Somatic Embryogenesis and Plant Regeneration in Diploid Banana Cultivars (*Musa acuminata* cv. Chingan and *Musa acuminata* cv. njalipoovan) from Kerala

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Plant regeneration by somatic embryogenesis was attempted with diploid banana cultivars *Musa acuminata* cv. Chingan (AA) and *Musa acuminata* cv. Njalipoovan (AB). *In vitro* plants grown on solid MS medium supplemented with BA (4.4-17.6 µM) and TDZ (0.45-9 µM) were used as explant source. The basal meristem including 3-4 leaf primordial and excised apices were inoculated on MS medium supplemented with 2,4-D (0.225-9 µM) and BA (0.044-8.8 µM) for callus induction. Pale yellow embryogenic callus initiated after three weeks of inoculation and subsequent subculture were done in the same medium to enhance callus proliferation. Maximum callus proliferation was observed in 2,4-D (4.5 µM) along with BA (2.2 µM). Embryogenic calli were isolated and transferred to 100 ml erlenmeyer flask with 20 ml of liquid MS medium with zeatin (0.46-4.56 µM) and malt extract (100 mg/l) or malt extract and ascorbic acid (0.01-0.5 mg/l). The culture medium was refreshed every third week, embryogenic complexes and compact structures were removed. Small embryo-like structures and embryogenic calli were transferred to fresh medium. After 4-5 subculture the embryos were isolated and inoculated on petridish containing MS basal medium. Leaf and root initials were developed after 2-3 weeks. These were then transferred to same medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 per cent survival rate.

Key Words: Somatic embryogenesis, *Musa acuminata* cv. Chingan, *Musa acuminata* cv. Njalipoovan

Introduction

Edible bananas (*Musa* spp.) are the major staple food for rural and urban consumers in the tropical and sub tropical countries and an important source of rural income. However, in the banana production system, the non-availability of disease free, true-to-type planting material, low fertility due to triploidy, slow propagation and long time span from one generation to the next are the major constraints. Local or indigenous cultivars are generally more useful and tolerant to local conditions and have lesser growth requirements to obtain satisfactory yield. Most of cultivars in Kerala, South India are adapted to domestic cultivation and have unique morphological features as well as fruit qualities. South India is well-known for the presence numerous diploid banana cultivars. Diploid banana varieties are a well known source of genetic resistance to many biotic stresses that have limited banana cultivation. Somatic embryogenesis offers an ideal system for the production of somatic embryos on a large scale for use in the preparation of synthetic seeds, propagation and genetic transformation. Somatic embryogenesis technique in the genus *Musa* developed new, high performance micropropagation techniques and that of cell regeneration system useful for genetic improvement. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules became an applied technique with potential for mass propagation of elite plant species. The advantages of using artificial seeds include ease of handling, transportation and potential storage, higher scale-up potential and low cost of production and subsequent propagation (Redenbaugh *et al.*, 1986). Synthetic seed technology will have a significant impact on crop production, in both vegetatively propagated and seed propagated crops. For the vegetatively propagated plants, synthetic seeds would allow direct planting material of clonal varieties and may provide a means for maintenance of elite germplasm. The objective of this work was to establish a protocol of cell suspension culture and plant regeneration via somatic embryogenesis of two diploid banana cultivars (*Musa acuminata* cv. Chingan (AA) and *Musa acuminata* cv. Njalipoovan (AB).

Materials and Methods

In vitro grown plants were established from inflorescence explants obtained from field grown plants at Department of Botany, University of Kerala, Kerala, India. The *in vitro* plants were obtained on MS medium supplemented 7.6 µM BA. The shoot buds were obtained on MS medium supplemented with 4.5 µM TDZ. The cultures were maintained at a temperature of 25±1°C, with 50-70% relative humidity and 16 h photoperiod. Thin longitudinal sections of approximately 1cm square pieces from leaf

base (basal meristem) were excised from *in vitro* grown plants, after the removal of leaves and roots from the plantlets. Shoot apices of approximately 1 cm square pieces were excised from *in vitro* grown shoot buds maintained on MS medium supplemented with TDZ. Approximately 6-8 sections were cut from the shoot apex and 2-4 sections were dissected from basal leaf meristem. The explants were inoculated on MS medium supplemented with 2,4-D (0.225-9 µM) and BA (0.44-8.8 µM) for callus initiation. These calli were subcultured at an interval of 3 weeks in the same medium for callus proliferation.

In 3-month-old embryogenic calli derived from both the explants were used to establish the suspension cultures. Approximately 100 mg of callus transferred to 100 ml Erlenmeyer flask containing 20 ml of liquid MS medium supplemented with zeatin (0.46-4.6 µM) or ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l) in a gyratory shakers at 80 rpm. Subcultures were done once every 21 days by adding 10 ml of fresh medium.

During subculturing compact structures were removed, small embryo like structures were isolated and transferred to fresh medium. After 4-5 subculture, these embryos were transferred to petri dish containing MS basal medium. After the development of shoot and root initials these were transferred to same medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 per cent survival rate.

Results and Discussion

The shoot apices and basal meristem showed callus development in the presence of 2,4-D (0.225-9 µM) and BA (0.44-8.8 µM) in various combinations. High frequency of embryogenic callus proliferation was obtained in presence of 2, 4-D (4.5 µM) along with BA (2.2 µM). Initially the callus morphology was pale yellow or white compact and later milky white globular structures developed along with white callus. Embryogenic tissues developed within 1-2 months after callus initiation (Fig. 1). The embryogenic tissues were subcultured and maintained on the same hormonal combination for 12 weeks for maximum proliferation. These embryogenic calli were transferred to liquid MS medium with zeatin (0.46-4.6 µM) or ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l). The suspension showed presence of free cells and cell aggregates in the liquid medium. Embryogenic cell aggregates were obtained after 3rd or 4th subculture. Both the shoot apices and leaf sheath derived calli from each cultivar showed maximum embryogenic potential in the presence of zeatin (1.84 µM) along with malt extract. In both the cultivar presence of ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l) showed development of embryogenic cell aggregates which later grown to plants/ shoots. However, only a little embryo development was observed in this medium.

The embryos developed in the liquid medium were isolated and transferred to petri dish containing basal MS medium (Fig. 2). Root and shoot initials were

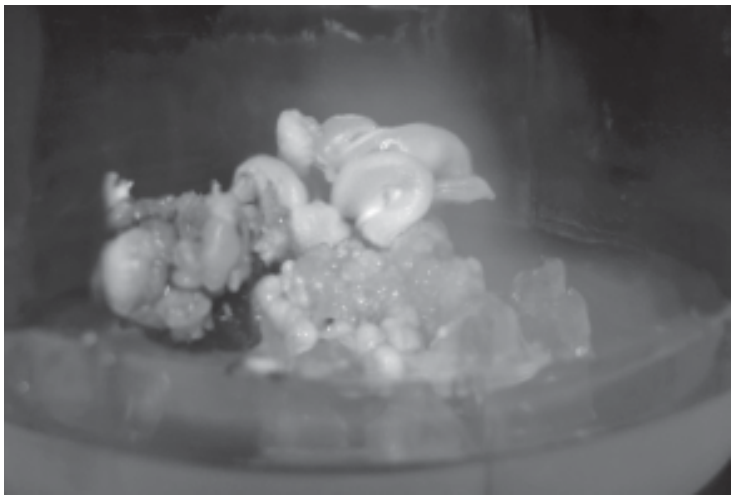

Fig. 1: White embryogenic callus

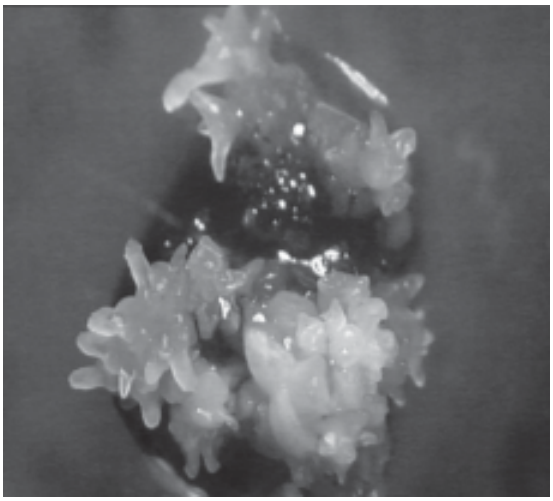

Fig. 2: Initiation of shoot primordial on MS basal medium

Table1. Induction of somatic embryo from the diploid banana cv. Njalipoovan and cv. Chingan

MS Medium		Average no. of embryos from shoot apices		Average no. of embryos from leaf sheath	
Zeatin (μM)	Ascorbic acid (mg/l)	Chingan	Njalipoovan	Chingan	Njalipoovan
0.4	–	33.5000±0.73c	10.8750±0.44d	23.5000±3.11cd	16.6250±0.18c
0.92	–	34.0000±0.53c	14.5000±1.95c	30.7500±0.36b	18.5000±0.18b
1.84	–	42.3750±0.49a	27.5000±0.18a	35.2500±0.31a	21.5000±0.18a
2.76	–	37.3700±0.18b	18.2500±0.16b	26.3750±0.77c	18.2500±0.6b
3.68	–	31.6200±0.41d	15.1250±0.35c	23.8750±0.61cd	16.5000±0.18c
4.6	–	28.0000±0.26e	14.6250±0.83c	21.3750±0.18d	13.8750±1.24d
–	0.01	16.5000±0.37d	8.0000±000e	7.5000±0.32de	9.8750±0.22d
–	0.02	13.2500±0.64e	8.8750±0.12d	13.1300±0.35c	12.0000±0.18b
–	0.04	13.3500±0.18e	10.6250±0.46c	14.7800±0.32b	11.2500±0.16c
–	0.06	11.8750±0.12f	9.7500±0.36d	17.4300±0.36a	12.2500±0.25b
–	0.08	18.6250±0.18c	11.2500±0.36bc	15.6300±0.18b	13.5000±0.18a
–	0.1	25.2500±0.36a	15.5000±0.32a	9.1300±0.12d	12.3750±0.26b
–	0.3	23.1250±0.39b	11.7500±0.16b	7.6300±0.41de	12.3750±0.18b
–	0.5	15.8750±0.29d	9.6250±0.26d	6.1300±0.29f	9.2500±0.16e

Significance was determined by ANOVA:significant at P<0.05
SE=Standard Error; Data represent the mean of eight replications

developed within 2-3 weeks. These plantlets were transferred to basal medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 % survival rate (Fig. 3).

Plant regeneration of banana has been reported from various explant sources and from a variety of cultivars. In cultivar Grand Nain, which has a genomic constitution of AAA, Novak *et al.* (1989) used bases of leaf sheath and corm sections from *in vitro* grown plants for embryogenesis. For the cultivar Bluggoe, which has an ABB genomic composition, Dheda *et al.* (1991) cultured thin sections from highly proliferate shoot tip cultures to produce embryogenic cell suspension cultures. Male flowers are used as the starting material for initiating embryogenic cultures in cv. Grand Naine (Escalant *et al*., 1994; Cote *et al*., 1996; Navarro *et al*., 1997; Becker *et al*., 2000).

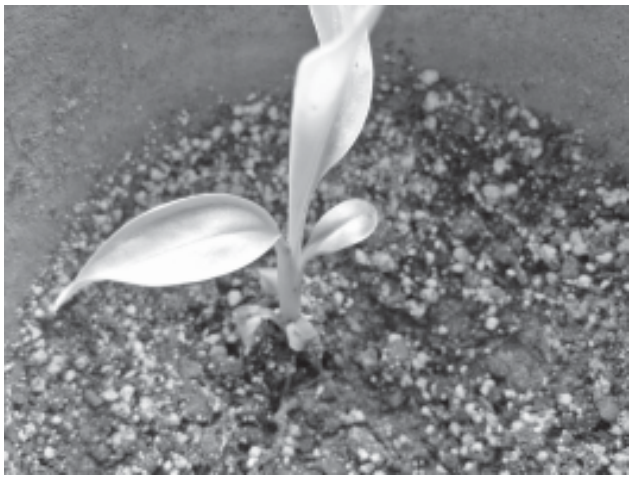

Fig. 3: Plantlet in vermiculite

Developing embryogenic culture systems with reliable regeneration efficiency from important varieties of banana is a pre requisite for realizing the potential of cellular and molecular tools of crop improvement (Smith *et al.*, 2005). Toward this goal, studies were made to develop protocols for somatic embryogenesis and plant regeneration from different banana cultivars. According to Dheda *et al.* (1991) elongated and highly vacuolated cells were apparently originated from the non-meristematic tissue. In the present study, elongated, thin, free cells appeared in the cell suspension was non meristematic, but cell aggregates showed embryogenic potential.

Somatic embryogenesis in the presence of malt extract was reported from different triploid banana cultivars (Khalil *et al*., 2002; Cote *et al*., 1996; Ganapathi *et al*., 2001). Similar type of observation was reported by Navarro *et al*. (1997) in a diploid and triploid Cavendish banana. Addition of ascorbic acid (0.01-0.5 mg/l) reduced blackening of medium. Only cell aggregate developed into embryos. Similar cell aggregates were described in suspension cultures of corn (Emons and Kieft, 1992) and wheat (Tabaeizadeh and Campeau, 1992). These cell clusters were called pro-embryogenic masses (Georget *et al*., 2000) and indicated the embryogenic potential of suspension culture. In the present study, the cvc. Chigan (AA) showed maximum embryo development in the medium supplemented with zeatin from both explants. The differential response of

two cultivars with respect to embryogenesis suggested that genetic factors affect the rate of embryogenic potential. The literature also revealed that somatic embryogenesis is genotype dependent phenomena (Assani *et al*., 2002; Gahan and George, 2008). However the embryogenic frequency in banana was not only dependent on the genome group, but it also varied with the variety with in the genome groups and even one experiment to another (Strosse *et al*., 2006).

In this procedure, the establishment of plant regeneration system for *Musa* spp. via somatic embryogenesis was achieved in a limited time period of 120-150 Days. Strosse *et al*. (2006) reported plant regeneration system for *Musa* spp. via somatic embryogenesis within a period of 14-42 months. Somatic embryogenesis was reported as time consuming, labour intensive and rather in efficient method for raising regenerable suspension cultures in case of banana. Several commercial and elite clones have been induced in to embryogenesis. A large number of banana genotype still need to be explored for embryogenic potential for use in propagation and genetic improvement. The protocol developed in this work is suitable for large scale propagation of diploid banana cultivars such as *Musa acuminata* cv. Chingan and *Musa acuminata* cv. Njalipoovan.

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