Highly Efficient Micropropagation of Grand Naine Banana

Ratnesh Kumar Singh[1] Dr. Prashant Kumar Mishra[2]
[1]Research Scholar [2]Associate Professor
[1,2]Department of Botany
[1,2]Vinoba Bhave Univeristy, Hazaribagh

Abstract— Efficient technique of micropropagation of the Grand Naine banana for the mass propagation has been developed. The explants were selected from the superior high yielding mother plants which were tested for viruses & Genetic fidelity. The sword suckers were selected as explants, trimmed properly to appropriate size, washed in detergent water followed by dipped in 70 % alcohol for 1 minute & after finally sterilized with 25 % Sodium hypochlorite for 15 to 20 minutes followed by distilled water wash for 4 to 5 times and finally treated with 0.1 % mercuric chloride for 5 to 7 minutes ,followed by distilled water wash, after proper sterilization the culture were inoculated in establishment medium with combination MS basal medium+ BAP, 2mg/l + Citric acid,40 mg/l + Sucrose, 20g/l + Agar, 5 gm/l. After successful establishment, the culture were transferred to Multiplication, medium with various trails of which trail code T8 (MS basal medium+ 4.5 mg/l BAP+ 0.45 mg/l IAA) was found the most appropriate for the efficient multiplication of cultures . For thin & week shoots elongation medium was tried with different combinations, out of them Trail code S2 was found the most appropriates for shoot elongation & healthy shoots in four week time. Well elongated shoot were directly rooted in rooting medium. For rooting, the trial code R2, MS basal medium ½ strength+ 1.5 mg/l IAA+ 0.3% activated charcoal, was found the best one. Finally the well rooted plants were taken for acclimatization. Initially plants were kept in high humidity of 90 % for primary hardening & then transferred to shade net house for the secondary hardening with different trails of potting mix out of them, the trail code H2 (Cocopeat+Sand+vermicopost) in the the ratio of 2:1:1 ,was found the most suitable. The plants well developed in 2.5 months for field plantations.

Key words: BAP- Benzyl Amino Purine, IBA- Indol-3-Butyric Acid, MS – Murashige and Skoog, Banana Micropropagtion

I. INTRODUCTION

Grand Nain banana is perennial, monocot & belong to a genus Musa. It is a cultivar to a well-known Cavendish Bananas. This group of banana is distinguished from the other group by their AAA genotype. This is world well accepted staple food in tropical countries. India the largest producer of banana contributes about 30 to 37 % of world production.

The banana cultivation in India has been doing mainly from the sword sucker plantation but various problems arises & there is always chance of viral disease, non-uniformity not surety of genetically identical so the sucker plantation has been decline in practice. Tissue culture has gained popularity among the cultivators because of the superiority, uniformity, Disease free, high yielding, & genetically identical & true to type. The rapid production of healthy planting material of demand clones of banana within a short time period facilitated by large scale micropropagation. Micropropagation has played a key role in banana and plantain breeding programs Worldwide (Rowe and Rosales, 1996; Vuylstekeet al., 1997). It is also stated earlier that the plantlets produced through micropropagation methods have been found to establish faster, healthier, stronger, shorter production cycles and higher yield than those of conventional methods; (Ortiz and Vuylstelce, 1996).

As regards yield performance in banana, tissues culture plants has been reported to produce 39 % high yield than plants from seed suckers; (Pradeeep et al; 1992). Micropropagation of banana is highly efficient allowing a large turnover of plants in a very short period of time within a very little space (Arias, 1992, Arvantnoyannis et al., 2007).

II. MATERIAL & METHODS

Collection of explants & Sterilization of the explants. The young sword suckers were collected from the well-developed mother plants, which had been tested for genetically true to type & all the probable known viruses. The collected suckers of size ,1 to 1.5 feet were taken, washed primarily for the removal of the mud, dipped in 70% solution of Isopropyl Alcohol for one minutes followed by DM water wash , as used earlier by various investigators for research purposes (Sita at el; 1998, Rahman et al ; 2002, Jalil et al ; 2003) and then the suckers were again trimmed with very carefully to the acceptable size of 2 to 3 inch size, washed again in detergent Water to remove the debris & then the explants were kept in 1 % solution of antifungal solution for one hour with Gentamycin 500 mg/l. As treated with the antifungal & antibiotics minimizes the contamination load in vitro cultures (Vanden Home 1998; Nandwani et al; 2000).

Finally the explants were again washed in DM water until the traces of antifungal and antibiotic solution removed completely. Finally the explants were taken to the laminar flow bench for the further sterilization. The explants were dipped in 25% solution of sodium hypochlorite & few drops of tween 20 for 15 to 20 minutes with continuous shaking followed by washed in two to three times in double distilled water. As stated earlier the sodium hypochlorite is the most commonly used disinfectant for the surface sterilization of banana explants (Cronauer & Krikorian 1984; Mendes et al; 1996; Mohamed et al; 2004). Finally the explants were washed in 0.1% solution of mercuric chloride for 5 to 7 minutes followed by 5 times washed in double distilled water. It was also advocated by some investigator to use of mercuric chloride in place of sodium hypochlorite in low concentration (Banarjee and Sharma 1988; Habib et al; 2002; Molla et al; 2004; Titov et al; 2006).

III. MEDIA PREPARATION

Medium for the different growth stages were prepared with basal MS medium with different combination of Growth regulators (discussed below), sterilized for 15 minutes with 121 degree centigrade, for 15 psi pressure. Ph was adjusted with 1 N HCL and 1 N KOH.

IV. ESTABLISHMENT OF CULTURES

After successful sterilization the culture were inoculated in Basal MS (Murashige & Skoog 1962) medium + BAP, 2 mg/liter + Citric acid, 40 mg/l + Sucrose, 20 g/l + Agar ,5 gm/l for establishment of cultures. The incubation was done in completes darkness for 2 weeks. Relative humidity of 60 %, temperature ranging from 25 to 28 degree centigrade.

V. MULTIPLICATION

Medium for multiplication of cultures was prepared with MS basal medium with different concentration of BAP as a potent cytokinin, (The high performance of the BAP over other cytokinins in the multiplication of the shoot tips has been reported in different cultivars of banana (Wong, 1986; Gilmar et al; 2000). In particular cytokinin have been found to reduce the dormancy of apical meristem & induce auxiliary shoot from meristemetic explants (Madhulatha et al; 2004).

The combination was MS basal medium + BAP ranging from 1 mg/l, 1.5mg/l, 2mg/l, 2.5mg/l,3mg/l, 3.5mg/l, 4mg/l, 4.5mg/l,5mg/l, 5.5 mg/l. + IAA (Indol acetic acid), concentration ranging from (0.1mg/l, 0.2mg/l, 0.3mg/l, 0.5mg/l,0.6mg/l, 0.7mg/l,0.8mg/l, 1mg/l, 1.5mg/l, 2mg/l) + Sucrose 30g/l +Agar 5.8 gm/l. The ph was adjusted to 5.8. The well-established cultures were taken, trimmed slightly in laminar flow bench and were transferred to fresh multiplication medium. Incubation of cultures was done in complete darkness for 4 weeks.

VI. SHOOT ELONGATION

After the four weeks , the thin cultures were transferred to the shoot elongation medium for further shoot development & girth , Three trials were conducted with combination of MS basal + BAP Conc. ranging from 0.5mg/l, 0.7mg/l, 1.5mg/l + IBA (Indol butyric acid) concentration ranging from 0.1mg/l, 0.2mg/l, 0.2mg/l+ sucrose 40 gm /l +Agar 5.8 gm/l. The ph was adjusted to 5.7. Light intensity of 1000 lux provided by cool white florescent lamp with a photoperiod of 16 hour with relative humidity of 55 to 60 %, temperature ranging from 25 to 28 degree centigrade. The four week duration was given for the incubation.

VII. ROOTING

Well elongated culture were selected & transferred to the rooting medium. Three rooting trials were conducted with combination of Basal MS medium of half strength +Activated charcoal of conc. 0.5 % + IBA conc. ranging from , 1mg/l, 1.5mg/l,2mg/l + Sucrose, 20g/l+ Agar 6gm/l with ph adjusted to 5.7.

All the cultures were incubated in relative humidity of 60 %, temperature ranging from 26 to 28 degree centigrade for the duration of 3 weeks. Light of 12 hours photoperiod were provided to the rooting culture.

VIII. ACCLIMATIZATION

The well elongated & rooted cultures were considered for the Primary hardening. The ex-agar cultures were transplanted in portray filled with decomposed, sterilized coco peat. The transplanted plants were kept in high humidity of 90 % in poly tunnel with temperature ranging from 30 to 38 degree centigrade. The 1 month duration was given for primary hardening. During this period the tunnel was open in 15 days interval & mild nutrients were applied as foliar spray. For the prophylactic measure systemic antifungal solution of 1% solution were applied as foliar spray at the time of tunnel opening.

The secondary hardening were conducted in three tails H1 - Coco peat + sand+ Vermicompost in the ratio of 1:1:2 , H2- Coco peat+ vermicompost+ sandy soil in the ratio of 2:1:1. , H3- Sandy soil+cowdung in the ration of 2:2. The duration given for the secondary hardening was about 1.5 month with proper fertilizer & prophylactic spray. The Plants were kept in 75% agro shade net house. Precaution had been made to avoid any fungal growth.

IX. RESULT & DISCUSSION

The observation of the growth of cultures was evaluated for all the three stages separately and best combination of medium was selected. For multiplication & establishment of culture, about ten trails were conducted, for each trial about 30 cultures had been taken for the experiment; among them the Trail code T8 of combination MS + BAP, 4.5 mg/l + IAA, 1 mg/l + Agar 5.8 g/l. , Was found the best combination which gave 98% well developed shoot, 2 % shoot primordia with multiplication rate of 4 to 4.5 fold. The cultures were incubated in complete darkness. Cultures were evaluated after four weeks. It was found that cultures were healthy & freed from somaclonal variation and verification.

X. SHOOT ELONGATION

For shoot elongation, well developed healthy shoots were selected & were transferred to the shoot elongation medium. Three trails was conducted with each of 30 cultures were taken for the experiment, among them trail code S2 of combination of MS basal medium+ BAP, 0.7mg/l + IBA,0.2 mg/l + Agar,6g/l + Ph, 5.8; was found most appropriate, which was having well developed elongated shoot with four to five open leaves , shoot girth was also very Appreciable.

Cultures were observed well developed after five weeks under incubated in light of photoperiod of 12 hours with relative humidity of 60%, and temperature ranging from 26 to 28 degree centigrade.

XI. ROOTING

The well-developed elongated cultures with good morphological characteristic had been taken for rooting.

Three trails were conducted each of 30 cultures were taken for the experiment. Among them the trail code R2, with combination of MS basal medium with half strength + IBA, 1.5mg/l + 0.5 % activated charcoal + Agar, 5.8 g /l + 5.8 ph. The cultures were kept in full light of the

photoperiod of 12 to 15 hours. The roots were developed in 2 to 2.5 weeks in 99 % of the cultures.

XII. ACCLIMATIZATION

The proper acclimatization, two stage hardening had been conducted the primary & secondary hardening. In primary hardening the result was excellent with coco peat used in portray kept in 98 % relative humidity for One month. The successful primary harden cultures were taken for the secondary hardening. In secondary hardening, three trails were conducted among them Trail code H2 was found most appropriate, for the excellent growth of plants, with well-developed root & shoot system was noted. The five open Leaves developed & shoot girth was also very appreciable. The secondary hardening was done for 1.5 months.

XIII. CONCLUSIONS

The above experiments were found very important to find the efficient protocol of fast micropropgation of the Grand Nain banana for the commercial purpose. The multiplication of the cultures can be carried out in the similar manner for 7 to 9 multiplication cycle. This method can also be implemented for the mass propagation of the planting material for the easily avavability of the disease free & true to type of planting material to the farmers in minimal cost in less time & can help in socio economic development of the nation.

Trail code	Basal Medium	BAP Conc. mg/l	IAA Conc mg/l	% of Culture response	% of Healthy Shoot	MR
T1	MS	1	0.1	70	.60	1
T2	MS	1.5	0.15	80	60	1.2
T3	MS	2	0.2	80	50	1.8
T4	MS	2.5	0.25	85	70	2.5 to 3
T5	MS	3	0.3	85	58	2..5
T6	MS	3.5	0.35	85	60	2.6
T7	MS	4	0.4	90	50	3.8
T8	MS	4.5	0.45	98	90	4.5
T9	MS	5	0.5	90	50	3.5
T10	MS	5.5	0.55	90	40	2.5

Table 1: Culture Multiplication Trails
*MR - Multiplication ratio

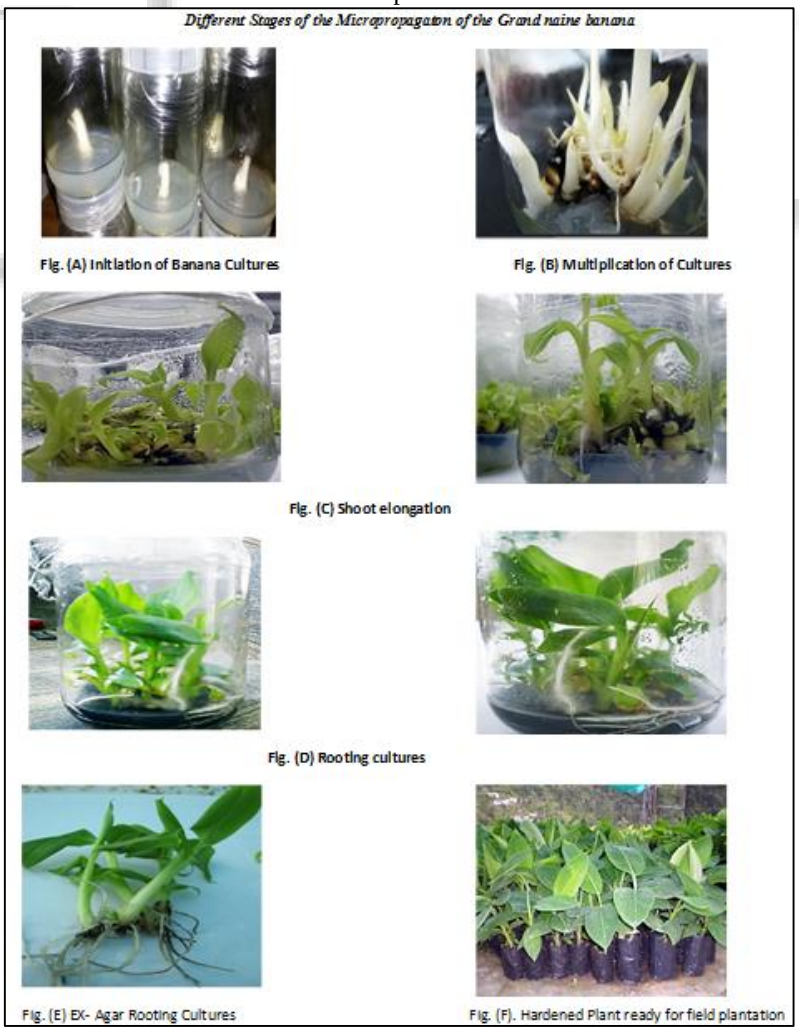

Fig. 1: Snapshots

Trail code	Basal Medium	BAP Conc. mg/l	IBA Cong mg/l	% elongated healthy shoots
S1	MS	0.5	0.1	50
S2	MS	0.7	0.2	98.9
S3	MS	0.8	0.3	75

Table 2: Shoot Elongation

Trail code	Basal Medium	IAA Conc. mg/l	Activated Charcoal	% Rooting Cultures
RI	1/2MS	1	0.3%	90
R2	1/2MS	1.5	0.3%	98.9
R3	1/2MS	2	0.3%	75

Table 3: Rooting trails

Trail code	Substrate Used	Ratio used of the Substrates	Fertilizers Used	% Hardened & Elongated, With five opened leaves
HI	Coco peat + sand+Vermicompost	1:1:1	NPK 19:19:19 2gm/l as Foliar spray	70
H2	Coco peat + sand+ Vermicompost	2:1:1	½ MS medium as foliar spray	98.9
H3	Sandy Soil+CowDung	2:2	NPK 19:19:19 2gm/l as Foliar spray	75

Table 4: Hardening Trails

REFERENCES

[1] Arias O. (1992). Commercial micropropagation of banana. In: Biotechnology application for banana and plantain improvement. Inibap, San Jose, Costa Rica. pp. 139-142.
[2] Arvanitoyannis, I. S., Mavromatis, A.G., Grammatikaki Avgeli, G. amd Sakellariou. M. (2007). Banana: cultivars, biotechnological approaches and genetic transformation. Int.J. Food Sci. Tech. 43: 1871-1879.
[3] Banerjee, N, & Sharma, A.K (1988) In vitro treasons as a reelection of genomic diversity in long –term cultures of Musa. Theor. Appl. Genet., 76: 733-736.
[4] Gilmar Cronauer- Mitra SS, Krikorian AD (1984). Multiplication of Musa from excised stem tips. Ann. Bot. 53: 321-328.
[5] Habiba U. , Reza, S., Saha, M.L., Khan, M R, & Hadiuzzamaan, S. (2002) Endogenous bacterial contamination during invitro cultures of table banana identyification and prevention. Plant Tisusue cult. 12(2):117-124.
[6] Jalil, M., Khalid, N., & Othman, R.Y. (2003). Plant regeneration from embryonic suspension culture of Musa accuminata cv. Mas.(AA), Plant Tissue cell Tissue organ cult. 75: 209-214.
[7] Madhulatha P, Anbalagan M, Jayachandran S, Sakthivel N (2004). Infulence of liquid pulse treatment with growth regulators on in vitro propagation of banana (Musa sp. AAA). Plant cell organ Cult. 76:189-191.
[8] Mendes B. M. J., Mendes F. J., Tulmann Neto A., Demetrio CGB, Pieske OR. (1996). Efficacy of banana plantlets production by microprogation. Pesqui Agropecu Bras 31: 863-867.
[9] Molla M, M.H., Khanam M. Dilafroza, Khatun M.M., Amim M. al . & Malek M . A. (2004). In vitro rooting and ex- vitro plantlets establismnt of BARI banana 1 (Musa sp) as influenced by different concentration of the IBA. Assian Journal of Plant Sciences 3(2): 196-199.
[10] Muhamad Aish, Hussian Iqbal, Naqvi S.M. Saglan and Rashid Hamid. (2004). Banana plantlets production through Tissue culture. Pak . J. Bot., 36(3): 617-620.
[11] Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum 15: 167-211.
[12] Nandwani, D., Zehr, U., Zehr, B. E. , & Barwale , R . B. (2000). Mass propagation & ex vitro survival of banana cv. Basrai through tissue culture. Garten Bauwissen Chaft. , 65 (6):237-240.
[13] Rahman Md. Moshium, Rabbani Md. Golam, Rahman Mohamamd Atikur & Uddin Md. Farid. (2002). In vitro shoot multiplication and rooting of banana cv. Sabri. Pakistyan Jornal of Biological Sciences. 5(2): 161-164.
[14] Rsshid, H., Toriyama, K., Qureshi, A., Hinata, K. and Malik, A.K. 2000. An improved method for shoot regernertaion from calli of indica rice (Basmati). Pak. J. Bio. Sci. 3: 2229-2231.
[15] Ortiz R, Vuylsteke D (1996). Recent advances in Musa genetics, breeding and biotechnology, Plant Breed, Abs.66: 1355-1363.
[16] Rowe P, Rosales FE (1996). Bananas and plantains. Fruit Breed. 1:167-211.
[17] Titov Soubir, Bhowmik Salil kumar, Mandal ajoy, Alam Md, Sadrul& Uddin Sarder Nasir, (2006). Control of phnolic secretion and effect of growth regulatiors for organ formation from Musa Spp .cv Kanthali Floral buds explants. American jornal of Bichemistry & Biotechnology 2 (3) :19-107
[18] Vanden Houwe, I., Guns, J., & Swennen, R. (1998). Bacterial contaminations in Musa shoot tip cultures, Acta Horticul. 490-485-492.
[19] Vuylsteke D, Ortiz R, Ferris RSB, Crouch JH (1997). Plantain improvement. Plant Breed. Rev. 14:267-320.
[20] Wong WC (1986). In vitro propagation of banana (Musa sp): initiation, proliferation and development of shoot –tip cultures on defined media. Plant cell tissue Organ Cult. 6: 169-166.

