml NAA produced the shortest roots at 30 days (0.1). As discussed above, media supplemented with higher auxin content compared to cytokinin promotes root initiation and development, it also enhances elongation of the roots. Al-Amin *et al.,* (2009) reported maximum length of roots in the same treatment that produced maximum number of roots.

Acclimatization

Rooted plantlets of each treatment were transplanted in sterilised river sand medium. Fig.1D exhibited good survival rate of plantlets with no significant difference among the treatments. Usman *et al.,* 2013 reported the use of riverside sand as a cost effective substrate for hardening *in vitro* pineapple plantlets. Success of plantlets acclimatization using sand medium was reported by Jose *et al.,* (1996). Similar result was also found by Sharma *et al.,* (1997) in hardening of Dwarf Cavendish plantlets in sand culture.

The observations concluded with treatment combination 2mg/ml BA + 2mg/ml NAA as most potential for Kew pineapple multiplication shoot regeneration and good crop vigor after hardening in river sand medium.

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References

Akbar, M. A., Biplab, K. K., and Shyamal, K. R. (2003). Callus Induction and High-frequency Plant Regeneration of Pineapple (*Ananas comosus* (L.) Merr.). *Plant Tissue Cult*. 13(2):109-116.

Al-Amin, Md., Karim, M. R., Amin, M. R,

Rahman, S., and Mamun, A. N. M. (2009). *In vitro* Micropropagation of Banana (*Musa spp.*). *Bangladesh J. Agr. Res.* 34(4):645-659.

Al-Saif, A.M., Sharif Hossain, A. B. M., and Taha, R. M. (2011). Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro. Afr. J. Biotechnol.* 10(27):5291-5295.

Barboza, S. B. S. C., Caldas, L. S., and Souza, L. A. C.(2004). Micropropagation of pineapple hybrid PExSC-52 and cultivar Smooth Cayenne. *Pesqui. Agropecu. Bras.* 39 (8):725-733.

Be, L. V., and Debergh, P. C. (2006). Potential low-cost micropropagation of pineapple (*Ananas comosus*). *South Afr. J. Bot.* 72: 191-194.

Bhatia, P., and Ashwath, N. (2002). Development of rapid method for micropropagation of a new pineapple (*Ananascomosus* (L.)Murr) clone, 'Yeppooon Gold'. *ActaHort.* 575:125-131.

Danso, K. E., Ayeh, K. O., Oduro, V., Amiteye, S., and Amoatey, H. M. (2008). Effect of 6-Benzylaminopurine and Naphthalene Acetic Acid on *in vitro* Production of MD2 Pineapple Planting Materials. *World Appl. Sci. J.* 3(4):614-619.

Gomez, K. A., and Gomez, A. A. (1984). Statistical procedures for agriculturalresearch. John Wiley and sons, Inc. London, UK (2nd edtn) pp 13-175.

Hamad, A. M., and Taha, R. M. (2008a). The effect of different hormones and Incubation Periods on *in vitro* proliferation of pineapple [(*Ananas comosus* L. Merr) cv. Smooth Cayenne] Shoot-tip culture. *Pakistan J. Biol. Sci.* 11(3): 386-391.

Hamad, A. M., and Taha, R. M. (2008b). Effect of sequential subcultures on *in vitro* proliferation capacity and shoot formation pattern of pineapple (*Ananas comosus* L. Merr.) over different incubation periods. *Sc.Hort.* 117: 329-334.

Hussain, A., Qarshi, I. A., Nazir, H., and Ullah, I. (2012). Plant tissue culture: current status and opportunities. In: Leva A, Rinaldi LMR (eds) Recent advances in plant in vitro culture. InTech, Rijeka, pp 1–28. ISBN:978-953-51-0787-3.

Jose, J. O., Radha, C. T., and Aravindakshan, K. (1996). *In vitro* multiplication of pineapple through enhanced release of axillary buds. *J Appl Hortic* (Navsari) 2 (1/2): 82-85.

Kothawale, S. P., Kshirsagar, A. B., and Zahid, I. H. (2015). Response of BA and NAA on shoot proliferation of *Punica Granatum L. Bionano Frontier*. 8(1).

Macedo, C. E. C. de, Silva, M. G. da, Nobrega, F. S. da, Martins, C. P., Barroso, P. A. V., and Alloufa, M. A. I. (2003). The effect of NAA and BAP concentrations on the micropropagation and hydroponic cultures of pineapple. *Rev Bras Frutic*. 25(3): 501-504.

Nekumbhe, P. H., Sonavane, P. N., and Sable, P. A. (2014). *In vitro* technology for Propagation of pineapple (*Ananas comosus* L.) cv. Kew. *Internat. J. agric. Sc.* 10(1):172-175.

Rao, Y. S., Mathew, M. A., Madhusoodanan, K. J., and Naidu, R.(1993). Multiple shootregeneration in vanilla (*Vanilla planifolia*).*J. Plantation Crops.* 21: 351-354.

Sharma, G. L., Tiwary, B. L., and Pandey, S. D. (1997). Rapid *in vitro* mass propagation of banana and changes in bio-chemical constituents at various cultural stages. *Indian J. Hort.* 54 (2):

128-131.

Staden, J. van, Zazimalova, E., and George, E. F. (2008) Chapter 6: Plant Growth Regulators II: Introduction, Cytokinins, their Analogues and Antagonists. In: George EF, Hall MA and De Klerk G-J (eds): Plant Propagation by Tissue Culture 3rd Edition. Volume 1 The Background, Springer, Dordrecht, The Netherlands, pp. 205–226.

Usman, I. S., Abdulmalik, M. M., Sani, L. A., and Muhammad, A. N. (2013). Development of an efficient protocol for micropropagation of pineapple (*Ananas comosus* L. Var. Smooth cayenne). *Afri. J. Agric. Res.* 8(18): 2053-2056.

Waseem, K., Jilani, M. S., Jaskani, M. J., Khan, M. S., Kiran, M., and Khan, G. U. (2011). Significance of different plant growth regulators on the regeneration of chrysanthemum plantlets (*Dendranthema morifolium* L.) through shoot tip culture. *Pak. J. Bot.* 43(4): 1843-1848.

Yapo, E.S., Tanoh, H.K., Mongomaké, K., Justin, Y.K., Patrice, K., and Jean-Michel, M. (2011). Regeneration of Pineapple (*Ananas comosus* L.) Plant through Somatic Embryogenesis. *J. Plant Biochem. Biotech*. 20(2):196-204.

Zuraida, A. R., Nurul Shahnadz, A. H., Harteeni, A., Roowi, S., Che Radziah, C. M. Z. and Sreeraman, S. (2011). A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus* L.) shoots using liquid shake culture system. *Afr. J. Biotechnol*. 10(19): 3859-3866.

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