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Effect of Growth Regulators, Sterilization Treatments, and Potting Mixtures on *in vitro* Micropropagation of Red Banana (*Musa acuminata*)

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ABSTRACT: Bananas are popular, nutritious food, and rich in carbohydrates, vitamin C, potassium, and iron. Which are affected by several devastating diseases and cause a 30–50% loss in production. Micropropagation of shoot tips *in vitro* has become a significant application of biotechnology in agriculture and offers rapid multiplication, disease-free, and year-round availability of banana plants. The present investigation aimed to develop a simple and efficient protocol, which focused on the effect of plant growth regulators, sterilization treatments, and potting mixtures on the micropropagation of red banana. The meristem tip was used as an explant. Treatment T9 demonstrated the highest (67%) aseptic culture percentage focusing on bacterial control. The shoot induction with MS + 5 mg/L BAP showed 89.533% of explants showed growth in 39 days. The highest rooting (94%) was obtained with MS + 2 mg IBA + Activated charcoal and the average number of roots per explant (13.7).The highest survival (84.33%) was found in a potting mixture with red soil, Cocopeat, and Vermicompost (1:1:1).The above findings showed that an efficient and feasible technique has been established for the effective regeneration of new red banana plantlets. The importance of using specific pre-treatment methods, media combinations, and potting mixtures improved the success of plant regeneration and growth in terms of aseptic culture, shoot induction and multiplication, shoot length, rooting, and survival rate.

Keywords: Red banana, Musa acuminata, Micropropagation, Plant growth regulators, sterilization treatments.

INTRODUCTION

The ‘Banana’ belongs to the genus Musa of the family Musaceae. It is the most important and most widely grown fruit crop in India (Al-Amin *et al*., 2009; Lohidas and Sujin 2015). Itis the fourth most important food commodity globally. It has socio-economic importance in India (Aquil *et al*., 2012) and can be found in orchards across the country. It is an evergreen plant that can grow up to 30 feet tall and 15 feet wide. It is a nutritious food, rich in carbohydrates, vitamin C, potassium, and iron.

The red banana, known as "Red Dacca," a triploid cultivar, belonging to the AAA group, is a variant derived from the wild banana species Musa acuminata. It is a rich source of phenolic compounds, tannins, anthocyanins, minerals, and other natural antioxidants that contribute to its color, pleasant fragrance, and health benefits. Which are cultivated in South America and East Africa (Ghodake *et al*., 2023). It is a favorite in the Central East due to its taste, as they are sweeter than bananas and have a raspberry flavor (Ayur Times, 2014). They are smaller, plumper, softer, and have a unique flavor. This fruit has a soft and creamy texture with distinctive red to purple skin; the red color comes from beta-carotene, and the aroma reminds of sweet strawberries (Healthy Food House, 2013).

Bananas affected by several devastating diseases like a banana bunchy top virus, fusarium wilt, and black sigatoka cause significant losses in production (30–50%). Despite the sterility and polyploidy of edible banana types, breeding Musa continues to be a challenging attempt (Vuylsteke *et al*., 1993). Disease-free plantlet production is required for commercializing the cultivation of red bananas for which micropropagation is the effective way of multiplying large amounts of genetically identical plantlets. Micropropagation became an alternative technique for rapid multiplication, disease-free, and year-round availability of banana planting material (Ray *et al*, 2006, Kiran *et al*., 2015). The success of the *In vitro* regeneration technique involves the maintenance of aseptic conditions for microbial contamination-free explants. Thus, there is a need to establish a

micropropagation protocol for the banana cultivar not only for propagation, multiplication, and preservation but also for the enhancement of secondary metabolites (Anonymous, 2002).Several investigators have reported the in vitro propagation of bananas utilizing various explant sources and practices: embryogenic suspension cultures (Jalil *et al*., 2003); shoot meristem (Strosse *et al*., 2006); embryogenic cell (Wong *et al*., 2006); shoot and leaf (Venkatachalam *et al*., 2006, 2007); male inflorescence tips (Resmi and Nair 2007); shoot tips (Shirani *et al*., 2009).

The current study was undertaken to minimize the above-mentioned problems with the major objective to develop a protocol for *In vitro* micropropagation followed by the effect of plant growth regulators (PGRs) and standardizing the surface sterilization protocol. The success of *In vitro* multiplication is based on the differentiation of plant tissues, by the addition of required growth hormones in appropriate quantities (Gaspar *et al*., 2003). Henceforth this study was conducted to find out the best combination of growth regulators and to optimize its concentration for a better regeneration rate of red banana.

MATERIALS AND METHODS

A. Collection of explant and chemicals

Healthy three-month-old sword suckers of the red banana used as an explant were collected from the field-grown plants at "Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli". Shoot tips were prepared by trimming roots and outer sheaths from the suckers and such meristem after sterilization used for the experiment.

B. Sterilization of explant

The suckers were cleaned and cut into small (5 to 10 cm) pieces. Then treated with surfactants for 10 min., i.e.,0.5% Tween-20 and 1% Savlon, to remove any contaminants. Fungicides, like Bavistin or carbendazim (0.50% for 30 min), were also applied to eliminate any fungal growth. The suckers were washed with sterile distilled water (4 to 5 times) to ensure no chemical residues remained. After sterilization, they were placed in a sterilized chamber with UV lights to create a pathogen-free environment. The explants were further treated with 5% sodium hypochlorite, 0.1% mercuric chloride, and 70% ethanol for disinfection. Finally, the explants were thoroughly rinsed with sterile water, dried, and trimmed to a shorter length.

C. Choice of culture media and culture conditions

To initiate the shoot growth, the MS basal media (Murashige and Skoog 1962) was used with different combinations of BAP. Similarly, for root development in the sterile culture, the media was supplemented with different combinations of IBA. The pH was maintained at 5.8 and agar was added as a gelling agent before autoclaving at 120°C and 15 p.s.i pressure. The explants were then placed in the medium and kept in a culture room at a temperature of 25 ± 28°C with uniform light provided by fluorescent tubes. Observations such as days for shoot initiation, shoot length, root length, and other parameters were recorded regularly.

RESULTS

A. Standardization of sterilization technique for In vitro regeneration

In vitro culture requires aseptic explants, often from field-grown suckers. Surface sterilization is crucial for obtaining aseptic cultures, with effectiveness varying based on agent concentration, treatment duration, and sequence. Multiple sterilization is generally effective.

Treatment T9 demonstrated the highest aseptic culture percentage at 67%, focusing on bacterial control and T1 had the lowest contamination percentage at 13%, employing a combination of Carbendazim, Ethanol, and 5% Sodium Hypochlorite for fungi and bacteria control. The concentration and duration of exposure to Sodium Hypochlorite influenced results. Higher concentrations (10%) and longer exposure times (15 minutes) generally correlated with improved aseptic culture percentages. The treatment T7 and T8, which included Mercuric Chloride along with other agents, showed a noticeable decrease in contamination, suggesting potential effectiveness against contaminants. These results highlight the importance of optimizing sterilization protocols for treatment effectiveness.

B. Effect of dark and light conditions on explants

The dark preconditioning of explants reduces bacterial contamination and increases survival rates. If the dark period exceeds 8 days, explants turn brown, reducing regeneration and bud formation. Dark preconditioning decreases phenol content and polyphenol oxidase activity controls phenolic compound exudation and increases survival rate. Thus, dark preconditioning promotes bud formation by reducing bacterial contamination and phenolic compound synthesis (Mitsukuri *et al.,* 2009).

Mitsukuri *et al.* (2009) showed that dark preconditioning reduces browning and bacterial contamination in *Habenaria radiate.* Strosse *et al.* (2004); Koli *et al.* (2014) also kept explants in the dark for 8 days to reduce bacterial contamination and browning.

C. Effect of media on the establishment of explants

After 21 days established explants (Fig. 3B) became globular and round-shaped. Various combinations of growth regulators viz. BAP, NAA, and IAA showed different results on the establishment of shoot tips of red bananas. It has been recorded that the maximum shoots (89.53%) were initiated in treatment containing MS + 5 mg/L^{-1} BAP. The lowest initiation of shoots was observed in (23%) containing MS + 1 mgL^{-1} BAP.

Iqbal *et al.* (2013) found MS + 5 mgL^{-1} BAP+2 mgL^{-1} IAA best for establishment. Ahmed *et al.* (2014) observed 100% establishment of explants on media combination MS + 4 mgL^{-1} BAP+ 2 mgL^{-1} IAA. Kumari and Misra (2017) also found a 75% establishment of banana explants on MS + 3 mgL^{-1} BAP + 0.2 mgL^{-1} IAA with 30 mg ascorbic.

D. Effect of PGRs on Shoot Regeneration

The combination and concentration of auxin and cytokinin in culture media plays a crucial role in the successful regeneration of shoots. The suckers were sterilized and then inoculated on MS medium with

varying amounts of BAP to analyze their impact on shoot initiation. The best results (Table 3 and 4) for shoot initiation (Fig. 1) were seen with MS medium supplemented with 5 mg/l of BAP, while Ms+6 mg/l of BAP showed promising outcomes for shoot multiplication. The results revealed significant variations in shoot multiplication percentages and average shoot lengths across the treatments. The treatment with MS + 6 mg/L BAP exhibited the highest shoot multiplication percentage (3.7%) and a corresponding average shoot length of 3.633, while MS basal demonstrated the lowest shoot multiplication percentage (1.533%) with an average shoot length of 3.533. Moreover, the shoot tip responses are represented as percentages. The treatment with MS + 5 mg/L BAP demonstrated the highest response, with 89.533% of explants showing positive growth in 39 days for shoot induction. In contrast, the MS basal treatment exhibited the lowest response at 12.133%.

Overall, the findings suggest that the concentration of BAP in the growth medium significantly influences in vitro shoot multiplication and shoot tip culture responses, with higher concentrations leading to increased shoot production and positive responses in the evaluated plant explants. Rajoriya *et al.* (2018) reported the highest shooting with 3 mg/l BAP + 0.2 mg/l NAA among the investigated combinations and concentrations, and 2 mg/l IBA with 0.5 and 1 mg/l IAA as well. Moreover, Rahul *et al.* (2023) recorded after 55 days, the shoot with the maximum number (6.16) and length (5.76) cm was seen in MS media supplemented with 4.0 mg/1 BAP.

E. Effect of PGRs on rooting and hardening

The *In vitro* regenerated shoots were excised from and transferred onto the MS medium supplemented with different combinations of IBA (0.5, 1, 1.5, and 2 mg/l) for the root induction as depicted in Table 5 and Fig. 3(D). The observations were recorded based on no. of roots and rooting percentage. The results (Fig. 2) indicated a significant influence on rooting percentages and the average number of roots per explant. Among the treatments, MS basal + Activated charcoal exhibited the lowest rooting percentage (22%) and average number of roots per explant (4.567). Conversely, the treatment with MS + 2 mg IBA + Activated charcoal demonstrated the highest rooting percentage (94%) and the greatest average number of roots per explant (13.7). Overall, the findings suggest that the concentration of IBA in the growth medium positively correlates with increased in vitro rooting performance in the tested plant explants.

F. Effect of different potting mixtures on hardening of red banana plantlets

The survival of rooted plants in different potting mixtures was conducted. The mixtures included red soil, Cocopeat, and Vermicompost in varying proportions. The observations showed (Table 6, Fig. 4) that the highest survival rate of plantlets (84.33%) was achieved in a potting mixture with an equal proportion of these three components (1:1:1). Rout *et al.* (2009) washed and planted rooted micropropagules thoroughly in the earthen pot containing a mixture of sand, soil and cow dung manure in the ratio of 1:1:1 which showed 94% survivability, Meenakshi *et al.*, (2011) were transferred the rooted plantlets into a poly bag containing fine sterilized sand, sterilized soil, and farmyard manure (1:1:1) mixture, Surabhi and Pattanayak (2015) were thoroughly washed and planted rooted plantlets in an earthen pot containing a mixture of sand: soil: cow dung manure in the ratio of 1:1:1, Nandhakumar *et al.* (2017) studied acclimatization by transferring sufficiently grown plants into poly bags containing sterilized pot mixture 1:1:1 ratio of sand: red soil: FYM observed maximum survival.

DISCUSSION

Conventional propagation of red banana (*Musa acuminata*) is generally done vegetatively through suckers as it is seedless. The vegetative production of suckers is low i.e., 5-10 suckers per plant per year (Vuylsteke and De Langhe 1985) and it leads to the transmission of soil-borne diseases through rhizome and viral infection causing bunchy top, resulting in a significant loss in productivity (Qazi, 2016). Besides, this vegetative method of propagation is slow, and season bound. All these limitations can be reduced by the propagation of bananas through tissue culture technique i.e., micropropagation which is the process of rapidly multiplying stock plant material to generate progeny plants. Through this biotechnological tool, it is possible to get bacteria and other microbes free plantlets (Cronauer and Krikorian 1984; Vuylsteke and Oritz 1996). Also, the technique produces genetically uniform plants.

In the Konkan region, there is limited area under red banana plantations due to the low availability of quality red banana plantlets. The availability of plantlets is obstructed due to the unavailability of a highly efficient micropropagation technique. Contamination during micropropagation reduces the plants during tissue culture techniques. Hence, it is necessary to control the contamination by standardizing the surface sterilization technique. In tissue culture, plant growth regulators (PGRs) play a critical role in media components in figuring out the plant cells' growth route. Hence, it is necessary to optimize the concentration of growth regulators with MS media. DNA-based molecular markers have become popular for easy and precise detection and better understanding of somaclonal variation. These markers provide valuable data to assess the genetic homogeneity and true-to-type nature of micropropagated plants (Rai *et al.*, 2012).

Hence, the present investigation aimed to develop surface sterilization techniques, optimization of growth hormones, and potting mixtures on hardening for the *in vitro* regeneration of red bananas. Efforts were made to study the effective concentrations and time of exposure of different sterilization agents and also concentrations of growth hormones viz. auxins and cytokinin for the *In vitro* regeneration of red bananas.

Table 1: Details of Sterilization treatments for *In vitro* regeneration.

Treatments	Sterilization agents	Conc.	Exposure Time (min)	% aseptic culture	Occurrence of Contamination
T1	Carbendazim	0.50%	30	13 (21.11)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	5%	5		
T2	Carbendazim	0.50%	30	13.333 (21.38)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	5%	10		
T3	Carbendazim	0.50%	30	22 (27.95)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	5%	15		
T4	Carbendazim	0.50%	30	33 (35.04)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	10%	5		
T5	Carbendazim	0.50%	30	33 (35.04)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	10%	10		
T6	Carbendazim	0.50%	30	42 (40.38)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	10%	15		
T7	Carbendazim	0.50%	30	47 (43.26)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	5%	10		
	Mercuric chloride	0.1%	2		
T8	Carbendazim	0.50%	30	53 (46.70)	Fungi and bacteria
	Ethanol	70%	1		
	Sodium hypochlorite	5%	10		
	Mercuric chloride	0.1%	4		
T9	**Carbendazim**	**0.50%**	**30**	**67 (54.91)**	**Bacteria**
	Ethanol	**70%**	**1**		
	Sodium hypochlorite	**5%**	**10**		
	Mercuric chloride	**0.1%**	**6**		
			C.D.	**2.104 (1.419)**	
			S.E.	**0.703 (0.474)**	
			F-test	**SIG**	

Table 2: Composition of modified Murashige and Skoog medium.

Constituents	Conc. in mg/l^{-1}	Constituents	Conc. in mg/l^{-1}
Macronutrients		**Vitamins/ amino acids**	
Ammonium nitrate (NH_4NO_3)	1650	Glycine	2.0
Potassium nitrate (KNO_3)	1900	Nicotinic acid	0.5
Magnesium sulphate ($MgSO_4$. $7H_2O$)	370	Thiamine HCl	0.1
Calcium chloride ($CaCl_2$. $2H_2O$)	440	Pyridoxine HCl	0.5
Potassium dihydrogen phosphate (KH_2PO_4)	170	Folic acid	1
Micronutrients		Tyrosine	1.875
Manganese sulphate ($MnSO_4.4H_2O$)	22.30	**Supplements**	
Boric acid (H_3BO_3)	6.20	Agar	8200
Zinc sulphate ($ZnSO_4.7H_2O$)	8.60	Sucrose	30000
Sodium molybdate ($Na_2MoO_4.2H_2O$)	0.25	Myo-inositol	100 mg
Copper sulphate ($CuSO_4$.$5H_2O$)	0.025	Auxins	2
Cobalt chloride ($COCl_2$.$6H_2O$)	0.025	Cytokinin	6.5 and 5
Potassium iodide (KI)	0.83	**pH** (adjusted after addition of PGR)	5.8
Iron source			
Ferrous sulphate ($FeSO_4.6H_2O$)	27.80		
Disodium Salt of EDTA (Na_2EDTA)	37.25		

Table 3: Effect of different concentrations of growth regulators on shoot tip culture after 4 weeks.

Sr. No.	Media combination	No. of explants inoculated	Shoot tip % response	No. of days forshoot induction
1	MS basal	10	12.133(20.37)	55
2	MS + 1 mg/L BAP	10	23.5(28.98)	52
3	MS + 2 mg/L BAP	10	34.667(36.05)	49
4	MS + 3 mg/L BAP	10	54.033(47.29)	48
5	MS + 4 mg/L BAP	10	59.067(50.20)	42
6	**MS + 5 mg/L BAP**	**10**	**89.533(71.11)**	**39**
7	MS + 6 mg/L BAP	10	74.033(59.34)	43
		C.D.	**2.479(1.59)**	
		S.E.	**0.809(0.52)**	
		F-test	**SIG**	

Table 4: Effect of different concentrations of growth regulators on *In vitro* shoot multiplication.

Sr. No.	Media combination	Shoot multiplication%	Average shoot length
1.	MS basal	1.533(7.04)	3.533(10.8)
2.	MS + 1 mg/L BAP	1.277(6.48)	3.767(11.1)
3.	MS + 2 mg/L BAP	1.863(7.84)	4.533(12.2)
4.	MS + 3 mg/L BAP	2.51(9.11)	3.767(11.1)
5.	MS + 4 mg/L BAP	2.953(9.89)	3.733(11.1)
6.	MS + 5 mg/L BAP	3.633(10.9)	3.7(11.0)
7.	**MS + 6 mg/L BAP**	**3.7(11.0)**	**3.633(10.9)**
8.	MS + 7 mg/L BAP	3.6(10.9)	2.867(9.74)
	C.D.	**0.458(0.891)**	**0.499(0.758)**
	S.E.	**0.152(0.295)**	**0.165(0.251)**
	F-test	**SIG**	**SIG**

Table 5: Effect of different concentrations of growth regulators on *In vitro* rooting.

Sr. No.	Media combination	No. of explants	Rooting%	Average no. of roots/ explant
1.	MS basal + Activated charcoal	100	22(27.94)	4.567(12.3)
2.	MS + 0.5 mg IBA + Activated charcoal	100	56.333(48.62)	7.6(15.9)
3.	MS + 1 mg IBA + Activated charcoal	100	74(59.33)	9.433(17.8)
4.	MS + 1.5 mg IBA + Activated charcoal	100	84.333(66.67)	11.6(19.9)
5.	**MS + 2 mg IBA + Activated charcoal**	**100**	**94(75.82)**	**13.7(21.7)**
6.	MS + 0.5 mg NAA +Activated charcoal	100	44.333(41.72)	6.633(14.9)
7.	MS + 1 mg NAA +Activated charcoal	100	58(49.58)	7.567(15.9)
8.	MS + 1.5 mg NAA +Activated charcoal	100	66(54.31)	8.433(16.8)
9.	MS + 2 mg NAA +Activated charcoal	100	78(62.00)	8.8(17.2)
		C.D.	**2.641(1.818)**	**0.505(0.545)**
		S.E.	**0.882(0.607)**	**0.169(0.182)**
		F-test	**SIG**	**SIG**

Table 6: Effect of different potting mixtures on Hardening.

Sr. No.	Potting mixture	Survival %
1.	Red soil 100%	43(40.9)
2.	Cocopeat 100%	72(58.0)
3.	Vermicompost 100%	56.333(48.6)
4.	Red soil + cocopeat (1:1)	73(58.6)
5.	**Red soil + cocopeat + Vermicompost (1:1:1)**	**84.333(66.6)**
	C.D.	**2.815(1.795)**
	S.E.	**0.882(0.562)**
	F-test	**SIG**

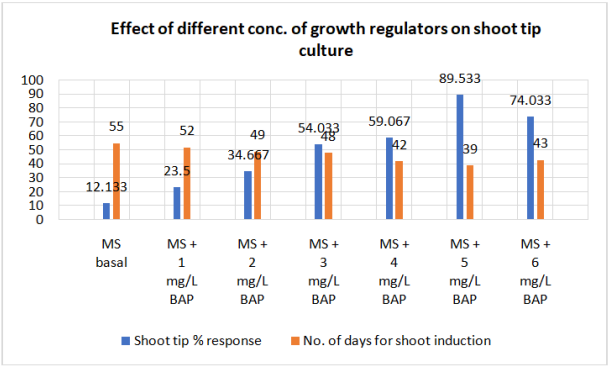

Fig. 1. Effect of different conc. of growth regulators on shoot tip culture after 4 weeks.

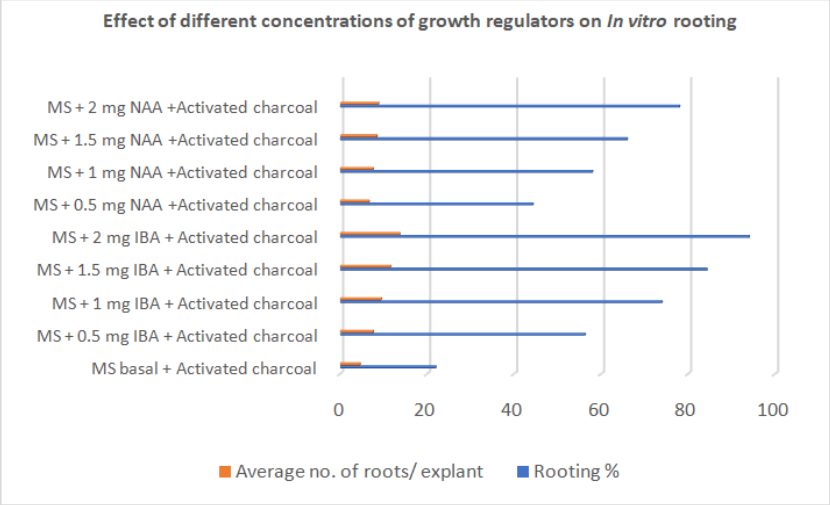

Fig. 2. Effect of different concentrations of growth regulators on *In vitro* rooting.

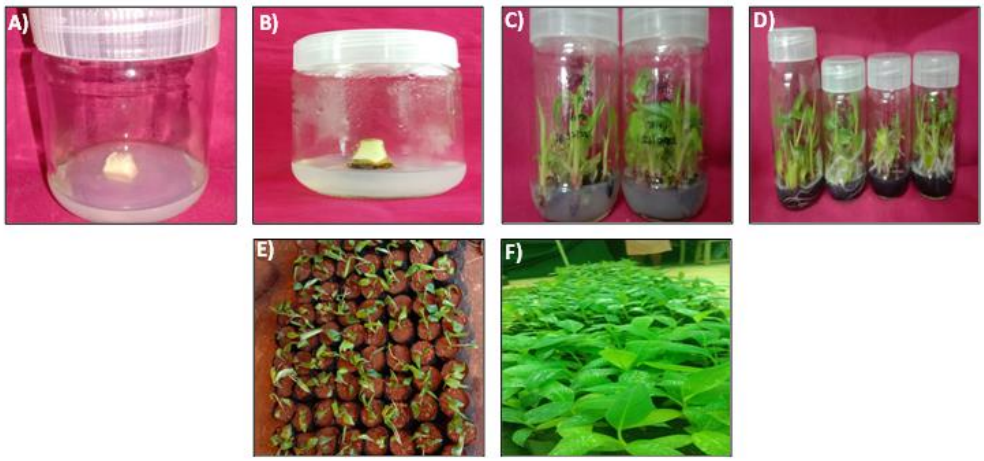

Fig. 3. Micropropagation stages of red banana: A) Inoculation of explant, B) Initiation, C) Multiplication, D) Rooting of *in vitro* shoots, E) Hardening of *in vitro* rooted plants, and F) Acclimatization in the greenhouse.

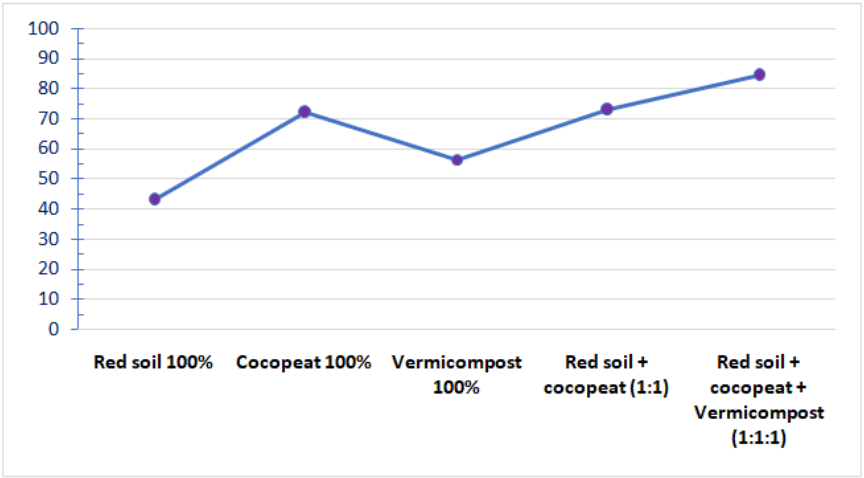

Fig. 4. Effect of different potting mixtures on hardening.

CONCLUSIONS

The pre-treatment of 10ml/L Tween 20 for 10 min, 10ml savlon/L for 10 min, 0.50% Carbendazim for 30 min, 70% Ethanol for 1 min, 5% NaOCl for 10 min, and 0.1% $HgCl_2$ for 6 min proved to be instrumental in optimizing the growth conditions for plant regeneration. The media combination of MS with 5 mg/L BAP demonstrated a remarkable multiplication rate of 89.53%, underscoring its efficacy in fostering robust plant development. Furthermore, the inclusion of MS with 2 mg IBA and activated charcoal emerged as a potent combination, yielding an impressive 94% root regeneration. The subsequent hardening of plantlets in a potting mixture comprising red soil, cocopeat, and vermicompost further contributed to their optimal

growth. These findings collectively underscore the significance of the proposed pre-treatment and media combinations in enhancing the efficiency of plant regeneration and growth, paving the way for advancements in plant tissue culture methodologies.

FUTURE SCOPE

Micropropagation offer the potential for scalability and efficiency, reducing labor requirements. Climate-responsive protocols tailored to specific environmental conditions could broaden the adaptability of micropropagation practices. Moreover, the economic viability and commercialization of micropropagated red banana plants need exploration, fostering accessibility for farmers. Embracing sustainable agriculture practices and promoting collaborative research efforts will contribute to the holistic development of optimized protocols, ensuring future success and widespread adoption of red banana micropropagation. Micropropagation enhancing the efficiency of plant regeneration and growth, paving the way for advancements in plant tissue culture methodologies.

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Conflict of Interest. None.

REFERENCES

Ahmed, E. A., Hassan, E. A., El Tobgy, K. M. K., & Ramadan, E. M. (2014). Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. *Annals of Agricultural Sciences*, *59*(2), 273-280.

Al-Ami,n Md., Karim, M. R., Amin, M. R., Rahman, S. and Mamun, A. N. M. (2009). *In vitro* micropropagation of banana (*Musa* spp.). *Bangladesh J. Agril. Res., 34*(4), 645-659.

Anonymous, Red Banana Benefits. *Ayur Times*, 2014.

Anonymous, Red Bananas – Nutrition Facts & Health Benefits. *Healthy Food House*, 2013.

Aquil, B., Jan, A. T., Sarin, N. B., & Haq, Q. M. R. (2012). Micropropagation and genetic transformation of banana for crop improvement and sustainable agriculture. *Journal of Crop Science*, *3*(2), 64.

Cronauer, S. S. & Krikorian, A. D. (1984). Multiplication of Musa from excised stem tips. *Annals of Botany*, *53*, 321-328.

Cronauer, S. S., & Krikorian, A. D. (1984). Rapid multiplication of bananas and plantains by *In vitro* shoot tip culture. *HortScience*, *19*(2), 234-235.

Gaspar, T. H., Kevers, C., Faivre-Rampant, O., Crèvecoeur, M., Penel, C. L., Greppin, H., & Dommes, J. (2003). Changing concepts in plant hormone action. *In vitro Cellular & Developmental Biology-Plant*, *39*, 85-106.

Ghodake, B. D., Kadam, M., & Mane-Deshmukh, V. A. (2023). Micropropagation: role in propagation of red banana. *Krishi Science – eMagazine for Agricultural Sciences*, *4*(07), 34-38.

Iqbal, M. M., Muhammad, A., Hussain, I., & Bilal, H. (2013). Optimization of in vitro micropropagation protocol for banana (*Musa sapientum* L.) under different hormonal concentrations and growth media. *Intl. J Agric. Innov. Res*, *2*(1), 23-27.

Jalil, M., Khalid, N., & Yasmin Othman, R. (2003). Plant regeneration from embryogenic suspension cultures of Musa acuminata cv. Mas (AA). *Plant Cell, Tissue and Organ Culture*, *75*, 209-214.

Kiran, U., Moahnty, S. K., Roy, P. S., Behera, L., & Chand, P. K. (2015). Genetic diversity among banana cultivars from Odisha using RAPD markers. *Sci Res Rep*, *5*(2), 118-124.

Koli, M. N., Sawardekar, S. V., Gokhale, N. B., & Patil, D. M. (2014). Optimization of in vitro sterilization technique in local cv.(Safed Velchi) along with Grand Naine of banana. *Int J Biotechnol Biosci*, *4*, 224-230.

Lohidas, J., & Sujin, D. (2015). Effect of growth hormones in the micro propagation of banana CV. Matti. *Plant Archives*, *15*(1), 307-314.

Meenakshi, S., Shinde, B. N., & Suprasanna, P. (2011). Somatic embryogenesis from immature male flowers and molecular analysis of regenerated plants in banana 'Lal Kela'(AAA). *Journal of fruit and ornamental Plant Research*, *19*(2), 15-30.

Mitsukuri, K., Arita, T., Johkan, M., Yamasaki, S., Mishiba, K. I., & Oda, M. (2009). Effects of type of explant and dark preconditioning on bud formation in Habenaria radiata (Thunb.) in vitro. *Hort Science*, *44*(2), 523-525.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497.

Nandhakumar N., Sundaram K. S., Sudhakar D. & Kumar K. K. (2017). Genetic Fidelity Analysis in the Micropropagated Banana Derived from Immature Primordial Male Flower Bud. *International Journal of Current Microbiology and Applied Sciences*, *6*, 1759-1769.

Qazi, J. (2016). Banana bunchy top virus and the bunchy top disease. *Journal of general plant pathology*, *82*, 2-11.

Rahul, R. Rodge, Anis Mirza, Harjinder Kaur, Lakhesing Girase and Khan Jabroot J. (2023). In vitro Regeneration of Musa spp. Plantlet CV. Grand Naine by Plant Tissue Culture Technique. *Biological Forum – An International Journal*, *15*(4), 533-538.

Rai, M. K., Phulwaria, M., Harish, Gupta, A. K., Shekhawat, N.S. and Jaiswal, U. (2012). Genetic homogeneity of guava plants derived from somatic embryogenesis using SSR and ISSR markers. *Pl. Cell Tiss. Org. Cult., 111*(2), 259-264.

Rajoriya, P., Singh, V. K., Jaiswal, N., & Lall, R. (2018). Optimizing the effect of plant growth regulators on in vitro micro propagation of Indian red banana (*Musa acuminata*). *Journal of Pharmacognosy and Phytochemistry*, *7*(1S), 628-634.

Ray Tui, Dutta I., Saha P., Das S. & Roy S.C. (2006). Genetic stability of three economically important micropropagated banana cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell, Tissue and Organ Culture*, *85*, 11-21.

Resmi, L., & Nair, A. S. (2007). Plantlet production from the male inflorescence tips of Musa acuminata cultivars from South India. *Plant cell, tissue and organ culture*, *88*, 333-338.

Rout, G. R., Senapati, S. K., Aparajita, S., & Palai, S. K. (2009). Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers. *Plant Omics*, *2*(6), 250-285.

Shirani, S., Mahdavi, F., & Maziah, M. (2009). Morphological abnormality among regenerated shoots of banana and plantain (Musa spp.) after in vitro multiplication with TDZ and BAP from excised

shoottips. *African Journal of Biotechnology*, *8*(21), 5755-5761.

Strosse, H., Schoofs, H., Panis, B., Andre, E., Reyniers, K., & Swennen, R. (2006). Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (Musa spp.). *Plant Science*, *170*(1), 104-112.

Strosse, H., Van den Houwe, I., & Panis, B. (2004). Banana cell and tissue culture-review. *Banana improvement: Cellular, molecular biology, and induced mutations*, 1-12.

Surabhi, G. K., & Pattanayak, S. (2015). Deciphering the genetic identity and fidelity of banana through inter simple sequence repeats fingerprinting. *Horticultural Biotechnology Research*, *1*, 16-22.

Venkatachalam, L., Sreedhar, R. V., & Bhagyalakshmi, N. (2007). Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regulation*, *51*, 193-205.

Venkatachalam, L., Thimmaraju, R., Sreedhar, R. V., & Bhagyalakshmi, N. (2006). Direct shoot and cormlet regeneration from leaf explants of 'Silk' banana (AAB). *In vitro Cellular & Developmental Biology-Plant*, *42*, 262-269.

Vuylsteke, D. R., & Ortiz, R. (1996). Field performance of conventional vs. in vitro propagules of plantain (Musa spp., AAB group). *HortScience*, *31*(5), 862-865.

Vuylsteke, D., & De Langhe, E. A. L. (1985). Feasibility of *In vitro* propagation of bananas and plantains. *Tropical Agriculture*, *62* (4), 323-328.

Vuylsteke, D., Ortiz, R., & Swennen, R. (1993). Genetic improvement of plantains and bananas at IITA. *InfoMusa*, *2*(1), 10-12.

Wong, W. C., Jalil, M., Ong-Abdullah, M., Othman, R. Y., & Khalid, N. (2006). Enhancement of banana plant regeneration by incorporating a liquid-based embryo development medium for embryogenic cell suspension. *The Journal of Horticultural Science and Biotechnology*, *81*(3), 385-390.

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