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Micropropagation of Native Cultivars of Banana- A Critical Review

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ABSTRACT

Bananas are important tropical fruit which stands second in area and production of fruit crops. Most of the indigenous commercial cultivars of banana are derivatives of natural hybrids from Musa balbisiana. Genetic diversity and diverse agro climatic conditions prevailing in Indian subcontinent have encouraged the development and sustenance of a large number of varieties catering to local needs. Even though the fruit characters like flavour, size, shape, tastes are superior its production levels are meagrely contributing for countries banana fruit production. Perpetuation of planting material by tissue culture in native cultivars is difficult because of the occurrence of apical dominance mediated growth arrest. In vitro regeneration in banana can be achieved through shoot tip culture as a direct organogenesis. Different kinds of cytokinins and auxins have been used for micropropagation of banana cultivars and shoot proliferation rate is significantly affected by type of banana cultivars and their genomic constitution. Morphologically abnormal plants in local banana cultivars are observed during hardening period which is strongly influenced by the genetic stability of each cultivar, and that its frequency is amplified by culture -induced factors.

Key words: *Micropropagation, Native cultivars, Shoot tip, Somaclone.*

INTRODUCTION

Banana and Plantains (*Musa* spp.) are some of the earliest crop plants having been domesticated by humans. Bananas and plantains constitute the fourth most important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes.

Botany:

Bananas and plantains are monocotyledonous plants in the genus Musa (Musaceae, Zingiberales). They are giant herbs, commonly up to 3 m in height, with no lignification or secondary thickening of stems that is characteristic of trees[32].

The term banana is used throughout the text to refer to all types of bananas including cooking bananas and plantains.

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There is no straightforward botanical distinction between bananas and plantains but, in general, bananas refer to the sweeter forms that are eaten uncooked, while starchy fruits that are peeled with a knife when unripe and then cooked are referred to as plantains and cooking bananas[7].

Bananas with all its species, varieties or hybrids belong to the genus *Musa*, order Zingiberales, family Musacea*e*[4]. The genus *Musa* contains 30-40 species, with all wild species being diploids (2n=2x= 14, 18, 20, 22) and native to South East Asia[31].

Centre of origin:

Bananas originated from South East Asia, a region considered as the primary centre of diversification of the crop and where the earliest domestication has occurred[28]. This is an area bordered on the west by India and on the east by Samoa, Fiji and other South Pacific islands[29]. *Musa acuminata*is said to have originated from Malaysia, while the hardy *Musa balbsiana*originated from Indochina.

Native cultivars of India:

The Indian subcontinent is thought to have been the major centre for hybridisation of *acuminata* types with the indigenous *M. balbisiana* and the region is noted for the wide variation of (AAB) and (ABB) cultivars[21]. Cultivated bananas and plantains evolved from the intra and/or interspecific hybridisation of two wild species, *Musa accuminata* Colla. that originates from Malaysia and *M. balbsiana* Colla. originating from Indochina[34]. As a result of natural hybridisation, a range of diploid cultivars designated as AA evolved. Triploids AAA were generated from AA by chromosome restitution while inter specific hybridizations between *Musa acuminata* (AA) and *M. balbisiana* (BB) gave rise to various groups like AB, AAB and ABB. Hybridization has been accomplished artificially by cross-pollination to produce genome groups like AABB, ABBB, and AAAB. In such cases no flow of transgenes is possible among the triploids since they are generally sterile while the developed hybrids (tetraploids) are clonally propagated.

India has been considered as one of the important centres of biodiversity for banana, having about eight species and 120 distinct clones. Banana is well adapted to diverse regions varying from humid tropics to humid sub tropics and semi-arid sub tropics upto an elevation of 2000 m above mean sea level. The wide range of adoption of banana is due to its appreciable genetic diversity and suitability to varied agro climatic conditions. The diverse agro climatic conditions have encouraged the development and sustenance of a large number of varieties catering to local needs[23].

The micro propagation in native cultivars is of limited success mainly because of the occurrence of hormone-mediated apical dominance of the mother plant.A plant produces only 5-20 suckers during its life time of 12-14 months. Even though there is a huge demand for local cultivars across the country supply of tissue culture plantlets is meagre. For accelerating the propagation rate, suckers with growing buds or cut rhizomes called ‘bits’ and ‘peepers’ are used. Several good bits, each with a centrally placed germinating eye can be cut from an unbunched rhizome after trimming the roots. Selection of appropriate mother plant for raising new propagules through *in vitro* methods is important.

Shoot tip culture

The earliest reports of *in vitro* culture of bananas came from Taiwan in the 70’s [13,14]. Shoot tip (meristem plus a few attached leaf primordia) culture of *Musa* may be considered simple, easy, and applicable to a wide range of musa genotypes[2,34]. *In vitro* propagation has many advantages, such as higher propagation rates for multiplying clean (pest and pathogen free) planting materials, small space requirements regardless of season, and short time requirements[9]. Till date, protocols have been standardized for *in vitro* propagation of a wide range of *Musa* species and cultivars belonging to various ploidies and genomes[27]. Shoot tips can be extracted from the pseudostem, suckers, peepers, lateral buds or even small eyes which contain a shoot meristem[11,33]. Though all of them behave

similarly under *in vitro* conditions, peepers and sword suckers are highly preferred because of their ease of handling and the minimum damage caused to the parent stool during their removal. It is always better to collect the explants from flowering plants so as to ascertain their trueness to type. The steps followed for production of micropropagation based banana planting material are:

Initiation of shoot culture

Shoot cultures of banana start conventionally from any plant part that contains a shoot meristem, i.e. the parental pseudostem, small suckers, peepers and lateral buds. For rapid *in vitro* multiplication of banana, shoot tips from young suckers of 40–100 cmheight are most commonly used as explants. From the selected sucker a cube of tissue of about 1–2 cm^3 containing the apical meristem is excised. This block of tissue is dipped in 70% ethanol for 10 seconds; Sodium hypochlorite at higher concentration (1.0 %) has turned out to be a better sterilant than mercuric chlorite alone at 0.1 % for 5 minutes treatment time. However, a treatment combination of Sodium hypochlorite (1.0%) for 15 minutes followed and $HgCl_2$ (0.1%) for 7 minutes resulted the highest percentage (85, 75 and 90 %) of aseptic culture establishment in banana cultivars Amritsagar-AAA, Malbhog-AAB and Chenichampa-AAB respectively in *in vitro* condition followed by Sodium hypochlorite (1.0%) for 10 minutes and $HgCl_2$ (0.1 %) for 7 minutes and Sodium hypochlorite (1.0%) for 15 minutes alone[5].

Subsequently a shoot tip of about 3 × 5 mm, consisting of the apical dome covered with several leaf primordia and a thin layer of corm tissue is aseptically dissected. Larger explants have the merit of consisting of a shoot apex bearing more lateral buds[12] which rapidly develop into shoots. The explant is further reduced to a size of 0.5–1 mm length, leaving a meristematic dome with one or two leaf primordia.

Success of *in vitro* culture depends largely on the choice of nutrient medium, including its chemical composition and physical form[17]. Several media formulations has been reported for banana shoot tip culture but nearly half of them are modified MS media[1]. For banana micropropagation, MS-based media[18] are widely adopted. Generally, they are supplemented with sucrose as a carbon source at a concentration of 30–40 g/l. Banana tissue cultures often suffer from excessive blackening caused by oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier round the tissue, preventing nutrient uptake and hindering growth. Therefore, during the first 4–6 weeks, fresh shoot-tips are transferred to new medium every 1–2 weeks. Alternatively, freshly initiated cultures can be kept in complete darkness for one week.

Browning or oxidation of phenolic compounds which is a common problem in the establishment of plantain *in vitro* culture may be reduced with the use of antioxidants, such as Potassium citrate-citrate (0.2 mg/L) combination for excised plantain tissue proved to be the best[20]. The explants are dipped in antioxidant solution (cysteine 50mg/l) prior to their transfer to culture medium will substantial reduce blackening in the medium.

Generally, the cultures are established on a separate initiation medium, which has a lower concentration of cytokinin than the multiplication medium to which the cultures are subsequently transferred[11,19]. Their concentration and ratio determines the growth and morphogenesis of the banana tissue.

In most banana micropropagation systems, semi-solid media are used. As a gelling agent agar (5 –8 g/l) is frequently added to the culture medium but Gelwell which was stated equalent to Phytagel has beneficial effect on shoot multiplication and growth in the micropropagation of banana var. Poovan[24]. The accelerated shoot growth in the gel medium may be due more availability of water in the media than in agar. The most prominent distinction among the gelling agents which influences the *in vitro* growth characters is the water retention capacity of the gels and the availability of nutrients to the cultured tissue.

Banana shoot-tip cultures are incubated at an optimal growth temperature of 28± 2°C in a light cycle of 12–16 h with a photosynthetic photon flux (PPF) of about 60 $\mu E/m^2s^1$

Culture proliferation

Multiplication of shoot-tip cultures is of most important step in maximizing the number of plantlets. Virus-free explants were transferred onto multiplication medium. The rate of multiple shoot formation during first and second subculture ranges from 1 to 5, which mainly depends on clones and its genomic constitution. In general, diploids like Matti-AA, Anaikomban-AA and SennaChenkadali-AA produce more buds than commercial cultivars. Among the latter, the number of buds produced during subculture is high in Cavendish (Robusta, Grand Naine – AAA genome) group followed by Plantain (Nendran – AAB genome) and Monthan (ABB genome) types.

The formation of multiple shoots and buds in banana cultivar Basrai (AAA) is promoted by supplementing the medium with relatively high concentrations of cytokinins. With increase in BAP concentration, numbers of shoots/ explant increased and a maximum of 5.4 shoots/explant was achieved when the liquid medium contained 4.0 mg/L BAP[14]. Maximum number of shoots proliferation per explant was found from 5.0 mg/l BAP (3.50 in Amritasagar (AAA) and 2.00 in Sabri (AAA)) at 30 days after subculture. The number of shoots increased with the increase of BAP concentration up to 5.0 mg/l and then decreased[3].With BAP at 8.0 mg/L, the number of shoots regenerated from the single explant decreased, which indicated that BAP had an adverse effect when used at a higher-than optimal concentration[14].

Average number of multiple shoots/bottle after the 14th subculture was maximum in Basrai-AAA (12.33) followed by LalKela-AAA (10.72) and minimum in Nendran-AAB and SafedVelchi-AB (8.63). Multiple shoot formation rate declined eventually with increase in the number of subcultures in all four clones. The growth of the clones as measured by stem height, girth, number of leaves and leaf size declined after the 8th subculture, with some plants exhibiting very stunted growth after the 14th subculture[10]. This indicates that the potentiality of a shoot tip to proliferate multiple shoots decreases as the sub culture cycles increase beyond the optimal level. Generally, minimize somatic variation; the subculturing is restricted to a maximum of seven cycles.

Regeneration of plants:

Individual shoot or shoot clumps are transferred to a nutrient medium which does not promote further shoot proliferation but stimulates root formation. To initiate rhizogenesis IAA, NAA (α-naphthalene acetic acid) or IBA (indole -3-butyric acid) are commonly included in the medium at between 0.1 and 2mg/l. In rooting medium, MS medium fortified with indole butyric acid (IBA) 0.5 mg l^{-1} + naphthalene acetic acid (NAA) 1.0 mg l^{-1} + activated charcoal (AC) 250 mg l^{-1} (Saraswathi *et al*.[26]). Activated charcoal at 250 mg l^{-1} of rooting medium invariably increases rooting per cent, because besides acting as an antioxidant it also promotes the root growth and development in banana[6].

Hardening

During hardening, the plantlets undergo physiological adaptation to changing external factors like water, temperature, relative humidity and nutrient supply. Primary hardening should be done in a controlled environment of 24-26°C temperature and more than 80 % humidity. Planting media for primary hardening range from sieved sand augmented with nutrients to mixtures of cocopeat and Soilrite with fine sand in equal proportions. Cocopeat + vermiculite (1:1) showed the optimum growth and development in cultivar Udhayam-ABB[26] . The reason might be that cocopeat + vermiculite would have improved the water retention ability and aeration to the growing plant as well as altered anchorage and nutrient content of the medium thereby promoting the growth and development of nursery plants.

Whereas, in secondary hardening NPK is provided in liquid form on weekly basis.

Press mud cake (PMC) mixed with soil as the optimal medium may be used for producing sturdy uniform sized banana plantlets.

Somaclonal variation

Variation among plants regenerated from tissue culture is termed 'somaclonal variation'. In banana, somaclonal variants of different types have been reported with regard to plant morphology[25]. The incidence of somaclonal variation is strongly influenced by the genetic stability of each cultivar, and that its frequency is amplified by culture -induced factors. There is no evidence that growth regulators routinely used in tissue culture directly affect the rate of variation, but it has been found that the rate of somaclonal variation is positively related to the generation number. In banana, off-types can be visually detected during acclimatization in the green house before transplanting to the field. BAP at 5.0 mg/L, TDZ at 0.2 mg/L and NAA at 0.2 mg/L and BAP at 2.0 mg/L, TDZ at 0.2 mg/L and NAA at 0.2 mg/L were assumed to be the most suitable for commercial micropropagation system with low frequency of abnormal shoot production for local banana cultivars[15]. Variability rates of up to 20 % for a Cavendish variety and of 19% for Grande Naine were recorded in Jamaica[30] and Puerto Rico[22], respectively. Whether the kinds of medium used, the incubation conditions, or the number of sub culturing also affect the range of variability in each variety remains to be studied[8].

REFERENCES

1. Brown, D.C.W., Finstad, K.I. and Watson, E.M., Somatic embryogenesis in herbaceous dicots. In: Thorpe, T.A. (Ed). *In vitro* embryogenesis of plants.Kluwer Academic Publishers. Dordrecht, The Netherlands, pp. 345-416 (1995).
2. Cronauer, S.S. and Krikorian, A.D., Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture. *Hort. Science.*, **19:** 234-235 (1984).
3. Ferdous, M.H., Billah, A.A.M., Mehraj, H., Taufique, T. and Uddin, A.F.M.J., BAP and IBA pulsing for in vitro multiplication of banana cultivars through shoot-tip culture. *J. Biosci. Agric. Res.*, **3(2):** 87-95 (2015).
4. Gill, L.S., Taxonomy of Flowering Plants. Ibadan: Eds Africana Fep. Publishers Ltd. pp. 106–109 (1988).
5. Goswami, N.K. and Handique, P.J., *In Vitro* Sterilization Protocol for micropropagation of Musa (AAA group) 'Amritsagar' Musa (AAB group) 'Malbhog' and *Musa* (AAB group) 'Chenichampa' Banana. *Ind. J. App. Res.*, **3(6):** 51-54 (2013).
6. Gubbuk, H. and Pekmezci, M., In vitro propagation of some new banana types (*Musa* spp.). *Turkish J. Agric. Forest*., **28:** 355-61 (2004).
7. Heslop, J.S., Harrison and Schwarzacher, T., Domestication, Genomics and the Future for Banana. *Ann. of Bot.*, **100:** 1073–1084 (2007).
8. Hwang, S.C. and Ko, W.H., Somaclonal Variation of Bananas and Screening for Resistance to Fusarium Wilt. Banana and Plantain Breeding Strategies Proceedings of an International Workshop held at Cairns, Australia, 13-17 October. pp. 151-156 (1986).
9. Jain, S.M. and Ishii, K., Micropropagation of woody trees and fruits. *For. Scie.*, 353-380 (2003).
10. Jambhale, N.D., Patil, S.C., Jadhav, A.S., Pawar, S.V. and Waghmode, B.D., Effect of number of subcultures on in vitro multiplication of four banana clones. *Infomusa*., **10(1):** 38-39 (2001).
11. Jarret, R.L., Rodriguez, W. and Fernandez, R., Evaluation, tissue culture propagation and dissemination of 'Saba' and 'Pelipita' plantains in Costa-Rica. *Scie. Horti.*, **25:** 137-147 (1985).
12. Lee, S.W., Hwang, S.C., Banana micropropagation system in Taiwan, Proc. Int. Symp. Recent Developments in Banana Cultivation Technology, Valmayor, R.V., *et al*., Eds, Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan. (1992).

13. Ma, S.S. and Shii, C.T., Growing banana plantlets from adventitious buds. *J. Chin. Soci. Horti. Scie.*, **20:** 6-12 (1974).
14. Ma, S.S., Shii, C.T. and Wang, S.O., Regeneration of banana plants from shoot meristem tips and inflorescence sections *in vitro.* In: Abstract of the 20th Int. Hort. Congress, Sydney, Australia, August 15-23, Abstract No. 1639 (1978).
15. Manjula, R., Jholgiker, P., Subbaiah, K.V., Prabhuling, G., Swamy, G.S.K. and Kumar, Y.L., Morphological Abnormality among Hardened Shoots of Banana cv. Rajapuri (AAB) after in vitro Multiplication with TDZ and BAP from Excised Shoot Tips. *Int. J. Agri, Env. Biotechnol*., **7(3):** 465-470 (2014).
16. Muhammad, A., Hamid, R. and Iqbal, H., Proliferation-rate effects of BAP and Kinetin on Banana (Musa spp. AAA Group) Basrai. *Hort. Sci.*, **42(5):** 1253–1255 (2007).
17. Murashige, T. Plant propagation through tissue culture. *Ann. Rev. Plant Physi.*, **25:** 135 (1974).
18. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiol. Plant*., **15:** 473–497 (1962).
19. Novak, F.J., Afza, R., Van Duran, M., Perea-Dallas, M., Conger, B.V. and Xiaolang, T., Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA, AAA) and cooking (ABB) bananas. *BioTechnology*, **7:** 154-159 (1989).
20. Onuoha, C.I., Eze, C.J., Unamba, C.I.N. and Ugochukwu, C.K., *In Vitro* Prevention of Browning in Plantain Culture. *Rom. J. Biol. Plant Biol*., **56(2):** 77–152. (2011).
21. Ortiz, R., Morphological variation in *Musa*germplasm, *Gen. Res. and Crop Evol.*, **44:** 393–404 (1997).
22. Pool, D.J. and lrizarry., 'Off-type' banana plants observed in a commercial planting of Grand Nain propagated via tissue culture (Abstract). In: Proceedings, Seventh Meeting, ACORBAT. San Jose, Costa Rica, 23-27 September (in press). (1985).
23. Rajamanickam, C., Rajmohan, K., Parthiban, S. and Venkateshan, K., Performance of triploid banana (*Musa* spp.) cultivars of Kerala. *South Indian Hort*., **55(1-6):** 119-132 (2006).
24. Ramesh, Y. and Ramassamy, V., Effect of gelling agents in in vitro multiplication of Banana var. Poovan. *I. J. A. B. R.*, **4(3):** 308-311 (2014).
25. Sahijram, L., Jaya, R., Soneji, and Bollamma, K.T., Invited Review: AnalyzingSomaclonal Variation in Micropropagated Bananas (*Musa* Spp.) *In Vitro Cell. Devel. Biol. - Plant*., **39(6):** 551-556 (2003).
26. Saraswathi, M.S., Praveena, S., Uma, S., Thangavelu, R., Kannan, G., Backiyarani, S. and Arivazhagan, T., Development of an efficient micropropagation technique for Musa cv. Udhayam (ABB). *Ind. J. Hort*., **71(4):** 452-457 (2014).
27. Sathiamoorthy, S., Uma, S., Selvarajan, R., Shyam, B. and Singh, H.P., Multiplication of Virus Free Banana Plants through Shoot Tip Culture. NRCB, Tiruchirapalli, Tamil Nadu, India. pp. 60 (1998).
28. Simmonds, N.W., The Evolution of the Bananas. Longmans, London **(**1962).
29. Simmonds, N.W., Bananas 2nd Edition. Longmans, London (1966).
30. Stover, R.H. and Buddenhagen, L.W., Improvement 156 of the banana tree: polyploid, resistance to diseases and productivity. *Fruits.*, **41:** 175-91 (1986).
31. Stover, R.H. and Simmonds, N.W., Bananas. Third Edition, Longman, Harlow. pp. 172-191 (1987).
32. Tomlinson, P., Anatomy of the monocotyledons. III. Commelinales (1969).
33. Vuylsteke, D. and De Langhe, E., Feasibility of *in vitro* propagation of bananas and plantains. *Trop. Agric.*, **62:** 323-328 (1985).
34. Vuylsteke, D., Ortiz, R., Shaun, B.F. and Crouch, J.H., Plantain Improvement. *Plant breed. rev*., **14:** 267-320 (1997).