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In Vitro Mutiplication of *Musa Laterita* Roxb.

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Abstract This study was done to investigate the best culture media for multiplication and proliferation shoot tip of *Musa laterita* Roxb. A micro- propagation protocol for *Musa laterita* was established. Shoot tip was obtained by removing leaf sheaths from suckers and were cultured aseptically in Murashige and Skoog (1962) medium supplemented with 0, 1, 3, 5 and 7 ppm BA (6-Benzyladenine). Planned trial was laid – out in Completely Randomized Design (CRD). Subculture was undertaken every 4 weeks for 6 months. Results showed that treatment with 3 ppm BA got the most number of shoots (2.80) which is significantly higher than the other treatments. For plant height, the treatment with 0 ppm BA got the longest with 8.16 cm. As the width of the explants addition of 5 ppm BA to the MS medium got the highest with an average of 4.30 cm. which is significantly higher than all other treatments. For the number of leaves, no significant difference was observed in treatment with 0, 1, 3 and 7 ppm. The root primordial in treatment with 0 ppm BA got the highest with an average of 4.60 roots which is significantly higher than all other treatments.

Keywords: Banana, *Musa laterlita Roxb*, *In vitro* multiplication, Benzyl adenine(BA).

Introduction

Musa laterita is called Heliconia, Bird of Paradise Banana or Bronze Banana It is a tropical ornamental plant species (Hakkinen, 2001) in the banana family native to the Indian Subcontinent (Northeastern India) and Indo-China (Myanmar and Thailand) (Kew Bull,1949). and is from north-eastern India, Burma (Myanmar). This banana tree belongs to the Musaceae family. This plant is a superb new ornamental species with elegant, slender upright leaves with dark midribs and produce suckers from the base. It has large, colorful flowers with bright salmon-red bracts. (*Musa laterita* is a small section of the Rhodochlamys species).

This plant sends up suckers that are borne on long rhizomes at long distances from the parent main stem base and forms only lax open stools. The green pseudostems are slender, reaching up to 3 - 6 ft (1 - 2 m) in height, and

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they are devoid of any perceptible wax bloom. The leaves are about 3 ft (1 m) long and 12 in (30.48 cm) wide and taper gradually to the slender petioles. The leaf blades are bright green on top, scarcely paler under, truncate at the apex, with a reddish midrib and taper very gradually to an acute base into the leaf stalk or petiole. The petioles are about 15 – 20 in (40 - 50 cm) long, and they closely clasp the pseudostem base and soon become rare at the region of junction. *Musa laterita* may not be a hardy type, but is notable for its extreme upright habit, that is, the leaves do not spread, but point upwards.

As the stem reaches maturity, when it is about 3 – 5 ft high, an erect inflorescence develops from the apical portion of the stem. The inflorescence is upright and has brick-red bracts, similar in color to the tropical soil laterite that gives the species its name, subtending yellow female flowers are borne at the end of little green bananas. The first sterile bract is usually a foliage leaf with a broadened petiole developing the red color, and then, it is followed by one sterile true bract 8 – 12 in (20 - 30 cm) long. Small green fruits develop which sometimes mature and produce seeds.

The family of banana is Musaceae genus Musa has more than 50 species, with some of these species having numerous subspecies. This diversity has lead to the genus being divided into two sections from the five traditional section: Musa (2n=22, incorporating Rhodochlamys), Callimusa (2n=20, incorporating Australimusa) and single species section Ingentimusa (2n=28). Among these sections, there are wild, seeded plants and edible clones, with overlapping geographical distributions. Ensete, the other genus in the family, ranges from Asia to Africa, while Musa ranges from Africa though Asia to the Pacific .

The flowers are bright yellow on opening and rather conspicuous. The first flowers of the basal bract to open are functionally female and they must be pollinated and fertilized before any fruits will develop, usually about 4 "hands" of 4 - 6 flowers each. The female flowers are 2.8 – 3.1 in (7 - 8 cm) long. Self fertilization is prevented by the male flowers, darker in color than the rest, developing on the inflorescence after the female flowers are no longer receptive. The male bud, in advanced blooming, is ovate, and the bracts slightly imbricate at the tip. The bracts are slightly glaucous on the outside and rather strongly sulcate, without wax on the inside and transversely corrugated between the ridges. The male flowers are grouped 6 - 10 per bract in two rows, and the compound tepal is about 4 cm. long, 1.8 cm. wide, orange-yellow, with its tip and lobes slightly darker. The fruit bunch is very compact because the fruits are almost appressed to the rachis. Individual fruits are about 8 - 10 cm. long, 2 cm. in diameter (fresh), on a very short pedicel and with a short but pronounced acumen, and they remain strongly angled, even at full ripeness. The fruits

become yellowish when ripe but are not edible, the flesh being insipid and full of small black seeds.

Many pest and diseases especially banana mosaic virus constrains banana production which resulted in serious consequences for environment through the application of pesticides. Thus, major constraints in the banana production system are the non-availability of disease-free, true-to-type planting material, slow propagation and long time span from one generation to the next generation. Classical breeding is difficult Because of its high degree of sterility and polyploidy of the edible varieties (Stover and Simmonds, 1987). Bananas belong to group of crops which are normally propagated through vegetative parts of the plant because almost all cultivated banana cultivars are triploid, seedless, or seed sterile.

The materials used for conventional propagation include corms, large and small suckers, and sword suckers (Cronauer and Krikorian, 1984; Arias, 1992; Haq and Dahot, 2007). Mass propagation of selected genotypes, somaclonal variation techniques, genetic engineering and other biotechnological applications can be utilized for banana crop improvement which is based on reliable plant regeneration protocols. Tissue cultures were used in the distribution of germplasm, conservation, safe exchange of internal planting material and rapid propagation of newly selected hybrid cultivars. Several researchers have reported the regeneration of *Musa* spp. via micro propagation (Cronauer and Krikorian, 1986; Jarret, 1986; Diniz *et al*., 1999;; Krishnamoorthy *et al.,* 2001; Kagera *et al*., 2004 and Muhammad *et al*, 2004). But, propagation percentage and repeatability of the method are matters of concern which ultimately need a comprehensive, repeatable and applied method for a wide range of genotypes to facilitate disease free production of banana crop on commercial scale. For *in vitro* micropropagation of banana, bacterial contamination is a big problem. Although initially surface sterilization works, latter on microbial contamination at the base of the explant appears within 7 to 15 days after inoculation. Huge number of explants is destroyed in the culture due to endogenous bacteria (Habiba *et al*., 2002).

Banana Tissue Culture

Banana (*Musa spp.*) are large herbaceous plants with their center of diversity in Southeast Asia. The main method of propagation is by means of daughter suckers formed at the base of the pseudostem. Traditionally, sword suckers with narrow leaves are the preferred planting material for vegetative propagation. The major constraint for conventional propagation of banana is the lack of ready availability of large quantities of sword suckers at any given time. The problem is felt more acutely in non-availability of sword suckers

consistently. Banana plants produced from tissue culture are free from diseases at the time of supply and they give high yields since they are made from selected high yielding mother plants. If proper care is taken, as per instructions, they grow into strong healthy plants and give high yields of good quality fruits. Since they are produced under controlled laboratory conditions using selected nutrients, they usually give yields one or two month earlier than conventionally propagated plants. Shoot-tip cultures of *Musa* cultivars (both banana and plantain) are induced by culturing small excised shoot apices on modified MS semisolid medium supplemented with various concentrations and combinations of auxins and cytokinins. (Israeli *et al.*, 1995). But in This research we studied about effects of cytokinins concentrations in the medium as well as the genotypic configuration of the cultivars on the rate of shoot-bud proliferation have been tested. The established shoot-tip cultures grown on modified MS semisolid medium supplemented with IAA (0.18 mg/l) and BA (2.30 mg/l) have been successfully stored at 15 ℃ with 1000 lux light intensity up to 13–17 months depending on the cultivar. The cultivars tested in the present investigation seem to vary in their ability to withstand minimal growth temperature.

In micropropagated bananas somaclonal variation detectable at the level of the phenotype has been reported to vary between 1 and 50% (Israeli *et al.*, 1995). This variation is one of the major drawbacks that have limited the expansion of the tissue culture technology. Various factors such as genotype, origin of shoots *in vitro* (adventitious or axillary buds), number of subcultures, the choice of explant and the degree of dedifferentiation of the tissues in culture have all been shown to influence both the quantity and the type of somaclonal variation in micropropagated bananas (Swamy and Sahijram,1989). Somaclonal variants have been observed mainly in plant stature, inflorescence morphology and leaf abnormalities. For commercial propagation of Cavendish bananas the vegetative apex of the sucker is most frequently used as an explant, however it is possible to obtain reversion of floral to vegetative apices *in vitro* (Cronauer,1986)**.** Perceived advantages for the use of the inflorescence as an explant include : decontamination is easier as there is no contact with soil; in the case of suspected chimeric clones it is possible to retain fidelity of the fruit bunch characteristics by propagation from the male floral apex; a lower incidence of virus infection in regenerants.

Materials and methods

Plant material

Suckers of selected plants from *Musa = laterita* Roxb. were collected and the meristem was isolated from *in situ* plants. They were washed in running tap water for 20 min. The shoot tips were surface sterilized successively in rectified spirit and in 15% sodium hypochlorite solution for 15 minutes and were followed by repeated washing with sterile distilled water. Finally, shoot meristems of 2-3 mm size along with primordia was exised aseptically in laminar flow and immediately paced onto Murashige and Skoog (1962) medium supplemented with different concentrations (0, 1, 3, 5 and 7 ppm) of 6- benzyl adenine (BA) for multiplications.

Preparation of culture medium

The explants were inoculated aseptically in MS medium (Murashige and Skoog, 1962) containing 30- g/l sucrose for culture establishment. The MS medium was variously supplemented with benzyl adenine (BA) in various combinations as shown in Table 1. After 1 month, the established shoots were sub cultured to the same media.Explant were culture in modified MS medium supplemented with BA at 5 levels of concentration as 0, 1, 3, 5 and 7 ppm. Sub- culture was undertaken for 4 weeks in the same medium. The culture medium used contains 0.8% agar and the pH was adjusted to 5.8 prior to autoclaving at 121°C 15 pound/inch for 20 minutes. The cultures were incubated at 25°C under constant light of 3000 lux intensity provided by white fluorescent lamp for 16 hours photoperiod and 70% relative humidity.

Statistical analysis

Experiment was set up in Completely Randomized Design (CRD) with 5 treatments and 10 replications. Observations were recorded on the number of shoots (shoots), the height of shoot (cm), circumference of pseudostem (cm), number of leaves, root primordial. Analysis of variance was statistically computed. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01.

Results and discussions

In vitro shoot-tip culture is a suitable alternative to the traditional methods of propagation of banana (*Musa* spp). In the present study, Banana cultivar *Musa laterita* Roxb. was tested for *In vitro* multiplication. The study revealed that shoot-tip culture technique can be used for mass propagation of the local cultivars of banana. A variation in multiplication rate was seen not only among different genomic groups but also among cultivars of the same group. In general, from about 7-8 weeks after inoculation, shoot-tip proliferation could be seen. The meristem of the explant multiplied produced several meristems/shoots. Each can be considered as a bud due to its ability to develop into a plant.The results shows that the number of shoots and the height of shoots of *Musa laterita* Roxb. at 6 months of incubation varied among treatments. In terms of the number of shoots, treatments having 0, 1 and 5 BA is significantly lower than treatment with 3 and 7 ppm BA. The height of shoots at six months was found to be highest (8.16 cm) in treatment with zero or no BA. The treatment with 0 ppm BA is significantly higher than treatment with 5 ppm BA. Treatments with 0, 1, 3 and 7 ppm BA do not vary significantly but significant with 5 ppm BA (Table1,Fig 1).The number of leaves at sixth months of incubation.There was significant difference observed among treatments in terms of the number of leaves, treatments with 0, 1,3 and 7 ppm BA produced more leaves and vary significantly from treatment with 5 ppm BA. However, treatments with 0 1 3 and 7 ppm ppm BA do not vary significant. Finally for the root primordia, treatments with 0 ppm BA produced high number of root primordia and these treatments is vary significantly from each other. On the other hand treatments with 5 ppm BA produced lower root primordia but have the width of explant higher than the others treatment than the others treatment and these treatments is vary significantly from each other.

The present study suggests a rapid banana multiplication protocol from shoot meristem by using a medium with optimized concentration of cytokinins. Many reports are available on *In vitro* propagation with complicated protocols but less shoot proliferation percentage which eventually yield less number of regenerated plants per culture. Here, we reported a very simple, efficient, economical, rapidly multiplying and highly reproducible protocol for the micro-propagation of banana on commercial scale.

Table 1. Effect of different concentrations of benzyladenine (BA) on shoot proliferation from shoot tip explants of *Musa laterita* Roxb. at 6 months.

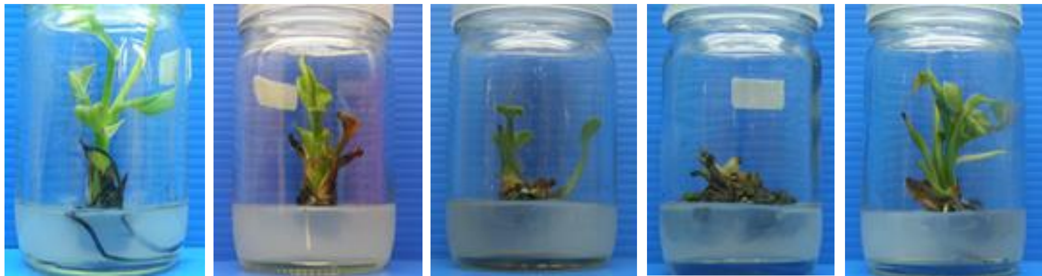
Culture medium	Number of shoots (shoots)	The height of shoot (cm)	The width of explant (cm)
MS+BA 0ppm	1.00 c1/	8.16 a1/	1.90 b1/
MS+BA 1ppm	1.00 c	6.38 a	2.00 b
MS+BA3ppm	2.80 a	6.28 a	2.40 b
MS+BA 5ppm	1.00 c	3.38 b	4.30 a
MS+BA 7ppm	2.20 b	8.06 a	2.84 b

*means with the same superscript do not vary significantly 5% level of significance using DMRT.

Table 2. Effect of different concentrations of benzyladenine (BA) on number of leaf and root primordia(leafs) of *Musa laterita* Roxb. from shoot tip explants at 6 months

Culture medium	Number of leaves (leafs)	Root primordial (roots)
MS+BA 0 ppm	5.20a1/	4.60a1/
MS+BA 1 ppm	4.60ab	2.00b
MS+BA3 ppm	4.40ab	1.80b
MS+BA 5 ppm	3.00b	0.00c
MS+BA 7 ppm	3.60ab	1.40b

*means with the same superscript do not vary significantly 5% level of significance using DMRT.



MS+0 ppm BA MS+1 ppm BA MS+3 ppm BA MS+5 ppm BA MS+7 ppm BA

Fig. 1. *In vitro* shoot tip culture of *Musa laterita*. cultured in MS medium supplemented with with 0,1,3 ,5 and 7 ppm BA

Conclusion

In conclusion the best culture media for micro propagation of *Musa laterita* Roxb. for multiple shoot regeneration directly from shoot tip, the suitable medium was MS +3 ppm BA for shoot multiplication. MS medium with 3 ppm BA has the most number of shoots (2.80 shoots).These media

composition might be applied for large scale propagation of healthy and disease free banana(*Musa laterita* Roxb). Treatment with 3 ppm BA got the most number of shoots (2.80) which is significantly higher than the other treatments.

For plant height, the treatment with 0 ppm BA got the longest with 8.16 cm. As the width of the explants addition of 5 ppm BA to the MS medium got the highest with an average of 4.30 cm. which is significantly higher than all other treatments. For the number of leaves, no significant difference was observed in treatment with 0,1,3 and 7 ppm. The root primordial in treatment with 0 ppm BA got the highest with an average of 4.60 roots which is significantly higher than all other treatments.

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