Response of Two Banana Cultivars Basrai and Grandnaine to *in vitro* Propagation

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Abstract- **Banana and plantain is the most important fruit crop of India having great socio-economic significance. The use of *in vitro* plants obtained by *in vitro* budding is one of the main improvements in the cultivation of banana. Banana tissue culture plantlets have several advantages like higher survival rate than suckers, reduce the cost of disease and pest control, show uniform and vigorous growth, and have a shorter harvesting period. India still lags behind in research and development, regarding micro-propagation of banana because low cost protocol for commercial production of *in vitro* plantlets has not been established. The present investigation therefore attempts to see the response of two banana cultivars Basrrai and Grandnaine to *in vitro* propagation. The application of somaclonal variation, tissue culture and micropropogation seems to be the best option available; not only for quality improvement but also for commercial exploitation of this crop that has great economic potential. Micropropogation of banana is currently being done to produce large population of clones that are disease free. However, the multiplication rates vary with genotype or races. Hence, in the present investigation, attempts were made to see the Response of Two Banana Cultivars Basrai and Grandnaine to *in vitro* Propagation that can be used for rapid multiplication of banana cultivar, Basrai.**

***Key words*:** **Micropropagation, cultivars, explant, somaclonal, *in vitro*.**

I. INTRODUCTION

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 (Tomlinson, 1969; see Fig. 1 A) The centre of origin of the group is in South-East Asia, where they occur from India to Polynesia (Simmonds, 1962). The centre of diversity has been placed in Malaysia or Indonesia (Daniells *et al.,* 2001), although considerable diversity is known throughout the range. The plants are distributed mainly on margins of tropical rainforests (Wong *et al.,* 2002).

There are a number of horticultural species in the genus *Musa,* and *Strelitzia reginae* (bird-of paradise) lies in the sister familiy Strelitziaceae. The leaves of *Musa* are used for their fibre content: when fresh as plates for eating or wrapping food parcels for steaming, or when dry as strips for weaving into various articles and for roofing shelters. Specific names such as *M ornata* and *M. textilis* reflect these uses.

The vast majority of the cultivated bananas (Pollefeys *et al.,* 2004) are derived from inter- and intraspecific crosses between two diploid (2n = 2x = 22) wild species, *Musa acuminata* and *Musa balbisiana* (Simmonds and Shepherd, 1955). In terms of the chromosome sets, these are designated as having the genome constitution AA *(M acuminata)* or BB *(M balbisiana).* These diploid *Musa* species have seeded fruit with little starch and only a small amount of fleshy pith, and are of no value as a crop.

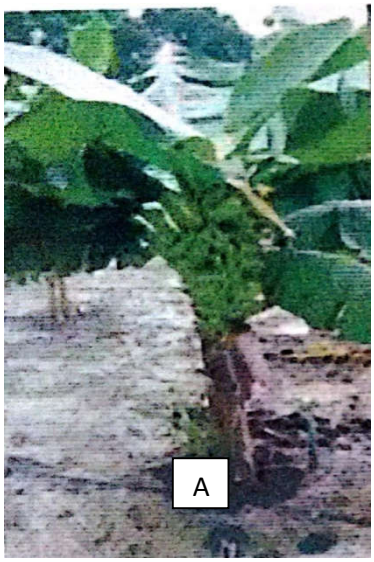
The cultivated bananas and plantains differ from their wild relatives by being seedless and parthenocarpic. Most of the cultivars are wild collections made by farmers of spontaneously occurring mutants with parthenocarpic fruit production, which were brought into cultivation and then multiplied and distributed by vegetative propagation. 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, while some cultivars are 'beer bananas' for fermentation of the juice, or used for deep frying as banana chips. Simrnonds (1962) considered five plant characteristics that lead to farmers picking plants for cultivation: plant vigour, yield, seedlessness, hardiness and fruit quality, the first four ofwhich are related to polyploidy (triploidy).

In *Musa,* Faure *et al.* (1994) made controlled and reciprocal crosses that demonstrated strong bias towards maternal transmission of chloroplast DNA, but showed the unusual phenomenon of paternal transmission of mitochondrial DNA in *Musa acuminata.* The study was extended by Carreel *et al.* (2002) to analyse the origins of more

than 300 *Musa* genotypes, leading to the conclusion that most cultivars are linked to two subspecies of *M. acuminata, M acuminata banksii* and *M acuminata errans,* through their mitochondrial genomes.

In vitro tissue culture propagation systems are very efficient in *Musa.* These can give high-quality, uniform plants free of disease and nematodes, and much of the planting material used in commercial plantations, and increasingly in smallholder production, comes from mass micropropagation. Shoot tip cultures have been most widely used (Strosse *et al.,* 2004), but suspension cultures are also being developed (Roux *et al.,* 2001). In some tissue culture system, high levels of chimerism are found, where chromosome number and genotype vary (Roux *et al.,* 2001) in the resulting plants. The valued South Indian 'Red' sweet banana (see Fig. 2) shows regular reversion of the colour character to green, particularly in tissue-culture propagated plants but also in the field (Stover and Simmonds, 1987), although the basis of this has not be confirmed. A programme checking varietal characteristics of material grown up after a decade of storage *in vitro* is showing that very few morphological or ploidy variants have been induced (van den Houwe *et al.,* 1995). Applications of molecular markers do show some DNA changes (Ray *et al.,* 2006) arising following tissue culture. Notably, Oh *et al.* (2007) demonstrated that some genomic regions of *Musa* show higher rearrangements and mutation rates than others under the stresses imposed during tissue culture.

Some 1000 *Musa* cultivars and 180 wild species, representing much of the diversity of the banana crop, are maintained in tissue culture at the Biodiversity International Transit Centre (ITC) in Belgium, and these provide a valuable reference collection that is mostly in the public domain and freely accessible for research and breeding, and distributed as tissue-culture plantlets.



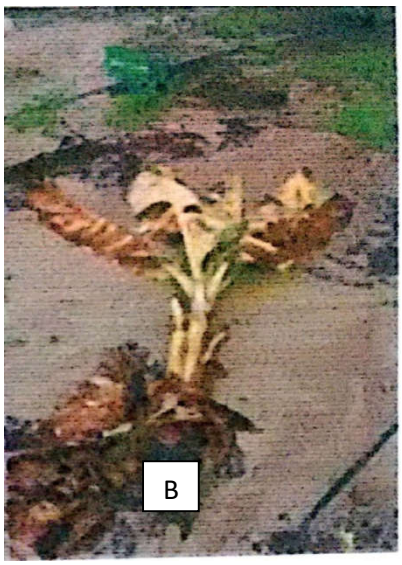


Fig. 1 . A, A banana plant with ripening fruit bunch. B, The dessert banana killed by Panama disease.

Fig. 2. The diversity of banana and plantains on sale in a shop in south India (Varkala, Kerala State)

In Vitro Propagation

In addition to the advantages of using little space and providing continuous availability, the plants produced by this technique have a very high sanitary quality. If combined with appropriate agronomic practices, the vitro plants may produce high-yielding crops while reducing pesticide use (nematicides). However, the cost of the vitro plant

produced by *in vitro* budding remains high compared to conventional methods. This high cost is the most significant obstacle to its common use. Somatic embryogenesis is likely to significantly reduce such a cost (reduction of labor cost). In the near future, this technique will be applied to traditional cultivars but also to new hybrids from genetic improvement programs in order to satisfy the probable high demand for varietal reconversion.

II. MATERIALS AND METHODS

Procurement of Germplasm

The germplasm of banana comprising of different cultivars was procured in the form of suckers collected from the nearby fields and from the germplasm collection available at Anand Niketan College, Anandwan, Warora, District Chandrapur, Maharashtra State. The cultivars selected were Basrai and Grandnaine (Table 1).

Table 1 Salient features of Banana genotypes

Accession Number	Common name	Genome	Souirce / Place of collection	Salient features
	Ardhapuri	AAA	Akola	Similar to Cavendish dwarf dessert type
	Basrai	AAA	Jalgaon	Leading commercial cultivar selection of Cavendish dwarf dessert type
	Grandnaine	AAA	BARC Mumbai	Cavendish banana from Central America
	Hatti	AAA	Akola	Similar to Cavendish thick pseudo stem dessert type
	Shreemanti	AAA	Jalgaon	Slightly high yielding selection from Basrai

Exotic obtained through the international network for the improvement of Banana and Plantain, France

Shoot-Tip Cultures

Shoot cultures of banana are conventionally taken from any plant part that contains a shoot meristem, i.e. the parental pseudostem, small suckers. peepers and lateral buds (V uylsteke, 1989). The apex of the inflorescence and axillary flower buds (Cronauer and Krikorian, 1985) are also suitable explants for tissue culture initiation. Overall, it is important to select explant material from preferably mature individuals whose response to environmental factors is known, and whose quality traits governed by genotypic and environmental effects have been identified. For rapid *in vitro* multiplication of banana, shoot tips from young suckers of 10-50 cm height were used as explants. From the selected sucker a cube of tissue of about 1-2 cm^3 containing the apical meristem was excised. This block of tissue was dipped in 70% ethanol for 60 seconds, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water. Subsequently a shoot tip of about 1 x 3 mm, consisting of the apical dome covered with several leaf primordia and a thin layer of tissue was aseptically dissected. The explant was placed directly on a multiplication-inducing culture medium. For banana micropropagation, MS—based media (Murashige and Skoog, 1962) was adopted (Table 2). The media was supplemented with sucrose as a carbon source at a concentration of 30 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 were transferred to new medium every fifteen days. Antioxidants, such as ascorbic acid or citric acid in concentration 50 mg/1, were added to the growth medium to reduce blackening, or the explants were dipped in antioxidant solution (cysteine 50 mg/l) prior to their transfer to culture medium (Janet, 1985).

Usually two types of growth regulators, a cytokinin and an auxin, are added to the banana growth medium. Their concentration and ratio determines the growth and morphogenesis of the banana tissue. In the present study 2, 4, 6, and 8 mg/l 6-benzyl amino Purine (BAP) and 0.5 mg/1 Kinetin was added to the initiation medium.

Table -2 Composition of Murashige and Skoog basal tissue culture medium

Essential Elements	Concentration in medium mg/l	Essential Elements	Concentration in medium mg/l
Macroelements		**Iron Source**	
NH_4NO_3	1650	$FeSO_4.7H_2O$	27.8
KNO_3	1900	$Na_2EDTA.2H_2O$	37.3
$CaCl_2.2H_2O$	440	**Organic Suppliment**	
$MgSO_4.7H_2O$	370	Myoinosiltol	100
KH_2PO_4	170	Nicotinic acid	0.5
Microelements		Pyridoxine HCl	0.5
KI	0.83	Thyamine HCl	0.5
H_3BO_3	6.2	Glycine	2
$MnSO_4.4H_2O$	22.3	**Carbon Source**	
$ZnSO_4.7H_2O$	8.6	Sucrose	30,000
$Na_2MoO_4.2H_2O$	0.25		
$CuSO_4.5H_2O$	0.025		
$COCl_2.6H_2O$	0.025		

In most banana micro-propagation systems, semi-solid media are used. As a gelling agent agar (3g/l) was added to the culture medium but our preference was for Gelrite (2g/l) because of its higher transparency, allowing much earlier detection of microbial contamination. However, higher cost limited its use.Banana shoot-tip cultures were incubated at an optimal growth temperature of 26 ± 2°C in a light cycle of 16 h with a photosynthetic photon flux (PPF) of about 60 E/m^2s^1.

Multiplication of shoot-tip cultures

The formation of multiple shoots and buds was promoted by supplementing the medium with relatively high concentrations of cytokinins. In banana, BA is the preferred cytokinin and is usually added in a concentration of 0.1-20 mg/l (Banerjee and Langhe, 1985). For the multiplication of propagules, MS medium containing 2, 4, 6 and 8 mg/1 BAP and 0.5 mg/1 Kinetin was used. Clusters were separated, trimmed and repeatedly subcultured at 4-6 week intervals.

Regeneration of plants

Individual shoot or shoot clumps were transferred to a nutrient medium which does not promote further shoot proliferation but stimulates root formation. The cytokinin in the regeneration medium is greatly reduced or even completely omitted. Within 2 weeks, shoot tips develop into shoots. To initiate rhizogenesis IAA, NAA (a-naphthalene acetic acid) or IBA (indole-3-butyric acid) are commonly included in the medium between 0.1 and 2 mg/1. However, for the current study, IAA was used at concentrations ranging between 1-2 mg/l. Activated charcoal (0.1%) was added to the rooting medium to enhance rooting. After rooting, plants were hardened *in vitro* for 2 extra weeks on the rooting medium prior to transplantation to soil.

Acclimatization in the Nursery

After removing the plantlets from the culture vessel, the clusters were washed to remove the medium, separated into individual plants, and sorted according to size (large: more than 5 cm high; medium: 3 to 5 cm and small: less than 3 cm). They were then transplanted into soil boxes. Planting density was high, with 126 plantlets in a box measuring 58 x 36 x 12.5 cm. After planting, the box was covered with a perforated transparent plastic cover for seven to ten days, to conserve moisture.

The plastic was then removed, and two or three weeks later, the rooted plantlets were removed from the box and transferred to the field shed house. Here, the plantlets were transplanted into plastic pots 9 cm high and 10 cm in diameter. They were kept in the shed house for six to eight weeks until they reach a height of around 100 cm. At this stage, they were released for planting, out in the field.

Fig.3: Regeneration of Plants

Fig. 4: Acclimatization in the Nursery

III. RESULTS AND DISCUSSION

To initiate micro-propagation of banana cultivars, hardy mother plants that were able to tolerate high summer temperatures were selected. Suckers from such mother plants that were at least 10 months old were removed for experimentation. The response of such suckers on establishment media was investigated.

Response of Banana Cultivars in establishment medium

The response of sucker length on the establishment medium was investigated. Sucker of three different sizes were selected for the purpose. Out of the sucker sizes selected, the best response was given by suckers of length between 21-35 cms, for all the cultivars tested. Similar observation regarding the proper age or size of sucker was reported by Okole and Schultz, 1996. With increase in sucker size the response for multiple shoot initiation decreased probably due to the reduction of meristematic zone and increase in the differentiated zone of the shoot apical meristem.

When the response of different cultivars on the establishment medium comprising of BAP and Kinetin was studied, it was found that in general BAP favored better establishment of the shoot tips over kinetin (data not provided here). The present findings are in agreement with those of Miller and Muraishige, 1996; Bamasco and Barke, 1985 and Lad, 1982.

Amongst the different hormone combinations tried, the performance of Grandnaine cultivar was the best with highest percentage of successful established cultures (98%), the response of Basrai was lowest (97%) (Table-3).

Table 3 Response of Banana cultivars in establishment medium

Cultivar	Length of sucker	0.5mg/l BAP	1.0 mg/l BAP	1.5 mg/l BAP	2.0 mg/l BAP
Basrai	10-20cm	No Response	84%	95%	89%
	21-35cm	38%	65%	97%	91%
	36-45cm	23%	48%	76%	78%
Grandnaine	10-20cm	32%	75%	64%	52%
	21-35cm	46%	98%	79%	65%
	36-45cm	41%	78%	62%	55%

Different hormone combinations were tried for successful establishment of apical meristems. The response shown by different cultivars was found to vary considerably. The response of Basrai was maximum 97 in 1.5mg/1 BAP. While the cultivar Grandnaine, responded more efficiently ie 98% to l mg/1 BAP. Similar result were reported by Banerjee *et al;* 1986, Naqndi and Choudhary 1998, Bekheet and Saker 1999, Schorfs et al 1999, Oliveira *et al.,* 2001.

In general the overall response of the cultivar Grandnaine was more superior over all other cultivars tested.

Induction of shoots in banana cultivars

After successful establishment of the apical meristems, the established meristerns were split vertically and the resulting halves were transferred aseptically to the shoot proliferation medium comprising of different combinations of BAP and Kinetin. Four different concentrations of BAP were tested (2, 4, 6 8 mg/1), keeping the concentration of Kinetin constant to 0.5mg/l.

The higher ratio of BAP to Kinetin in general favoured higher proliferation rates. The response of banana cultivar Grandnaive was maximum with around eight multiple shoots per meristem while the response of Basrai was the least with about 7.6 multiple shoots per meristem (Table-4). A positive correlation between increase in shoot multiplication and BAP concentration was also reported by Arinaithe 2000, Hirimburegama 1996, and Dhumale *et al.*, 1997.

Amongst the different hormone combinations tried, the cultivars Basrai and Grandnaive responded maximally to 6 mg/1 BAP and 0.5mg/1 Kinetin and the number of multiple shoots induced in these cultivers were 8.2 and 7.6 respectively. (Table-4).

Table 4 Induction of Multiple shoots in Banana cultivars

Cultivars	BAP mg/l	Kinetin mg/l	Number of shoots
Basrai	2.0	0.5	5.46
	4.0	0.5	7.25
	6.0	**0.5**	**7.64**
	8.0	0.5	5.30
Grandnaine	2.0	0.5	6.4
	4.0	0.5	7.5
	6.0	**0.5**	**8.2**
	8.0	0.5	7.6

Induction of roots in half strength MS medium

The response of different banana cultivars for the induction of roots in all the concentration of IAA was cultivar specific with respect to the days required for induction and success rate With alt the concentration of IAA tried, maximum rooting was induced when the exPlants were incubated on 1.5 mg/I for all the four cultivars. Maximum roots for explants were induced in this concentration by Grandnaine (7.50) while that induced in Srimanti (4.25) were the least (Table 5) A minimum of 14-20 days were required for root induction, depending upon the cultivaf. Earliest rooting was induced by 140 day in Grandnine while the slowest response was shown by Srimanti (20 days) (Table 5). Grandnine proved to be superior in performance with success rate of 97% for root induction, while the least response of 92% was given by Srimanti.

Bekheet and Saker 1999, Fitchet and Wimmar 1987, Cronaner and Kirkorian 1985 also reported such rapid and profuse root induction and proliferation in excised shoots.

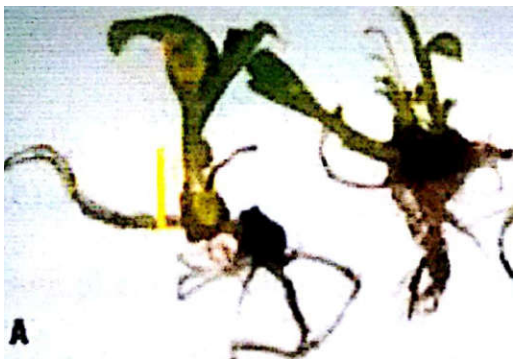


Fig 5 Induction of Roots in Basrai on different concentration of IAA

Table 5- Induction of roots in half strength MS medium

Cultivars	IAA mg/l	No. of roots	Days to induction	Success %
Basrai	1.0	3.72	18	93
	1.5	4.78	17	95
	2.0	3.75	19	94
Grandnaine	1.0	6.25	15	96
	1.5	7.50	14	95
	2.0	5.45	17	89

Survival percentage:

Well grown Plantlets with a mean shoot height of 6cm and root length of 8cm were removed from the culture tube or bottle, washed and transferred to sand, soil, and cow dung mixture in a ratio of 1:1:1. The plantlets were hardened in the culture room under conditions of high humidity for 15 days and then transferred to green house. The survival percentage ranged between 75-82% depending up on the cultivar. The survival Percentage of Grandnaine was maximum and that of Basrai was the least. Similar results were reported by Arvirldakasham and Shanmugavelu 990.The application of somaclonal variation, tissue culture and micropropogation seems to be the best option available, not only for quality improvement but also for commercial exploitation of this crop that has great economic potential. Micro propogation of banana is currently being done to produce large population of clones that are disease free (Cote *et al;* 1990, Buah *et al;* 2000). However, the multiplication rates vary with genotype or races (Jambhule *et al;* 2001). Hence, in the present investigation, attempts were made to develop a suitable micropropagation protocol that can be used for rapid multiplication of different banana cultivars. The results were highly promising and it can therefore be concluded that a successful protocol can be developed for commercial exploitation of the banana cultivars.

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