Plant Science Research 41 (1&2) : 8-11, 2019

Plant Science Research

ISSN 0972-8546

Substitution of BAP with meta-Topolin (m-T) in multiplication culture of *Musa* species

Nimisha Mohapatra and Bandita Deo[Ψ]

Plant Physiology and Biochemistry Division, Regional Plant Resource Centre, Bhubaneswar - 751 015, Odisha, India

ARTICLE INFO

Article history:
Received : 24 October 2019
Revised : 22 November 2019
Accepted : 18 December 2019

Keywords:

Meta-Topolin
Benzyladenine
Micropropagation
Banana
In vitro.

ABSTRACT

Meta-Topolin (mT), a benzyladenine analog [N 6-(3-hydroxybenzylamino) purine], is a highly active cytokinin. Benzyladenine (BA) is the most widely used cytokinin in the micropropagation industry due to its effectiveness and affordability. The effect of the cytokinin (meta-Topolin) on micropropagation of banana cultivar Patakpura was studied and compared to BA (6-benzylaminopurine). *In vitro* cultures were sub-cultured on MS media containing BAP and m-T. Results obtained after six weeks of growth showed that there were statistically significant differences among the parameters analyzed for different treatments. Higher multiplication rates were recorded with cultures treated with m-T compared to BAP.

1. Introduction

Bananas and plantains (*Musa* spp.) are among the most important fruit crops in the world and are staple food for millions across the globe (FAO, 2010). Presently banana is grown in around 150 countries across the world on an area of 50.34 million hectare producing 106.84 million tonnes (FAO, 2015). World total banana and plantain production ranks at the 5th place after cereals, and there is still much scope for yield improvement (Jain *et al.*, 2004). It represents an essential source of nutrients for millions of people, particularly in tropical and subtropical regions, as well as a cash crop in many developing countries. In general, banana cultivars are considered as good sources of carbohydrates, proteins, vitamins and minerals (Anbazhagan *et al.,* 2014). Banana is generally propagated vegetatively through suckers, which grow from lateral buds originating from corms and suckers. Thus, banana propagation through conventional method using young shoots or part of the tuber is not an ideal method. This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material (Hussein, 2012).Tissue culture plants have been reported to produce 39% higher yield than plants from sword suckers (Pradeep *et al*., 1992) likely performed better than in banana (Faisal *et al*., 1998). Shoot apex, nodal segments and root segments were successfully used for callus induction and regeneration (Jatoi *et al*., 2001) and cytokinin helps in shoot multiplication (Cronauer and Krikorian, 1984a). Growth regulators namely auxin, cytokinin, gibberellin and abscisic acid like kinetin, indole-3-acetic acid, benzyl-aminopurine etc were used for the *in vitro* regeneration of various plants (Ali *et al*., 2014 and 2015; Momena *et al*., 2014). Cytokinins such as benzyl-aminopurine (BAP) are known to reduce apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana (Madhulatha *et al*., 2004). Effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different banana cultivars (Buah *et al*., 2010; Farahani *et al*., 2008; Rahman *et al*., 2006; Resmi and Nair, 2007).Meta-

Topolin is first isolated from poplar leaves. Meta-Topolin is a natural constituent of plant tissues, together with its 9-â-D-ribofuranosyl and 9-â-v-glucopyranosyl derivatives. Meta-Topolin is more active than zeatin and benzyladenine in the promotion of shoot formation in plant tissue cultures (Werbrouck *et al*., 2008). The aim of this work was to assess the potential of meta-Topolins as alternatives to BA in banana tissue culture.

2. Material and methods

The present investigation was carried out to study the effect of meta-Topolin during multiplication culture of *Musa* species through tissue culture in the Banana Tissue culture Laboratory, Regional Plant Resource Centre, Bhubaneswar.

Patakpura variety is a locally available cultivar of banana found in coastal region of Odisha. It is very popular for its sweet taste and pleasant flavour. Tissue cultured plants of banana variety Patakpura were taken as source of explants. Aseptically established *in vitro* plantlets were cut transversely to separate leaves and produce a section of pseudostem approximately 1 cm in length, including an intact vegetative bud. The lower part of the pseudostem was trimmed to remove darkened or necrotic tissues and the sheath removed carefully by peeling. The explants were then cut into half longitudinally.These explants were cultured in bottles containing modified Murashige and Skoog (1962) including macro- and micro-elements (Vuylsteke, 1998) with growth regulators such as auxin (IAA), cytokinin (BA) and (meta-Topolins) m-T. After adjusting the pH to 5.8, the media was autoclaved at 121°C and 103 kPa for 20 min. An air conditioned culture room with controlled temperature of 25 ± 2ºC and light intensity of 2000-3000 lux for a photo-period of 16 h of light by cool white fluorescent tubes was used to incubate the tissue cultured plantlets. The photo-period was controlled manually by turning on / off the light switches. Artificial lighting was provided by providing cool-white, fluorescent tubes to carry out most of the micropropagation work. Sub-cultures were made at intervals. After inoculation, observationsof the growth responses, multiplication efficiency of BAP and m-T were compared.

3. Results and discussion

In this experiment, banana var. Patakpura was used to study the effect of naturally occurring PGR (Plant Growth Regulator) and synthetic PGR on multiplication culture. During this 4th multiplication culture of the Patakpura variety, eight treatments (T1 to T8) of Basal MS Medium was used with different concentrations of BAP+ IAA and BAP +m-T. During 7-14 days of multiplication culture, less contamination of cultures was observed. During this period explants turned green in colour and clusters of 3-4 proliferating buds with 1-3 auxiliary buds were regenerated from the basal parts of explants around the meristematic region, which is shown in Table 1.

After 15 days of inoculation, a combination of BAP + IAA or BAP + m-T) in 5th multiplication culture of Patakpura exhibited differential effects on shoot length, number of shoots and fresh weight. Interactive effect of BAP and synthetic hormone m-T was observed among different parameters in multiplication culture. The MS basal media containing BAP+ mT at a concentration of 3mg + 0.1mg showed significant increase in high fresh weight (6.75 gm) compared to treatment of BAP + IAA (2.96gm). In terms of shoot number, medium with 3 mg BAP + 0.1 mg m-T resulted in production of 3.79 shoots in comparison with the combination BAP + IAA (3.1).

Table 1

Effects of different concentration of BAP, m-T and IAA on multiplication culture of banana var. Patakpura

Code	Treatment	Concentration	Mean Fresh Weight (gm)	Mean Shoot Length (cm)	Mean no. of shoots/explant
T1	BAP + m-T	1mg + 0.1mg	3.90 ± 0.54	2.14 ± 0.54	6.10 ± 1.19
T2	BAP + m-T	2mg + 0.1mg	4.25 ± 0.47	2.75 ± 0.18	6.78 ± 1.15
T3	BAP + m-T	3mg + 0.1mg	6.75 ± 0.28	3.79 ± 0.60	7.80 ± 1.13
T4	BAP + m-T	4mg + 0.1mg	3.65 ± 0.64	2.79 ± 0.69	6.4 ± 1.30
T5	BAP + m-T	1mg + 0.1mg	2.96 ± 0.79	2.89 ± 0.15	5.10 ± 0.69
T6	BAP + m-T	2mg + 0.1mg	3.16 ± 0.69	2.23 ± 0.45	5.40 ± 1.71
T7	BAP + m-T	3mg + 0.1mg	3.80 ± 0.53	3.14 ± 0.78	5.95 ± 1.05
T8	BAP + m-T	4 mg + 0.1mg	3.76 ± 0.46	3.18 ± 0.57	5.20 ± 0.69

Figure 1: Effect of meta-Topolin with BAP in 5th subculture of multiplication stage of Patakpura variety of banana

Figure 2: Effect of BAP with IAA in 5th subculture of multiplication stage of Banana var. Patakpura

After 21 days of culture, it was observed that the Patakpura explants produced large numbers of shoot buds during multiplication culture. The culture showed higher regeneration capacity in medium containing m-T compared to IAA supplementation with BAP. From the study it was found that MS medium supplemented with BAP at the concentration of 3mg, showed highest average fresh weight (6.75 gm), highest shoot length (4.23 cm) and more number of shoot per explants (7.8) as compared to the other combinations of BAP supplemented with m-T and IAA. The combination of BAP (3 mg) and mT (0.1 mg) showed maximum proliferation compared to the treatment combination (BAP+ IAA) in 5th subculture of multiplication culture (**Figure 1 & 2**).

Meta-topolin is reported to promote *in vitro* shoot proliferation and improve quality of shoots in many plant species (Aremu *et al*., 2012). With application of such cytokinins, Mongomake *et al*. (2015) and Taylor *et al*. (2012) have found enhanced germination of somatic embryos and induction of shoot organogenesis from cotyledon explants in Cassav (*Manihot esculenta*) by Li *et al*. (1998). The effect of *meta-Topoline* on micro-propagation of banana cv. Patakpura as assessed during the present study is comparable to the results obtained by Mongomake *et al*. (2015 and Raemakers *et al*. (1993) in Cassava.

Conclusion

From the above result and observation during multiplication culture of banana var. Patakpura, it was observed that the presence of BAP along with IAA in the culture medium induced efficient shoot multiplication. The use of combination BAP along with IAA and m-T in MS medium was found to enhance fresh weight, average number of shoots and shoot length at a concentration of 3mg/l BAP + 0.1 mg m-T. Though the cultural processes described above have to be fully optimized, it is observed that use of m-T brings important and open up new opportunities for development of rapid plant regeneration systems with potential applications. From this experiment, it was concluded that meta-Topolin was quite effective in inducing multiple shoots, which needs further study.

Acknowledgement

The authors are thankful to the Chief Executive, Regional Plant Resource Centre, Bhubaneswar for providing required laboratory and field facilities to carry out the research work.

References

Ali, M. R., Akand, M. H., Hoque M. E., Homayra Huq, Mehraj, H. and Jamal Uddin, A. F. M. (2015). *In vitro* regeneration and rapid multiplication of tuberose. Int. J. Bus. Soc. Sci. Res. 3(1): 35-38.

Ali, M. R., Mehraj, H. and Jamal Uddin, A. F. M. (2014). Kinetin (KIN) and Indole-3- Acetic Acid (IAA) on *in vitro* shoot and root initiation of tuberose. Int. J. Sustain. Agril. Tech. 10(8): 1-4

Anbazhagan, M., Balachandran, B. & Arumugam, K. (2014). *In vitro* propagation of banana *Musa* spp. Int. J. Curr. Microbiol. Appl. Sci. 3:399-404.

Aremu, A. O., Bairu, M. W., Doležal, K., Finnie, J. F., Van Staden, J. (2012). Topolins: a panacea to plant tissue culture challenges? Plant Cell Tiss. Organ Cult. 108: doi:10.1007/s11240-011-0007-7.

Buah, J. N., Danso, E., Taah, K. J., Abole, E. A., Bediako, E. A., Asiedu, J. and Baidoo, R. (2010). The effects of different concentration cytokinins on the *in vitro*

multiplication of plantain (*Musa* sp.). Biotechnology 9(3): 343-347.

Cronauer, S. S. and Krikorian, A. D. (1984a). Multiplication of *Musa* from excised stem tips. Annals Bot. 53(3): 321-328.

Faisal, S. M., Haque, M. A. and Quasem, A. (1998). Field performance of *in vitro* plantlets against normal suckers of banana (*Musa sapientum*) cv. Champa. Plant Tissue Cult. 8(2): 125-129.

FAO (2015). FAO website, February, 2015. Food and Agricultural Organization, Rome, 2015.

FAO (2010). The second report on the state of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization, Rome, Italy, PP. 511.

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

Guohua, M. (1998). Effects of cytokinins and auxins on cassava shoot organogenesis and somatic embryogenesis from somatic embryo explants. Plant Cell Tiss. Org. 54:1–7. doi:10.102 3/A:1006065120629.

Hussein, N. (2012). Effects of nutrient media constituents on growth and development of Banana *Musa* spp. shoot tips cultured *in vitro*. Afr. Journ. Biotech.11:9001-9006.

Jain, S. M. & Swennen, R. (2004). Banana Improvement: Cellular, Molecular Biology and Induced Mutations. Science Publishers, New Hampshire, USA.

Jatoi, S. K., Sajid, G. M., Sappal, H., Baloch, M. S., Qureshi, A. and Anwar, R. (2001). Differential *in vitro* response of tomato hybrids against a multitude of hormonal regimes. Online J. Biol. Sci. 1: 1141-1143.

Li, H., Guo, J., Huang, Y., Liang, C-Y., Liu, H-X., Potrykus, I., Puonti-Kaerlas, J. (1998). Regeneration of cassava plants via shoot organogenesis. Plant Cell Rep. 17:410–414. doi:10.1007/s002990050416.

Mongomake, K., Doungous, O., Khatab,i B. and Fondong, V. N. (2015). Somatic embryogenesis and plant regeneration of cassava (*Manihot esculenta* Crantz) landraces from Cameroon. Springer plus 4:477. doi:10.1186/s40064-015-1272-4

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

Momena, K., Adeeba, R., Mehraj, H., Jamal Uddin, A. F. M., Saiful Islam and Rahman, L. (2014). In vitro microtuberization of potato (Solanum tuberosum L.) cultivar through sucrose and growth regulators. Journal of Bioscience and Agriculture Research 2(2): 76-82.

Pradeep, K. P., Zachariah, G., Estelitta, S. and Suma. A. (1992). Field performance of banana tissue culture plants of variety Nendran (*Musa* AAB). South Indian Hort. 40(1): 1-4

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

Raemakers C., Amati, M., Staritsky, G., Jacobsen, E. and Visser, R. (1993). Cyclic somatic embryogenesis and plant regeneration in cassava. Ann. Bot. doi:10.1006/anbo.1993.1037

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

Strnad, M., Hanuš, J., Vanìk, T., Kamínek, M., Ballantine, J. A., Fussell, B. and Hanke, D. E. (1997). Meta-topolin, a highly active aromatic cytokinin from poplar leaves (*Populus* × *canadensis* Moench cv. Robusta). Phytochemistry 45(2):213-218.

Taylor, N., Gaitan-Solis, E., Moll, T., Trauterman, B., Jones, T., Pranjal, A., Trembley, C., Abernathy, V., Corbin, D., Fauquet, C. M. (2012). A high throughput platform for the production and analysis of transgenic cassava (*Manihot esculenta*) plants. Trop. Plant Biol. 5:127–139.

Werbrouck, S. P. O., Strnad, M., Van Onckelen, H. A. & Debergh, P. C. (2008). Meta-topolin, an alternative to benzyladenine in tissue culture? Physiol. Planta. 98(2):291 - 297