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An Alternate Protocol for in Vitro Propagation of Musa Accuminata

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Abstract: The present work elaborates an alternate protocol for rapid in vitro propagation of Musa accuminata (Grand naine) using sword suckers which were longitudinally slit into 3-5 parts each retaining a fragment of the shoot meristem and the rhizomatous base. These longitudinally slit suckers were cultured on Murashige and Skoog medium supplemented with BAP(3mg/l)+Kinetin(0.5mg/l)+IAA(0.5mg/l) to obtain multiple shoot buds within 2 weeks. These proliferating shoots were slit longitudinally as before and subcultured onto the same medium after 4 weeks of culture. The banana shoots were further transferred into half strength Murashige and Skoog medium containing IAA(1mg/l)+IBA(1.5mg/l)+ activated charcoal(0.2%) which induced rooting within 10 days. Further subculture was carried out in the same rooting medium to attain adequate elongation of the shoot and development of leaves. These shoots were hardened in soil : cocopeat: vermicompost(1:1:1) and later transplanted to soil after 3 weeks of secondary hardening.

Key words: Musa accuminata, Grand naine, In vitro propagation, suckers, BAP, Kinetin, Activated charcoal.

Introduction

Bananas are energy booster tropical fruits of high socio-economic importance most commonly available all over the globe. They are eaten as a whole fruit or by incorporating them into various culinary preparations and are much relished for their sweet, soft and easily digestible nature(Robinson, 1996;). Being high caloried they are a rich source of simple sugars, proteins, poly-phenolic antioxidants, vitamins and soluble dietary fibre(Castillo et al ,2016). Because of their high Potassium content they are highly recommended fruits for alleviating blood pressure and maintenance of cardiovascular health(Sinha RK et al, 2018, Tripathi L, 2003). In addition bananas are also indicated to be incredibly

beneficial in managing conditions like asthma, cancer,diabetes, and healthy gut(Falcomer AL et al, 2019).

The term "Banana" comprises many hybrid species in the genus Musa belonging to the family Musaceae. All of them are tropical or subtropical perennials, herbaceous psuedostems flourishing well in organic humid farms). The current in vitro research findings have been concentrated in Musa accuminata, a high yielding Cavendish banana variety which is commonly referred to as Grand naine (also called Grand Nain or G9) and is a AAA genotype. This plant is a herbaceous, medium height, high yielding tree very suitable for commercial plantations and therefore ranked fourth in terms of valuable food commodity cultivated worldwide(Rajoriya P et al,2018).

In vitro propagation technology offers many advantageous prospectives for banana cultivation over conservative agricultural practices. The plant tissue culture protocols facilitate rapid mass multiplication of Grand Nain banana plants and aid in maintaining steady supply of pathogen free in vitro raised plants(planting material) round the year .Several researchers have designed and standardized tissue culture protocols for efficient micropropagation of Grand Nain banana plants(Hansuek S et al, 2023;Maharana K et al, 2017; Rajoriya P et al,2018). Working in the same lines, the present work has been targeted to establish an alternate strategy to use suckers for rapid in vitro propagation of Grand Naine banana plants. Unlike in previous methodologies, the explant(suckers) in the current protocol have been longitudinally slit before inoculation so that each fragment retains a part of the shoot meristem and a rhizomatous base that aids in development of shoot buds. This way it has been possible to obtain banana multiple shoots by utilizing explants(suckers) in a novel and efficient manner.

Material and Methods

Selection of explant

Healthy Grand Naine sword suckers about 1.5-2 months old, which grew around the mother plant were collected from from banana plantations of Moodalabeedu, K R Nagara and Hunsur regions of Karnataka, India. The sucker explants were carefully transported to the laboratory by storing them in sterile polypropylene bags.

Explant preparation

The suckers were trimmed by removing outer layers and washed in running tap water for 30 minutes to remove the adhering soil particles. They were treated with 0.1% Mercuric chloride for 15 minutes and washed in tap water thrice, two minutes each. They were immersed in 0.1% Bavistin for 3 hours and rinsed in

water four to five times. Further treatment with an antibiotic (Cefatoxime 0.1%) for 1hour followed and were carried into the laminar air flow unit. Inside the laminar air hood they were rinsed with sterile distilled water twice for 1minute each. Treatment with 0.1% Mercuric chloride for 2 minutes and washing in sterile distilled water 4 times for 1 minute each followed.

Inoculation of the explant (sucker): The surface sterilized meristem tissue blocks(suckers) were then prepared by further removal of outer tissue of meristem with the help of sterile scalpel under aseptic condition inside the laminar airflow cabinet until the length reached to 2- 3 cm of its base. They were further subjected to a treatment with 0.1% Mercuric chloride for 10 seconds and rinsed in sterile distilled water twice. Each of these surface sterilized suckers were blot dried on sterilized blotting sheets and cut longitudinally into 4-6 slits (depending on the size of the sucker) and given a final rinse in sterile water. They were directly inoculated onto MS medium (Murashige and Skoog, 1962) supplemented with membrane filtered growth regulators viz. BAP (3mg/l), Kinetin(0.5mg/l) and IAA(0.5mg/l). The bottles were then sealed with parafilm or clingfilm and incubated.

Conditions of culture: The culture bottles were transferred to the culture room to grow in controlled environment. The temperature of the culture room was maintained with in 25 ± 2^0 C. A 16-h light period was maintained with the intensity of 2000 lux for the growth and culture of explants. Relative humidity (RH) was maintained between 40-45%.

Subculturing of culture stocks: The well developed shoots formed from the sucker were subcultured onto same medium after about 4 weeks of culture. The multiple shoots which had grown from a few suckers were subcultured after 8 weeks of culture. The shoots were excised using a sterile scalpel and separated from the main shoot. Leaf and blackish/ browned basal tissues were removed to expose the meristem. Each shoot was further inoculated into a similar fresh MS medium.

Root induction in regenerated shoots: After 6 weeks of culture when the shoots had grown about 8-11 cm in length with 2-3 well developed leaves, they were rescued aseptically from the culture vessels and transferred into the rooting medium (Half strength MS medium supplemented with membrane filtered growth regulators viz. IAA(1mg/l), IBA(1.5mg/l) and 0.2% activated charcoal). The cultured conditions were maintained as described above.

Primary hardening: The primary hardening was carried out after about a month of culture in rooting medium. The shoots with well developed tufts of roots were carefully removed from the rooting medium and washed in sterile water to

remove the media and charcoal particles. They were then placed in bottles containing sterile water for a week before transferring into pots containing cocopeat. To overcome the problem in hardening and acclimatization, the in vitro raised plantlets were kept in polythene mist chambers maintained at 80-90% humidity. The humidity was gradually reduced and plantlets were kept outside the mist house.

Secondary hardening: After 20 days, the primary hardened plants with good root ball and shoots were transferred to poly bags/pots filled with potting mixture consisting of garden soil, cocopeat and vermicompost(1:1:1).

Results

Standardization of surface sterilization protocol

A successful protocol for surface sterilization of suckers was the first prerequisite required for in vitro propagation of Musa accuminata. Therefore initial efforts were directed to standardize surface sterilization procedures using treatments with Mercuric chloride, Bavistin and Cefotaxime. The first protocol included treatment of suckers with 0.1% Mercuric chloride(15 min) and overnight treatment with 0.1% Bavistin. The suckers upon culture failed to elicit a positive response and were heavily contaminated with bacteria within a week. In the subsequent protocols the suckers were treated with 0.1% Mercuric chloride(2min) after trimming subsequent layers. Additional treatments with 0.1% Cefatoxime and reduction of exposure time to 0.1% Bavistin(18 hours to 3 hours) helped to establish the cultures without further contamination(Table 1)

Culture establishment, proliferation and in vitro rooting

The slit suckers, inoculated on the MS medium supplemented with BAP (3mg/l), IAA(0.5mg/l) and Kinetin(0.5mg/l) started showing positive response for growth after 3-4 days. The tip of the suckers turned green (photosynthetic) and looked fresh (Fig.8). The innermost layer of the sucker opened out exposing the shoot meristem (Fig.8). Shoot buds initiated from the tips after 5-8 days which was followed by formation of leaf buds after a few days was (9-12 days)(Fig.8). Stem elongation and uncurling of leaves were initiated at 10 -12 days (Fig.7). A few suckers produced multiple shoots (Fig.8.) which were subcultured on the same multiplication medium after 4 weeks of culture. When the shoots had grown 8-10 cm and had given out 3-4 leaves they were carefully removed from the medium and transferred to the rooting medium(after 40-45 daysof culture) containing half strength MS medium ingredients supplemented with IAA (0.5mg/l), IBA(1.0mg/l) and 0.2% activated charcoal. Initiation of whitish hairy like roots was observed at the submerged end of the shoots after about 10 days of transfer into rooting medium (Fig.8).The shoots showed well developed, highly branched roots after 4 weeks of culturing in rooting medium. They were then hardened and

acclimatized initially in mist chambers and later in pots containing soil:cocopeat/sand: manure (1:1:1). (Fig.8). The plantlets showed good sustenanace and development during secondary hardening indicated by formation of 2-3 new leaves and stem elongation after 3 weeks.

Discussion

Regeneration of banana plantlets through in vitro technique offers a unique scope of developing disease free planting materials against bunchy top, cucumber mosaic virus and panama wilt. This method also ensures product uniformity, season independent banana yields, rapid multiplication and advantageous agronomy (Manokari M et al, 2022). Production of plants by tissue culture provides safe movement and easy handling of germplasm between laboratories within and across countries. Since the micropropagation based progeny is genotypically and phenotypically similar to the mother plant, which is often a superior selection, the yield and returns are expectedly higher (Singh HP et al, 2011). Despite the superiority of tissue culture plants, high initial cost is one of the main reasons for their low adoption. However, the gross and net income from tissue cultured bananas is higher than those from conventional planting material by 35.4 % and 42.4 %, respectively which indicate the economic advantage of tissue cultured bananas. The production cost of tissue culture plants can also be reduced through the adoption of improved technologies like drip irrigation, high density planting and integrated nutrient and pest management strategies (Alagumani, 2005).

Keeping in view of all the facts discussed above, an initiative was taken up to establish a protocol for in vitro propagation of Musa accuminata. Though many protocols have been already standardized, a dedicated effort was made to repeat the protocol with minor modifications to suit our culture conditions and convenience. As a result it has been possible to successfully regenerate Musa accuminata plantlets in a short duration of time.

References

1. Castillo C, Fuller DQ (2016) Bananas: the spread of a tropical fruit as an agricultural staple. In: Lee-Thorp J, Katzenberg MA (eds) The Oxford handbook of the archaelogy of diet. Oxford University Press, Oxford.
2. Robinson JC (1996) Bananas and plantains. CAB International ,Wallingford, p 238
3. Rabindra Kumar Sinha, Puja Rani Saha, Anath Bandhu Das, Satya Narayan Jena, Sangram Sinha;In Vitro Clonal Propagation of Musa Sp. Cultivar Gopi: A Palatable Banana of Tripura, India ; 2018. American Journal of Plant Biology; 3(1): 12-6, www.sciencepublishinggroup.com

4. L. Tripathi ;Genetic engineering for improvement of Musa protection in Africa. 2003. African Journal of Biotechnology, 2(12), pp. 503- 508.
5. Falcomer AL, Riquette RFR, de Lima BR, Ginani VC, Zandonadi RP. Health Benefits of Green Banana Consumption: A Systematic Review. Nutrients. 2019. 11(6):1222.
6. Preeti Rajoriya, Vivek Kumar Singh, Nancy Jaiswal and Rohit Lall; Optimizing the effect of plant growth regulators on in vitro micro propagation of Indian red banana (Musa acuminata)2018. Journal of Pharmacognosy and Phytochemistry SP1: 628-634
7. Hansuek, S , Liamnimitr, N. and Khawniam, T; In vitro propagation of Musa acuminata (AAA group) cv. 'Kluai Nak';2023; International Journal of Agricultural Technology 2023 19(4):1541-1552 ; www.ijat-aatsea.com ISSN 2630-0192.
8. Mani Manokari, Mahesh Kumar Badhepuri, Mathiyazhagan Cokulraj, Bharat S. Rajput, Abhijit Dey, Mohammad Faisal, Abdulrahman A. Alatar, Anshu Alok, Mahipal S. Shekhawat,; 2022; High-throughput in vitro propagation and evaluation of foliar micro-morpho-anatomical stability in Musa acuminata cv. 'Grand Nain' using 6-benzoyladenine (BOA) in the nutrient medium, Scientia Horticulturae, 304,111334.
9. H.P. Singh, S. Uma, R. Selvarajan and J.L. Karihaloo. 2011. Micropropagation for Production of Quality Banana Planting Material in AsiaPacific. Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), New Delhi, India. P.:92.
10. Alagumani, T., 2005. Economic Analysis of Tissue-cultured Banana and Sucker-propagated Banana; Agricultural Economics Research Review, Agricultural Economics Research Association (India); 18(1)-81-89.

Table 1: Standardization of surface sterilization protocols for suckers of Musa accuminata

Sl no	Sterilization protocol	Contamination (%)	Suckers showing positive response(%)
1	0.1% HgCl2-15 min 0.1% Bavistin-18 h	90	-
2	0.1% HgCl2- 2 min after removal of each sucker layer 0.1% Bavistin- 18 h	80	-
3	0.1% HgCl2- 15 min 0.1% Bavistin-12 h 0.1% Cefatoxime-1 h	80	-
4	0.1% HgCl2-15 min 0.1% Bavistin- 3 h 0.1% Cefatoxime-1 h 0.1% HgCl2-2 min	0	70%, The suckers turned green after a week and started proliferation.

Table 2: Effect of various concentrations of growth regulators(mg/l) on shoot proliferation of Musa accuminata.

Sl No.	Growth regulators (mg/l)	Number of suckers showing positive response(%)	Time taken(days)	Length of shoot(cm) (Mean ±SD)	Number of leaves per shoot(cm) Mean ±SD
1	BAP(1 mg/l)+Kn(1mg/l)+IAA(0.5mg/l)	No response	-	-	-
2	BAP(1.5mg/l)+Kn(1mg/l)+IAA(0.5mg/l)	14	07	2.5±0.37	1.16±0.4
3	BAP(2mg/l)+Kn(0.5mg/l)+IAA(0.5mg/l)	48	07	3.0±0.42	1.6±0.80
4	BAP(3mg/l)+Kn(0.5mg/l)+IAA(0.5mg/l)	92	04	4.2±0.28	3.6±0.33

Table 3: Effect of activated charcoal and various concentrations of growth regulators on rooting of M.accuminata shoots

Sl. No	Growth regulators (mg/l)	Time taken (days)	Number of roots/shoot (Mean ±SD)	Length of roots(cm) (Mean ±SD)	Number of leaves/shoot (Mean ±SD)	Length of shoot(cm) (Mean ±SD)
1	IAA(0.5mg/l)+IBA(0.5 mg/l)+Activated charcoal(0.1%)	18	2.5 ±0.50	3.0±0.63	1.30±0.8	3.1±0.32
2	IAA(1mg/l)+IBA(1mg /l)+ Activated charcoal(0.1%)	17	3.6±0.81	6.0±1.4	2.1±0.3	7.6±0.24
3	IAA(1mg/l)+IBA (1.5mg/l)+ Activated charcoal(0.2%)	10	6.0±1.40	10.0±2.3	3.8±0.6	11.2±2.1

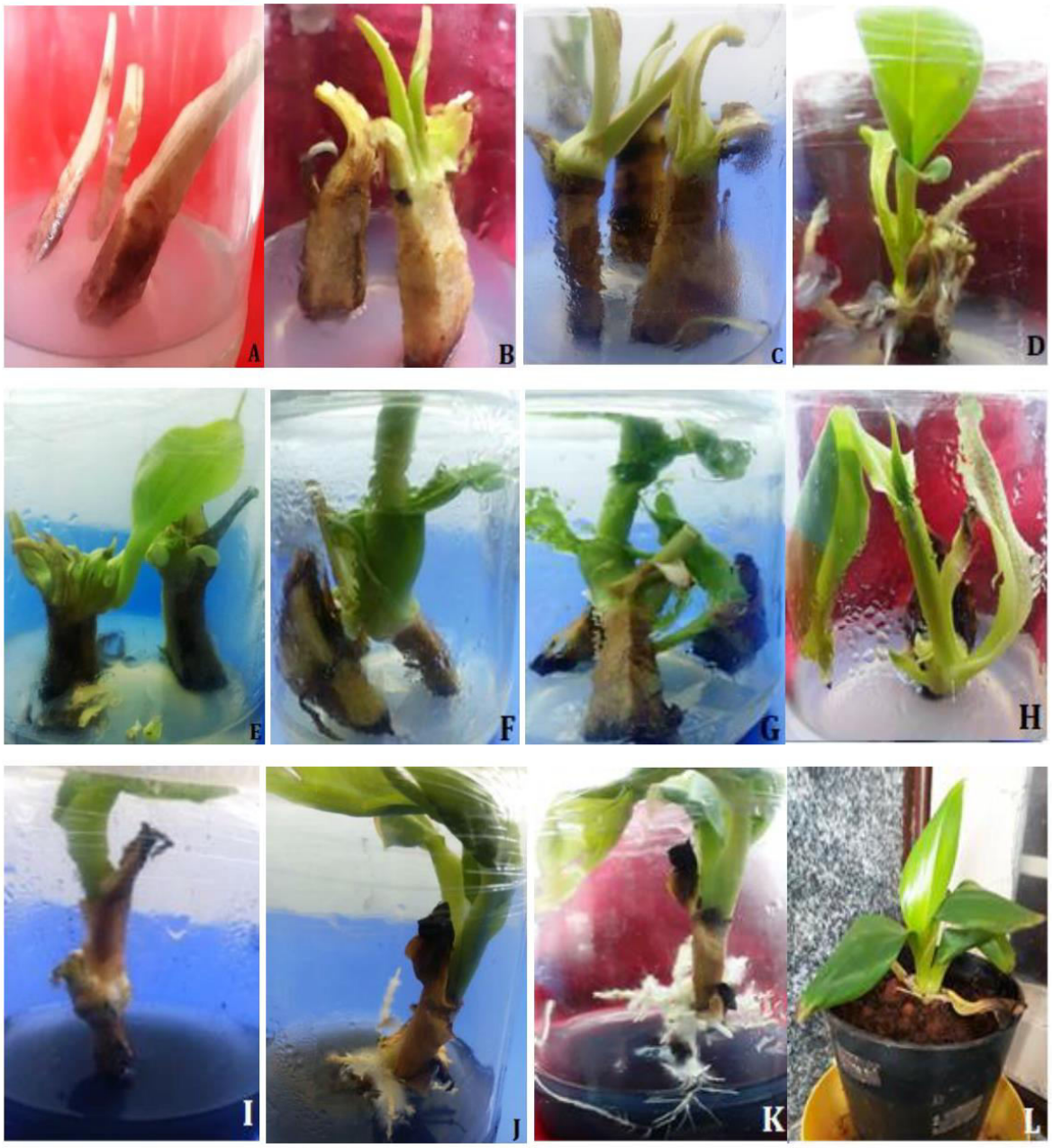

Figure 8: Growth stages of *Musa accuminata* under *in vitro* conditions

A:Longitudunally slit suckers inoculated in MS medium; B:Emergence of apical meristem; C: Initiation of shoot buds; D:Uncurling of leaf; E:Developmemt of multiple shoots; F: Formation of new leaves; G:Stem elongation; H: Shoots after first subculture; I:Shoots tranferred into rooting medium; J:Initiation of roots; K: Shoots with well developed roots; L: Hardened and acclamatized plantlet of *Musa accuminata*