The Pharma Innovation Journal 2023; 12(11): 332-336

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 332-336

www.thepharmajournal.com
Received: 20-09-2023
Accepted: 25-10-2023

Aparna K Gokul
Ph.D., Scholar, College of Agriculture, Vellanikkara, Kerala Agricultural University, India

Vimi Louis
Professor and Head, Banana Research Station, Kannara, Kerala, India

Anita Cherian K
Former Professor and Head, Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Kerala, India

Soni KB
Professor and Head, Department of Biotechnology, College of Agriculture, Vellayani, Kerala Agricultural University, Kerala, India

R Selvarajan
Director, National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India

Sible GV
Professor and Head, Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Kerala, India

S Backiyarani
Principal Scientist, National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India

Corresponding Author:
Aparna K Gokul
Ph.D., Scholar, College of Agriculture, Vellanikkara, Kerala Agricultural University, India

Development of somatic embryos in banana cultivar Nendran (*Musa* AAB)

Aparna K Gokul, Vimi Louis, Anita Cherian K, Soni KB, R Selvarajan, Sible GV and S Backiyarani

Abstract
Banana, a widely grown fruit crop worldwide faces challenges due to various abiotic and biotic factors. Being a vegetatively propagated crop, the production of high-quality, disease-free planting materials is vital to mitigate these challenges. Micropropagation, especially somatic embryogenesis, is a widely accepted technique for the mass multiplication of quality planting materials. However, the success of somatic embryogenesis varies with the genome type and also within the same genome groups. Therefore, standardizing culture conditions for each banana cultivar is essential. In this study, we successfully induced embryogenic calli from immature male flowers of the banana cultivar Nendran (*Musa* AAB). MS medium supplemented with different concentrations of 2,4-D, IAA, NAA and picloram was evaluated for callus development and embryogenesis. The explants were maintained on the same media, leading to the induction of friable calli over a span of approximately five months. These somatic embryos matured on the same medium. Subsequently, the mature somatic embryos were transferred to MS medium with Morel vitamins, BAP (0.2 mgL^{-1}), IAA (1 mgL^{-1}) and GA_3 (0.5 mgL^{-1}) which gradually led to the germination of shoot primordia. The regenerated plantlets exhibited no morphological abnormalities.

Keywords: Banana, explant, MS medium, calli, somatic embryos

Introduction
Banana is a highly nutritious and commercially significant fruit crop cultivated in over 130 countries, with more than 1200 varieties (Siddiq *et al*., 2020) [1]. In India, it ranks as the second most cultivated fruit crop after mango, covering 877,000 ha with a production of 31,779,000 MT in 2019-20 (NHB, 2019-20) [2].
However, banana cultivation is impeded by various abiotic and biotic factors, adversely affecting the livelihood of farmers. Availability of good quality planting materials is crucial to combat these stresses, especially since banana is a vegetatively propagated crop. Ensuring the quality of suckers is not easy mainly due to the prevailing viral diseases of banana that can get transmitted through infected suckers. The major viral diseases of banana are Banana bunchy top disease, Banana bract mosaic, Banana streak mosaic and infectious chlorosis. Mass multiplication of banana through tissue culture techniques helps to overcome this issue to a great extent. However, the genome and ploidy variations of bananas necessitate the establishment of regeneration protocols for location specific farmer-preferred varieties of banana. Nendran is a commercial variety consumed as fresh fruit, used for culinary purposes and is even more popular for making chips and other value added products. In this study, an attempt was made to develop somatic embryos from the male flowers of the Nendran cultivar.

Materials and Methods
Collection and preparation of explant
Healthy flower buds were collected from Nendran plots, maintained at Banana Research Station, Kannara. The male buds were trimmed to five to six centimetres in length by removing the outer floral bracts. The buds were then washed in sterile water to remove all the excess stain and dust particles sticking onto the surface. The trimmed flower buds were surface sterilized in the laminar chamber with cotton dipped in 90 per cent ethanol. After surface sterilization, the remaining younger bracts were removed carefully with a sterile scalpel. For removing the bigger bracts, scalpel blades of 20 mm size were used and 10 mm blades were used for removing the thin inner bracts. Immature male flowers from the zeroth position, *i.e.*, closest to the apical dome to the fifteenth position were used as explant material

(Lekshmi *et al*., 2016) [3]. The size range of the explants varied from a few millimetres to less than 0.5 cm (Plate 1).

Preparation of medium

MS medium (Murashige and Skoog, 1962) [4] was used as the basal medium, to which suitable hormones were added for the development and regeneration of somatic embryos. The MS stocks and hormones were prepared in advance and stored at 4 °C. Ascorbic acid (25 mgL^{-1}) was added to the medium to reduce the effect of phenolic exudates. The pH of the medium was adjusted to 5.7 with KOH or HCl. Gelrite or phytagel at the rate of 2 gL^{-1} was used in place of agar as a solidifying agent. The medium was dispensed into tissue culture bottles and test tubes and then autoclaved at 121 °C for 20 min. When the explants were cultured in Petri plates, the medium was sterilized in bulk and later dispensed into Petri plates when the medium was cooled to about 50 °C.

Medium composition

To standard MS medium, different combinations and concentrations of hormones *viz*., 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), naphthalene acetic acid (NAA) and picloram were added to understand the effect on callus development and embryogenesis. The combination and concentration of the hormones used are given in Table 1. For embryo germination, MS salts with Morel vitamins (Morel and Wetmore, 1951) [5] along with 6-benzylaminopurine (BAP, 0.2 mgL^{-1}), indole-3-acetic acid (IAA, 1 mgL^{-1}) and gibberellic acid GA_3 (0.5 mgL^{-1}) was used. Once the embryos germinated, they were subcultured to MS medium along with BAP (0.2 mgL^{-1}) and IAA (1 mgL^{-1}).

Development of somatic embryos

The explants were maintained in the same medium, *i.e*., MS medium with different hormonal combinations, without subculturing, at 24-25 °C, under dark conditions till they developed friable embryogenic calli. The embryogenic calli were allowed to grow in the same medium for the development of somatic embryos. The per cent of embryogenesis of Nendran in different hormonal combinations were noted. After 15 days, the embryos were transferred to the germination medium.

Regeneration of plants from somatic embryos

The somatic embryos that developed on the calli were transferred to the embryo germination medium, at 24-25 °C and kept under light conditions. The embryos were transferred to the germination medium, according to the method used by Escalant *et al*. (1994) [6] with slight modification. The germination medium was poured into Petri plates and allowed to solidify. On the top of solidified germination medium, a sterile filter paper disc of 60 cm diameter, moistened with liquid germination medium was placed. The somatic embryos were then placed on this filter paper. Once the shoot primordia emerged from the embryos, they were subcultured onto fresh medium without filter paper. Regular sub culturing was done at an interval of 20-25 days, till the plants attained three to four leaf stage.

Table 1: Combinations of growth hormones used for somatic embryo development

Treatment	Hormone concentration (mgL^{-1})			
	2,4-D	IAA	NAA	Picloram
T_1	2	1	1	-
T_2	2	-	1	-
T_3	2	1	-	-
T_4	4	1	1	-
T_5	-	1	1	2
T_6	-	-	1	2
T_7		1	-	2

Results and Discussion

Establishment of explant

The inner male flower clusters were obtained without damage, by using blades of different sizes for explant preparation. The explants released phenols and showed darkening at the base within four to five days. Browning of the explants due to phenolic exudates was a severe problem during explant establishment and was slightly reduced with the addition of ascorbic acid. Lekshmi *et al*. (2016) [3] observed the same issue in their study in Nendran cultivar. The use of antioxidants like cysteine and methionine (Khatri *et al*., 2005) [7], ascorbic acid (Namanya *et al*., 2004; Lekshmi *et al*., 2016) [8, 3] and citrate (Titov *et al*., 2006) [9] had resulted in the reduction of phenolic exudation. Also, studies have shown that replacing agar with other solidifying agents like gelrite has helped in the reduction of browning in the medium (Khatri *et al*., 2005) [7].

The explants placed on medium (Plate 2) developed cauliflower like structures within two weeks (Plate 3), which later enlarged in size. After three months, calli development was initiated. Pale white, yellow globular and hard calli were observed initially (Plate 4A-C). The embryogenic calli development from the explant took another six to eight weeks. The frequency of callus development was high in all treatments containing, 2,4-D. However, the development of embryogenic calli was low. The highest per cent of embryogenic calli development (8%) was in T_3 which contained 2,4-D (2 mgL^{-1}) and IAA (1 mgL^{-1}). This was followed by T_1 (5%) and the remaining treatments showed only about 1 per cent of embryogenic calli development. Previous studies have shown long duration for the development of embryogenic calli in different Musa genotypes. Strosse *et al*. (2006) [10] have maintained explants for up to 12 months without subculturing for induction of embryogenesis. Similarly, there are reports of obtaining ideal embryogenic calli after maintaining the explants for three to six months (Ghosh *et al*., 2009 and Tripathi *et al*., 2012) [11, 12] without subculturing. Mohandas *et al*. (2011) [13] observed that in the *Musa* AAB group, ideal embryogenic calli developed from immature male flower clusters after about

seven to nine months in MS medium supplemented with 2,4-D, IAA and NAA. Ghosh *et al*. (2009) [11] used MS medium supplemented with 2,4-D, IAA, NAA and biotin and obtained embryogenic calli in the Robusta cultivar.

Wei *et al*. (2005) [14] observed 7.45 per cent of embryogenic callus development from male floral hands of *Musa acuminata* when grown on a medium containing 2,4-D. Lekshmi *et al*. (2016) [3] observed that callus formation was high in a medium supplemented with auxins like 2,4-D along with IAA and in a medium supplemented with picloram alone. However, somatic embryogenesis was recorded from callus initiated on a medium containing picloram and 6-benzylaminopurine (BA). Similarly, Remakanthan *et al*. (2014) [15] reported direct induction of somatic embryos, *i.e*., without callus formation, from split shoot tips of Grand Naine cultivar in MS medium supplemented with different combinations of picloram and BA. Khatri *et al*. (2005) [7] in their study using a diploid and triploid banana cultivar, observed that callus induction was affected by genotype and ploidy in banana. One of the major limitations of indirect somatic embryogenesis in banana is the necessity to initiate hundreds of explants to obtain one ideal embryogenic callus (Strosse *et al*., 2003) [16].

Plate 1: Male flower clusters used as explant

Plate 2: Explants on medium

Plate 3: Formation of cauliflower like structures

Plate 4A: Pale white globular callus

Plate 4B: Pale white hard callus

Plate 4C: yellow globular callus

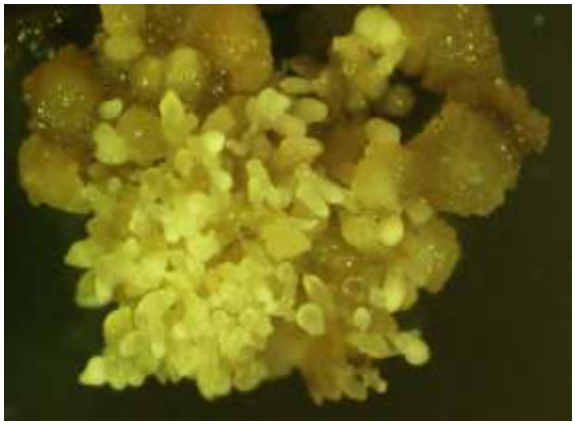

Plate 4: D. Development of somatic embryo on embryogenic callus (under stereo microscope)

Development of somatic embryos

Somatic embryos were observed after about six months of maintaining the explant (Plate 4D). The explants were maintained in the same medium till they showed signs of embryo development. The embryos initially appeared as translucent round bodies giving a pearl like appearance as previously described by Cote *et al*. (1996) [17]. These translucent somatic embryos later matured and developed into white polar shaped embryos. Strosse *et al*. (2006) [10] observed variation in embryogenic frequency within the same genome groups depending on the variety.

Regeneration of plants from somatic embryos

The matured embryos on transferring to filter paper discs placed on MS medium supplemented with BAP, IAA and GA_3, germinated and developed green shoot like primordia within three weeks. Previous studies have recorded the success of BA and IAA containing medium for embryo germination (Cote *et al*., 1996) [17]. Escalant *et al*. (1994) [6] observed that somatic embryos germinated to produce plumule like structures in a medium containing BAP and IAA. They also noted increased germination when embryos were transferred to filter paper on a semisolid medium. The positive influence of gibberellic acid on embryo germination was recorded by Pancholi *et al*. (1995) and Uma *et al*. (2012) [18, 19]. According to Pancholi *et al*. (1995) [18], 82 per cent germination of banana zygotic embryos was obtained on a medium containing gibberellic acid. From the second subculture, GA_3 was removed from the culture medium as extensive shoot elongation was observed in this medium. The germinated plantlets took another 60 days to attain three to four leaf stage.

Conclusion

The success of somatic embryogenesis in bananas varies with the cultivars and requires unique standardization protocols. This study achieved embryogenic calli of Nendran from male flower clusters in MS medium containing 2,4-D (2 mgL^{-1}) and IAA (1 mgL^{-1}). The somatic embryos were directly transferred to the embryo germination medium and subsequently subcultured to develop into whole plants. Moreover, the Nendran embryogenic calli obtained in this study can be used for the development of embryogenic cell suspensions, further facilitating biotechnological applications like genetic engineering in Nendran.

Acknowledgments

We thank the faculty from NRCB for the continuous guidance in tissue culture techniques. We also acknowledge the help of Dr. Sreelatha U., of the Department of Floriculture and Landscaping, Kerala Agricultural University. This work was supported by the INSPIRE fellowship of DST.

References

1. Siddiq M, Ahmed J, Lobo MG. Handbook of banana production, postharvest science, processing technology, and nutrition. John Wiley and Sons; c2020.
2. NHB 2019-20 [https://nhb.gov.in].[Visited on 15 October 2023].
3. Lekshmi RS, Soni KB, Alex S, Nair DS, Sreekantan L, Reghunath BR. A rapid protocol for somatic embryogenesis mediated regeneration in banana (*Musa* spp.) cv. Nendran. Journal of Horticulture Science

2016;11(2):116-123.
4. Murashige T, Skoog FA. revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia plantarum. 1962;15(3):473-497.
5. Morel G, Wetmore RH. Tissue culture of monocotyledons. American Journal of Botany. 1951;38(2)1:38-140.
6. Escalant JV, Teisson C, Cote F. Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* spp.). *In Vitro* Plant 1994;30:181-186.
7. Khatri A, Khan IA, Dahot MU, Nizamani GS, Siddiqui MA, Raza S, *et al*. Study of callus induction in banana (*Musa* sp). Pakistan Journal of Biotechnology. 2005;2(1-2):36-40.
8. Namanya P, Magambo SM, Mutumba G, Tushemereirwe W. Somatic embryogenesis from immature male inflorescences of East African highland banana CV 'Nakyetengu'. African Crop Science Journal 2004;12(1):43-49.
9. Titov S, Bhowmik SK, Mandal A, Alam MS, Uddin SN. Control of phenolic compound secretion and effect of growth regulators for organ formation from *Musa* spp. cv. Kanthali floral bud explants. American Journal of Biochemistry and Biotechnology. 2006;2(3):97-104.
10. Strosse H, Schoofs H, Panis B, Andre E, Reyniers K, Swennen R. Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). Plant Science 2006;170(1):104-112.
11. Ghosh A, Ganapathi TR, Nath P, Bapat VA. Establishment of embryogenic cell suspension cultures and Agrobacterium-mediated transformation in an important Cavendish banana cv. Robusta (AAA). Plant Cell, Tissue and Organ Culture 2009;97:131-139.
12. Tripathi JN, Muwonge A, Tripathi L. Efficient regeneration and transformation of plantain *cv*. Gonja manjaya (*Musa* spp. AAB) using embryogenic cell suspensions. *In vitro* Cellular and Developmental Biology Plant. 2012;48:216-224.
13. Mohandas S, Sowmya HD, Manjula R, Pratibha KY, Meenakshi S, Ajay KM. Development of highly regenerative embryogenic cell suspensions of 'Nanjangud Rasbale' (syn. 'Rasthali', *Musa*, AAB, Silk subgroup) and transformants with AMP gene. Acta Horticulturae. 2009;897:245-253.
14. Wei YR, Huang XL, Li J, Huang X, Li Z, Li XJ. Establishment of embryogenic cell suspension culture and plant regeneration of edible banana *Musa acuminata cv*. Mas (AA). Chinese Journal of Biotechnology 2005;21(1):58-65.
15. Remakanthan A, Menon TG, Soniya EV. Somatic embryogenesis in banana (*Musa acuminata* AAA cv. Grand Naine): effect of explant and culture conditions. *in vitro* Cellular and Developmental Biology Plant 2014;50:127-36.
16. Strosse H, Domergue R, Panis B, Escalant JV, Cote F, Vezina A, *et al*. Banana and plantain embryogenic cell suspensions. INIBAP technical guidelines. 2003.36p
17. Cote FX, Domergue R, Monmarson S, Schwendiman J, Teisson C, Escalant JV. Embryogenic cell suspensions from the male flower of *Musa* AAA cv. Grand Nain. Physiologia Plantarum 1996;97(2):285-90.
18. Pancholi N, Wetten A, Caligari PD. Germination of *Musa velutina* seeds: comparison of *in vivo* and *in vitro* systems. *in vitro* Cellular and Developmental Biology Plant. 1995;31:127-30.
19. Uma S, Lakshmi S, Saraswathi MS, Akbar A, Mustaffa MM. Plant regeneration through somatic embryogenesis from immature and mature zygotic embryos of *Musa acuminata* ssp. *burmannica*. *in vitro* Cellular and Developmental Biology Plant. 2012;48:539-45.