



Explants Size Response to *in Vitro* Propagation of Musa (Aaa Group) 'Amritsagar' Musa (Aab Group) 'Malbhog' and Musa (Aab Group) 'Chenichampa' Banana

KEYWORDS

Explant, sterilization, *in vitro*, microshoot, initiation, micropropagation, culture

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ABSTRACT *In vitro* propagation of Musa (AAA group) 'Amritsagar', Musa (AAB group) 'Malbhog' and Musa (AAB group) 'Chenichampa' was carried out using sword sucker explants. The effect of three different sizes of explants (5, 10 and 20 mm) on the establishment of banana in micropropagation was investigated. Three cultivars (Amritsagar, Malbhog and Chenichampa) were used for the study with an average of 12 explants per treatment per cultivar. Initiation media- MS Basal, MS+BAP 0.2 mg L⁻¹, MS+BAP 0.3 mg L⁻¹ and MS+BAP 0.5 mg L⁻¹ were used to evaluate the explants size response for initiating and establishing banana cultures in *in vitro* condition. The larger explants (20 mm) responded well with regard to survival of explants, days to swelling and greening of explants, emergence of leaf and days to multiple bud initiation under *in vitro* condition as compared to smaller explants.

INTRODUCTION

In vitro propagation of banana involves various aspects that include the selection, sterilization and sizing of explants (suckers) which are vital in response to initiation and establishment of cultures, survival of explants, shoot proliferation and rooting of microshoots. Explant size play an important role in getting healthy cultures under *in vitro* condition. Various sterilizing agents are used to make the explants free from contaminants. However, these sterilants have some toxic effect on the plant tissues and the effect is variable on different sizes of explants. The sizes of explants play an important role in getting cultures with minimum injury as well as in establishing cultures for subsequent multiplication. Three different sizes of explants viz., 5 mm, 10 mm and 20 mm were used in the present work to examine the effect of explant size on survival and establishment of banana shoots of Musa (AAA group) 'Amritsagar' Musa (AAB group) 'Malbhog' and Musa (AAB group) 'Chenichampa' Banana.

MATERIAL AND METHOD

The study was carried out in the Department of Biotechnology, Gauhati University, Assam and in The Energy and Resources Institute, Guwahati, Assam with the objective to evaluate the response of different explant sizes on *in vitro* propagation of banana. Three popular and commercial banana cultivars of Assam (India) namely, Amritsagar, Malbhog and Chenichampa were considered for the present work. Amritsagar (AAA) is a good table banana cultivar with medium sized plant, good size fruit and medium thick rind, and the ripe banana develops a bright yellow colour. Malbhog (AAB) is one of the most popular table banana cultivar of Assam and has a high demand on market due to its sweet aroma, taste and higher post harvest life. On the other hand Cheni Champa (AAB) is one of the hardiest medium tall banana cultivar, resistant to Fusarium wilt and fairly resistant to bunchy top disease with small size fruits, thin peel, creamy pulp and sub-acid taste.

The sword suckers of all the three cultivars of 2-3 month old and around 80-100 cm in length were collected from field grown healthy mother plants with good bearing capacity and prepared the explant for *in vitro* study (Plate 1). The suckers were washed under running tap water for 30 minutes to 1 hour to remove the dirt followed by trimming into 3-4 inched sizes. The trimmed explants were further treated with Savlon (Johnsons & Johnsons) for 15

minutes followed by with a mixture of 2% Sodium Hypochlorite + 1 g L⁻¹ Captan or Dithane M-45 and Rifampicin (0.1%) for 45 minutes with Tween-20 as surfactant to increase the efficiency of sterilizing agents by enhancing the maximum penetration of the sterilizing agents. Thereafter, the explants were rinsed with clean water for 4 times and a quick dip (15 sec) in 70% alcohol was given before transferring the explants to Laminar Air Flow Cabinet. Explants were then dipped in double distilled water, Ascorbic Acid, Citric Acid and a solution of Ascorbic acid and Citric acid at 100 mg L⁻¹ for 1 hour before surface sterilization. Similar antioxidants i.e., Ascorbic Acid and Citric acid were also used at a concentration of 50 mg L⁻¹ and a solution of Ascorbic acid and Citric acid at 100 mg L⁻¹ for 10 minutes after trimming and sterilization of the explants in Laminar Air Flow Cabinet. After that explants were taken out from the solution and washed with sterile water and then treated with Sodium Hypochlorite solution (0.5-1.0 %) for 7, 10, and 15 minutes exposure time followed by 4 times washing with sterile water. The treated suckers were further peeled up by removing one more scale and treated with 0.1 % HgCl₂ for different period of exposure (5 and 7 minutes) and washed with sterile water for 4 times. Finally the trimming was done to a sizes of 5 mm, 10 mm and 20 mm (Table 1) and dipped in a sterile solution of FL Cystine (15 mg L⁻¹) for 30 minutes and then explants were directly inoculated in MS media (Murashige and Skoog, 1962) without or with hormones (BAP) at 0.2 mg L⁻¹, 0.3 mg L⁻¹ and 0.5 mg L⁻¹. The cultures were incubated in the Plant Growth Room (PGR) at 25°C ± 2°C with 16 hours illumination and 8 hours dark phases. Inoculated cultures were monitored periodically at 2 weeks intervals to observe the parameters like days to greening of explants, days to swelling of explants, development of green globular hard coat structures, days to emergence of leaf and days to multiple bud initiation.

Table 1: Different treatment (explant size) used for the study of *in vitro* culture establishment and development of banana shoots

Treatment	Replication	Explant size (mm)
R1	12	5
R2	12	10
R3	12	20



Plate 1: Steps in preparation of banana explants for in vitro initiation

RESULTS

The present study was conducted to evaluate the response of different explant sizes on in vitro propagation of *Musa* cv. Amritsagar, Malbhog and Chenichampa. Three different explant sizes (Table 1) were used for the study. Explant size 20 mm (R3) in all the three cultivars showed the best results with regard to number of days required to show greening by the inoculated explants, number of days for swelling of explants, number of days taken for emergence of leaf and number of days to multiple bud initiation under in vitro condition.

After initiation the explant base swelled considerably from the 3rd week onwards due to the development of leaf primordia. The colour of the explants changed from white cream to green color and multiple buds developed from these swollen buds within 29-33 days and developed shoots from these buds. The similar kind of results were also reported by Kanchanapoom and Promsorn (2011), where they observed bud swelling, greening and shoots development from the swollen and green buds within forty-nine days.

Effect on explants size on days to greening of inoculated explants:

Days required for greening of inoculated explants was lowest in the treatment R3, i.e., explant size 20 mm followed by R2 and R1 respectively. A minimum of 11.00, 13.17 and 14.42 days were found for initial greening of inoculated explants of 20 mm size (Fig. 1 and Table 2). Among the three cultivars Amritsagar took the lowest time to show explant greening symptoms indicating the success of inoculation of the explants (Plate 2a).

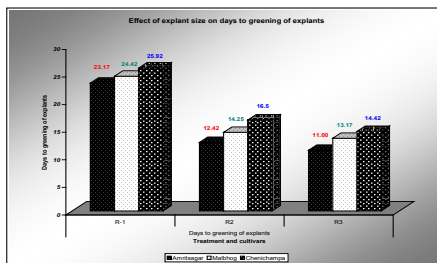


Fig 1: Effect of explant size on days to greening of banana explants

Effect of explants size on days to swelling of inoculated explants:

Days required for swelling (Plate 2b) of inoculated explants was again lowest in the treatment R3, i.e., explant size 20 mm followed by R2 and R1 respectively. A minimum of 16.08, 18.67 and 20.75 days were observed with regard to swelling of in vitro culture of explants of 20 mm size. Among the three cultivars Amritsagar responded very well taking a minimum of 16.08 days for swelling of in vitro cultures (Fig. 2 and Table 2).

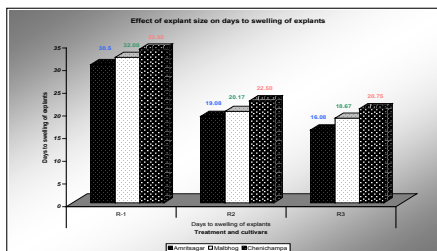


Fig 2: Effect of explant size on days to swelling of banana explants

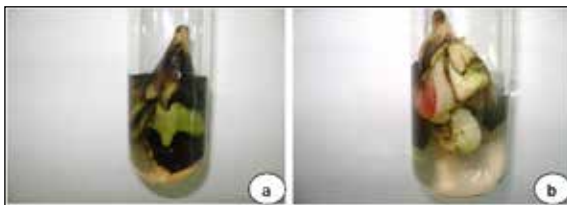


Plate 2: Greening (a) and swelling (b) of banana explant under in vitro condition

Effect of explants size on survival percentage of in vitro cultures:

Significant differences with respect to survival of in vitro cultures pertaining to inoculation of explants of different sizes were observed when explants were cultured in MS medium. Smaller explants (5 mm) showed lower survivability rate (50.00%, 41.67% and 58.33 %) as compared to the larger explants (20 mm), where it was 91.67%, 83.33% and 91.67% for cultivars Amritsagar, Malbhog and Chenichampa respectively (Fig 3). The details of results obtained were presented in table 3.

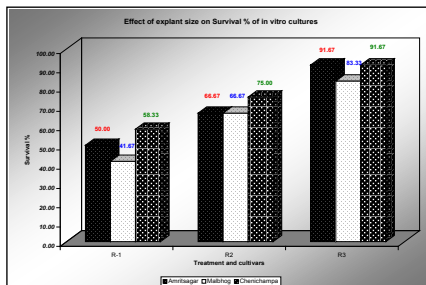


Fig 3: Effect of explants size on survival of in vitro cultures of banana

Effect of explants size on days to emergence of leaf:

A minimum of 21.42, 25.17, and 25.92 days were recorded for banana cultivars Amritsagar, Malbhog and Chenichampa with regard to emergence of leaf (Plate 3) from the initiated cultures in treatment R3, i.e. culture of 20 mm explant size (Fig 4 and Table 2).

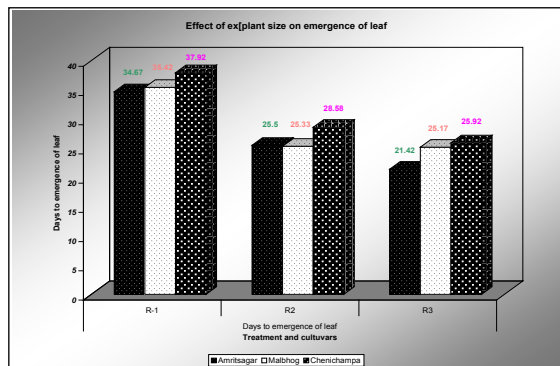


Fig 4: Effect of explant size on emergence of leaf in vitro initiated cultures of banana



Plate 3: Emergence of leaf from initiated cultures of banana (a, b, c)

Effect of explants size on days to multiple bud initiation:

The in vitro culture grew into a green globular hard coat structures after 29-33 days and from those green globular structures adventitious plantlets were developed in due course of time (Plate 4). In the present study treatment R3 (20 mm explants size) showed minimum period for initiation of multiple bud (Plate 5) in the in vitro cultures (28.92 32.42 and 32.92 days) for Amritsagar, Malbhog and Chenichampa cultivars respectively which was followed by treatment R2, where it is 32.42 33.75 and 32.92 days respectively (Fig 5 and Table 2).

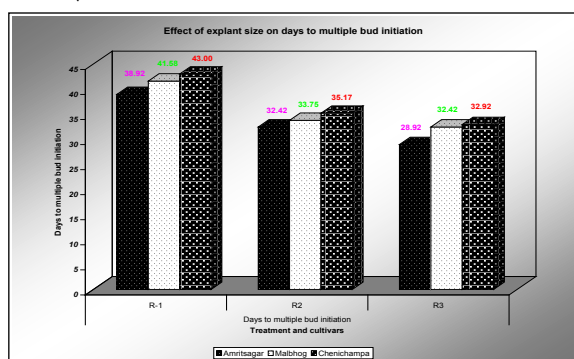


Fig 5: Effect of explant size on days to multiple bud initiation in vitro cultures of banana



Plate 4: Development of green globular hard coat structures in vitro cultures

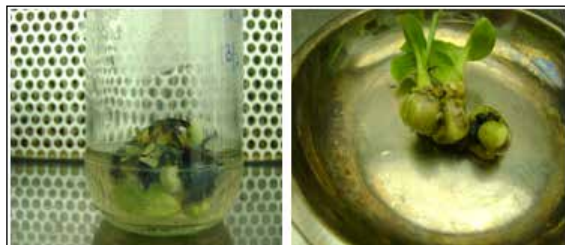
tures in in vitro cultures

Plate 4: Development of green globular hard coat structures in vitro cultures

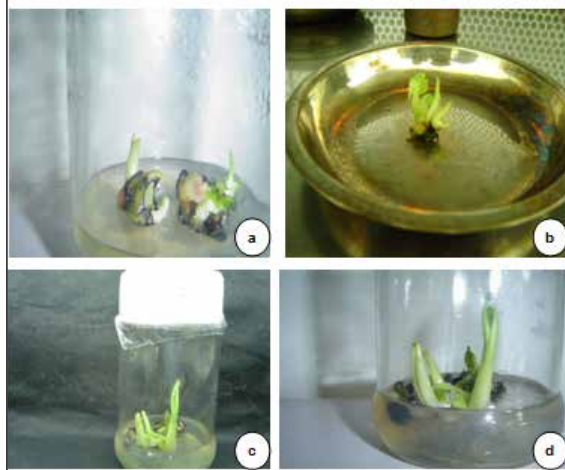


Plate 5: Multiple bud initiation in in vitro cultures of banana (a, b, c, d)

Table 2: Effect of explant size on establishment of banana shoots in in vitro condition

Cultivar	Explant size	Days to green-ing of explants	Days to swelling of explants	Days to emer-gence of leaf	Days to mul-tiple bud initiation
Amritsagar	R-1	23.17 _a	30.50 _a	34.67 _a	38.92 _a
	R-2	12.42 _b	19.08 _b	25.50 _b	32.42 _b
	R-3	11.00 _c	16.08 _c	21.42 _c	28.92 _c
	S.Ed.±	0.39	0.54	0.53	0.57
	CD _{0.05}	0.80	1.11	1.09	1.16
Malbhog	R-1	24.42 _a	32.08 _a	35.42 _a	41.58 _a
	R-2	14.25 _b	20.17 _b	25.33 _b	33.75 _b
	R-3	13.17 _b	18.67 _b	25.17 _b	32.42 _b
	S.Ed.±	0.43	0.59	0.69	0.46
	CD _{0.05}	0.88	1.20	1.41	0.94
Cheni-champa	R-1	25.92 _a	33.92 _a	37.92 _a	43.00 _a
	R-2	16.50 _b	22.50 _b	28.58 _b	35.17 _b
	R-3	14.42 _c	20.75 _c	25.92 _c	32.92 _c
	S.Ed.±	0.51	0.78	0.53	0.37
	CD _{0.05}	1.03	1.58	1.09	0.75

Table 3: Effect of explant size on survivability of in vitro culture of banana cultivars (Amrit Sagar, Malbhog and Chenichampa)

Cultivar	Treatment	No. of explants inoculated	No. of explants survived	Survival % of in vitro cultures
Amrit Sagar	R1	12	6	50.00
	R2	12	8	66.67
	R3	12	11	91.67
	Mean			69.44
	SD			20.97
Malbhog	R1	12	5	41.67
	R2	12	8	66.67
	R3	12	10	83.33
	Mean			63.89
	SD			20.97

Cultivar	Treatment	No. of explants inoculated	No. of explants survived	Survival % of in vitro cultures
Chenichampa	R1	12	7	58.33
	R2	12	9	75.00
	R3	12	11	91.67
	Mean			75.00
	SD			16.67

DISCUSSION

The use of smaller explants resulted in surviving of fewer explants, probably due to tissue damage upon excision and treatment with sterilants during the process of sterilization of the explants. Domingues et al. (1995) observed that explants of 1 cm long and 0.7 cm diameter obtained from banana cv. Maca gave the highest number of buds on nutrient solution containing 5.0 mg L⁻¹ BA for 45 days. Hirimburegama and Gamage (1996) used explants of about 2-3 cm in length and about 2.5 cm in diameter for sterilization and in vitro multiplication of local cultivars of banana (*Musa* spp.) through shoot-tip culture. Jafari et al. (2011) used the explants of size 3 to 4 cm in length and 2 to 3 cm in diameter after trimming to study the effect of BAP on in vitro multiplication of *Musa acuminata* (banana) cv. Berangan. Morfeine (2013) also used the banana explants of 1.5-2.0 cm in length after removing the outer leaves to initiate the cultures in in vitro condition. Significant differences in results due to explants size was observed when cultures in the uniform MS media. In the present study smaller explants (5 mm) showed slow response to the swelling, greening, survival of explants, emergence of leaf and multiple bud initiation under in vitro condition as compared to the larger explants (20 mm). Survival percentage of 5 mm explants was 50.00%, 41.67% and 58.33 %, whereas, 20 mm explants showed 91.67%, 83.33% and 91.67% for cul-

tivars Amritsagar, Malbhog and Chenichampa respectively. Dore Swamy et al. (1983) and Epp (1987) reported that larger explants, consisting of the apical dome with 6-8 overlapping leaf bases, developed into multiple shoots more readily because they contained more lateral buds. However, Sandoval and Muller (1992) reported that initiating cultures from such large explants increases explants and medium blackening, thereby reducing their survival rate. In the present study the larger explants (10 mm and 20 mm) responded very well and performed well under in vitro condition, which indicate the deviation from the findings of Sandoval and Muller (1992). Larger explants (10 mm and 20 mm) showed better results with regard to days required for greening and swelling of explants base, emergence of leaf from the initiated explants and multiple bud initiation under in vitro condition in all the three banana cultivars studied. The culture meristem first turned brown in colour in 5-6 days, which eventually grew into a green globular hard coat structures after 29-33 days and from that green globular structure adventitious plantlets were developed. The similar results were also observed by Amin et al., (2009). Uddin et al., (2006) reported the swelling of explants and some colour changes from pale white to light/deep green, which substantiate the results of the present investigation. Mukunthakumar and Seeni (2005) noticed the swelling of explants up to 1.5 cm in diameter even while a marginal increase in height (0.7 cm) and greening of the outer leaf sheath surrounding the shoot apex during the first 3 weeks the explants. Jaisy and Ghai (2011) also found that after few days of initiation the explants swell and turn green and produce shoots within 4 weeks. All the above findings with regard to swelling of explant base, greening, development of green globular structures and development of shoots in in vitro condition for banana micropropagation corroborate the results of the present investigation.

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