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In vitro conservation, regeneration and evaluation of genetic fidelity of threatened Banana cv. Nanjanagud Rasabale (AAB)

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ABSTRACT

Banana cv. Nanjanagud Rasabale (AAB, Silk subgroup) is one of the unique cultivars of Karnataka, India is now failed to revive its GI (Geographical Indication) tag, the major bane of the cultivar is high susceptibility to Fusarium wilt caused by Fusarium oxysporum f. sp. cubense (Foc). Hence, there is a need for the conservation of this elite cultivar from the threat of extinction. Plant germplasm can be conserved by different approaches one of the valuable biotechnological approaches is in vitro conservation. Laboratory studies were undertaken to come out with an effective protocol for conserving plantlets through a slow growth process by media manipulation, viz., by adding different growth retardants. Growth retardants such as ancymidol, paclobutrazol, abscisic acid, and DMSO at three different concentrations each were supplemented to MS medium. Among different treatment combinations, MS medium supplemented with DMSO 2.25 ml/l was found to be most effective by prolonging the period of successive sub-culture (154.53 days) and enabling mid to long-term conservation of shoot tips. The conserved cultures were regenerated using a standardized medium comprising MS Basal + 4 mg/l BAP + 0.5 mg/l NAA + 3% sucrose + 0.6% agar-agar. Growth was retrieved successfully with parameters evaluated. Assessment of genetic stability was performed by using Inter Simple Sequence Repeat amplification (ISSR) markers. All the 15 ISSR primers amplified unambiguous indicating monomorphic bands with 100 % monomorphism. Overall, the cultures were conserved by extending the sub-culture period and prolonged storage life without any genetic variation by using growth retardants.

Keywords: *Banana, Geographical Indication, Growth retardants, ISSR and In vitro Conservation*

Introduction

Banana (*Musa* sp.) is the world's most important fresh fruit commodity in terms of volume of trade. It is the oldest tropical fruit cultivated by a man from prehistoric times with great socio-economic significance. It is 1st plant cultivated, 1st food of man, and 1st fruit offered to babies. Bananas have grown for its versatility and adaption to varied agro-climatic conditions and also resilience to climatic changes. Botanically, it is a monocotyledonous, monocarpic giant herbaceous plant belonging to the section Eumusa placed in the family Musaceae under order Zingiberales with chromosome numbers 22, 33, and 44. South-East Asian countries, especially eastern Malaysia is believed to be the center of origin of bananas [1]. Banana is considered an antique fruit crop. Fruits are consumed fresh, cooked, steamed, roasted, and brewed.

The cultivar Nanjanagud Rasabale (AAB, Silk sub-group) is known for its place of origin, unique taste, aroma, color, fibrous texture, and nutritional content for which it was provided Geographical Indication (GI) tag in 2005 under the Goods (Registration and Protection) Act,1999. Once a leading cultivar of the Mysore district, Karnataka is now failed to revive its GI tag, the major lacuna of this cultivar is that it is highly susceptible to soil-borne Panama wilt disease caused by *Fusarium oxysporum* f. sp. *cubense.* The symptoms aggravate and start showing just before the flowering stage. The leaves turn yellow and wither, the base of the stem splits and the root rot leading to the destruction of the whole plant. Hence, there is a need for its conservation from the threat of extinction [3].

Conservation of plant germplasm can be carried out either *in situ* (in the natural habitat) or *ex-situ* (outside the natural habitat). The major obstacles to *in situ* conservation practices are the requirement of larger space, the high cost of operation, complicated management and the risk of damage by both biotic and abiotic factors under changing climate situations. Therefore, risks involved in field maintenance have led us to search for secure, efficient *in vitro* protocols for the effective conservation of plant germplasm [23]. Among the different methods of conservation available for banana germplasm, *in vitro* conservation is still advantageous over the other methods. *In vitro* preservation enables the safeguarding of genetic material within a controlled condition, facilitating sterile plant propagation, disease-free plantlets, safe and convenient global or local exchange of genetic resources, and a relatively reduced cost of preservation.*In vitro,* conservation is carried out by inducing slow growth conditions. The aim of slow or minimal growth is to decrease the frequency of sub-culturing to a level that doesn't have a lasting negative impact on the genetic material or the stability of the regenerated plants. This conservation method is carried out by different techniques, wherein one of the techniques is by modifying the medium

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composition using growth retardants, which results in the inhibition of cell division thereby limiting both callus formation and shoot development. These slow-growth procedures allow plant conservation for several months to years under aseptic conditions [10]. During the course of *in vitro* conservation, plants are exposed to a variety of stresses, both physico-chemical and physiological. These may cause a loss of genetic stability of conserved plants. Morphological and molecular markers provide the most accurate means of evaluating the variability and instability of the plantlets. Identifying somaclonal variants at an early stage of development is considered to be very useful for quality control in germplasm conservation. It is necessary to carefully examine the morphology of *in vitro* raised plantlets to distinguish between normal plants and somaclonal or epigenetic variants [26]. Hence, the present study was undertaken to standardize the *in vitro* conservation protocol in an endangered banana cv. Nanjanagud Rasabale.

2. Material and Methods

The present experiment was carried out in 2021 & 2022 in the Centre for Horticultural Biotechnology Research, Department of Biotechnology and Crop Improvement, College of Horticulture, Bagalkot.

2.1 Plant material

Shoot tips were used as the explant since they resist genetic variability. Stem bases were collected from the healthy sword suckers from the mother block of cv. Nanjanagud Rasabale is grown at Nanjanagud Taluka of the Mysore district, Karnataka. The macro stem-cuttings were processed following the standard procedure to excise the meristem as given below.

2.2 Establishment of aseptic culture

Explants were washed in running tap water followed by dipping in solution having detergent. The stem disc was then cut with a scalpel and transferred to a pre-treatment solution having 0.50% carbendazim + 0.05% streptomycin sulphate solution (8 h) and thereafter soaked in 1% carbendazim solution (30 min) followed by two rinsing with sterilized double-distilled water. The explants were transferred to the laminar airflow and agitated in 0.05% cetrimide for 30 min followed by 2-3 rinsing with sterile double-distilled water (Fig. 1).

The explants were surface sterilized with 0.10% mercuric chloride for 10 min after the removal of the outer scales and were rinsed thoroughly 5-6 times with sterilized double-distilled water and air-dried on a sterilized tissue paper cushion. Shoot tip explants were grown for two weeks under standard culture conditions (25±1°C, 70% relative humidity and a photoperiodic cycle of 16/ 8 h of light and dark cycle). Greening and swelling of the explants indicated the establishment success of the cultures. These were then excised by trimming the discolored tissues and transferred placed in glass jars containing *in vitro* conservation medium comprising of growth retardants (ancymidol, paclobutrazol, abscisic acid, DMSO) as per treatment details.

2.3 Regeneration of the conserved Meristem cultures

Aseptic shoot tip/ meristem conserved cultures were regenerated as per the protocol standardized [15]. Further, the cultures were incubated in a growth chamber maintained at 25±1°C temp., 70±2% RH, and a photoperiodic cycle of 16/8 h of light and dark cycle.

2.4 Genetic stability test of regenerated plantlets

The regenerated plants were assessed for genetic stability by using morphological and ISSR markers. Over 15 ISSR primers were selected for screening and the reproducibility of the PCR amplification was assessed using selected primers with different DNA samples, which were isolated using the trimethyl Ammonium Bromide (CTAB) method [13].

Statistical analysis

Complete randomized design (CRD) was adopted to zanalyze the experiment's statistical analysis which was done by using Wasp 2 statistical software (Anon, ICAR-Research Complex, Goa). All the percent data were subjected to Arc Sin transformation before subjecting to ANOVA. The data in decimals were transformed using the square root method [14]. The mean data in a column were separated using Duncan's Multiple Test (DMRT) for their significance.

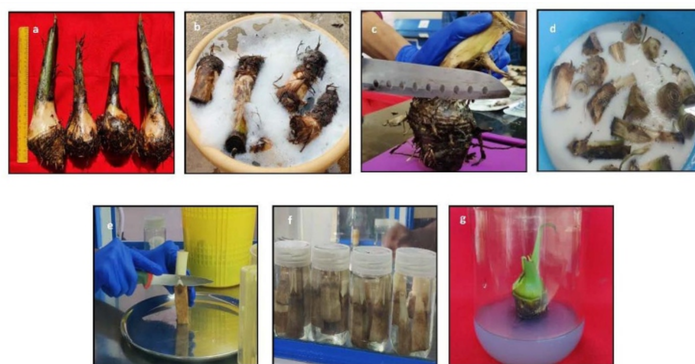

Fig. 1 Establishment of aseptic culture in banana cv. Nanjanagud Rasabale (AAB) : **a** Sword sucker.**b** Washing with water and tween 20. **c** Removal of roots and outer leaf sheaths. **d** Treatment with 0.5% carbendazim and 0.5% streptomycin. **e** Reducing explant size in laminar airflow. **f** Treatment with 0.10% mercuric chloride. **g** Established cultures.

Results and Discussion

Effect of growth retardants on *in vitro* conservation of banana *cv.* Nanjanagud Rasabale (AAB)

Among different growth retardants, dimethyl sulfoxide (DMSO) treated media showed the highest tissue storage period (154.53 days) since it had the most delayed bud sprout on the medium comprising of MS B + DMSO 2.25 ml/l. The explants were observed green and alive without any visible phenotypic variations. At the end of the sub-culture cycle, shoot tip browning (154.53 days) was noted in some of the cultures. Generally, tissue culture--raised banana plantlets are sub-cultured every 4-5 weeks. However, in the present investigation DMSO DMSO-supplemented culture media, the sub-culture period was prolonged (20-22 weeks) [Table 1, Fig 2]. Medium exhaustion is directly proportional to *in vitro* storage period. Hence, days taken for media exhaustion was highest in DMSO treated media combinations. It is a wellestablished fact that DMSO induces reduction in cell volume and water content [12] and also elicits membrane configuration [11]. In these conditions, the cells adjust to the osmotic imbalance between outer and inner parts by electrolyte accumulation within the cells [30] and the injurious level of dehydration is prevented by the modified cell membrane [17]. These findings are in close agreement with the reports of [7] on carnation, where the best results were noted due to supplementation of 3% DMSO, which helped in prolonged *in vitro* conservation for 10-12 months.

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Similarly, abscisic acid (ABA) is one of the important growth retardants used for restricting tissue growth *in vitro* conservation. ABA at 3.50 mg/l concentration showed no bud sprouting but the cultures were healthy and conserved upto 140.96 days by extending sub-culture period. Earlier [4] conducted a study on *in vitro* conservation of cassava by application of abscisic acid and reported 20 and 30 μM ABA to induce bud dormancy and delayed bud sprouting thus extending storage life for 3 months. The results upon supplementation had moderate effects.

Table 1. Effect of growth retardants on culture storage period in banana cv. Nanjanagud Rasabale (AAB)

Treatment	Culture conservation period (Days)
T_1: MS B* (Control)	39.70^{j}
T_2: MS B + ANC 2.25 mg/l	83.40^{i}
T_3: MS B + ANC 2.50 mg/l	91.73^{h}
T_4: MS B + ANC 2.75 mg/l	96.90^{g}
T_5: MS B + PBZ 2.50 mg/l	95.80^{g}
T_6: MS B + PBZ 3.50 mg/l	102.23^{f}
T_7: MS B + PBZ 4.50 mg/l	106.73^{e}
T_8: MS B + ABA 3.00 mg/l	109.56^{d}
T_9: MS B + ABA 3.25 mg/l	123.96^{c}
T_{10}: MS B + ABA 3.50 mg/l	140.96^{b}
T_{11}: MS B + DMSO 2.25 ml/l	154.53^{a}
T_{12}: MS B + DMSO 2.50 ml/l	154.36^{a}
T_{13}: MS B + DMSO 2.75 ml/l	153.96^{a}
S. Em ±	0.61
CD at 1%	2.40

Note: MS B- Murashiage and Skoog basal medium*

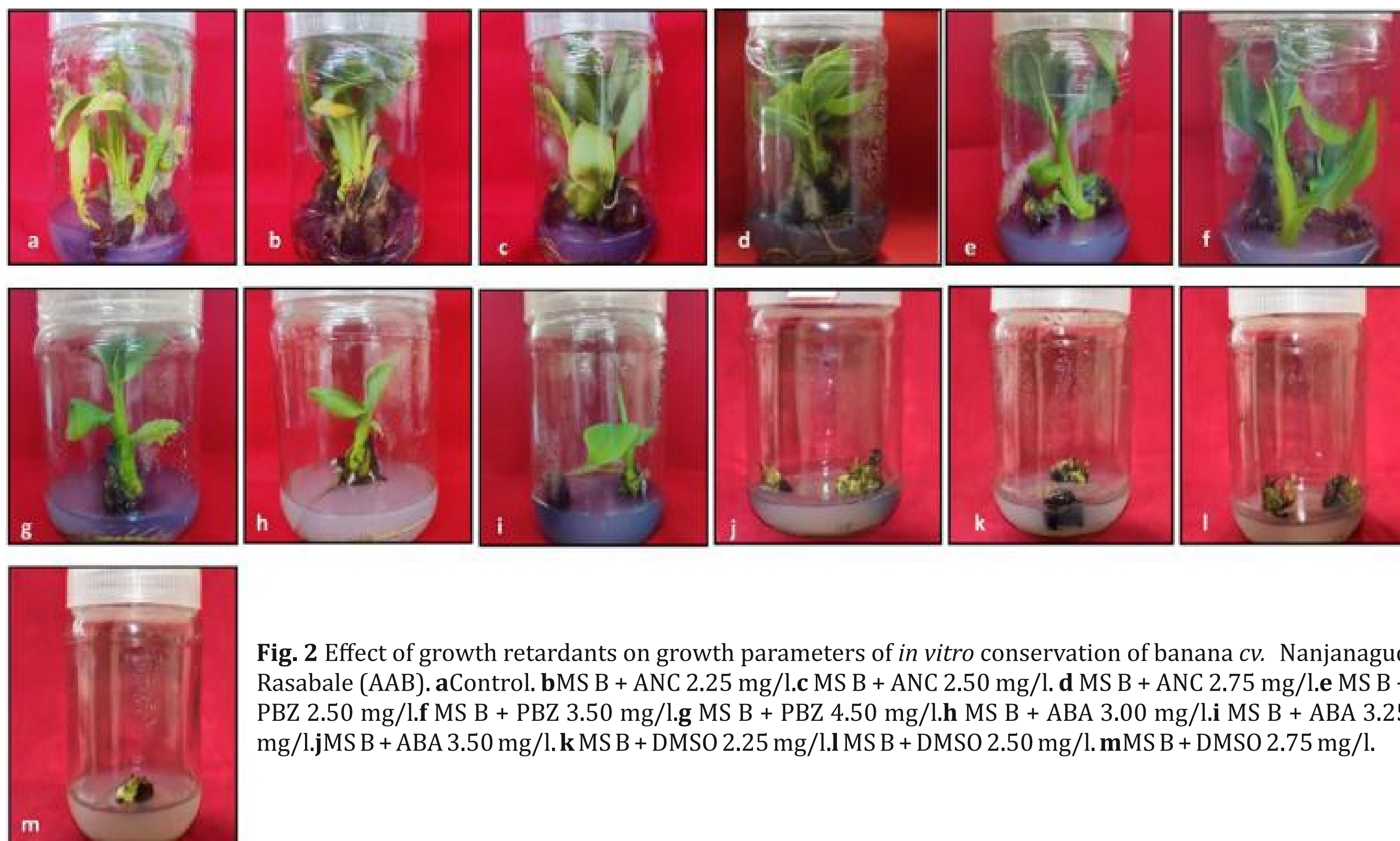

Fig. 2 Effect of growth retardants on growth parameters of *in vitro* conservation of banana *cv.* Nanjanagud Rasabale (AAB). **a**Control. **b**MS B + ANC 2.25 mg/l.**c** MS B + ANC 2.50 mg/l. **d** MS B + ANC 2.75 mg/l.**e** MS B + PBZ 2.50 mg/l.**f** MS B + PBZ 3.50 mg/l.**g** MS B + PBZ 4.50 mg/l.**h** MS B + ABA 3.00 mg/l.**i** MS B + ABA 3.25 mg/l.**j**MS B + ABA 3.50 mg/l. **k** MS B + DMSO 2.25 mg/l.**l** MS B + DMSO 2.50 mg/l. **m**MS B + DMSO 2.75 mg/l.

Lethal browning of the tissue, results in the death of the cultured explants, which in turn depends upon the rate of phenolic exudation as well as the quantity of the total phenols, which are the main problems in tissue culture systems [22]. Phenolic compounds are secondary metabolites that are frequently produced in large quantities in response to tissue injury and are released from injured explants. The oxidation of liberated phenolic compounds from the damaged tissue by polyphenol oxidase leads to the production of quinones causing the blackening of tissue [19] and ultimately cell death (necrosis) [22].

The findings of the present investigation revealed that culture media browning [Table 2] was found highest as the concentration of growth retardant was increased especially in conservation medium supplemented with PBZ (4.50 mg/l) and ABA (3.25 mg/l and 3.50 mg/l). Numerous internal and external factors frequently have an impact on phenol concentration and cultured media browning [29].

Concentrations of various growth retardants may affect the rate of phenolic exudation since phenols are reactive compounds [21]. Concentration of phenols in plants are influenced by certain nutrient elements and environmental stress factors such as drought, salinity, heavy metals, irradiation, pathogen infection, etc. from the damaged tissues. Since ABA is a stress hormone it may have led to an increase in the phenol concentration in the explant, which on exudation had a negative effect on the culture media browning leading to explant necrosis.

Vitrification (Hyperhydricity) is termed as glassy shoot syndrome where the affected shoots are swollen (pale green) and their leaves have a translucent, watery, or glass-like appearance with dark green leaves on stunted shoots. In the present study, vitrification symptoms were not observed in any of the treatments[Table 2]. Factors influencing vitrification are providing sub-optimal growth conditions (temperature, light, relative humidity), prolonged exposure to liquid media [9], nutrient composition (symptoms occur on high nutrient concentrated media than that of low concentration media), lack

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of oxygen, change in the media pH [6]. While growth retardants used in the present study restricted the growth of the cultures and reduced the nutrient availability to the developing plantlets. Moreover, the cultures were maintained under optimal growth conditions and also the solid media (8% agar-agar) was used with the pH adjusted to 5.74. Therefore, it may be possible for the almost negligible occurrence of vitrification symptoms in the *in vitro* conserved cultures. These findings are in close agreement with an earlier report on potatoes [27] where vitrification though frequently observed in micro plant cultures due to prolonged storage, could be controlled by supplementing ancymidol in the conservation medium.

Among the different treatments, the highest microbial contamination (11%) was recorded in T_2 and T_8 [Table 2]. Tissue culture plants may be contaminated due to endogenous fungi including yeasts, bacteria, and bacteria-like organisms [8] [16]. Most of the problems caused due to microbial contamination in the cultures is mainly due to human error such as unhealthy aged explants (high microbial load) which have been collected from: (1) plant tissues exposed to or near to soil; (2) plants grown in tropical conditions; or (3) overhead irrigated stock plants. Inappropriate surface sterilization protocol, aseptic handling of cultures, and the sub-standard laboratory environment (especially the cleanliness of floors, work surface inside the laminar flow cabinets, and the laboratory air) have also been shown to affect the rate of contamination [20]. Inappropriate sterilization and preparation of media along with some heat-resistant *Bacillus* sp. have been reported to survive even after autoclaving (121°C) the growth media.

Table 2. Effect of growth retardants on microbial contamination, media exhaustion, vitrification and media browning in banana cv. Nanjanagud Rasabale (AAB)

Treatment	Level of medium browning	Vitrification of tissue	Microbial contamination (%)	Days taken for media exhaustion/ sub-culture
T_1: MS B* (Control)	2	-	5.33 (13.34) b*	29.90 l
T_2: MS B + ANC 2.25 mg/l	1	-	11.00 (19.35) a	67.40 k
T_3: MS B + ANC 2.50 mg/l	1	-	5.33 (13.34) b	76.16 j
T_4: MS B + ANC 2.75 mg/l	1	-	0.00 (1.17) c	80.76 i
T_5: MS B + PBZ 2.50 mg/l	1	-	5.33 (13.34) b	81.60 i
T_6: MS B + PBZ 3.50 mg/l	1	-	0.00 (1.17) c	86.63 h
T_7: MS B + PBZ 4.50 mg/l	2	-	0.00 (1.17) c	91.70 g
T_8: MS B + ABA 3.00 mg/l	1	-	11.00 (19.35) a	95.06 f
T_9: MS B + ABA 3.25 mg/l	2	-	5.33 (13.34) b	105.23 e
T_{10}: MS B + ABA 3.50 mg/l	2	-	0.00 (1.17) c	121.36 d
T_{11}: MS B + DMSO 2.25 ml/l	1	-	0.00 (1.17) c	136.33 c
T_{12}: MS B + DMSO 2.50 ml/l	1	-	0.00 (1.17) c	139.00 b
T_{13}: MS B + DMSO 2.75 ml/l	1	-	0.00 (1.17) c	140.00 a
S. Em ±			0.30	0.47
CD at 1%			1.23	1.87

Note: MS B- Murashiage and Skoog basal medium*
Visual scoring (Vitrification): (+) Presence, (-) Absence
Visual scoring (Media browning)- 0: No browning, 1: Browning only at explant base, 2: Browning extended beyond explant base, 3: Complete browning of the medium

Analysis of genetic fidelity of regenerated plants

In the present study, normal healthy-looking shoots were retrieved from the *in vitro* conserved slow-growth cultures. For retrieval of growth, the cultures were grown in standard regeneration media [15]. In most of the culture media supplemented with growth retardants, the vegetative growth was normal[Table 3 Fig. 3]. However the growth parameters such as the number of shoots, shoot length, number of leaves, number of roots, and length varied due to the residual effect of growth retardants. The appearance of plants was normal except in the case of ABA (T_{10}) treated media. This may be due to the carryover effect of growth retardants from the conservation media. Similar results were reported [1] in Grand Naine banana after *in vitro* conservation using growth retardant supplements.

Table 3. Analysis of growth parameters and morphology of plantlets regenerated from culture conserved by usinggrowth retardants

Treatment	No. of shoots per explant	Shoot length (cm)	No. of leaves per shoot	No. of roots per shoot	Longest root length (cm)	Appearance of plant
T_1 (Control)	1.70a	7.20a	5.20a	0.00e	0.00c	+
T_2: MS B + ANC 2.25 mg/l	1.60ab	7.11a	5.00abc	4.50a	3.12a	+
T_3: MS B + ANC 2.50 mg/l	1.40ab	6.90a	4.90abc	4.20a	3.10a	+
T_4: MS B + ANC 2.75 mg/l	1.20ab	6.85a	4.90abc	4.10a	2.96a	+
T_5: MS B + PBZ 2.50 mg/l	1.40ab	7.09a	5.10ab	4.00ab	2.92a	+
T_6: MS B + PBZ 3.50 mg/l	1.20ab	7.00a	5.00abc	3.40bc	2.35b	+
T_7: MS B + PBZ 4.50 mg/l	1.20ab	6.82a	4.90abc	3.20c	2.31b	+
T_8: MS B + ABA 3.00 mg/l	1.20ab	5.43cd	4.70abc	3.20c	2.26b	+

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T_9: MS B + ABA 3.25 mg/l	1.00^{b}	5.24^{d}	4.20^{bc}	3.10^{c}	2.20^{b}	+
T_{10}: MS B + ABA 3.50 mg/l	1.00^{b}	3.90^{e}	3.00^{d}	2.10^{d}	2.01^{b}	++
T_{11}: MS B + DMSO 2.25 ml/l	1.40^{ab}	6.12^{b}	4.50^{abc}	3.10^{c}	2.35^{b}	+
T_{12}: MS B + DMSO 2.50 ml/l	1.20^{ab}	5.92^{bcd}	4.20^{bc}	3.10^{c}	2.24^{b}	+
T_{13}: MS B + DMSO 2.75 ml/l	1.00^{b}	5.87^{bc}	4.10^{c}	3.00^{c}	2.11^{b}	+
S. Em ±	0.14	0.13	0.20	0.15	0.11	
CD at 1%	0.56	0.53	0.79	0.62	0.45	

Note: + = Normal, ++ = Subnormal, +++ = Abnormal

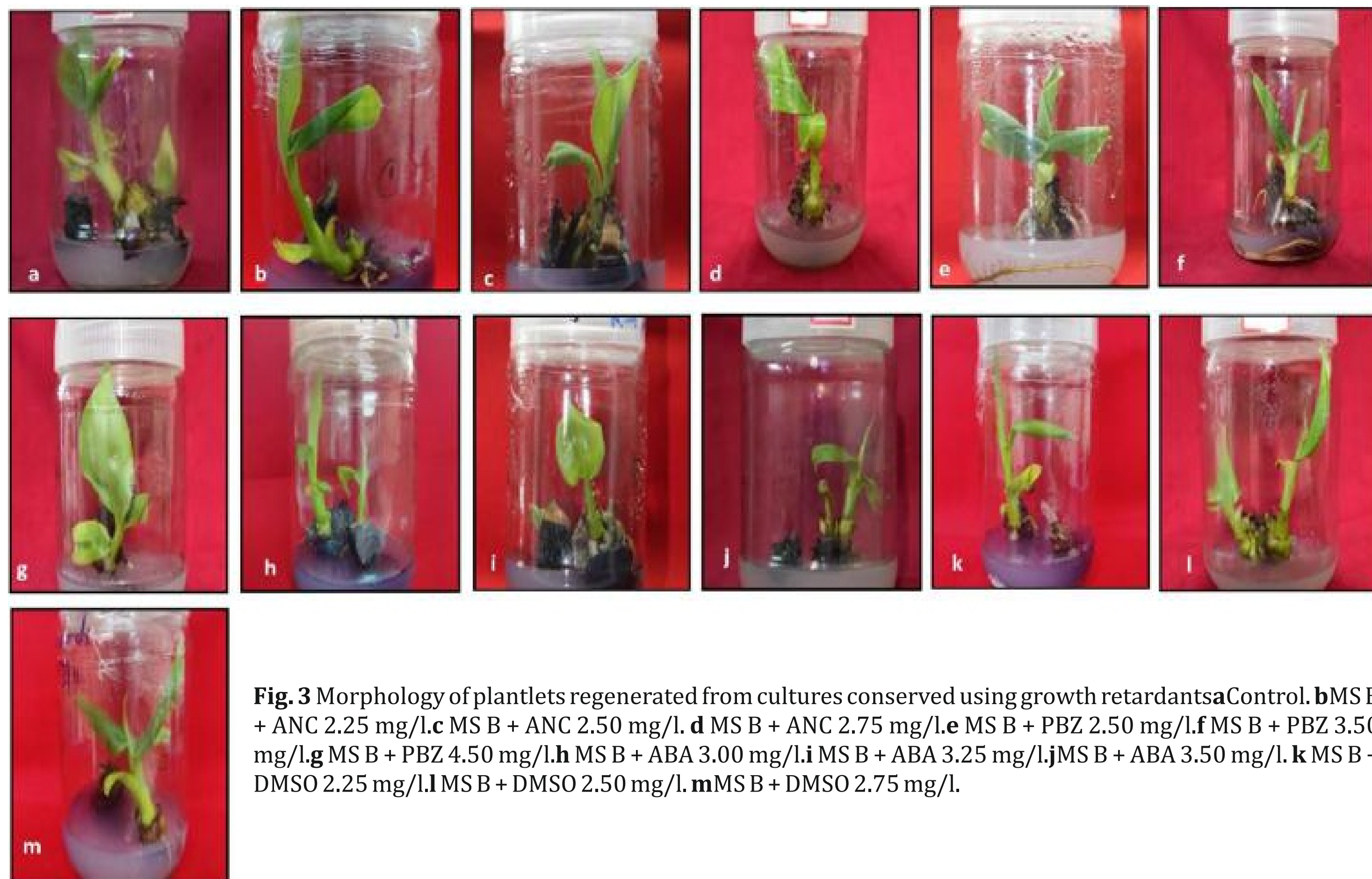

Fig. 3 Morphology of plantlets regenerated from cultures conserved using growth retardants**a**Control. **b**MS B + ANC 2.25 mg/l.**c** MS B + ANC 2.50 mg/l. **d** MS B + ANC 2.75 mg/l.**e** MS B + PBZ 2.50 mg/l.**f** MS B + PBZ 3.50 mg/l.**g** MS B + PBZ 4.50 mg/l.**h** MS B + ABA 3.00 mg/l.**i** MS B + ABA 3.25 mg/l.**j**MS B + ABA 3.50 mg/l. **k** MS B + DMSO 2.25 mg/l.**l** MS B + DMSO 2.50 mg/l. **m**MS B + DMSO 2.75 mg/l.

During *in vitro* conservation, the plants may lead to genetic changes and the plantlets may not be identical to the mother plant. The failure to assess the morphological polymorphism in plants does not negate the possibility of genetic variations and it requires critical analysis. Unambiguous identification is crucial in a crop that is clonally propagated such as banana. *In vitro* raised and conserved plants can be monitored using various techniques. The most reliable method is the use of molecular marker techniques. For quality control in plant tissue culture, it is believed that early identification of potential somaclonal variations is very important. Among the numerous molecular markers available, inter-simple sequence repeat (ISSR) is cost-efficient and requires lower amounts of DNA [31]. ISSR markers have been frequently used in establishing the genetic stability of several micropropagated plants in horticultural crops especially bananas, potatoes etc.

All the 15 ISSR primers amplified unambiguous, readable, and showed monomorphic bands. A total of 193 scoreable bands were produced with an average of 12.8 bands per primer. The results obtained from ISSR primers showed 100% monomorphism. The samples showing monomorphic banding patterns is presented [Table 4 Fig. 4]. Thus, the regenerated plantlets maintained the genetic stability even after long-term conservation on the same medium without sub-culturing. The degree of genetic stability brought on by *in vitro* culture has been linked to genotype rather than culture conditions in *Musa* [24]. The degree of genetic stability in bananas on the other hand, was shown to depend on the interaction between the genotype and the *in vitro* culture conditions [28]. These findings are in close congruence with reports of [2] on *Reseda pentagyna* -an endemic plant where 14 out of 15 ISSR primers amplified and produced monomorphic bands. Similar results have also been reported in *Populus alba* [25], *Taraxacum biennibiennium* [18], and *Vanilla* [5].

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Table 4. Analysis of genetic stability of in vitro regenerated plants of banana cv. Nanjanagud Rasabale by using ISSR markers

Primer No.	Nucleotide sequence (5' to 3')	No. of monomorphic bands	No. of polymorphic bands	Per cent Monomorphism	Per cent Polymorphism
UBC 829	ACACACACACACACACG	14	0	100	0
UBC 850	CTCTCTCTCTCTCTCTRC	14	0	100	0
UBC 840	TGTGTGTGTGTGTGTGG	13	0	100	0
UBC 852	GTGTGTGTGTGTGTGTYA	12	0	100	0
UBC 808	AGAGAGAGAGAGAGAGC	13	0	100	0
UBC 864	TCTCTCTCTCTCTCTCRT	14	0	100	0
UBC 815	CTCTCTCTCTCTCTCTG	12	0	100	0
UBC 836	TGTGTGTGTGTGTGTGC	12	0	100	0
UBC 855	ACACACACACACACACCTT	13	0	100	0
UBC 813	CTCTCTCTCTCTCTCTT	13	0	100	0
UBC 821	CACACACACACACACAG	13	0	100	0
UBC 841	GAGAGAGAGAGAGAGAY	14	0	100	0
UBC 899	GAGCAACAACAACAACAA	12	0	100	0
UBC 901	GAGCAACAACAACAACAA	11	0	100	0
UBC 862	AGCAGCAGCAGCAGCAGC	13	0	100	0
Total		193	0	1500	0
Average		12.8	0	100	0

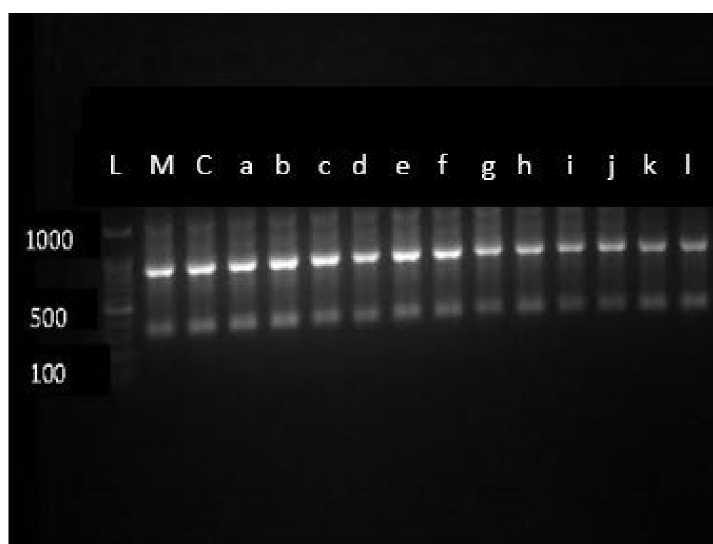

Fig. 4 Genetic fidelity test of *in vitro* conserved banana *cv.* Nanjanagud Rasabale (AAB) showing ISSR based DNA amplification pattern on agrose gel electrophoresis for UBC 850. **L** Ladder. **M** Mother plant. **C** Control. **a** MS B + ANC 2.25 mg/l. **b** MS B + ANC 2.50 mg/l. **c** MS B + ANC 2.75 mg/l. **d** MS B + PBZ 2.50 mg/l. **e** MS B + PBZ 3.50 mg/l. **f** MS B + PBZ 4.50 mg/l. **g** MS B + ABA 3.00 mg/l. **h** MS B + ABA 3.25 mg/l. **I** MS B + ABA 3.50 mg/l. **j** MS B + DMSO 2.25 mg/l. **k**MS B + DMSO 2.50 mg/l. **l** MS B + DMSO 2.75 mg/l.

Conclusion

For *in vitro* conservation of banana, media manipulation with growth retardants can be recommended for increasing the -sub-culture period and extending the storage period. In the present study among growth retardants and osmotic agents, growth retardant *i.e.* DMSO (2.25 mg/l) treated cultures were conserved for a longer period (154.53 days) without subculturing and regeneration was successful with normal morphology. Hence, DMSO was found to be best for *in vitro* conservation of banana *cv.* Nanjanagud Rasabale. ISSR markers are useful for testing the genetic fidelity of the conserved cultures.

Future Thrust

Further it is also worthwhile to study the *in vitro* response for conservation under the combined influence of different growth retardants and osmotic agents. The utility of encapsulation technology in extending the storability of cultures can also be explored.It is important to ensure that the germplasm conservation is not only viable but also has the potential to regenerate into complete plantlets in field condition, in sufficient numbers for any future needs. The molecular markers that have shown good amount of monomorphism can be used in other *Musa* spp. assessment of genetic stability.

Author contribution

All authors contributed to the study conception and design. The first draft of the manuscript was written by A and all authors commented on previous versions of the manuscript. All authors read and agreed to the published version of manuscript.

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Conflict of Interest Statement

The authors declare that they have no competing interests

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