Proceedings of Pakistan Society for Horticultural Science
2nd International Conference on Horticultural Sciences, February 18-20, 2016
Theme: Production Challenges and Food Security
Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Punjab 38040, Pakistan

Optimization of Protocol for *In Vitro* Regenration of Selected Cultivars of Banana

Arslan Bashir[1], Shazia Erum*[2], Aish Muhammad[2], Mustafa sajid[2]
[1]International Islamic University, Islamabad
[2]National Agriculture Research Centre, Islamabad
Emails: arslanbashir29@yahoo.com (A.B), shazia_rm@yahoo.com (S.E), aish.muhammad@yahoo.com (A.M), drmustafasajid@gmail.com (M.S)

Abstract

Banana is herbaceous, monocotyledonous and evergreen perennial plant. Most of the banana cultivars are susceptible to BBTV and it causes significant economic losses to banana production. Micropropagation is preferred over the conventional method of propagation in banana due to faster multiplication rate, uniformity in planting materials and production of disease-free planting materials. The present study comprises of three exotic banana genotypes viz: Wiallium-8818 hybrid, Pisang and Brazilian. Different combinations of IAA, NAA and BAP along with MS were prepared for optimizing tissue culture protocol. The Clorox at 50% concentration for 20 min give minimum contamination percentages, both fungal and bacterial, and optimum survival percentage. Seven different combinations (T_0-T_6) of BAP and IAA and five combinations (RT0-RT4) of NAA and IAA were used to optimize multiplication and rooting protocols. It was found that BAP and IAA at 2.5 mg/lt and 1 mg/lt respectively (T_5) gave maximum (6.53) mean number of shoots per explants while longest shoot length (7.14) was recorded at MS with BAP and IAA at 1 mg/lt and 1 mg/lt (T4) respectively. Maximum mean fresh weight value (12.96) was recorded in MS medium supplemented with BAP and IAA at 5.0 mg/lt and 1.0 mg/lt (T_6) respectively. The genotype Wiallum-8818 hybrid gained maximum value for mean number of shoots per explants (4.94) and longest shoot length (8.52) while the genotype Pisang gained maximum fresh weight value (8.95). It was found that NAA at 0.5 mg/lt (RT4) gave maximum (5.70) leaves per shoot while mean roots per shoot (4.67) was recorded at MS with 1 mg/lt NAA and 1 mg/lt IAA (RT3). Maximum mean value (5.58) for longest root length was recorded in MS medium supplemented with at 1 mg/lt NAA and 0,5 mg/lt (RT2) respectively. The genotype Wiallum-8818 hybrid gained maximum value for mean leaves per shoot (4.47) while variety Pisang showed maximum value for roots per shoot (4.11) and longest root length (5.48). The propagated germplasm under in vitro conditions were further acclimatized in the green house and transported in the research fields of Sind. Moreover, disseminated to the local farmers in Sind.

INTRODUCTION

Banana (*Musa spp*) is belonging to family *Musaceae*, originally belonging from Asia and Africa (Stover and Simmonds, 1987). Banana (*Musa spp*) is the 4th largest food crop in the world, a staple food for nearly 400 million people and an essential source of income for many national economies with world imports in 1995 valued at $5.3 billion (Vuylsteke, 1989; Sasson, 1997). The world production of banana is about 95 million tons per year, grown in 132 countries worldwide.

Banana is a major fruit crop of Pakistan. It is grown on 34,800 hectares with production of 154,800 tons. Banana is mainly grown in Sindh province where the soil and climatic conditions are favorable for its successful cultivation. The total share of sindh province alone in its cultivation is 87 per cent in Pakistan. Sindh covers about 91% of total areas under banana cultivation in the country. Major districts in sindh where banana is grown are Thatta, Hyderabad, Badin, Mirpurkhas, TandoAllahyar, Matiari, Tando Muhammad Khan, Sangar, NausheroFeroz, and Nawabshah.

Banana account for about 22% of the fresh production and are ranked as the second most important fruit crop (Pua, 2007). For commercialization, it is important to ensure constant supplies of good quality bananas through their production. This could be achieved through colonel planting materials obtained through tissue culture propagation technique. This technique provides planting materials with higher capabilities of multiplying and being genetically uniform and pest and disease free. Propagation of banana through in vitro techniques has been reported by several workers using different explants sources and methods (Jalil *et al.*, 2003; Madhulatha *et al.*, 2004; Strosse *et al.*, 2006).

Banana is one of the most important fruits in the world, both as a staple food as well as a major export commodity for many tropical and subtropical countries. Plants produced by tissue culture techniques have great potential being disease free, high yielding and carrying post harvest management. The combination of mutative breeding and in vitro culture has been suggested as an alternative approach for banana improvement (Novak *et al.*, 1990). Traditional method of banana production is generally through suckers but it is laborious, time consuming and less efficient method. Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest. Tissue culture technique produce 39% higher yield than conventional sword suckers (Farahani *et al.*, 2008). A large number of uniform disease free plants can be produced from a single plant or even a small plant tissue (explants) with good genetic potential and plant multiplication can be continued throughout the year irrespective of seasonal variation (Rahman *et al.*, 2006).

METHODOLOGY

Four week old suckers of selected varieties (8818-william, Pisang, Brazilian) of *Musa sapientum* were taken from from the banana fields of NARC at Thatta (Sindh).

These suckers were peeled off to the size of 4 cm at the base and 5 cm long consisting of single shoot tip. These explants were surface sterilized using different combination of commercial bleach (Clorox 5.75% NaOCl) for 0-15 mins. After complete washing with autocalved distilled water, explants were trimmed to final size of 3-5 mm in the laminar flow cabinet (Aish *et al.,* 2005). These explants were cultured culture initiation media i.e MS media containing 1 mg/l IAA and 6 mg/l BAP each. Cultures

were incubated in growth room at 25±2°C and 16 hours photoperiod. After 55-60 days the explants having 1-2 leaves and showing growth were selected and their leaves were aseptically excised in laminar flow hood. The excised leaves were put in polythene bag and PCR for virus indexing (BBTV) was performed. Only proliferated sucker free of any viral attack were subjected to optimization of plant growth regulator.

Explants was shifted to multiplication medium. Multiplication medium consisted MS salts and vitamins enriched with different concentrations of BAP and IAA (T0-T6). Subculturing will be done after every four weeks and black tissues were removed prior to subculturing. Data was collected after 60 days. The shoots were shifted to rooting medium for optimization of rooting hormones IAA and NAA (RT0-RT4). Rooting will be observed within 45 days and data were collected accordingly.

After removing the medium from roots under water, plants were shifted to polythene bags having piete moss. Water was given to these shifted plants immediately and humidity was maintained by covering them with polythene cover. These plants were acclimatized in green house for eight weeks.

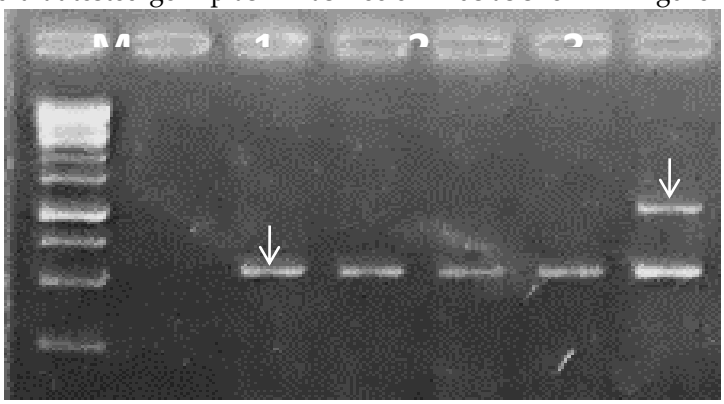
RESULTS

In the present study 3 banana genotypes (8818-william, Pisang, Brazilian) were used for optimization invitro multiplication.

Disinfection Test

Different concentrations of chlorox (0-50%) for different time intervals (5-15 mins). Among them best survival percentage (90%) was obtained using 50% chlorox for 15 mins.

For screening the presence of Banana bunchy top virus (BBTV), PCR was performed our results showed that tested germplasm was free of virus as shown in figure



Gel Doc Picture of multiplex PCR of Banana DNA samples with Rep B and Musa Actin Primers

M: 1Kb DNA ladder
Lane 1-5: Banana DNA samples amplified with Rep B and Musa Actin Primers
PC: Positive Control

Morphological Characters of Invitro Microplantlets

a) Effect of bap and IAA on shooting:

- Maximum mean number of shoots (6.53) was obtained using MS media supplemented with 2.5 and 1 mg/l BAP and IAA respectively (T5).
- Longest shoot length (8.95cm) was obtained using MS media supplemented with 1 mg/l BAP and IAA each (T4).
- Maximum fresh weight (12.96g) was obtained using MS media supplemented with 5 and 1 mg/l BAP and IAA respectively (T6).
- ***Effect of IAA and NAA on shooting:***
- Maximum mean number of leaves per shoots (5.70) was obtained using MS media supplemented with 0.5 mg/l NAA (RT4).
- Maximum mean number of roots per shoots (4.67) was obtained using MS media supplemented with 1 mg/l IAA and NAA each (RT3).
- Longest root length (5.58cm) was obtained using MS media supplemented with 0.5 and 1 mg/l IAA and NAA respectively (RT2).

Ex vitro hardening of plantlets

Our experiment showed that peat moss can be successfully used at commercial scale for successful hardening of banana plants with over 99% survival could be easily achieved by adopting the necessary precaution during hardening process

DISCUSSION

The present study was conducted using four levels of BAP (0, 1. 2.5 and 5.0 mg/L) and three levels of IAA (0, 0.5 and 1.0 mg/L) MS medium. These two hormones were employed using seven treatments viz: T0-T6 and mean values of different morphological traits viz: mean number of shoots, longest shoot length and fresh weight were calculated for each treatment.

In present study, maximum number of shoots, longest shoot length and fresh weight were increased as level of BAP (0-5 mg/L) and IAA (0-1.0 mg/L) was simultaneously increased in applied treatments. Maximum number of shoots per explants (7.33) for variety Wiallium-8818 hybrid was obtained in MS medium supplement with BAP at 2.5 mg/L and IAA at 1 mg/L while at control mean value (1.82) for same aforementioned trait of same variety was comparatively low (75.17%). Similar kind of results was obtained in an experiment to optimize best combination of different combinations of plant growth hormones (BAP, IAA and Kinetin). The combination of BAP and IAA at 2.0 mg/L and 0.5 mg/L along with MS gave highest number of shoots buds (7.85$\pm$0.26) for *Musa* species (Anbazhagan *et al*, 2014). Different kinds of results were reported by many other scientists as in a optimization study, maximum number of shoots (9.61) was found slightly higher (31.10%) as compared to our results, in MS medium with BAP at 0.56 mg/L (2.5 µM) concentration for Tantuk banana genotype (Elhory *et al.,* 2009). Similarly, in another study for optimizing invitro protocol for three banana exotic genotypes (GCTCV-215, Yangambi Km-5, FHIA-23), it was found that maximum number of shoots per explants value (8.75 shoots) was obtained in MS medium supplemented with BAP and IAA at 4 mg/L and 0.5 mg/L respectively for GCTCV-215 banana genotypes (Qamar *et al*, 2015). These different responses to aforementioned trait might be of variable genotypic responses to different levels of BAP.

We found that length of shoots was increased as level of BAP and IAA was simultaneously increased up to 1 mg/L concentration and maximum shoot length (12.20

cm) was obtained in MS medium supplemented with BAP and IAA each at 1 mg/L concentration. Similar kind of results were obtained in optimization study to explore the best combination of different combinations of plant growth hormones (BAP and Kinetin), found that combination of BAP and Kinetin at 0.5 mg/L and 1.0 mg/L respectively, along with MS gave highest length of shoots buds (8.9±1.142cm) for different *Musa* species (Miilon *et al.,* 2013). This showed the significance of using BAP along with Kinetin in optimizing banana invitro micropropagation protocols. Contrary to our findings, a experiment to optimize regeneration protocol for banana cultivar *Meitei hei*, it was found that highest mean value for maximum shoot length (6.25cm) was obtained in full strength MS medium supplemented with BAP at 0.5 mg/Land NAA at 1.0 mg/L concentration (Lalrinsanga *et al.,* 2013). This dissimilarity from our findings reflects presence of some genotypic differences or might be of reduced response of NAA as compared to IAA in our study of banana micropropagation.

In present study we found that highest mean values (12.96g) for fresh weight was obtained in MS medium supplemented with BAP and IAA at 5.0 mg/Land 1 mg/L respectively. These values were different as obtained from other studies. A study conducted to determine effect of BAP on *Musa acuminata* cv. Berangan, revealed that highest mean value (4.04±0.45 g) for fresh mass after 30 days was obtained in MS medium supplemented with BAP at 7.43 mg/L(33 µM) (Jafari *et al.,* 2010). Similarly, another study conducted on banana cultivar GCTCV-215, gained highest mean fresh value (8.77 g) after 45 days on MS medium supplemented with BAP and IAA at 4.0 mg/Land 0.5 mg/L respectively (Qamar *et al.,* 2015). On comparative analysis of results obtained by previous and present study revealed that mean fresh weight is directly proportional to number of days for recording aforesaid trait.

In the present study it was found that highest mean values (5.7 leaves) for maximum number of leaves per shoot was obtained in MS medium supplemented with NAA at 0.5 mg/L. The below mentioned previous studies showed that different combinations of plant growth regulators (auxins / cytokines / auxins + cytokines) were applied by different researchers to evaluate number of leaves per shoot. Our results are in accordance with an experimental study using auxins only in optimizing micropropagation protocol of Grand Naine banana genotype gained mean number of leaves per shoots (5.66 leaves) in half strength MS supplemented with IBA and NAA each at 1 mg/L concentration (Ahmed *et al.*, 2014). Contrary to present results, a study conducted to determine affect of BAP on *Musa sp.* cv. Banana BARI-1 revealed highest mean value for leaves per shoots (7.0 leaves) was obtained in MS medium supplemented with BAP and NAA at 7.5 mg/L and 0.5 mg/L respectively after thirty days (Al-amin *et al.,* 2009). This difference might be of using combination of cytokinins (BAP) along with the auxins (NAA) contrary to our treatments of using only auxins (NAA).

In the present study maximum number of roots per shoot (5.50) was obtained in MS medium supplemented with IAA and NAA each at 1 mg/L concentration. Our result is partially similar with a study conducted on optimization of root induction protocol for banana cv. Grand Naine and highest number of roots per shoot (6.4) using IAA at 1.0 mg/L concentration (Miilion *et al.*, 2013). This similarity is showing a positive contribution of IAA in increasing number of roots per shoot.

In our study maximum root length (6.25 cm) was obtained in MS medium with IAA at 0.5 mg/L concentration. Similar root length (7.80 cm) for banana Grand Naine genotype was obtained in another study by using half strength MS along with activated charcoal

(200 mg/*L*) and IBA (1.0 mg/L) concentration (Ahmed *et al*., 2014). This showed that root length is depended on number of factors including auxins (type and concentration), MS strength (full / half), concentration of activated charcoal and genotype. These similarities in previous and present studies also suggest that auxins (IBA and IAA) at 1 mg/L concentration might be useful in obtaining long roots for different banana genotypes

REFERENCES

Ahmed, S., A. Sharma, A.K. Singh, V.K. Wali and P. Kumari. 2014. In vitro multiplication of banana cv, Grand naine. J. Afr Jr of Biotech. 13(27):2696-2703.

Aish, M., M.M. Iqbal, I. Hussain. And H. Bilal. 2005. Optimization of In Vitro Micropropagation Protocol for Banana (*Musa Sapientum L.*) Under Different Hormonal Concentrations and Growth Media. J. Int. J. of Agric. Innovations and Res. 12(1):23-27.

Al-amin, M.D., M.R. Karim, M.R. Amin, S. Rahman and A.N.M. Mamun. 2009. In vitro micropropagation of banana (*Musa spp*.) Bag Jr of Agril Res. 34(4):645-659.

Anbazhagan, M., B. Balachandran and K. Arumugam. 2014. In vitro propagation of(*Musa sp*) banana. International Journal of Current Microbiology and Applied Sciences. 3(7):399-404.

Elhory, S.M.A., M.A. Aziz, A.A. Rashid and A.G. Yunus. 2009. Profilic plant regeneration through organogenesis from scalps of *Musa sp* cv Tanduk. Afr. J. of Biotech. 8(22):6208-6213.

Farahani, F., H. Aminpoor, M. Sheidai and Z. Noormohammadi. 2008. An Improved System for In Vitro Propagation of Banana (*Musa acuminate L.*) Cultivars. Plant Sci. 7(1):116-118.

Jafari, N., R.Y. Othman and N. Khalid. 2011. Effect of benzylaminopurine (BAP) pulsing on in vitro shoot multiplication of *Musa acuminate* banana cv. Berangan, Afr. J. of Biotech. 10(13):2446-2450.

Jalil, M., N. Khalid and R.Y. Othman. 2003. Plant Regeneration from Embryogenic Suspension Cultures of Acuminata cvmas. Plant Cell Tissue Organ Cult. 75:209-214.

Lalrinsanga, R., H. Vanlaldiki and W.I. Meitei. 2013. In vitro shoot tip culture of banana cultivar Meitei Hei, The Bioscan. 8(3):839-844.

Madhulatha, P., M. Anbalagan, S. Jayachandran and 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.

Miilon, P.M., G.K. Varsha and H.N. Bhagyshri. 2013. Effect of IAA and IBA on In vitro rooting of banana (*Musa paradisiaca*) cv. Grand Naine, International Journal of Science and Research. 4(5):959-962.

Novak, F.J., R. Afza, V.M. Duren, T. Xiaolang and V.B. Conger. 1990. Somatic Embryogenesis and Plant Regeneration in Suspension Cultures of Dessert (Aa and Aaa) and Cooking (Abb) Bananas (*Musa spp*.). Nature Bio-technology. 7:154-159.

Pua, E. C. Banana Biotechnology in Agriculture and Forestry. Transgenic crops. 60: 3-34.

Qamar, M., S.T. Qureshi, I.A. Khan and S. Raza. 2015. Optimization of in vitro multiplication for exotic banana *(Musa spp*.) in Pakistan. Afr. Jr. Biotech. 14(24):1989-1995.

Rahman, M.Z., M. Sharoar, M.N. Matin, M.H. Rahman, M.M. Rahman and M.R. Islam. 2006. High Frequency Plant Regeneration of a Dessert Banana Cv. Biotechnology. 5(3):296-300

Sasson, A. 1997. Future Prospects of Biotechnology in Tropical and Subtropical Horticulture Species. Acta Horticulture. 460:12-26.

Stover, R.H. and N.W. Simmonds. 1987. Bananas, 3rd Edition. Longman, london.

Strosse, H., H. Schoofs, B. Panis, E. Andre, K. Reyniers and R. Swennen. 2006. Development of Embryogenic Cell Suspensions from Shoot Meristematic Tissue in Bananas and Plantains (*Musa Spp*.). Plant Sci. 170:104-112.

Vuylsteke, D. 1989. Shoot Tip Culture for the Propagation, Conservation and Exchange of Musa Germplasm. Practical Manuals for Handling Crop Germplasm in Vitro.2: 82-89.