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VIRUS INDEXING, PCR DETECTION AND GENE FIDELITY IN TISSUE CULTURE RAISED BANANA PLANTS (*Musa spp.)*

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ABSTRACT:

In vitro banana propagation, especially meristem tip culture, has played a key role for obtaining a large number of virus free and homogenous planting material in Musa spp. The experiment was carried out in Botany section, Bajaj College of Agriculture, Wardha, Maharashtra India. The plantlet was regenerated using different concentration of BAP with MS medium. Maximum shoot multiplication observed at MS Medium + 3 mg l^{-1} BAP + 200 mg l^{-1} cefotaxime + 50 mg l^{-1} Bavistin. In vitro banana propagation, especially meristem tip culture, has played a key role for obtaining a large number of virus free and homogeneous planting material in Musa spp. In our previous study, we have standardized complete protocol for the banana meristem tip culture. The experiment was carried out in Botany Section, Bajaj College of Agriculture, Wardha (Affiliated to Dr. PDKV Akola, India). The plantlet was regenerated using different concentration of BAP with MS medium. Maximum shoot multiplication observed at 3 mg l^{-1} BAP + MS medium + 200 mg $^{-1}$ cefotaxime + 50 mg l^{-1} Bavistin. Tissue culture raised plantlet, its mother culture and mother plant tested against the four different viruses mostly found in banana plant. The virus indexing of banana leaf sample of Grand naine variety were carried out for four viruses i.e. cucumber mosaic virus (CMV), Banana bract mosaic virus (BBrMV) by DAS-ELISA and banana steak Mysore virus (BSMYV), bunchy top virus (BBTV) by Duplex PCR. None of the virus were detected in any of the sample. Additionally, genetic fidelity analysis was also carried out with ISSR primers and no polymorphism was detected..

Keywords: Micropropagation, Meristem tip culture, Virus indexing, Genetic fidelity and Hardening

INTRODUCTION

Banana is globally important food plant with 97.5 million tonnes of production in India it supports livelihood of millions of people with total annual production 62.97 million tonnes from 497.70 thousand hector with national average 33.5 tonnes per hectare. The Maharashtra ranks first in production with 60 tonnes per hectare, banana contribute 37% total food production. Banana occupied 20% area among the total area under the crop in India (National horticulture board database). The Jalgaon is major banana grower district of Maharashtra which occupied 50000-hectare area under banana but now most of the banana is grown by planting tissue culture raised banana plant. Grand naine is gaining particularly and most preferred variety due to tolerance to biotic stress and good quality bananas for banana micropropagation. MS based media widely adopted. The present study was done for production of diseased free and virus free banana plantlet at plant biotechnology

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laboratory, Bajaj college of Agriculture, Wardha, India. The present study was undertaken during 2022 to 2024 with the following objectives:

1. To study the effects of BAP and kinetin on in vitro regeneration of Grand nain-9 variety of banana
2. To study the effects of IAA and IBA on in vitro root regeneration of Grand nain-9 variety of banana
3. To develop an efficient protocol for in vitro rapid propagation of banana varieties
4. To test the tissue culture raised banana plants for most commonly found virus in banana plants

MATERIAL AND METHODS

Banana suckers aged 2–3 months were selected and collected from the experimental plot and farmers' fields in the Seloo region of Wardha district, Maharashtra. This region is well known for the cultivation of tissue-culture bananas. Explant isolation was carried out in the Plant Biotechnology Laboratory, Botany Section, BCA, Wardha. A single-layered meristematic dome was excised from each banana sucker and used as the explant for in vitro multiplication. Prior to inoculation onto Murashige and Skoog (MS) medium prepared in the laboratory, the explants were surface sterilized using ethanol, mercuric chloride ($HgCl_2$), and Taxim to eliminate microbial contamination. The experiment was conducted during two consecutive periods: September 2021 to April 2022 and September 2022 to April 2023. Regenerated plantlets were subsequently sent for virus indexing to the Vasantdada Sugar Institute, Pune, to assess their health status and ensure the production of virus-free planting material.

Table.1. The details of media used for meristem tip culture in banana for shoot regeneration

Sr. no	Treatments	Treatment details
1	MS1	MS+1 mg l^{-1} BAP+50 mg l^{-1} Bavistin+50 mg l^{-1}cefotaxime
2	MS2	MS+ 2 mg l^{-1} BAP +60 mg l^{-1} Bavistin+100 mg l^{-1} cefotaxime
3	MS3	MS+ 3 mg l^{-1} BAP + 50 mg l^{-1} Bavistin+ 200 mg l^{-1} cefotaxime
4	MS4	MS+4 mg l^{-1} BAP+ 50 mg l^{-1} Bavistin+200 mg l^{-1} cefotaxime
5	MS5	MS+5 mg l^{-1} BAP+50 mg l^{-1} Bavistin+200 mgl^{-1} cefotaxime
6	MS6	MS+1 mg l^{-1} kinetin+50 mg l^{-1} Bavistin+ 200 mg l^{-1} cefotaxime
7	MS7	MS+1 mg l^{-1} kinetin+50 mg l^{-1} Bavistin+ 200 mg l^{-1} cefotaxime

Table. 2. The details media used for meristem tip culture in banana for root regeneration

Sr. no	Treatments	Treatments details
1	R_1	MS+ 0.5 mg l^{-1} IAA+50 mg l^{-1} Bavistin+200 mg cefotaxime+2mg l^{-1} activated charcoal
2	R_2	MS+ 1.5 mg l^{-1} IAA+50 mg l^{-1} Bavistin+200 mg cefotaxime+2mg l^{-1} activated charcoal
3	R_3	MS+ 2.5 mg l^{-1} IAA+50 mg l^{-1} Bavistin+200 mg cefotaxime+2mg l^{-1} activated charcoal
4	R_4	MS+ 3.5 mg l^{-1} IAA+50 mg l^{-1} Bavistin+200 mg cefotaxime+2mg l^{-1} activated charcoal
5	R_5	MS+ 0.5 mg l^{-1} IBA+50 mg l^{-1} Bavistin+200 mg cefotaxime+2mg l^{-1} activated charcoal
6	R_6	MS+1 mg l^{-1} IBA+50 mg l^{-1} Bavistin+200 mg l^{-1} cefotaxime+2 mg l^{-1} activated charcoal
7	R_7	MS+ 1.5 mg l^{-1} IBA+ 50 mg l^{-1} Bavistin+200 mg l^{-1} cefotaxime+2 mg l^{-1} activated charcoal

The present study on gene fidelity studies of tissue culture raised Plant banana cultivar G-9 was conducted at GE & MB laboratory of Vasantdada Sugar Institute, Pune during 2023-2024, mother plant of banana cultivar grown in experimental field trial of Bajaj college of Agriculture, Wardha, Maharashtra. The in vitro regenerated plant let where used for extraction of DNA & further Characterization. Sample were tested for CMV and BBr MV virus by ELISA & Bsmys & BBTV by PCR.

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A. Establishment of explant in MS medium

B. **Effect of different levels of BAP on shoot multiplication**

C. Multiplication of buds

D. Rooting in shoot let

Plate. 1. In vitro regeneration of banana meristem at different concentration of BAP

RESULTS

Data observed significant in treatments and replication for multiplication of bud, shoot length and root length. The shoot length noticed very high in MS_2 and MS_3 and lowest in MS_7 and significantly different in MS_2 and MS_3 than other treatments. Shoot length observed during early days of initiation was rapid and in cycle 7 and 8 shoot length, root length and multiplication of buds noticed minimum. Multiplication of buds observed very high in MS3 and cycle2 and found lowest in MS_7. While data indicated for root length noticed maximum in R_3 and lowest minimum in R_7, whereas R_3, R_2, R_4 significantly differed among all treatments Khatan *et al* (2017) found that 2, 4 and 6 weeks after inoculation (WAI), the highest number of shoots (2, 3 and 3.40 shoots per plant) was observed in medium supplemented with 5.0 mg/l BAP. Ferdous *et al* (2015) observed maximum root length found from 3 mg l^{-1} IBA in Amritsagar (3.60 cm) and 3.10 cm in Sabri at 30 DAI.

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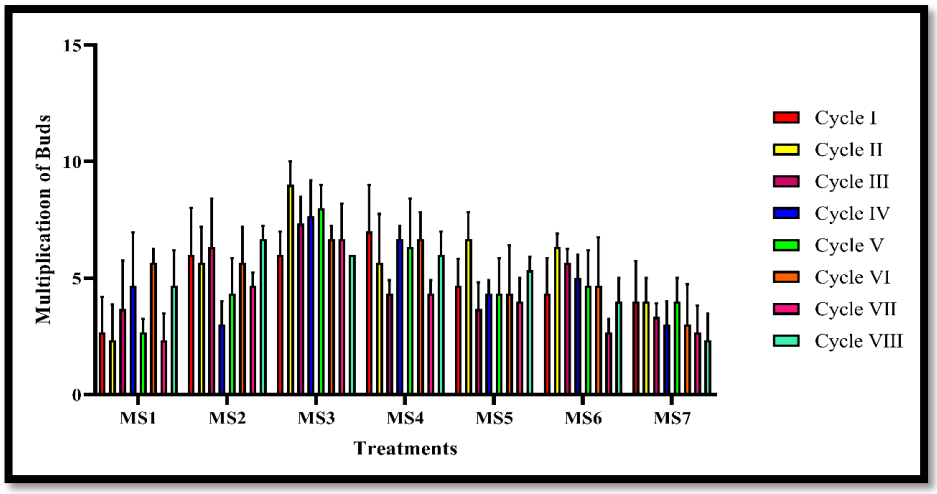

Fig.1. Multiplication of banana buds in response to different concentrations of BAP (6-benzylaminopurine) during eight successive subculture cycles.

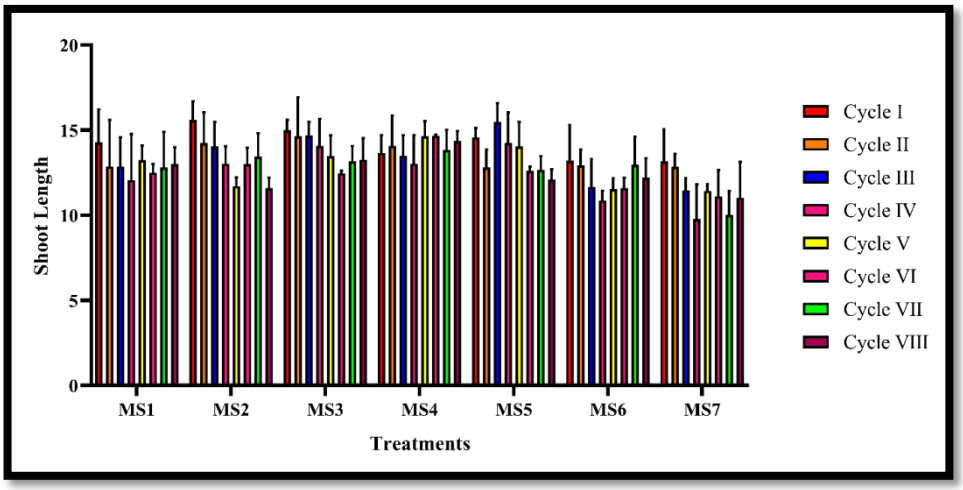

Fig.2. Shoot length of banana buds in response to different concentrations of BAP (6-benzylaminopurine) during eight successive subculture cycles.

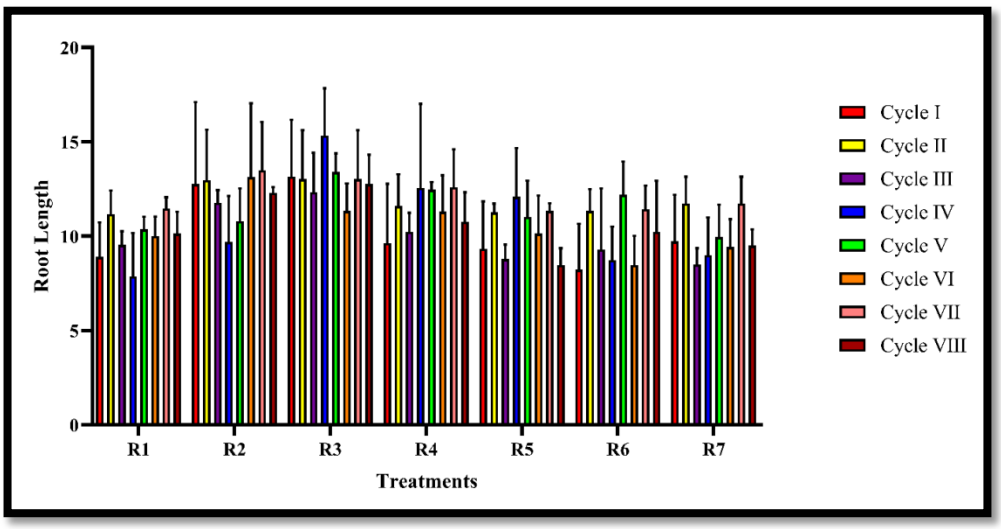

Fig.3. Shoot length of banana buds in response to different concentrations of BAP (6-benzylaminopurine) during eight successive subculture cycles.

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A wide range of molecular diagnostic technique are available for many plant viruses. It is important to test the tissue culture raised banana plant for virus indexing. The virus testing worked was done by Vasant dada sugar Institute Pune, in GE and MB lab for which virus specific primer were designed, tested & validated for pcr based detection.

Virus indexing of different banana leaf samples of grand nine variety value carried out for four different viruses, i. e., cucumber mosaic virus (CMV), banana bract mosaic virus (BBMV) by DAS-ELISA & banana streak Mysore virus (BSMYV) and banana bunchy top virus (BBTV) by duplex PCR. None of the virus were detected in any of the sample. Additionally, genetic fidelity analysis was also carried out with ISSR primers & no polymorphism was detected. Molecular cloning and characterization of coat protein gene of banana (AAB) showed BBrMV infected samples by DAC-ELISA (Darshan *et al.*, 2019)

Table. 3. CMV and BBrMV testing by ELISA from different tissue culture raised banana plants for 8 cycles of invitro regeneration

Treatments	Virus tested		Genetic fidelity testing protocol with ISSR primers
	Testing protocol		
	PCR		
	Name of the virus		
	BSMYS	BBTV	
Mother plants	Negative	Negative	True to type
Mother culture	Negative	Negative	True to type
C1B3	Negative	Negative	True to type
C2B3	Negative	Negative	True to type
C3B3	Negative	Negative	True to type
C4B3	Negative	Negative	True to type
C5B3	Negative	Negative	True to type
C6B3	Negative	Negative	True to type
C7B3	Negative	Negative	True to type
C8B3	Negative	Negative	True to type

Table. 4. BSMYS, BBTV viruses tested by PCR and gene fidelity from different tissue culture raised banana plant for 8 cycles of in vitro regeneration

Treatment	Virus tested	
	Testing protocol	
	ELISA	
	Name of the virus	
	CMV	BBrMV
Mother plant	Negative	Negative
Mother culture(02)	Negative	Negative
C1B3	Negative	Negative
C2B3	Negative	Negative
C3B3	Negative	Negative
C4B3	Negative	Negative
C5B3	Negative	Negative
C6B3	Negative	Negative
C7B3	Negative	Negative
C8B3	Negative	Negative

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HARDENING OF PLANTLETS

The initial selection of healthy plantlets is a crucial step for successful hardening and further growth. After selection, the plantlets are transferred to pots containing a potting mixture of cocopeat and soil. These pots are kept inside the laboratory for approximately 8 days to allow the plantlets to acclimatize. After this period, the plantlets are moved to an open area with adequate ventilation and indirect sunlight. During the primary hardening stage, the plantlets should be maintained under cool conditions with high humidity. A relative humidity of 70–80% is recommended, which can be maintained using a humidifier. After 15 days of primary hardening, the one-month-old hardened plants are gradually exposed to direct sunlight as part of the secondary hardening process. During this stage, 2.5 g of urea is applied to each plant at 15-day intervals to promote healthy growth. Once the plantlets reach approximately three months of age and are well established, they are ready for transplantation into the field.

A. Selected plantlet for Hardening

B. Transfer of plantlet in pot contained mixture of cockpit, soil and vermicompost

C. Hardening of plant without greenhouse at Botany section, BCA, Wardha

D. 8-month-old tissue cultured plant at BCA Farm

Plate.2. Hardening of tissue culture banana plantlet without greenhouse

Recently genetic transformation, genome editing, genomics, nano biotechnology, molecular marker technology used for banana crop improvement revealed by Chukwu and his coworker (2025).

CONCLUSION:

In present study we found that MS+ 3 mg l^{-1} BAP + 50 mg l^{-1} Bavistin+ 200 mg l^{-1} cefotaxime and MS+1 mg l^{-1} IBA +50 mg l^{-1} Bavistin+200 mg l^{-1} cefotaxime

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+2 mg l^{-1} activated charcoal performed best in vitro shoot and root regeneration followed by other treatments. In future more varieties have been standardized for in vitro regeneration. There was no virus detected in any of the samples tested for CMV, BBMV, BSMYV and BBTV by PCR and ELISA. The study of gene fidelity also done showed true to type for all tested samples. This plant hardened without greenhouse facility and hardening protocol standardized completely in open area where direct sunlight reach. This work can support to many tissue culture-based industries those have not greenhouse facility for hardening of tissue culture plants.

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