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Research Article

In vitro Multiplication and Conservation of a Diploid Landrace of Banana

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Abstract

South India is well-known for its diverse *Musa* genotypes and diploid landraces, which can be found in cultivated or wild pockets. Young and healthy sword suckers of *Musa acuminata* landrace Chingan (AA) were collected from Nagercoil, Tamil Nadu, and used as explants for *in vitro* regeneration in the current study. After 60 days of culture, MS medium supplemented with 0.1 or 0.2 mg/l TDZ induced numerous tiny shoot initials, which increased to an average of 37.29 ± 1.08 and 29.57 ± 0.84, respectively, after 120 days. When 2iP or Kinetin was combined with BAP, it was much more effective than when used alone in the formation of shoots. For the diploid landrace Chingan, the current study established an efficient and reproducible micropropagation protocol (AA). The current study highlighted the potential of TDZ to induce *in vitro* multiplication in banana shoot tip cultures. The conservation and genetic improvement of native diploids is critical to preventing genetic erosion caused by repeated cloning of only a few selected high yielding clones, making them susceptible to many diseases. Banana diploid land races are important in breeding programmes, and their preservation is urgently needed.

Keywords: *Banana, diploid, Musa, South India.*

Introduction

Bananas and plantains, the world's largest tropical perennial herbs of the Musaceae family and the genus *Musa*, originated in South East Asia (Simmonds, 1962). With an annual production of about 100 million tonnes, they are important staple crops that provide a good source of nutrients to millions of people in developing countries. India is one of Asia's major banana producing countries, with over 1, 64,000 ha under cultivation. Bananas, the country's leading fruit crop, have significant socioeconomic significance because they contribute to food security while also providing income and employment to rural populations.

Cultivated bananas are seed sterile diploid, triploid or tetraploid clones containing various combinations of the A and B genomes coming from the two diploid wild progenitors of *Musa*, *M. acuminata* Colla and *M. balbisiana* Colla (Simmonds and Shepherd, 1955). Edible bananas are propagated vegetatively by means of suckers, however, conventional propagules are not ideal because they carry weevils, fungal pathogens, nematodes and viruses and also suffer from slow multiplication, bulkiness and poor phytosanitary quality (Gubbuk and Pekmezci, 2004). Therefore, banana breeding possess a lot of constraints such as high frequency of sterility, low fertility due to polyploidy and seedlessness, non-availability of disease free, true-to-type planting material, slow propagation and long time span from one generation to the next.

With rising demand and vast export potential, as well as farmers' desire to grow bananas in large quantities, *in vitro* propagated plants are increasingly becoming the planting material of choice for rapid multiplication of economi-

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cally important commercial cultivars. *In vitro* propagation of bananas has several advantages over traditional propagation, including a higher multiplication rate, physiological uniformity, year-round availability of disease-free material, rapid dissemination of new elite plant materials around the world, uniformity of shoots and shoot harvest intervals, and faster growth in the early growing stages (Gubbuk and Pekmezci, 2004).

South India is well known for its diverse *Musa* genotypes and diploid landraces, which can be found in cultivated or wild pockets. When compared to many other commercial cultivars, edible diploid cultivars have numerous desirable characteristics such as disease resistance, flavour, and taste. Furthermore, diploids with fertile pollen can be used in hybridization programmes as male parents. However, due to their low yield potential, many of them are kept out of the mainstream of commercial cultivation. The conservation and genetic improvement of native diploids is critical to preventing genetic erosion caused by repeated cloning of only a few selected high yielding clones, making them susceptible to many diseases.

Chingan is a male fertile diploid Musa acuminata cultivar native to Malabar (Kerala, India) and parts of Tamil Nadu. It is a medium-sized slender plant with black blotches on the green pseudostem. The naked axis is clothed with dry bracts and male flowers, which is a distinguishing feature of the variety. The fruits have prominent apices and small bunches. The rind is thin, and when ripe, it turns a yellowish green colour. The flesh is creamy white, soft, and sweet, with a slightly subacid taste and a distinct flavour (Nayar, 1962). The current study was designed with an objective to conserve the valuable diploid genetic sources of banana through *in vitro* multiplication.

Materials and Methods

In vitro regeneration

Young and healthy sword suckers of *Musa acuminata* cultivar Chingan (AA) were collected from Nagercoil, Tamilnadu and used as explants for *in vitro* regeneration. The suckers were washed thoroughly in tap water and trimmed to a size of 4-5 cm in height after the removal of leafy top and roots. The explants were then immersed in 10% (v/v) labolene (Qualigens, India) for 15 minutes and placed under running tap water. The explants were surface sterilized sequentially treating in 4% commercial bleach solution (final conc. 0.1% NaOCl) for 20 minutes and in 0.1% (w/v) mercuric chloride for 7 minutes. Each treatment was followed by three rinses in distilled water, five minutes for each time. Then, the primordial leaves were removed carefully until the shoot apex of about 5-10 mm size was exposed. Without damaging the shoot apex, surrounding corm tissue was trimmed and implanted vertically into MS medium containing 3% sucrose (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of various plant growth regulators. The pH of the medium was adjusted to 5.8, gelled with 0.7% agar and autoclaved for 15 min. under 121°C temperature and 15 lbs pressure. The cultures were maintained at a temperature of 25± 2^0C with a photoperiod of 16 hrs/day under 2500 lux light intensity.

Subculture and Hardening

The explants were subcultured, after the transverse removal of primary shoot in order to obtain further multiplication. After 4-6 weeks, individual shoots of 4-5 cm height were separated and transferred to MS basal medium for root proliferation and shoot elongation. Adequately rooted plantlets were transferred to plastic cups filled with vermiculite covered with transparent plastic bags. After 2-3 weeks hardened plants were potted in a mixture of soil: sand: farmyard manure (1:1:1) and maintained in greenhouse conditions.

Experiments were set up with 7 replications for each treatment. Number and length of

shoots were recorded after 60 and 120 days. Statistical analysis was performed on the results of each experiment with the software SPSS/PC Version 4.0 (SPSS Inc., Chicago, USA). Mean and SE were calculated and differences between means were tested using Scheffes' Multiple Comparisons at the level of p=0.05.

Results and Discussion

The diploid land races of bananas find significance in breeding programmes. The conservation of diploids is the need of the hour to prevent genetic erosion of invaluable genotypes. Standardization of an efficient micropropagation protocol is a prerequisite for the rapid multiplication as well as the genetic improvement of cultivars. Chingan (AA), a favourite cultivar of Southern Tamil Nadu and Northern Kerala, exhibited a significant *in vitro* regenerative potential. Sterile explants established under the conditions of aseptic culture were creamy white when inoculated, turned green and bulged within one or two weeks. Primary shoots emerged within one month, which on transverse removal induced multiple shoots in culture. TDZ induced a stronger effect in shoot proliferation compared to all other cytokinins used in the study. MS medium supplemented with 0.1 or 0.2 mg/l TDZ induced numerous tiny shoot initials after 60 days and the number increased to an average of 37.29 and 29.57 respectively, after 120 days of culture (Table 1). However, the length of shoots did not show any enhancement even after 120 days. Further increase or decrease in the concentration of TDZ reduced the number of shoots, though the shoots obtained were of greater length. TDZ (Thidiazuron), a substituted phenyl urea is primarily used as a cotton defoliant and has been shown in various cytokinin bioassays to exhibit cytokinin like activity. TDZ is known to non-competitively inhibit cytokinin oxidase activity, thereby enhancing the availability of endogenous cytokinins (Eapen, *et al*., 1998). TDZ shows higher cytokinins activity than BA, zeatin, 2-iP or kinetin and can be used at a much lower concentration than BA (Arinaitwe, G. *et al*., 2000). Compared to most other compounds with cytokinin activity, lower concentrations of TDZ can stimulate axillary shoot proliferation, whereas higher TDZ concentrations may result in the formation of both axillary and adventitious shoots (Chalupa, 1988). Moreover, the concentrations at which TDZ is most effective are 10 to 1000 times lower than other plant growth regulators. Thidiazuron is persistent in plant tissues, and since most cultures are transferred to fresh medium monthly, or more frequently, the plant parts are continually exposed to fresh TDZ (Huettman and Preece, 1993). High rates of shoot proliferation, often desirable for efficient micropropagation may include both axillary and adventitious shoots. However, if clonal fidelity is desired, TDZ or other cytokinins may be used at levels that stimulate only axillary shoot growth, thereby avoiding the potential somaclonal variants derived from adventitious shoots (Kim. *et al*., 1997). Lowest concentrations of TDZ (0.005 or 0.01 mg/l) produced better shoots in the present study, though the number was less. However, in the optimum concentration of TDZ, shoot proliferation was manifested in the appearance of numerous fleshy bulbous structures each of them producing several tiny adventitious buds on the surface. Cytokinins commonly stimulate shoot proliferation and inhibit their elongation. Therefore, inhibition of shoot elongation by TDZ may be consistent with its high cytokinin activity.

In the present study, 2 mg/l BA induced an average of 29.83 shoots of moderate length after 120 days. Though, increase in the concentration of BA slightly reduced the number of shoots, an enhancement in shoot length was noticed. However, increase in the concentration of BA beyond 3 mg/l reduced the number of *in vitro* shoots, though the shoots possessed an average of 8.72 cm height. Micropropagation protocols for banana via shoot tip culture invariably use BA as plant growth regulator (Vuylsteke, 1989). 2iP and Kin were not found to be effective in shoot multiplication; however, 2iP was better when compared to Kin in shoot proliferation. 2iP or Kin in combination with BA was much

effective in the formation of shoots than when used alone. Individual shoots of 8-9 cm height were transferred to MS basal medium and thick, white, hairy roots were produced within 15 days. The rooted plantlets were transferred to vermiculite and maintained at a temperature of 25± 2^0C for initial 10 days and then at room temperature for 1-2 weeks. The plantlets transferred to potting mixture of soil: sand: farmyard manure (1:1:1) showed 100% survival.

Diploid landraces appear to be the primary source of crop improvement efforts in bananas for biotic and abiotic stress resistance. In this context, genetic resource conservation and redistribution are critical, and *in vitro* mass multiplication will provide a means to prevent the drastic erosion of valuable genotypes.

Table 1: Influence of different hormone concentrations and combinations on multiple shoot proliferation from shoot tip explants of diploid banana cultivar Chingan (AA)

Hormones (mg/l)				Number of Shoots		Length of Shoots	
BA	2-iP	Kin	TDZ	After 60 days	After 120 days	After 60 days	After 120 days
2	-	-	-	22.14 ± 0.88^{ab}	29.43 ± 1.07^{b}	3.01 ± 0.56^{abcdef}	5.81 ± 0.36^{abcd}
3	-	-	-	16.71 ± 0.42^{bcd}	22.57 ± 0.97^{bcd}	5.23 ± 0.40^{abcd}	7.73 ± 0.51^{ab}
5	-	-	-	6.14 ± 0.46^{fgh}	11.71 ± 0.84^{efghij}	5.17 ± 0.31^{abcde}	8.59 ± 0.34^{a}
-	2	-	-	7.71 ± 0.47^{fgh}	9.14 ± 1.71^{fghij}	3.50 ± 0.23^{abcdef}	5.13 ±0.33^{bcd}
-	3	-	-	11.29 ± 0.68^{def}	14.14 ± 0.51^{efghij}	3.60 ± 0.37^{abcdef}	6.20 ± 0.49^{abcd}
-	5	-	-	4.00 ± 0.31^{gh}	6.86 ± 0.67^{ghij}	4.49 ± 0.39^{abcdef}	6.34 ± 0.39^{abcd}
-	-	2	-	2.29 ± 0.36^{h}	5.00 ± 0.31^{ij}	1.79 ± 0.24^{ef}	3.63 ± 0.26^{de}
-	-	3	-	3.57 ± 0.37^{gh}	5.14 ± 0.34^{ij}	1.63 ± 0.20^{f}	5.70 ± 0.32^{abcd}
-	-	5	-	4.58 ± 0.43^{gh}	6.71 ± 0.42^{hij}	1.59 ± 0.21^{f}	5.77 ± 0.32^{abcd}
2	1	-	-	6.43 ± 0.72^{fgh}	10.71 ± 0.57^{efghij}	2.70 ± 0.38^{abcdef}	4.84 ± 0.39^{bcd}
2	2	-	-	6.71 ± 0.64^{fgh}	7.29 ± 0.68^{ghij}	2.44 ± 0.31^{bcdef}	4.23 ± 0.29^{cde}
2	3	-	-	4.14 ± 0.40^{gh}	4.28 ± 0.42^{j}	4.30 ± 0.34^{abcdef}	7.07 ± 0.40^{abc}
2	-	1	-	5.57 ± 0.69^{fgh}	8.70 ± 0.52^{ghij}	5.24 ± 0.39^{abc}	7.20 ± 0.42^{abc}
2	-	2	-	6.28 ± 0.68^{fgh}	14.43 ± 0.78^{efg}	4.02 ± 0.33^{abcdef}	7.25 ± 0.25^{abc}
2	-	3	-	8.30 ± 0.71^{efg}	17.69 ± 0.75^{de}	3.21 ± 0.50^{abcdef}	5.87 ± 0.31^{abcd}
3	1	-	-	6.57 ± 0.65^{fgh}	12.72 ± 0.68^{efghi}	4.74 ± 0.28^{abcdef}	7.70 ± 0.34^{ab}
3	-	1	-	11.00 ± 1.91^{def}	14.00 ± 0.73^{efgh}	1.92 ± 0.26^{cdef}	7.92 ± 0.51^{ab}
-	-	-	0.005	6.00 ± 0.55^{fgh}	10.60 ± 0.75^{efghij}	4.44 ± 0.31^{abcdef}	6.60 ± 0.43^{abcd}
-	-	-	0.01	7.75 ± 0.0.85^{fgh}	14.25± 1.10^{efgh}	5.88 ± 1.20^{a}	8.73 ± 0.30^{a}
-	-	-	0.05	15.71 ± 0.42^{cd}	25.43 ± 1.48^{bc}	3.39 ± 0.49^{abcdef}	5.98 ± 0.39^{abcd}
-	-	-	0.10	24.57 ± 0.78^{a}	37.29 ± 1.08^{a}	-	1.10 ± 0.18^{e}
-	-	-	0.20	19.72 ± 0.75^{abc}	29.57 ± 0.84^{b}	-	1.26 ± 0.23^{e}
-	-	-	0.30	7.50 ± 0.42^{fgh}	13.88 ± 0.61^{efgh}	1.83 ± 0.30^{def}	5.44 ± 0.17^{abcd}
2	-	-	0.005	13.86 ± 0.80^{cde}	18.00 ± 1.51^{cde}	3.57 ± 0.56^{abcdef}	4.81 ± 0.39^{bcd}
2	-	-	0.01	8.71 ± 0.42^{efg}	16.71 ± 0.61^{def}	5.64 ± 0.48^{ab}	6.30 ± 0.66^{abcd}

Means within the column followed by different letters significantly different according to Scheffe's Multiple Comparison Test (P = 0.05)

SE = Standard Error; Data represent the mean of seven replications

Conclusion

Habitat fragmentation, combined with farmers' preferences for supreme varieties, causes valuable genetic resources in bananas to vanish, potentially jeopardising ongoing efforts to improve breeding for biotic and abiotic stress tolerance. For this vegetatively propagated crop that requires large field areas, *in vitro* conservation is the most feasible and alternative mode of multiplication and conservation.

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