DEVELOPING SOMATIC EMBRYOGENIC CULTURE SYSTEM AND PLANT REGENERATION IN BANANA

Meenakshi Sidha, P. Suprasanna, V. A. Bapat, U.G. Kulkarni and B.N. Shinde
Nuclear Agriculture & Biotechnology Division
Bhabha Atomic Research Centre

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Abstract

Bananas and plantains are one of the major fruit crops and a staple food in the developing world. Most of the edible bananas are triploid, highly sterile and hence integration of in vitro techniques banana improvement becomes crucial. In this milieu, technique of somatic embryogenesis in combination with genetic manipulation, has become pertinent. In the present study, results are presented on developing embryogenic culture systems in banana. Immature Male Flowers (IMFs) of different banana cultivars (Ardhapuri, Basrai, Grande Naine, Lalkela, Mutheli and Shrimanti) were tested for callus induction on medium containing 2,4-D, IAA and NAA. In terms of callus induction from IMFs of AAA genomic group, Lalkela showed highest response (77.7%) followed by medium response in Shrimanti (52.2%), Basrai (51%), Grande Naine (42.5%), Ardhapuri (42%) and Mutheli (40%). Compared to this group, Safed Velchi (BB) was found to be highly responsive to IMF culture (70%) similar to Lalkela. Embryogenic response was higher in Lalkela (83.3%), Grande Naine (62%) Ardhapuri (50%), Basrai and Mutheli (45%) and Shrimanti (40%) while Safed Velchi showed less response (20%). Experiments with different auxins (2,4-D & its analogs, Dicamba, Picloram and PAA) showed varied response among the cultivars tested. This is a first step in optimizing in vitro culture conditions for somatic embryogenesis in diverse Indian banana cultivars belonging to different genomic groups.

Bananas and plantains are one of the world’s important food commodity and rank fourth in terms of gross value, exceeded only by rice, wheat and milk/milk products. These are important staple food crops in the humid and sub-humid tropical regions of the world. Banana provides nourishment and a well-balanced diet to millions of people around the globe and contributes to livelihood through crop production, processing and marketing (Sundararaju, 1999). India is the largest producer of banana with an annual production of 11.7 million tonnes on 404,000 ha, contributing to 27% of the world production and about 37% of the total fruit crop production in the country (FAOStat, 2006). India has a rich genetic diversity of banana with more than 90 distinct clones. Depending on the contribution of Musa acuminata and Musa balbisiana,
the cultivars have been classified into genomic groups (AAA, AAB, ABB, BB, AB, BBB, AAAA, ABBB).

Banana is grown under diverse conditions and production systems and hence selection of varieties is based on needs and situations. Around 20 cultivars viz. Dwarf Cavendish, Robusta, Monthan, Poovan, Nendran, Red banana, Safed Velchi, Basrai, Ardhapuri, Rasthali, Karpurvalli, Karthali and Grande Naine etc. are commercially cultivated.

Banana cultivation and production are threatened by many pests and diseases (weevil borers, nematodes, sigatoka complex, fusarium wilt, moko disease and viruses) resulting in the application of high doses of pesticides with serious consequences for the environment. Because of its high sterility (i.e., seedlessness) and polyploidy of the edible varieties (Stover and Simmonds, 1987), classical breeding is difficult (Gany, 1990) and have resulted in increasing efforts to genetically improve the crop. In this regard, plant tissue culture and molecular genetic techniques have shown great potential to overcome some of the factors limiting traditional approaches to banana and plantain improvement. Such procedures largely depend on successful regeneration of plants from cultured banana cells. Therefore the integration of biotechnology into banana and plantain breeding programmes require access to reliable cell culture protocols.

![Diagram of somatic embryogenesis and plant regeneration in banana](image)

**Fig. 1 : Potential pathways for somatic embryogenesis and plant regeneration in banana**
Somatic embryogenesis is one of the important prerequisites for genetic engineering, however, this has been successful with limited numbers of banana cultivars such as Bluggoe (ABB) (Novak et al, 1989; Dheda et al, 1991; Panis et al, 1993); Grand naine (AAA) (Novak et al, 1989; Escalant et al, 1994; Cote et al, 1996; Becker et al, 2000); Rasthali (AAB) (Escalant et al, 1994; Ganapathi et al, 2001) and Mas (AA) (Jalil et al, 2003). The banana explants that have been used in somatic embryogenesis studies have been proliferating meristems (Cronauer-Mitra and Krikorian, 1983); zygotic embryos (Cronauer-Mitra and Krikorian, 1988; Escalant and Teisson, 1989; Marroqin et al, 1993); rhizome leaf sheaths (Novak et al, 1989); immature male flowers (Escalant, 1994; Cote et al, 1996; Grapin et al, 1996) and female flowers (Grapin et al, 2000). Among the explants that have been used, immature male flowers and scalps are the most responsive starting material, for initiating regenerable, embryogenic cell suspension cultures (Fig. 1). Considering the need to optimize culture protocols for diverse Indian banana cultivars, there have been efforts to establish regenerable cultures (Ganapathi et al, 2002, Suprasanna et al, 2002, Kulkarni et al, 2006). Herein, we present our results on the induction of somatic embryogenesis and plant regeneration in different commercial cultivars of banana: AAA genotype (Lalkela, Grand naine, Ardhapuri, Shrimanti, Basrai, Mutheli) and BB genotype (Safed Velchi).

**Materials and method**

Inflorescence male flowers of banana cultivars (Grand naine, Ardhapuri, Basrai, Shrimanti, Mutheli, Lalkela and Safed Velchi; obtained from Banana Research Station, Jalgaon, Maharashtra) were used as explant material. The male bud was shortened to 6-8cm in length by removing the enveloping bracts and these explants were sterilized in 70% ethanol for about 5 minutes and rinsed three to five times with sterile water. The male buds were further reduced to 1.5 cm in length for culture and immature male flower clusters from position 0-15 were removed and cultured on Murashige and Skoog medium (MS) supplemented with 18.10µM 2,4-dichlorophenoxy acetic acid (2,4-D), 5.71µM indole 3-acetic acid (IAA) and 4.09 µM d-Biotin, 3% sucrose and 0.2% gelrite for callus induction and for proliferation of callus d-Biotin, 2.4-D, malt extract and glutamine were used. For somatic embryo induction the medium the embryogenic callus was grown on Schenk and Haberlandent (SH) and MS (vitamin) supplemented with glutamine (100mg), malt extract (100mg) and picloram (1mg) and for development of somatic embryos, 0.22µM-benzyl amino purine (BA) and 1.14µM IAA, 3% sucrose, glutamine and malt extract were used.

Callus was also cultured on the different analogs of 2,4-D, (2,4-Dichlorophenoxyacetic acid) 2,4,5-P (2,4-5 -trichloropropionic acid), 2,4,5T (2,4,5-trichloro-phenoxyacetic acid), CPA (p-chlorophenoxyacetic acid), Dicamba (3, 6-Dichloro-o-anisic acid), PAA (phenylacetic acid) and Picloram (4-amino-3, 5, 6-trichloropicolinic acid). The pH of the medium was adjusted to 5.7 prior to autoclaving. All culture media were autoclaved at 121°C for 20 min. Cultural conditions for initiation of embryos, maintenance of suspension culture and development of embryos were maintained at 28°C under a 16h-photoperiod with light intensity of 31.4µmol·m⁻²·s⁻¹. Cell suspensions were initiated from embryogenic callus and maintained at weekly intervals at 70 rpm continuously on a shaker under above cultural conditions. For conversion of somatic embryos into plantlets, ½ strength MS basal medium with 0.5% ME + 0.1% AC and 0.2% gelrite was used and after plantlets emerged, these were separated and transplanted into paper cups and then into pots in the green house for further growth.

**Results and discussion**

Developing embryogenic culture systems with reliable regeneration efficiency from important varieties of banana is a prerequisite for realizing the potential of cellular and molecular tools of crop improvement (Smith et al 2005). Towards this goal, studies were made to develop protocols for somatic embryogenesis.
Young flowers responded after 2 to 3 months of culture by forming a small yellow callus, and during the following 3 to 5 months, a white and translucent callus formed on the yellow callus and development of somatic embryos appeared on its surface. Fig 2. (A-F) shows different developmental stages from the callus induction to embryo development in different cultivars. In terms of callus induction from IMFs, Lal-kela showed highest response (77.7%) among AAA genomic group followed by Shrimanti and Basrai (52.2 and 51%), Grand Naine, Ardhapuri and Mutheli (42.5, 42 and 40%). Compared to this, Safed velchi (AB) was found to be highly responsive (70%) to IMF culture. The original explants became brown at the base within a week of culture and began to swell and the size also increased after 2-3 weeks. In terms of embryogenic callus induction, it was highest in Lal-kela 83.3% than other cultivars which were in the range (50-40%) and in Safed velchi it was the lowest (20%). Upon transfer to suspension medium, friable embryogenic callus released embryogenic cells with dense cytoplasm. Suspension cultures obtained consisted of heterogeneous cells: the embryogenic cells were smaller, more spherical in shape and had dense yellow cytoplasm with very few small vacuoles; the non-embryogenic nature was distinct with large vacuolated cells with meager cytoplasm and irregularly shaped cells.

In general, the cultivars exhibited differential response in terms of the time taken for callus proliferation, embryo development and embryo conversion to plants. Cultivars like Grand naine, Lal-kela, Basrai completed all these stages till regeneration while in case of Ardhapuri, Shreemanti, Mutheli the embryo to plant conversion was slow. Compared to these cultivars of AAA genotype, in case of Safed Velchi (AB), callus initiation was found to be good, however, the nodular callus developed only into mucilaginous type and further development into other stages of friable or loose callus was not observed.
This is suggestive of the fact, that the cultivars of the same genotype onto the same medium do not behave similarly. For this differential behavior, the different analogs of 2,4-D, picloram, dicamba and PAA were used, to see the proliferation (Fig. 4) and embryo initiation. Of all the auxins tested, 2,4,5-P and 2,4,5 T showed good response towards the initiation of embryos while Dicamba and Picloram showed highest rate of proliferation. Safed velchi did not show any response with any of the auxins, which could be attributed to the genotypic variation. Among the rest of the cultivars (AAA), Ardhapuri displayed superiority of 2,4-D. Hormones are the most likely candidates in the regulation of morphogenetic and developmental cues. Auxins and cytokinins are the principal growth regulators in plants in the regulation of cell division and differentiation (Feher et al 2003). In banana, for the initiation of callus, medium is supplemented with three auxins IAA, NAA, 2,4-D and different auxins for the proliferation while a cytokinin with auxin (IAA+BA) is necessary for embryo development (Navarro et al 1997; Ganapathi et al 1999).

The features of somatic embryos (translucent and whitish) obtained in this study, were similar to those obtained by (Cote et al 1996, Escalant et al 1994). In general, higher numbers of somatic embryos per flower cluster were obtained in LK (4-10) as compared to other cultivars (4-6).

The embryos passed through different developmental stages and finally green plumules emerged from these embryos. Somatic embryos developed into the plantlets (Fig. 4 :A-C) (highest in LK 70% followed by Grand Naine) on ½ strength MS basal medium with 0.5%ME+0.1% AC and 0.2% gelrite and the time span for the development of shoot and root was within 6 to 8 weeks. Once the plantlets developed, these were separated and transplanted into paper cups (Fig. 4D).

Among the different explants tested for somatic embryogenesis, immature male inflorescences have mostly been used, to initiate embryogenic cultures of several banana and plantain cultivars (Escalant et
Genetic manipulation using Embryogenic Cell Suspensions (ECS) has become a tangible and useful approach for an integrated genetic improvement via different biotechnological approaches. Protocols have been developed for the induction of somatic embryogenesis using immature male flowers as well as proliferating in vitro cultures (Dheda et al. 1991, Ganapathi et al. 1999, Suprasanna et al. 2001, Kulkarni et al. 2006). Immature male inflorescences have also been used to initiate cultures with the objective of developing a protocol for primary and secondary somatic embryogenesis (Khalil et al. 2002). In our study, we have also observed secondary embryos on pre-existing somatic embryos (Fig. 2:E). Secondary embryos typically appeared directly from primary somatic embryos and were in clusters at the globular stage. Secondary embryogenesis is a process of induction of new somatic embryos from existing embryos and since new embryos are continually formed from existing embryos, this method has the potential to produce many plants over a long period of time. The use of secondary embryogenesis could also offer an efficient solution, to the problems limiting plant regeneration in some banana cultivars.

Somatic embryogenesis is an established method for raising regenerable suspension cultures in case of banana. Although several commercial and elite clones have been induced into embryogenesis, a large number of banana genotypes still need to be explored for embryogenic potential for use in propagation and genetic improvement. Developing protocols for in vitro plant regeneration from diverse banana genotypes, as attempted in this study, will usher efforts in conservation, propagation and for genetic manipulation for improving desirable traits.

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References


**About the Authors**

**Miss Meenakshi Sidha** is a research fellow doing her Ph.D in Pomology from Marathwada Agriculture University (MAU), Parbhani. This is a collaborative research programme on banana between MAU and BARC. She obtained her M.Sc degree in Horticulture with specialization in Pomology from MAU, Parbhani. She was a University topper and second in state (among all the four agriculture universities in the state) during her graduation and University topper during post graduation. Her main area of research is in the field of plant biotechnology.

**Dr Penna Suprasanna** joined BARC in 1991 after obtaining his Ph.D. degree in Genetics from Osmania University, Hyderabad. He is working in the Plant Cell Culture Technology Section of NA&BTD and is engaged in plant biotechnological research on rice, banana and sugarcane. His contributions have been in the areas of cell and tissue culture, somatic embryogenesis, *in vitro* selection and transgenics. Dr. Suprasanna has to his credit, more than 100 research publications in journals and books published by national and international publishers. His interests are in using plant cell cultures for cellular and molecular genetic manipulation.
Dr Vishwas A. Bapat is working in the area of plant tissue culture for the last 31 years. Currently he is heading the Plant Cell Culture Technology Section. The main thrust area of his work is on Micropropagation and Genetic Transformation of Plants. He has extensively contributed in the areas of clonal propagation, cell and protoplast culture, synthetic seeds, disease resistance and molecular farming. He has several publications in national and international journals to his credit. He is a Fellow of the National Academy of Sciences, India and the Maharashtra Academy of Sciences.

Dr Umakant G. Kulkarni did his Ph. D in Genetics & Plant Breeding from University of Agricultural Sciences, Dharwad. He is responsible for the release of groundnut cultivars LGN-1 & LGN-2. He has served as the Head of Agricultural Botany and Biotechnology Department at Marathwada Agricultural University, Parbhani and Officer In-Charge, Tissue Culture Project. Presently he is the Associate Dean and Principal, College of Agriculture, Osmanabad, MAU, Parbhani.

Dr B.N. Shinde did his Ph.D in Horticulture from Marathwada Agricultural University (MAU), Parbhani. Presently he is working as a Senior Research Officer in Pomology in Horticulture Research Scheme at MAU Parbhani. Dr. Shinde has 40 research papers, 35 articles in Marathi and two books to his credit.