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Standardization of *In Vitro* Regeneration Techniques in Red Banana and Fidelity Testing of Tissue Culture Raised Plantlets of Red Banana

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Abstract The present study was carried out to standardize surface sterilization techniques and optimization of growth hormones for *in vitro* regeneration of red banana and fidelity testing of tissue cultured red banana plantlets through molecular markers. The treatment in the sequential order of Tween 20 (5 ml/lit) 10 min Dettol+Savlon (5 ml + 45 ml/lit respectively) 30 min followed by carbendazim 1%, ethanol 70%, sodium hypochloride 5% and cefotaxime I–250 mgL^{-1} and cefotaxime II-250 mgL^{-1} for the period of 30, 1, 10 and 20 min and 40 min resp produces 67.8% aseptic culture. The maximum shoots were established in media containing MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid + 1gL^{-1} activated charcoal i.e. 78%. The media MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA +20 mgL^{-1} ascorbic acid was found effective for maximum number of shoots initiation (96.67%) and maximum rate of multiple shooting (560.29). MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid + 1 gL^{-1} activated charcoal was also found effective for highest percentage of root regeneration (93.33) and maximum number of roots per shoot with an average of 15.34 roots per shooted plant. The plantlets hardened in the potting mixture (1:1:1) of soil, FYM and sand and ISSR primers produced monomorphic banding pattern in 10 DNA lots of 100 plantlets. Average percent polymorphism was zero. Range of size of product within bulked DNA was 200—2000 bp. Average number of alleles produced per marker was 4.81.

Keywords Red banana, Surface sterilization, Shooting and rooting media, Hardening, Fidelity testing.

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Introduction

Red banana is a triploid cultivar (AAA Group). Its official designation is *Musa acuminata*. Red bananas have a distinctive red to purple skin. This fruit has soft and creamy texture in cream to slightly pink color (Anonymous 2013). This fruit has high nutritional and calorific value, containing large amounts of potassium, vitamin C and beta-carotene. Medium sized fruits contain 400 mg of potassium, which covers body's daily needs of this mineral and electrolytes and is necessary for proper function of the heart muscle and the digestive system (Anonymous 2013).

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 significant loss in productivity (Javaria 2015). Besides, this vegetative method of propogation is

Table 1. Effect of sterilization treatment combination on percent aseptic culture.

Treatments	Sterilizing Agents	Concentration	Exposure time (min)	Percent aseptic culture
T_1	Carbendazim	0.5%	45	45%
	Ethanol	70%	1	(42.10)
	Sodium Hypochlorite	5%	5	
	Cefotaxime	150 mgL^{-1}	15	
T_2	Carbendazim	0.50%	60	56%
	Ethanol	70%	1	(48.43)
	Sodium Hypochlorite	5%	15	
	Cefotaxime	200 mgL^{-1}	30	
T_3	Tween 20	5 ml/L	10	67.8%
	Dettol+Savlon	5 ml+45 ml/L	30	(55.44)
	Carbendazim	1%	30	
	Ethanol	70%	1	
	Sodium Hypochlorite	5%	10	
	Cefotaxime (I)	250 mgL^{-1}	20	
	Cefotaxime (II)	250 mgL^{-1}	40	
T_4	Tween 20	5ml/L	10	
	Dettol + Savlon	5 ml + 45 ml/L	30	
	Carbendazim	1%	30	
	Ethanol	70%	1	
	Sodium Hypochlorite	5%	10	
	Cefotaxime (I)	250 mgL^{-1}	20	65.8%
	Cefotaxime (II)	250 mgL^{-1}	40	(54.19)
	Cefotaxime (III)	250 mgL^{-1}	60	
			SEm	1.205
			CD at 1%	3.99

slow and season bound. This all limitations can be reduced by propogation of banana through tissue culture technique i.e. micropropogation which is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants. Through this biotechnological tool, it is possible to get plantlets free from bacteria and other microorganisms (Cronauer and Krikorian 1984, Vuylsteke and Oritz 1996). The process also produces genetically uniform plants. Micropropagation can be rewarding only if complete genetic fidelity of micropropagules is maintained. Genetic fidelity is the maintenance of genetic constitution of a particular clone throughout its growth span (Chatterjee and Prakash 1996). Molecular marker techniques have proven to be powerful in estimation of genetic fidelity / uniformity in tissue culture grown long-term cultures of economically important fruit plants (Chittora et al. 2015). Among different molecular markers, the ISSR markers are powerful, rapid, simple, reproducible and inexpensive way to assess genetic stability or to check uniformity in between tissue culture plantless, including banana. Hence, it is necessary to standardize surface sterilization method, concentration of media for establishment, shoot induction and root development. Also for commercial micropropogation, genetic fidelity testing is necessary.

Materials and Methods

Preparation of explants

The plant material obtained from field grown suckers of local farmers of shrivardhan (District–Raigad). It was thoroughly washed under running tap water. The explants were trimmed away using stainless steel knife until the length of explants is about 4 to 6 cm and diameter at leaf base is 2 to 4 cm. These shoot tips were collected in a tray and kept in water for 5 min, then explants were pre-treated with dettol, savlon and tween 20 as per following Table 1 then explants were placed in running tap water for 1 h. Transfer the explants in laminar air flow and various pre-treatment steps given in Table 1 were followed.

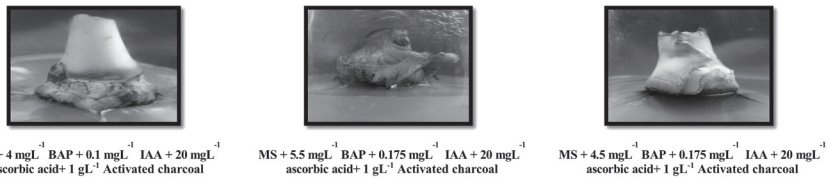


Fig. 1. Explant establishment after 21 days. MS+4 mgL^{-1} BAP+0.1 mgL^{-1} IAA +20 mgL^{-1} ascorbic acid + 1 gL^{-1} activated charcoal, MS+5.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid + 1 gL^{-1} activated charcoal, MS+4.5 mgL^{-1} BAP +0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal.

Inoculation and incubation of explants

The usual procedure of dissection and disinfection as described by Cronauer and Krikorian (1984) was used. The suckers were trimmed further to the final size of 2-3 cm^3 and inoculated in bottles containing MS medium supplemented with various growth hormones using aseptic culture technique. The cultures were incubated in a culture room. Initially the cultures were kept up to 21 days on the establishment medium (Fig. 1). For initial 8 days cultures were incubated in dark and then transferred in light (1600 lux) condition for 15 days. The culture in establishment medium was transferred to the multiplication medium after 21 days for shoot initiation. Following media combination was used for establishment and proliferation of shoot tips. Explants subcultured after every 15 days on new media containing same media combination for multiplication. After development of multiple shoots, the well grown elongated shoots were separated from each other and transferred to the rooting media MS containing different concentrations of auxin (NAA, IAA), cytokinin (BAP) and activated charcoal for further root development. The rooted shoots were transferred to various combinations of potting mixture. As all the studies were done in laboratory under well defined conditions which was maintained throughout the study. The experiment was conducted in completely randomized design.

Plant material

For the present experimental study, 100 tissue cultured plantlets of red banana were obtained from Plant Biotechnology Center Dapoli, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Dist Ratnagiri (MS) which was obtained from single mother plant used in above experiment. The DNA was isolated by the protocol of Kadam et al. (2017) i.e. rapid method. Extracted Genomic DNA samples were bulked into 10 lots and used for further analysis.

Table 2. Effect of media combination on establishment of explants.

Tr. No.	Media combinations	Percent establishment
E_1	MS	0.00 (00)
E_2	MS+2 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	0.00 (00)
E_3	MS+2.5 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	0.00 (00)
E_4	MS+3.0 mgL^{-1} BAP+0.175 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	0.00 (00)
E_5	MS+3.5 mgL^{-1} BAP+0.175 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	32.00 (34.42)
E_6	MS+4 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	37.33 (37.64)
E_7	MS+4mgL^{-1} BAP +0.1 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	44.67 (41.92)
E_8	MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	78.00 (62.39)
E_9	MS+5 mgL^{-1} BAP+0.1 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	55.33 (48.04)
E_{10}	MS+5.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	40.33 (39.41)
E_{11}	MS+6 mgL^{-1} BAP+0.1 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	35.67 (36.64)
	SEm	1.45
	CD at 1%	4.28

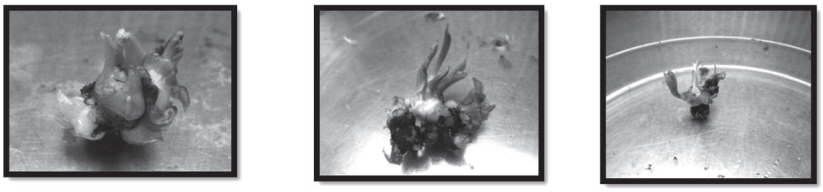


Fig. 2. Shoot proliferation. MS+4 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal, MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal, MS+6 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal.

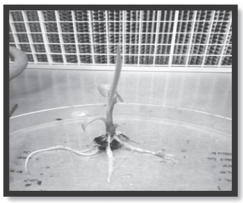
PCR amplification

Eleven inter-simple sequence repeat (ISSR) markers, composed of short, predefined tandem repeat sequences with an anchor sequence and representing different di- and tri-nucleotide microsatellites were used for PCR amplification. The ISSR markers were used for molecular characterization and to measure genetic fidelity between and within samples of the single explants of red banana. A PCR protocol was standardized for all ISSR markers. Each 20 µl PCR contained 25 ng template DNA, 2.5 ml of 10×PCR buffer, 0.5 µl of 15 mM Mg C12, 1 µl of 10 mM dNTPs (Bangalore Genei Pvt Ltd, Bangalore, India), 10 pmol of each ISSR or 5 pmol of each SSR primer (Bioresource Biotech Pvt Ltd, Pune, India) and 3.0 units of Taq polymerase (Bangalore Genei Pvt Ltd). Thermal profiles were referred from Kadam et al. (2017).

ISSR primer (i.e. marker) based on its melting temperature using a Master Cycler 2231 gradient-PCR machine (Eppendorf, Hamburg, Germany). The standard annealing temperatures of all ISSR primers are given in Table 2. The PCR-amplified products were separated by electrophoresis in 2% (w/v) agarose gels at 80 V. The gels were stained with 10 mg/ml ethidium bromide and visualized under UV light using a Fire Reader gel documentation system (Uvitec Ltd, Cambridge, UK) and the data were stored for further analysis.

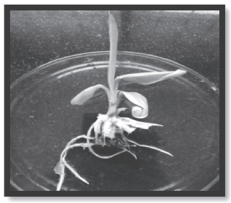
Table 3. Shoot induction response to different growth hormones, days to shooting and average number of shoot induction.

Tr. No.	Treatment details	Percent shooting	No. of days to shooting	No. of shoots regeneration
S_1	MS	0.00 (00)	–	0.00
S_2	MS+2mgL^{-1} BAP+20 gmL^{-1} ascorbic acid	0.00 (00)	–	0.00
S_3	MS+3 mgL^{-1} BAP 20 gmL^{-1} ascorbic acid	36.67 (37.13)	28.53	36.75
S_4	MS+3 mgL^{-1} BAP+0.1 mgL^{-1} IAA+20 gmL^{-1} ascorbic acid	43.33 (41.14)	22.07	49.91
S_5	MS+3.5 mgL^{-1} BAP+0.15 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	46.67 (42.98)	23.76	58.65
S_6	MS+4.0 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20mgL^{-1} ascorbic acid	53.33 (46.90)	26.38	111.37
S_7	MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	96.67 (83.85)	21	560.29
S_8	MS+5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	76.67 (61.20)	25.37	250.27
S_9	MS+5.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	73.33 (58.98)	26.33	226.52
S_{10}	MS+6 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	70.00 (56.98)	25.75	147.24
S_{11}	MS+6.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid	56.67 (48.83)	24.45	96.73
	SEm	3.22		21.582
	CD at 1%	9.51		63.706



MS + 1 mgL^{-1} BAP + 0.1 mgL^{-1} NAA + 20 mgL^{-1} Ascorbic acid + 1 gL^{-1} Activated charcoal

MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid+ 1 gL^{-1} Activated charcoal



MS + 4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid+ 1 gL^{-1} Activated charcoal

Fig. 3. Root Development. MS+1 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal, MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal, MS + 4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal.

Data analysis

ISSR markers across the 100 samples of single explants were scored for their presence (1) or absence (0) of bands for each primer. By comparing the banding patterns of those samples for a specific primer specific bands were identified. The binary data so generated was used to estimate the levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands.

Results and Discussion

Sterilization of explants

The surface sterilization is not effective, then the contamination by fungus and other microorganisms will profuse as the conditions in *in vitro* culture are most favorable for there growth and multiplication. The treatment combination T_3 comprising 5 ml//L Tween 20 for 10 min+(5 ml Dettol+45 ml savlon)/L for 30 min + 1% carbendazim for 30 min+70% ethanol for 1 min +5% NaoCl for 10 min+250 mgL^{-1} cefotaxime (I) for 20 min +250 mgL^{-1} cefotaxime (II) for 40 min

Table 4. Effect of different combinations of growth hormones on percent rooting, days to rooting and average root induction.

Tr. No.	Treatments details	Percent rooting	No. of days to rooting	No. of roots regenerated explant
R_1	MS	0.00 (00)	00	0.000
R_2	MS+1 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20 gmL^{-1} ascorbic acid	33.33 (35.20)	19.5	7.20
R_3	MS+1 mgL^{-1} BAP +0.5 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	43.33 (41.14)	17.66	7.26
R_4	MS+1 mgL^{-1} BAP+0.6 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	60 (50.83)	15	6.35
R_5	MS+1 mgL^{-1} BAP +0.7 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	66.67 (54.76)	16.33	9.00
R_6	MS+1 mgL^{-1} BAP+0.1 mgL^{-1} NAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	70 (56.97)	18	8.70
R_7	MS+1mgL^{-1} BAP+0.15 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	76,67 (61.90)	15.6	10.01
R_8	MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+ 1 gL^{-1} activated charcoal	93.33 (77.69)	14.56	15.34
R_9	MS+4 mgL^{-1} BAP+0.2 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal	83.33 (66.12)	15	10.430
	SE	3.628		0.790
	CD at 1%	10.864		2.366

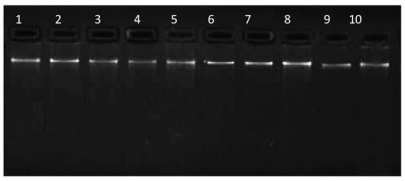


Plate 4 : 100 DNA samples in 10 bulked lots

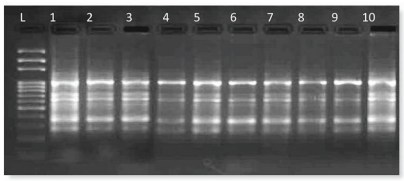


Plate 5 : Primer: UBC – 824,
L: Molecular Ladder (100bp)
1-10:Red Banana plantlets lots

Fig.4. 100 DNA samples in 10 bulked lots. **Fig. 5.** Primer: UBC–824, L: Molecular ladder (100 bp), 1-10: Red banana plantlets lots.

was found to be best combination to achieve highest percentage (67.80) of aseptic culture and treatment T_4 is at par with T_3. Koli et al. (2014) were used slightly similar surface sterilization treatment i.e. Tween 20 (10 min), dettol and savlon (30 min), 1% carbendazim (30 min), 70% ethanol (1 min), 5% sodium hypochlorite (10 min), 250 mgL^{-1} cefotaxime (30 min) with 96.67% aseptic culture.

In vitro establishment of the shoot tips

Treatment E_8 (78%) containing MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid was showed maximum establishment (Fig. 2). Iqbal et al. (2013) found MS+5 mgL^{-1} BAP + 2 mgL^{-1} IAA best for establishment. Ahmed et al. (2014) was observed 100% establishment of explants on media combination MS+4 mgL^{-1} BAP + 2 mgL^{-1} IAA. Kumari and Misra (2017) also found 75% establishment of banana explants on MS+3 mgL^{-1} BAP+0.2 mgL^{-1} IAA with 30 mg ascorbic.

Days to shooting and average shoots per explants

The endogenous level of auxin and cytokinin are important for morphogenesis. Higher the level of cytokinin help to multiple shoot induction. Since it is local cultivar the level of cytokinin may be different than other hybrid cultivars. Hence combination of auxin and cytokinin for shoot induction needs to be optimize. It was observed that the maximum number of shoots were initiated in treatment S_7 (96.67%) containing of MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA + 20 mgL^{-1} ascorbic acid (Table 3). Iqbal et al. (2013) showed observed best shoot proliferation (95%) on MS +5 mgL^{-1} BAP+ 1 mgL^{-1} IAA. Ahmed et al. (2014) got 99% shooting on media combination MS+4 mgL^{-1} BAP+2mgL^{-1} IAA. Koli et al. (2014) observed 99% shooting on media MS+4 mgL^{-1} BAP +2 mgL^{-1} IAA. Earlier it was observed that 95% explants formed multiple shoots in MS medium supplemented with 10 µm BAP and 1 µm IAA. Kumari and Misra (2017) found 80% explants forming shoots on media combination on MS+4, 5 mgL^{-1} BAP+0.2, 0.5 mgL^{-1} IAA with 30 mg ascorbic. It indicates that BAP is commonly used cytokinin for multiple shoot induction.

Table 5. Effect of different potting mixtures on hardening of red banana plantlets.

Treatments	Potting mixture	Survivability (%)
H_1	Soil + FYM + Sand (1 : 0 : 1)	25
H_2	Soil + FYM + Sand (1 : 1 : 0)	15
H_3	Soil + FYM + Sand (1 : 1 : 1)	90
H_4	Soil + FYM + Sand (1 : 0 : 0)	20

The treatment S_7 containing MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid (560.29) was significantly superior for multiplication of red banana explants over all media combinations. Iqbal et al. (2013) observed highest number of shoots produced (10 shoots per explant) on MS+5 mgL^{-1} BAP+1.5 mgL^{-1} IAA+10% CW. Ahmed et al. (2014) was observed maximum shoots 10.66 on media combination 4 mgL^{-1} BAP+2 mgL^{-1} IAA. Kumari and Misra (2017) observed 5 shoots/explant on MS+5 mgL^{-1} BAP+0.5 mgL^{-1} IAA + 30 mgL^{-1} ascorbic acid which approximately similar to findings in present investigation. According to observations it

was also showed that number of shoots increased upto 4.5 mgL^{-1} BAP and decreased after it. Earlier it was also reported that number of shoots increased with increasing concentration of BAP up to 22.2 µm with higher levels of BAP reduction in number of shoots formation. This showed that high concentration of BAP hamper the ability of multiplication of explants. From the data, it was observed that the maximum days to shooting were ranged between 21 to 30 days i.e. 3-4 weeks. Same result was obtained by Jaisy and Ghai (2011) who recorded 4 weeks to shoot production, Goswami and Handique (2013) recorded 21–34 days required to shoot induction.

Days to rooting and average roots per explants

Among the various media combinations treatment R_8 (MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} activated charcoal) recorded maximum (93.33%) frequency of rooting (Table 4). Iqbal et al. (2013) showed MS media supplemented with 2 mgL^{-1} IAA produced 60% root induction. It was observed that the maximum days to rooting ranged between 14 to 20 days. Roy et al. (2010) observed plantlets rooted *in vitro* within 2 weeks. Lalrinsanga et al. (2013) showed that 14.75 days required to root formation after inoculation on rooting media. Lohidas and Sujin (2015) observed that average days to rooting was 8–20 (Fig. 3). The rooting media S_7 containing MS+4.5 mgL^{-1} BAP+0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid was found significantly superior over all rooting media with an average of 15.34 roots per shooted plant. Roy et al. (2010) observed plantlets rooted *in vitro* with MS supplemented with 1 mgL^{-1} IBA (6 roots/plant). Kumari and Misra (2017) observed 5 roots / explant on 0.5 gmL^{-1} IAA+0.5 gmL^{-1} IBA.

Hardening

Maximum number of plantlets survived 90% in potting mixture containing all the three components in equal proportion (1:1:1) (Table 5). Sindha et al. (2011) were transferred the rooted plantlets into a poly bag containing fine sterilized sand, sterilized soil and a farmyard manure (1:1:1) mixture.

Marker analysis

Marker analysis helps to understand the genetic uniformity present among the all tissue cultured banana plantlets bulk wise. The present study employed ISSR markers technique to assess genetic fidelity / uniformity. Most of researchers, Rout et al. (2009), Nandhakumar et al. (2017) were used ISSR markers for fidelity testing of banana.

A total of 530 scorable DNA fragments were produced in 10 lots of 100 DNA samples. There was no any polymorphic fragment was observed among them. All 11 primers produced monomorphic banding pattern in all lots (Tables 6, 7). The average percent polymorphism across the 11 primers in between 10 lots of 100 DNA of red banana plantlets was 0%, indicating absence of variation among the micropropogated plants. Hrahsel et al. (2014), shows similar result i.e. no polymorphism for 30 primers. Rout et al. (2009) was observed a homogenous amplification profile for all the micropropagated plants of Bantala. Size of product ranged from 200 bp by the primers UBC 816, UBC 824, UBC 834 to 2000 bp by UBC 841 primer in 10 lots of 100 DNA samples (Figs. 4, 5). The product size range is similar with the result of Nandhakumar et al. (2017) (200 bp to 2000 bp) and corresponding to the result of Kadam et al. (2017) (200 bp to 1800 bp).

The number of alleles produced by the markers ranged from 2 to 8. While the average number of alleles per marker 4.81. Markers UBC 816, UBC 854 and UBC 879 showed two alleles i.e. minimum number of alleles and the maximum alleles (8) were produced by markers UBC 841 and UBC 891. Choudhary et al. (2015) also observed 2 to 8 scorable bands for primers they used.

Conclusion

From the present investigation it could be concluded that, for *in vitro* regeneration of cv Red banana the treatment in the sequential order of Tween 20 (5 ml/lit) 10 min. Dettol+Savlon (5 ml + 45 ml/lit respectively) 30 min. followed by carbendazim 1%, ethanol 70%, sodium hypochloride 5% and cefotaxime I–250 mgL^{-1} and cefotaxime II-250 mgL^{-1} for the period of

Table 6. Annealing temperatures of the 11 ISSR primers used in this study. B=C, G, T ; H = A, C, T ; V=A, C, G ; and D=A, G, T.

Primer	Primer sequence (5′—3′)	Annealing temp range (°C)	Stand annealing temp (°C)
UBC-807	AGAGAGAGAGAGAGAGT	45–55	45.5
UBC-814	CTCTCTCTCTCTCTCTA	40–50	49.0
UBC-816	CACACACACACACACAT	50–60	57.9
UBC-818	CACACACACACACACAG	45–55	47.9
UBC-824	TCTCTCTCTCTCTCTCTCG	50–60	56.7
UBC-825	ACACACACACACACACT	40–50	44.3
UBC-834	AGAGAGAGAGAGAGAGT	45–55	50.4
UBC-841	GAGAGAGAGAGAGAGAC	40–50	45.4
UBC-854	TCTCTCTCTCTCTCTCHG	45–55	54.8
UBC-879	CTTCACTTCACTCA	35–45	40.0
UBC-891	AGATGTGTGTGTGTGTG	45–55	53.8

Table 7. ISSR primer wise amplification and percent polymorphism in between 10 lots.

Sr. No.	Primer	No. of alleles	Total no. bands	Total no. of polymorphic bands	% Polymorphism	Range of amplification (bp)
1	UBC-807	7	70	00	00.00	300-1700
2	UBC-814	6	60	00	00.00	450-1500
3	UBC-816	2	20	00	00.00	200-300
4	UBC-818	3	30	00	00.00	400-1500
5	UBC-824	5	50	00	00.00	250-1000
6	UBC-825	4	40	00	00.00	500-1000
7	UBC-834	6	60	00	00.00	200-1000
8	UBC-841	8	80	00	00.00	300-2000
9	UBC-854	2	20	00	00.00	550-750
10	UBC-879	2	20	00	00.00	600-750
11	UBC-891	8	80	00	00.00	500-1000
	Total	53	530	00	–	–
	Average	4.81	48.18	0.00	0.00	

30, 1, 10 and 20 min and 40 min respectively produces 67.8% aseptic culture.

The media combination MS+4.5 mgL^{-1} BAP + 0.175 mgL^{-1} IAA+20 mgL^{-1} ascorbic acid+1 gL^{-1} charcoal was found to be best for establishment, proliferation and root regeneration. The plantlets hardened in the potting mixture (1:1:1) of soil, FYM and sand and it was found that the best month for inoculation was July for highest proliferation. *In Vitro* propogation were made useful in generating plantlets to meeting demands for large number of elite planting material. For commercial nursery, true to type plantlets were required. Sometimes due to improper tissue culture practices undesirable somatic variation was observed. Hence, detecting the variation at early stage is useful which is done through fidelity testing of tissue cultured plantlets.

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