

## EFFECTS OF 6-BENZYL AMINO PURINE (6-BAP) ON IN VITRO SHOOT MULTIPLICATION OF GRAND NAINA (MUSA SP.)

<sup>1</sup>\*Devi Reddy Dharaneeswara Reddy, Dasari Suvarna<sup>1</sup> and D. Muralidhra Rao<sup>2</sup>

<sup>1</sup>Plant Tissue Culture laboratory, Dept. of Biotechnology, Sree Vidyanikethan Engineering College,  
Sree Sainath Nagar, A.Rangampet, Chittoor Dist – 517 102, A P, India.

<sup>2</sup>Department of Biotechnology, SK University, Anantapuram, Andhra Pradesh, India.

\*Corresponding author: Email: devireddydh@gmail.com, Tel: +91 9000865648

[Received-12/01/2014, Accepted-22/02/2014]

### ABSTRACT:

The goal of this investigation was to study the effect of diverse concentrations of 6-benzyleaminopurine (6-BAP) on shoots induction of *Grand naine* plantlets (*Musa sp.*). Exercised cormlets from multistage (6 weeks) of *Grand naine* were inoculated onto modified MS 1962 medium supplemented with seven different concentrations of 6-Beznylaminopurine (BAP) (2 mgL<sup>-1</sup>, 0.5 mgL<sup>-1</sup>, 1.5 mgL<sup>-1</sup>, 3.7 mgL<sup>-1</sup>, 6.8 mgL<sup>-1</sup>, 8.7 mgL<sup>-1</sup>, 9.4 mgL<sup>-1</sup>) and the cultures were incubated at 24±2° C with 16 hours photoperiod (2000 flux). The effects of different concentration of regimes of 6-BAP on shoot multiplication was investigated. All the investigated concentrations of 6-BAP showed that the number of bud formation in shoot cultures of *Grand naine* during the process increased proportionately with the concentrations used however, the highest concentration of BAP simultaneously increased the formation of abnormal shoots. Out of all the investigated media, the best shoot induction of *Grand naine* plantlets were obtained on medium supplemented with 2.0 mg L<sup>-1</sup> of 6-BAP.

**Key words:** *Grand naine* (*Musa sp.*), Plant Tissue Culture, Micropropagation, 6-BAP.

### [I] INTRODUCTION

India is the largest producer of banana in the world with about 30% of total global production and in India banana is being cultivated in an area of about 500,000 hectares. At present, tissue culture propagated *Grand naine* (*Musa Sp.*) plants of banana have been widely plating in India. An increasing awareness of advantage of tissue culture plants, demand for tissue culture banana plants has increased at a high rate of

25-30%. The productivity can be enhanced (50 kg of yield per plant) from the same area by replacing the conventional breeding suckers with tissue culture banana plants. Tissue culture techniques have become an attractive field of biotechnological research and their roles are particularly esteemed in the areas of large scale clonal propagation and crop improvement. In the recent century, micropropagation technique is one of the key

tools of plant biotechnology and it has been broadly exploited to congregate the growing demands for selected planting material<sup>1</sup>. The main advantages of tissue culture technology are the production of high quality, disease free and uniform planting material. These plants can be multiplied on a year around basis under *in vitro* conditions, and supplied anywhere irrespective of the season and weather [2]. One of the most important practical considerations in commercialization of an *in vitro* system is the cost of *in vitro* plantlets as compared to conventional propagules [3]. In 1982, De Fossard was reported the cost of production per tissue culture plant will also be greatly affected by the rate of multiplication achieved for each species and on the inoculation rate. Micropropagation has become a routine procedure, but the high costs involved have prevented laboratories with limited resources from benefiting the tissue culture technology [4]. Hence, continuous efforts have been made to increase the effectiveness of the system through improvement in culture media and techniques [5].

Naturally, the cytokinins are present endogenously in the plants, many of the tissues and small organs (explants) are isolated from these plants (mother plants) and cultured under *in vitro* conditions. Under *in vitro* conditions, these explants are lack the capability to synthesis of enough quantities of cytokinins to retain their growth. In the tissue culture studies the effect of cytokinins is noticed, they are necessary in plant cell division. Hence, additions of cytokinins in low levels are essential for the culture medium. The effects of cytokinins on cell, tissue and organ culture vary according to the cytokinin used, its concentration, the type of culture and the variety from which the explant is derived. BAP has a marked effect

in stimulating the growth of auxiliary and adventitious buds and foliar development of shoot tip cultures [6,7]. The multiplication rate of adventitious buds under the influence of Benzylaminopurine (BAP) is one of the determining factors deciding on the efficiency of the micropropagation system. The most commonly used cytokinin is BAP at a range of 2-5 mg L<sup>-1</sup> in combination with an auxin, indole-3-acetic acid (IAA) at a concentration of 0.1-0.2 mg L<sup>-1</sup> [8].

The most established banana shoot tip culture system was achieved by using BAP as supplement to Murashige and Skoog, 1969 basal media [9]. Propagation of banana plants through the tissue culture techniques has been reported by several workers using different sources of explants and methods [10-15]. In 1984, Sandra and Krikorian was reported highest rate of *in vitro* multiplication (9.1 shoots/explant) of *Grand naine* plants was achieved on modified Murashige and Skoog [8] medium supplemented with 5.0 mg of 6-benzylaminopurine (6-BAP. But during the *in vitro* multiplication of cultivars of Bari-1 was achieved 4.52 shoots per plant multiplication on the same concentration of 6-BAP supplemented in modified Murashige and Skoog (1962) medium [16]. In 1986, Balakrishna Moorthy and Sree Rangaswamy was reported that the multiple shoot production can be induced by using shoot tip culture in MS medium supplemented with 2.5 to 5 mgL<sup>-1</sup> of BAP on cultivars of Matti, Robusta and Monthan under *in vitro* conditions. In different cultivars of bananas, the effectiveness of BAP over other cytokinins was reported in inducing multiplication by using shoot tip cultures under *in vitro* conditions [17-21].

Non true-to-type plants are produced due to cause of various chromosomal abnormalities

at higher concentrations of plant growth hormones is often disadvantages for *in vitro* propagation of plantlets (D'Amato, 1978). *In vitro Grand naine* plantlets growth is not only affected by type of cytokine used, and also affected by the concentration of BAP used in the MS 1962 medium. To obtain normal *Grand naine* plantlets, the application of BAP in the modified Murashige and Skoog 1969 media needs to be carefully monitored. BAP (*sigma made*) can be the most expensive plant growth hormone of plant tissue culture media. The BAP effects the growth of tissues in culture *i.e.* shoots elongation and proliferation of plantlets. Hence, an attempt is made in present study to determine the effects of BAP at different concentrations supplemented in modified Murashige and Skoog 1962 medium, on proliferation, multiplication and growth of *Grand naine* plantlets under *in vitro* conditions. This type of study was not carried out in the study area formerly, hence the present study is proposed with a specific objective to study the effect of BAP on *in vitro* plantlets of *Grand naine*.

### [II] MATERIALS AND METHODS

#### 2.1 plant material and explants source

The experiment was carried out at Plant Tissue Culture Laboratory in Department of Biotechnology, Sree Vidyanikethan Engineering College (Autonomous), Andhra Pradesh, India. The multiple shoots of *Grand naine* plantlets were separated and excised into small pieces and aseptically implanted into all types of media.

#### 2.2 culture medium and conditions

The base medium was modified Murashige and Skoog (MS,1962) medium supplemented with 30 gL<sup>-1</sup> sucrose, 2 mgL<sup>-1</sup> of BAP and 1 mgL<sup>-1</sup> of NAA (CBAM-1). Six different types of media were prepared according to 6-BAP concentrations (6BAM-2: 0.5 mgL<sup>-1</sup>; 6BAM-3: 1.5 mgL<sup>-1</sup>; 6BAM-4: 3.7 mgL<sup>-1</sup>;

6BAM-5: 6.8 mgL<sup>-1</sup>; 6BAM-6: 8.7 mgL<sup>-1</sup> and 6BAM-7: 9.4 mgL<sup>-1</sup>). 25 ml of distilled water was added in to each 250 ml conical flask. Sucrose was added and stirred it to dissolve completely, and then the stock solutions of major, minor, iron source, vitamins and hormones were added one by one. The final volume of 100 ml was obtained by adding sterilized double distilled water. Agar was added to the boiling media slowly and gradually with constant stirring to avoid formation of any clumps. All the media were adjusted the pH value between 5.75 - 5.80 by using of 1N HCl or 1N NaOH before autoclaving. Then the media were dispensed into culture vessels, plugged with polypropylene caps and autoclaved at 121°C temperature and 15 lbs pressure for 15 minutes. Sterilized media were allowed to cool, kept for contamination observations and used for inoculation.

The expurgate shoots of *Grand naine* (*Musa* sp.) plantlets were cultured on all types of media. Now, all the inoculated culture vessels were incubated in plant growth shelf at 24±2° C in growth room provided by fluorescent tubes (2000 Lux) and exposed to 16 hrs of photoperiod, 8 hrs of dark period and 60% of relative humidity.

These cultures were allowed eight weeks in the growth room for shoot proliferation and multiplication. Response of the explants and their growth, number of shoots, shoot length and multiplication rate were recorded. This experimental data was collected and recorded at the end of the 8<sup>th</sup> week from the subculture. The data were used to obtain mean of shoot length and number of shoots, and multiplication rate of *Grand naine* plantlets under *in vitro* conditions.

### [III] RESULTS AND DISCUSSION

The effect of different concentrations of 6-benzylaminopurine (6BAP) on *Grand naine* (*Musa* sp.) banana explants was investigated.

After eight weeks in all types of media, the response, proliferation and multiplication of *Grand naine* (*Musa sp.*) explants were observed and recorded. Less response of explants (9% & 12%) was observed in two types of media (6BAM-2 & 6 BAM-7) and recorded (fig-1, 2 & Table-1). High response of explants (95%) was observed in MS medium (6BAP-1) supplemented with 2.0 mg L<sup>-1</sup> of 6BAP (fig-1, 2 & Table-1) and recorded. Restrained response of explants (73% & 76%) was observed in two types of media (6BAM-3 & 6BAM-4) and recorded (fig-1, 2 & Table-1). The results indicated that the *Grand naine* (*Musa sp.*) plantlets proliferation and multiplication rate affected by various concentrations of 6-benzylaminopurine (6BAP) in MS media.

### 3.1 Shoot length

Explants were cultured on MS medium supplemented with different concentrations of 6-benzylaminopurine (6-BAP). All these treatments of explants were produced shoots throughout the culture period and observed the formation of new shoots from the explants in all the media. During the experimental period the shoots lengths of the *Grand naine* (*Musa sp.*) plantlets were observed, measured and recorded. There were clear *Grand naine* (*Musa sp.*) shoot lengths differences among all the various treatments of 6-benzylaminopurine (6-BAP) (fig.-1 and Table-1) in MS media. The 6BAM-1 medium did not affect the lengths of shoots, but remaining all the types media were affected the shoot lengths of the *Grand naine* (*Musa sp.*) plantlets. Overall, the highest mean shoot length (5.68 cm) was observed on 6BAM-1 medium, grow taller than the other cultures (fig.-1 & table-1).

The lengths found from the plants, which were cultured on different concentrations of 6-benzylaminopurine (6BAP) (6BAM-1: 2

mgL<sup>-1</sup>; 6BAM-2: 0.5 mgL<sup>-1</sup>; 6BAM-1.5 mgL<sup>-1</sup>; 6BAM-3.7 mgL<sup>-1</sup>; 6BAM-6.8 mgL<sup>-1</sup>; 6BAM-8.7 mgL<sup>-1</sup> and 6BAM-9.4 mgL<sup>-1</sup>) has the mean of shoot lengths 5.68±0.3cm, 1.92±0.6cm, 3.95±1cm, 3.05±0.8cm, 2.48±0.5cm; 1.53±0.4cm and 1.28±0.8cm respectively (fig.-1 & table-1). Media supplemented with 9.4 mg L<sup>-1</sup> of 6-benzylaminopurine give the least mean shoot height (1.8 cm) among all the type of media (fig.-1 & table-1). The result indicated that the highest length of shoots formed supplementing with 2 mgL<sup>-1</sup> of 6-benzylaminopurine (6BAP-1; 5.68 cm) (fig.-1 & Table-1). From the table-1 it appears that the mean of shoots lengths decreases by increasing the concentration of 6-benzylaminopurine in MS 1962 medium.

### 3.2 Multiplication

The trimmed explants were cultured on modified Murashige and Skoog medium 1962, supplemented with different concentration of BAP at the proliferation phase. M S medium supplemented with 2 mgL<sup>-1</sup> BAP induced the maximum number of normal and elongated shoots (Fig-1a), although more proliferated shoots were observed in MS medium supplemented with BAP at 9.4 mgL<sup>-1</sup>, but those were abnormal (Fig-1g). The lowest numbers of elongated shoots were observed MS medium supplemented with BAP at 0.5 mgL<sup>-1</sup> and 9.4 mgL<sup>-1</sup> (Fig-1b & 1g).

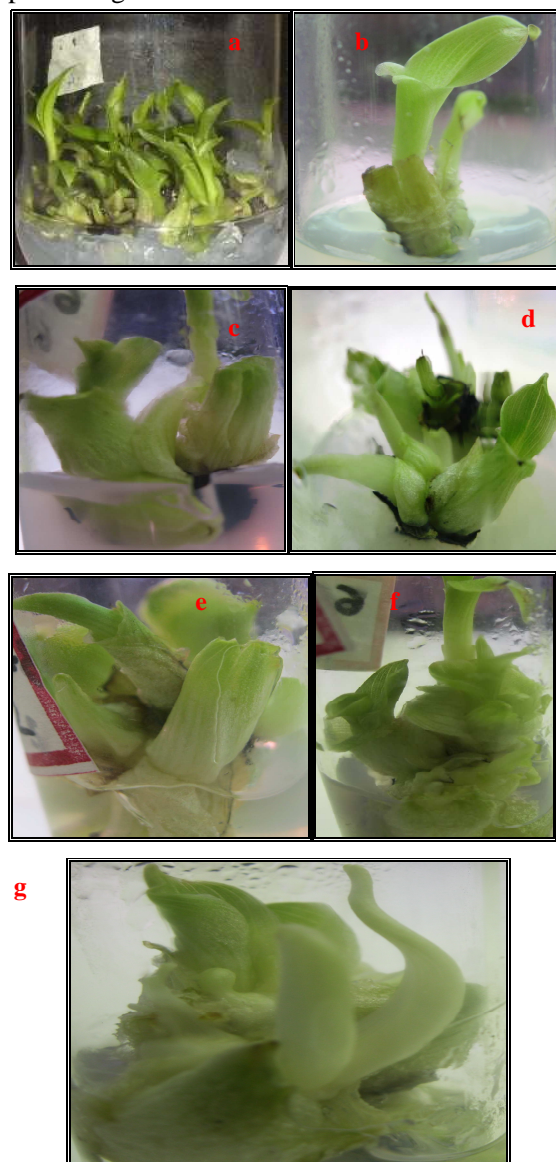
The results indicated that, the multiplication rate was decreased with decreasing the concentration of BAP in MS medium because less bud formation (cease of the cell division) and also the multiplication rate decreasing with increasing the concentration of BAP in MS medium due to abnormality development of the buds. The application of high BAP concentration, to initiate bud formation from the explants were reported by Zaffari *et al.*,

(2000) and Subramaniam *et al.*, (2008) in Cavendish banana cultivar Brasilian (AAA). Previous researchers (Vuylsteke and De Langhe, 1985; Bairu *et al.*, 2008) [17] indicated that  $5 \text{ mgL}^{-1}$  ( $22.2 \text{ }\mu\text{M}$ ) BAP was the optimum concentration for most banana cultivars. However, Arinaitwe *et al.*, (2000) reported that *in vitro* bud initiation from banana was cultivar dependent. Vidya and Nair (2002) reported that occurrence of somaclonal variants in red banana (AAA) is due to the presence of high concentration of BAP in the culture medium. This study suggested that high concentration of BAP after bud initiation was not essential for shoot propagation due to increase the abnormality of plantlets at multiplications level. High concentrations of BAP did not allow recovery of the explants in tissue cultures in becoming complete normal plants due to the habituation effect of BAP. From this study we observed that the optimal concentration of BAP ( $2 \text{ mgL}^{-1}$  of BAP) was required for normal proliferation, growth and multiple induction of *Grand nine* (*Musa* sp) in MS medium. Venkatachalam *et al.*, [17] reported a reduction in the number as well as length of shoot that occurred with exposure to high levels of BAP alone ( $44.44 \text{ }\mu\text{M}$ ) in banana cv. Nanjanagudu Rasabale (AAB).

The abnormality index was decreased with decreasing the concentration of BAP in MS medium. The highest abnormality index was noticed in MS medium supplemented with  $9.4 \text{ mgL}^{-1}$  of BAP. This type of *Grand naine* cultures were appears as a cluster of corms and produced small stunted shoot clusters. These results showed that the BAP decreasing to  $0.5 \text{ mg L}^{-1}$  significantly caused lower abnormality index than  $9.5 \text{ mgL}^{-1}$ .

In this study, the abnormality index increased with increasing concentrations of BAP with the highest value at  $9.4 \text{ mgL}^{-1}$  of BAP. This

study had shown that increasing the concentration of BAP during the initiation stage enhanced the fresh weight and percentage of buds formation.



**Fig-1:** *In vitro* development and shoot induction of *Grand naine* on the modified medium.

- Development of shoots on MS medium (6BAM-1) supplemented with  $30 \text{ g L}^{-1}$  of sucrose,  $2 \text{ mgL}^{-1}$  of BAP and  $1 \text{ mg L}^{-1}$  of NAA (Control medium).
- Development of shoots on MS medium (6BAM-2) containing  $0.5 \text{ mg L}^{-1}$  of 6BAP.
- Development of shoots on medium (6BAM-3) supplemented with  $1.5 \text{ mg L}^{-1}$  of 6BAP.

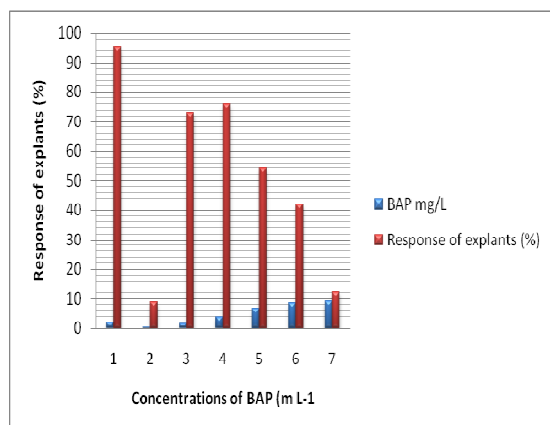
EFFECTS OF 6-BENZYL AMINO PURINE (6-BAP) ON IN VITRO SHOOT MULTIPLICATION OF GRAND NAINA (*MUSA SP.*)

- d. Development of shoots on MS medium (6BAM-4) containing 3.7 mg L<sup>-1</sup> of 6BAP.
- e. Development of shoots on MS medium (6BAM-5) supplemented with 6.8 mg L<sup>-1</sup> of 6BAP.
- f. Development of shoots on medium (6BAM-6) containing 8.7 mg L<sup>-1</sup> of 6BAP.
- g. Development of shoots on MS medium (6BAM-7) supplemented with 9.4 mg L<sup>-1</sup> of 6BAP.

**Table-1:** Effect of increasing concentration of 6BAP on shoot regeneration of Grand naine (*Musa sp.*) in MS 1962 medium.

BAP mgL <sup>-1</sup>	Number of explants inoculated	Response of explants (%)	Growth
2.0	34	95	+++
0.5	28	9	+
1.5	38	73	++
3.7	40	76	++
6.8	44	54	+
8.7	38	42	-
9.4	46	12	-

+++ : High; ++: Moderate; + : Low; - : Poor



**Fig-2 :** Response of *Grand naine* (*Musa sp.*) explants (%) in different concentrations of media under *in vitro* conditions.

**Table-2:** The affects of BAP on multiple shoot induction of *Grand naine* (*Musa sp.*)

Media Code	Concentration of BAP mgL <sup>-1</sup>	Number of shoots	Shoot length Mean	Multiplication rate
6BAM-1	2.0	21±2.6	5.68±0.3	6.12±0.6
6BAM-2	0.5	10±1.9	1.92±0.6	1.67±0.5
6BAM-3	1.5	13±2.1	3.95±1	4.98±0.1

6BAM-4	3.7	14±1.9	3.05±0.8	4.64±0.4
6BAM-5	6.8	9±1.6	2.48±0.5	3.66±0.2
6BAM-6	8.7	8±2.5	1.53±0.4	2.94±0.5
6BAM-7	9.4	4±1.2	1.28±0.8	2.01±0.6

Values are the mean ± SD. Of 12 samples

#### [IV] CONCLUSION

In this study, conclude that from the modified MS (1962) medium supplemented with different concentrations of BAP (2 mgL<sup>-1</sup>, 0.5 mgL<sup>-1</sup>, 1.5 mgL<sup>-1</sup>, 3.7 mgL<sup>-1</sup>, 6.8 mgL<sup>-1</sup>, 8.7 mgL<sup>-1</sup>, and 9.4 mgL<sup>-1</sup>), the applications of BAP in the Tissue culture media needs to be carefully monitored. To establish the normal growth rate and multiplications of plantlets of *Grand naine* (*Musa sp.*) *in vitro* conditions required 2 mgL<sup>-1</sup> of BAP was suitable in medium.

#### [V] REFERENCES

- Preece JE, Sutter EG (1991), In: Micropropagation Technology and Application. Edited by PC Debergh and RH Zimmerman, Martinus Nijhoff Publishers, Dordrecht, the Netherlands, pg 71-93.
- Geeta Singh, Sudheer Shetty (2011), Impact of Tissue Culture on Agriculture in India, Invited Review, Biotechnol.Bioinf.Bioeng, Vol-I, issue3, pg 279-288.
- George Ef, Sherrington PD (1984), Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories. Exegetics Ltd., Eversley, UK.
- Kodyma A and Zaoata-Arias FJ (2004), Low-cost alternatives for the micropropagation of banana, Plant Cell Tissue Organ Cult., issue66, pg 67-71.
- Lee S W (1993), Improvement of methods used in the regeneration of micropropagated banana plantlets, pg 179-192. In:R.V.Valmayor, S C Hwang, R Ploetz, S W Lee and N V Roa (eds.), proceedings of International Symposium on Recent Developments in Banana Cultivation Technology. Pingtung, Taiwan, 14-18 December, 1991. INIBAP/ASPNET.
- Abeyarante WM, Lathiff MA (2002), *In vitro* propagation of Rathambala (*Musa AAA*) and

- the occurrence of phenotypic variations in the pseudostem, Annals of the Sri Lanka Department of Agriculture (LKA), issue4 pg 191-197.
7. Buah JN, Danso E, Taah KJ, Abole EA, Bediako EA, Asiedu J, Baidoo R (2010), The effects of different concentration cytokinins on the *in vitro* multiplication of plantain (*Musa* sp.), Biotechnology, Vol-9 issue3, pg 343-347.
  8. Murashige T and Skoog F (1962), A revised medium for rapid growth and bioassay with tobacco tissue cultures, Physiol. Plant, issue15, pg 473-497.
  9. Vuylsteke D R (1998), Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. International Institute of Tropical Agriculture, Ibadan, Nigeria, pg 82.
  10. Resmi L, Nair AS (2007), Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India, Plant Cell Tissue Organ Cult, issue88, pg 333-338.
  11. Shirani S, Mahdavi F, Maziah M (2009), Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot tips, Afr.J.Biotechnol, vol-8, issue21, pg5755-5761.
  12. Madhulatha P, Anbalagan M, Jayachandran S, Sakthivel N (2004), Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA), Plant Cell Tissue Organ Cult., issue76, pg 189-192.
  13. Venkatachalam L, Thimmaraju R, Sreedhar RV, Bhagyalakshmi N (2006), Direct shoot and cormlet regeneration from leaf explants of "silk" banana (AAB), In *Vitro* Cell.Dev. Biol.Plant, issue42, pg262-269.
  14. Wong WC (1986), *In vitro* propagation of banana (*Musa* spp.): Initiation, proliferation and development of shoot-tip cultures of defined media, Plant Cell Tissue, Organ Culture, issue6, pg 159-166.
  15. Banerjee N, De Langhe EA (1985), Tissue culture techniques for rapid clonal propagation and storage under minimal growth conditions of *Musa* (Banana and plantain). Plant Cell Report, issue4, pg351-354.
  16. Rahman MZ, Sharoar MG, Matin MN, Rahman MH, Rahman MM, Islam MR (2006), High frequency plant regeneration of a dessert banana cv. Mehersagar for commercial exploitation, Biotechnology, vol-5, issue3, pg296-300.
  17. Venkatachalam L, Sreedhar RV, Bhagyalakshmi N (2007), Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers, Plant Growth Regul, issue51, pg192-205.
  18. Resmi L, Nair AS (2007), Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India. Plant Cell Tissue Organ Cult., issue88, pg 333-338;
  19. Farahani F, Aminpoor H, Sheidali M, Noormohammadi Z, Mazinani MH (2008), An improved system for *in vitro* propagation of banana (*Musa acuminata* L.) cultivars, Asian J. Plant Sci., vol-7, issue1, pg116-118.
  20. U P Bhosale, S V Dubhashi, N S Mali, H P Rathod (2011), *In vitro* shoot multiplication in different species of Banana, Asian J. Plant Sci. Res., vol-1, issue 3, pg23-27.
  21. Najmesh Jafari, Rofina Yasmin Othman and Norzulaani Khalid (2011), Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan, African Journal of Biotechnology vol-10, issue13, pg2446-2450.