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## RESEARCH ARTICLE

# EFFECTIVENESS OF BENZYLAMINOPURINE (BAP) CONCENTRATIONS ON *IN-VITRO* SHOOTS PROLIFERATION

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**ABSTRACT**

In present study benzylaminopurine efficiency was tested on shoot proliferation efficiencies under *in vitro* condition. About 45 explants were cultured on each of medium supplemented with different concentrations of 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L BAP. Concentration of BAP significantly affected the average of survival rate, shoot numbers, and shoot length per explants. Whereas, MS basal medium supplemented with 2.0mg/L concentration of BAP showed astonishingly survival rate and the maximum number of shoots (16shoots/explant). MS medium supplemented with 4mg/L of BAP followed by 3mg/L of BAP showed optimal efficiency. While, shoots regenerated on MS basal medium (control) without addition of BAP showed significantly lower survival rate (57.86%), and resulted lower number and length of shoots up to the end of experiments.



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## INTRODUCTION

In-vitro proliferation of shoots has been employed to aid in the clonal propagation of numerous plant species. The inherent advantage of tissue culture over field propagation is that it has greatest plant production potential from a single plant. Tissue culture techniques may offer a possible method to produce large numbers of genetically uniform palms. Several reports dealing with tissue culture have been appeared in the literature (Hussam *et al.,* 2007). The tissue culture technology has potential to produce maximum number of plants in limited time and space which are true to type and agronomically equal or superior to conventionally propagated plants. Thus so far,various reports have been published so far on the different hormonal efficiency which significantly affect the *In-vitro* multiplication of shoots such as medium enriched with BAP (Be and Debergh, 2006), naphthalene acetic acid (Firoozabady and Gutterson, 2003), indole acetic acid (IAA) (Hamad and Taha, 2008), indole butyric acid (Boxus *et al*., 1991) and 2,4-dichlorophenoxy acetic acid (Liu *et al*., 1989) and combination of different hormones (Soneji *et al*., 2002, IAA and IBA, Teixeira *et al*., 2006). Whilst, efficiency of BAP over other hormones in inducing shoot multiplication is surprisingly high which has been reported in different species (Buah *et al*., 2010; Farahani *et al*., 2008; Rahman *et al*., 2006; Resmi and Nair, 2007). The most established shoot-tip culture system was achieved by using BAP as a supplement to basal media (Murashige and Skoog, 1962) and its effect in stimulating growth of axillary and adventitious buds in shoot tips have also been reported under foliar applications (Buah *et al*., 2010). Thus, keeping in mind the above facts and potentiality of tissue culture and efficiency of BAP, the present study has been planned to evaluate the response of *in-vitro* proliferation of shoots under different concentrations of BAP.

## MATERIALS AND METHODS

### Plant material and growth conditions

The explants were screened outs and then surface sterilized with 10% of Sodium Hypochlorite by proper shaking about 20-30mints. After that plant samples were brought under the laminar airflow cabinet and washed thrice with sterilized distilled water. The soft buds were then cut off and placed on MS basal medium supplemented with different concentration of BAP. After thatthe plants were kept under growth condition at $25C^0$ and Phenotypes were observed.

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**Preparation of MS-Basal medium**

The required quantities of macronutrient, sucrose (30g), and agar (7g) were measured into conical flask and sterilized distilled water (900ml) was added after adding pre-prepared stock solution (1ml). Solution was stirred and remaining sterilized distilled water was added to final volume of solution (1L). pH of media was adjusted (5.8) using 1N NaOH and 1N HCl solution and microwaved (4min.) till it became transparent. Media was poured into bottles (100ml) and after plugging, it was autoclaved ($121^0$C) at 15 psi for 20 min. and stored in refrigerator for use.

**Preparations of different concentration of benzylaminopurine (BAP)**

BAP (0.1g) was poured into beaker and dissolved by adding drops of NaOH (1N). Some quantity of sterilized distilled water was added to beaker and mixed with magnetic stirrer. The solution was transferred to 100ml volumetric flask and made up volume up to mark with sterilized distilled water. It was then stored at $0^0$C till further use. Each of these supplemented with stock solutions with concentration of 0.5, 1.0, 2.0, 3.0 and 4.0 of BAP, respectively. Solution was stirred on magnetic stirrer and remaining sterilized distilled water was added to final volume of 1 liter. pH of media was adjusted (5.8) using 1N HCL and 1N NaOH solutions and boiled to transparent liquid using microwave oven. Media was transferred into bottles (100ml) and plugged properly. These were then autoclaved ($121^0$C) at 15 psi for 20 min. and were stored in refrigerator.

**Preparation media combination**

The media combinations were prepared given as B1=MS-Basal + 0.0 mg/L of BAP, B2=MS-Basal+ 0.5 mg/L of BAP, B3=MS-Basal+1.0 mg/L of BAP, B4=MS-Basal+2.0mg/L of BAP, B5=MS-Basal+3.0mg/L of BAP, B6=MS-Basal+4.0 mg/L of BAP.

**Statistical analysis**

Data so obtained were statistically analyzed according to the technique of analysis of variance (ANOVA). The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability (Waller and Duncan, 1969). All computational and statistical analysis were performed using student edition software package 8.1.

## RESULTS

**Effectiveness of BAP on survival rate of plant**

The survival rate of plant on medium supplemented with different concentrations of BAP was observed. The results showed that adventitious buds were cultured on MS medium without the addition of BAP showed significantly lower survival rate (57.86%) compared to explants on medium supplemented with various concentrations of BAP. Results further reveals that survival rate of explants cultured on media supplemented with either 0.5 mg/L or 1.0 mg/L each of BAP was relatively similar and were significantly moderate (63.2±1.18 or 64.3±0.10%, respectively) followed by cultured on medium supplemented with 2.0mg/L or 3.mg/L concentrations of BAP. It is really interesting to note that there were no significant differences amongst the survival rate cultured on medium supplemented with concentrations of 0.5 mg/L, 1.0 mg/L or 3.0 mg/L of BAP i.e. 63.2± 0.28, 64.3± 0.18 and 59.6±0.8, respectively (Figure 1). Contrast to that, 2.0mg/L showed consistently higher survival rate of shoot proliferations.

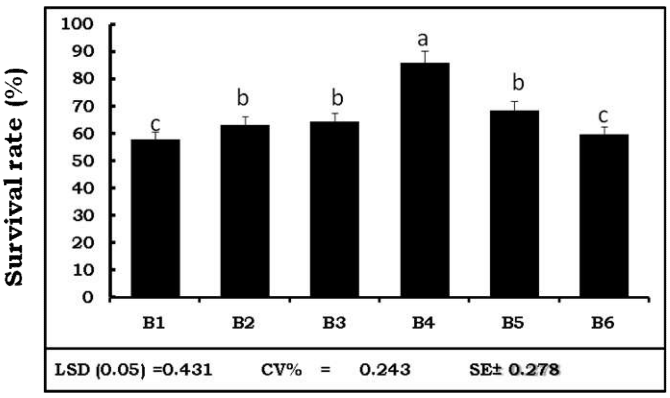


**Figure 1. Survival rates of shoots in percentage on different concentration of BAP under in vitro conditions**

**Effectiveness of BAP on initiation and number of shoots proliferation**

It was found that the buds cultured on medium without any supplementation has taken more time (28.76± 0.21 days) to initiate the shoot. While, on medium supplemented with 2.0mg/L has taken least time (14.43±0.15 days) to initiate the shoots (Figure 2). It was further noted that the explants cultured on 3.0mg/L and 4.0mg/L have taken similar time (i.e. 18.56± 0.12 and 18.87.45 ± 0.19 days). However, the buds cultured on media supplementation with 0.5mg/L and 1.0mg/L have taken significantly moderate time to initiate the shoots (23.50±0.23 and 24.56±0.21 days). Furthermore, regarding the shoots number regenerated on basal medium (control) showed slight response. However, supplementation with 0.5mg/L, 1.0mg/L, 3.0mg/L and 4.0mg/L showed more or less similar number of shoots per explant i.e. 9.5±0.15, 10.7±0.10, 10.6±0.11 and 10.5±0.10, respectively (Figure 3). Moreover, with 2.0mg/L concentration of BAP substantially increased the number of shoots (i.e. 16.6±0.23) per explant. Whereas, medium without any concentration of BAP (control) showed consistently slight growth.

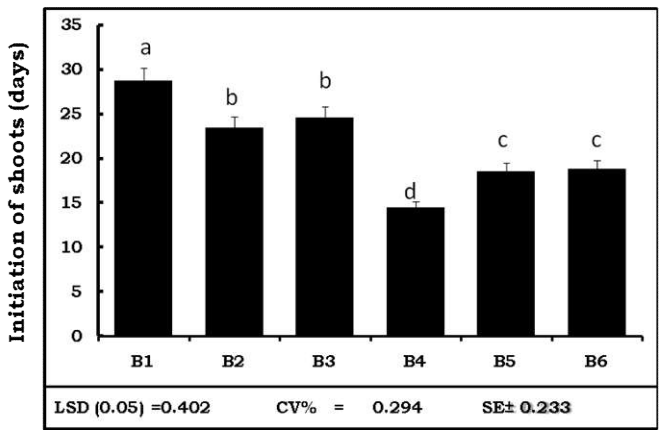


**Figure 2. Initiation of shoots on different concentration of BAP under in vitro conditions**

**Effectiveness of BAP on shoot length**

Length of shoot per explant cultured on medium supplemented with different concentrations of BAP was observed (Figure 4). Results showed that the shoot length was remarkably higher on 2.0mg/L of BAP concentration (8.36 ±0.26 mm) as compared to explants on other concentrations 0.5mg/L (3.02± 0.17 mm), 1.0mg/L (4.5± 0.14 mm), 3.0mg/L (5.42± 0.18 mm) and 4.0mg/L (5.44±0.07 mm). It was further noted that regardless the explants cultured on basal medium (control) was not died, the shoot trivial appear up to the end of experiment and also showed lower rate of growth circumstances.

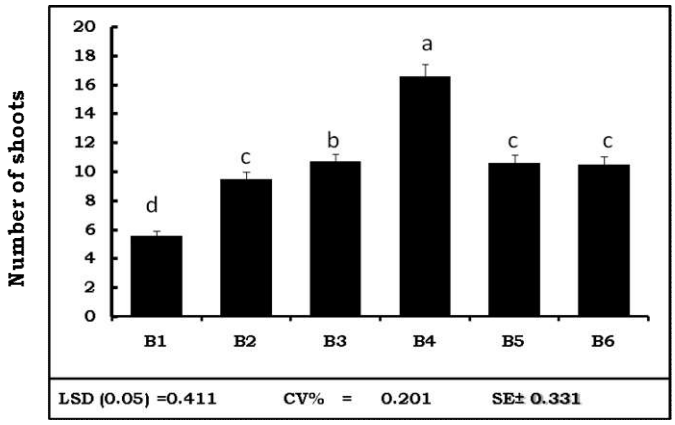


**Figure 3. Number of shoots on different concentration of BAP under *in vitro* conditions**

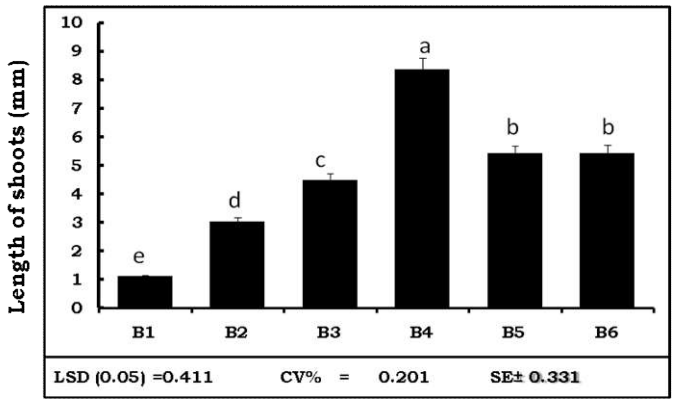


**Figure 4. Length of shoots on different concentration of BAP under *in vitro* conditions**

## DISCUSSION

*In-vitro* multiplication of shoots is very useful because it provides a means of overcoming difficulties of producing large numbers of relatively homogenous seedlings and system was extensively used in wide range of tissue culture investigations carried-out by many scientists (Rahman *et al*., 2006; Resmi and Nair, 2007; Farahani *et al*., 2008; Buah *et al*., 2010). The findings of these techniques provided an argument for the use of tissue and cell culture techniques as tools for shoot proliferations and its improvement.

***In-vitro* multiplication of shoots extensively important biological tool of clonal propagation**

Now a days, tissue culture or micro propagation which is frequently used in various reputed research institutes of the world. It is generally accepted that term plant tissue culture broadly refers to in vitro cultivation of all plant parts, tissues, meristem tips, buds, shoots, stem, flowers and embryo etc. Plant tissue culture is a technique, which has great potential as a means of vegetative propagating economically important species; a potential, which is being realized commonly at present. Micropropagation involves the production of plants from very small plant parts, tissues or cells grown especially in a test tube or other container where the environment and nutrition can be rigidly controlled. This technique is now often preferred to congenital practices of asexually propagation in several green house species because, only a small amount of plant tissues are needed as the initial explants for regeneration of millions of colonel parts.

**Benzylaminopurine promote shoot proliferations**

In techniques tissue culture techniques, plant growth regulators (PGR) are significantaly intermediate components in forming the developmental pathway of the plant cells. Cytokinins such as benzylaminopurine (BAP) generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants (Madhulatha *et al*., 2004). The most established shoot-tip culture system was achieved by using BAP as a supplement to Murashige and Skoog (MS) basal media (Murashige and Skoog, 1962). The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars. BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Abeyarante and Lathiff, 2002; Buah *et al*., 2010).

## REFERENCES

Abeyaratne W.M., Lathiff M.A. 2002. In-vitro propagation of 'Rathambala' (Musa AAA) and the occurrence of phenotypic variations in the pseudostem. Annas of the Sri Lanka Department of Agriculture (LKA), 4: 191-197.

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

Boxus, P., J.M. Terzi, C. Lieves, M. Pylyser, P. Ngaboyamahina and K. Duhem, 1991. Improvement and perspectives of micropropagation techniques applied to some hot climate plants. *ActaHortic*., 289: 55-59.

Buah J.N., Danso E., Taah K.J., Abole E.A., Bediako E.A., Asiedu J., Baidoo R. 2010.The effects of different concentration cytokinins on the in vitro multiplication of plantain(Musa sp.). *Biotechnology*, 9(3): 343- 347.

Farahani F., H. Aminpoor, M. Sheidai, Z. Noormohammadi, M.H. Mazinani 2008. An improved system for in vitro propagation of banana (Musa acuminate L.) cultivars. *Asian J. Plant Sci*., 7(1): 116-118.

Firoozabady, E. and N. Gutterson 2003. Cost-effective *in vitro* propagation methods for pineapple. *Plant Cell Rept*., 21: 844-850.

Hamad, A.M. and R.M. Taha, 2008. The effect of different hormones and incubation periods on *in vitro* proliferation of pineapple (*Ananascomosus* L.)Merr cv. Smooth Cayenne) shoot-tip culture. *Pak. J. Biol. Sci*., 11: 386-391.

Hussam, S.M., I. Khierallah and M.B. Saleh 2007. Action of several auxins and cytokinins on development of date seedling shoot-tips and apical meristems. Proc. IIIrd IC on Date palm Eds: Acta Hort., 736.

Liu, L.J., E. Rosa-Marquez and E. Lazardi 1989. Smooth leaf (spineless) red spanish pineapple (*Ananascomosus* (L.) Merr) propagated *in vitro*. *J. Agric. Univ. Puerto Rico*, 73: 301-311.

Madhulatha P., M. Anbalagan, S. Jayachandran, N. Sakthivel 2004. Influence of liquid pulse treatment with growth regulators on in vitro propagation of banana (Musa spp. AAA). *Plant Cell Tissue Organ Cult*., 76:189-192.

Mathews, V.H. and T.S. Rangan 1979. Multiple plantlets in lateral bud and leaf explant *in vitro* culture of pineapple. *Sci. Hortic*., 11: 319-328

Murashige T, F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497

Rahman M.Z., M.G. Sharoar, M.N. Matin, M.H. Rahman, M.M. Rahman, M.R. Islam 2006. High frequency plant regeneration of a dessert banana cv. mehersagar for commercial exploitation. *Biotechnology*, 5(3): 296- 300.

Resmi L., A.S. Nair 2007. Plantlet production from the male inflorescence tips of Musa acuminata cultivars from South India. *Plant Cell Tissue Organ Cult*., 88: 333-338.

Soneji, J.R., P.S. Rao and M. Mhatre, 2002. Somaclonal variation in micropropagated dormant axillary buds of pineapple (*Ananascomosus* L. Merr.). *J. Hort. Sci. Biotechnol*., 77: 28-32.

Teixeira, S.L., J.M. Ribeiro and M.T. Teixera, 2006. Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananascomosus* cv Smooth cayenne) behavior. *Plant Cell Tissue Org. Cult*., 86: 375-378.

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