World Journal of Agricultural Sciences 16 (1): 20-26, 2020
ISSN 1817-3047

DOI: 10.5829/idosi.wjas.2020.20.26

Conservation of Banana Germplasm under Minimal Growth Media and Low Light Conditions

El-Homosany Ahmed Abd El-Wahab

In vitro Storage and Cryopreservation Plant Laboratory,
National Gene Bank, Agricultural Research Center, Giza, Egypt

Abstract: Germplasm storage is an alternative for the conservation of plant genetic diversity, contributing to the improvement and maintenance of species. In this work, shoot tip explants of Grand Nain banana cultivar (about 2-2.5 cm in length) were excised from *in vitro* cultures and transferred to Murashige and Skoog (MS) media supplemented with different concentrations of sucrose or sorbitol (10, 20, 30, 40 or 50 gl^{-1}) and incubated under 23°C with photoperiod for 2 hours light with intensity of 500 lux for (2, 4, 6, 8 and 10 months). It was found that all shoot tip explants conserved on media with different concentrations of sucrose or sorbitol for 2 or 4 months were able to still alive as the survival percentage (100%). After 10 months of storage, the highest survival percentage (95.83%) were appeared when explants conserved on half strength MS medium with 10, 20 or 40 gl^{-1} sucrose and half strength MS media with 40 or 50 gl^{-1} sorbitol. Meanwhile, the lowest survival percentage (37.50 %) was noticed when explants conserved on full strength MS medium with 30 gl^{-1} sucrose (control). For conservation of microshoots in alginate beads, microshoots with five millimeter long were encapsulated with 3% sodium alginate and MS medium containing 1.0 $g1^{-1}$ Calcium chloride and placed into semi solid half strength MS medium at 23°C in complete darkness for 15, 30 and 45 days. Results showed the highest regrowth percentage (100%) of encapsulated microshoots were obtained with non encapsulated microshoots (control) and encapsulated microshoots after conserved for 15 days. While, the lowest regrowth of encasulated microshoots (66.66%) were observed when conserved for 45 days.

Key words: Banana · Encapsulation · *In vitro* conservation · Slow growth

INTRODUCTION

The banana belonging to the family Musaceae are one of the world's most important subsistence crops. Micropropagation has played a key role in banana and plantain breeding programs worldwide [1, 2]. Among others, *in vitro* culture is of great advantage for mass propagation of various vegetative propagated crops. Mass multiplication of tissue culture plants could be done in a short time. They are cheaper to transport than conventional suckers and the coupling with virus indexing allows for safe movement and exchange and conservation of germplasm [3].

Vegetatively propagated crops like banana are conserved in field gene banks. Field gene bank conservation also has tendency to undergo natural hazards and pest and diseases infestations. Therefore, *in vitro* conservation is an alternative tool that can be applied for vegetatively propagated crops including banana [4]. However, still the *in vitro* conservation is advantages over the other methods since it needs less labour and land requirement and easy germplasm exchange with other countries [5].

The storage of Musa germplasm has been carried out through *in vitro* conservation, which involves modifications of chemical and physical conditions that will reduce growth rate and enable long term storage without frequent explant transplantations. These include supplementing culture media with growth retardants, osmotic inhibitors or tissue dehydrators and using lower temperatures or lower light conditions [6-11]. Banana conservation under normal tissue culture condition showed very short conservation period. Therefore, frequent sub-culturing is needed. But it is very laborious, costly and there is a chance to human errors or mislabeling [12]. There are several reports of banana

Corresponding Author: El-Homosany Ahmed Abd El-Wahab, *In vitro* Storage and Cryopreservation Plant Laboratory, National Gene Bank, Agricultural Research Center, Giza, Egypt.

shoot cultures stored *in vitro*. Ko *et al*. [13] reported that banana shoot tips successfully *in vitro* conserved for 12 months on MS medium with addition of 3% sucrose under 15°C. Sunyoto [14] concluded that the salt modification and sucrose on MS medium *in vitro* can slow the explants growth, so it was appropriate for the purposes of banana cv. Ambon Kuning germplasm storage. Half strength MS + 6% sucrose is the better storage medium for banana explants *in vitro* than the most other storage tested media for 5 months at 16-18°C. Plantlets from the treatment of half strength MS + 6% sucrose is the lowest plantlets that had growth inhibition when compared to other treatments acclimatized (*in vivo*). Edirisinghe *et al*. [15] observed that period of *in vitro* cultures increases by reducing the mineral components in the medium. Furthermore, half strength MS media showed increase in conservation period than in full MS media.

The objective of this research was to study the effect of osmotic agents and low light on preservation of shoot tips of Grand Nain banana (*Musa* spp.) through tissue culture techniques.

MATERIALS AND METHODS

The present study was carried out during two years 2018 and 2019 in the *In Vitro* Storage and Cryopreservation Plant Laboratory of National Gene Bank, Agricultural Research Center. The study was performed through establishing rapid *in vitro* propagation of Grand Nain Banana cultivar and *in vitro* conservation using different media storage such as minimal growth medium with osmotic agents (sucrose and sorbitol). Planting materials of Grand Nain banana cultivar were collected from Behera governorate.

Explants Sterilization: The meristem was obtained from developing suckers and was brought to the laboratory. The suckers were washed thoroughly under running tap water and were cleaned by removing external pseudo stems and outer parts of the rhizomes. The explants were further cleaned and sized into 5-7 cm height. These explants were washed by keeping them samples under running tap water for one hour. Thereafter, explants were further trimmed and sterilized in 70% alcohol for two minutes under the aseptic conditions. Thoroughly washed explants were surface sterilized by immersing in Sodium hypochlorite solution was prepared using commercial bleach "Clorox" (5.25 % available chlorine) at 50 % concentration containing two drops of Tween 20 for 30 minutes. Finally, the explants were then rinsed three to four times with sterile distilled water.

Table 1: Composition of media tested for conservation of shoot tip explants.

No.	Treatments
1	Full MS + 30 g/l sucrose (control)
2	Half strength MS + 10 g/l sucrose
3	Half strength MS + 20 g/l sucrose
4	Half strength MS + 30 g/l sucrose
5	Half strength MS + 40 g/l sucrose
6	Half strength MS + 50 g/l sucrose
7	Half strength MS + 10 g/l sorbitol
8	Half strength MS + 20 g/l sorbitol
9	Half strength MS + 30 g/l sorbitol
10	Half strength MS + 40 g/l sorbitol
11	Half strength MS + 50 g/l sorbitol

Culture Media: MS medium Murashige and Skoog [16] supplemented with 3% sucrose and 8% agar was used as the culture medium. The pH of all media was adjusted to 5.7. Isolated explants of Grand Nain cultivar were cultured on MS medium supplemented with 5.0 $mg1^{-1}$ BAP and 0.1 $mg1^{-1}$ NAA as the culture initiation medium. Shoots were successfully multiplied on MS modified with adding 2.0 $mg1^{-1}$ BAP + 2.0 $mg1^{-1}$ Kin and 0.1 $mg1^{-1}$ NAA, four subcultures were done at 4 weeks intervals. The resulting *in vitro* shoots were placed on a half strength MS solid medium + 0.5 $mg1^{-1}$ IBA, 0.5 $mg1^{-1}$ NAA with 30 $g1^{-1}$ sucrose for 30-45 days to induce rooting. Potting mixture containing sand and peatmoss in the ratio of (1:1) by volume was mixed thoroughly and were placed into a 10 × 15 cm plastic pots for growing *in vitro* grown plantlets under *ex vitro* conditions.

Culture Environments: *In vitro* shoots were cultured in 350 ml glass jar each containing 25 ml of culture medium for shoot initiation and multiplication. All the cultures were maintained at 25±2°C in temperature by an air conditioner with photoperiod of 16 hours light with intensity of 3000 lux by white fluorescent lamps and 8 hours dark for the growth and development of culture.

Slow Growth Conditions: Media composition listed in the Table (1) were used to test the slow growth medium. Proliferated shoots (2.0-2.5 cm) at third subculture cycle in multiplication process were used. Isolated shoots were cultured in 150 mm height, 25 mm diameter test tubes, which contained 10 ml of medium and the test tubes were covered with double layer of polypropylene. Then the cultures were stored in the *in vitro* incubation unit at 23± 2°C temperature with photoperiod of 2 hours light with intensity of 500 lux by white fluorescent lamps and 22 h dark period. These cultures were maintained for ten months. Survival percentage of shoot tip explants of each treatment was recorded at the end of each conservation period (five conservation periods 2 months for each one).

Recovery of Conserved Explants: After 10 months of storage, shoot tip explants for all the treatments were transferred into the culture multiplication medium (MS medium with vitamins containing 2.0 $mg1^{-1}$ BAP + 2.0 $mg1^{-1}$ Kin and 0.1 $mg1^{-1}$ NAA, 30 $g1^{-1}$ sucrose and 8 $g1^{-1}$ agar) at 25± 2°C under a 16-h photoperiod for the recovery assessment. The cultures were considered to be survival and produced axillary shoots. Shoots recovered from slow growth culture were transferred to root induction medium consisting of half strength MS modified medium, 30 g/l sucrose, 0.5 $mg1^{-1}$ IBA, 0.5 $mg1^{-1}$ NAA and 8 $g1^{-1}$ agar. After promoting root initiation and development transferred and cultured on plastic pots filled with a mixture of peatmoss and sand (1:1 by volume) to acclimatization for 8 weeks in the green house.

Encapsulation in Alginate Beads: Excised microsoots with five millimeter long obtained from the multiple shoot production were placed into liquid MS medium containing half strength MS (macro nutrients) without calcium plus full strength MS (micro nutrients) supplemented with 3% sodium alginate (w/v) and 0.3% sucrose for 15 minutes. The mixture with microshoots was individually dispensed dropwise with a sterile pipette into liquid MS medium containing 1.0 $g1^{-1}$ Calcium chloride ($CaCl_2$) and 10 $g1^{-1}$ sucrose at room temperature for 30 minutes. Encapsulated microshoots were rinsed three times with sterilized distilled water. The beads were placed on semi solid half strength MS medium at 23°C in complete darkness for 15, 30 and 45 days. For plant regrowth after encapsulation in sodium alginate beads, the effects of duration of time in storage on regrowth of encapsulated microshoots were studied for a period of 45 days. Encapsulated microshoots were transferred to multiplication medium (mentioned previously). Plant conversion was calculated after 15 days of culture as the percentage of encapsulated microshoots that were able to grow after conservation.

Statistical Analysis: The experiments were arranged in completely randomized design (CRD) with 3 replications. Each treatment consisted of 8 culture tubes per replication. Duncan's multiple range test at 5% level was used to verify the differences between means of the treatments in all the experiments [17].

RESULTS AND DISCUSSION

The results in Table (2) show the effect of different conservation period and different sugar concentrations on survival percentage of "Grand Nain" banana shoot tip explants conserved at 23°C under low light (500 lux).

With regard to the effect of conservation period, results clearly showed that all shoot tip explants conserved for 2 and 4 months were able to still alive as the survival percentage was 100 %, this percentage reduced to 95.83, 91.67 % and 84.84 % when conservation period increased from 6, 8 to 10 months respectively without significant differences among them. The survival percentage decreased gradually when the conservation period gradually increased from 6, 8 to 10 months respectively without significant differences among them.

Concerning to the effect of different sucrose and sorbitol concentrations on conservation media, results clearly showed that conservation on half strength MS medium with 10, 20 and 40 gl^{-1} sucrose or half strength MS medium with 40 or 50 gl^{-1} sorbitol showed the highest survival percentage (99.17 %). This percentage decreased without significant differences with conservation on

Table 2: Effect of sugar types, concentrations and conservation periods on survival percentage of "Grand Nain" banana shoot tip explants conserved at 23º C under low light intensity for 10 months

	Period (month)					
Treatment	2	4	6	8	10	Mean
Full MS + 30 gl^{-1} sucrose (control)	100.00 a	100.00 a	58.33 f	50.00 g	37.50 h	69.17 B
Half strength MS + 10 gl^{-1} sucrose	100.00 a	100.00 a	100.00 a	100.00 a	95.83 ab	99.17 A
Half strength MS + 20 gl^{-1} sucrose	100.00 a	100.00 a	100.00 a	100.00 a	95.83 ab	99.17 A
Half strength MS + 30 gl^{-1} sucrose	100.00 a	100.00 a	100.00 a	91.66 bc	83.33 d	95.00 A
Half strength MS + 40 gl^{-1} sucrose	100.00 a	100.00 a	100.00 a	100.00 a	95.83 ab	99.17 A
Half strength MS + 50 gl^{-1} sucrose	100.00 a	100.00 a	100.00 a	95.83 ab	91.66 bc	97.50 A
Half strength MS + 10 gl^{-1} sorbitol	100.00 a	100.00 a	95.83 ab	87.50 cd	66.66 e	90.00 A
Half strength MS + 20 gl^{-1} sorbitol	100.00 a	100.00 a	100.00 a	87.50 cd	83.33 d	94.17 A
Half strength MS + 30 gl^{-1} sorbitol	100.00 a	100.00 a	100.00 a	95.83 ab	91.66 bc	97.50 A
Half strength MS + 40 gl^{-1} sorbitol	100.00 a	100.00 a	100.00 a	100.00 a	95.83 ab	99.17 A
Half strength MS + 50 gl^{-1} sorbitol	100.00 a	100.00 a	100.00 a	100.00 a	95.83 ab	99.17 A
Mean	100.00 A	100.00 A	95.83 A	91.67 A	84.84 A	

Means followed by the same letter (s) in each column are not significantly different from each other at 5 % level.

half strength MS medium with 50 gl^{-1} sucrose and half strength MS medium with 30 gl^{-1} sorbitol (97.50 %), conservation on half strength MS medium with 30 gl^{-1} sucrose, 20 and 10 gl^{-1} sorbitol which showed the survival percentage (95.00, 94.17 and 90.00 %), respectively. Explants conserved on full strength MS medium with 30 gl^{-1} sucrose (control) showed the lowest survival percentage (69.17 %).

With regard to the effect of interaction between conservation period and different sucrose and sorbitol concentrations on conservation media results revealed that all explants conserved on media with different concentrations of sucrose or sorbitol under investigation for 2 and 4 months were able to still alive as the survival percentage (100%).

After 6 months of storage, all explants conserved on media with different concentrations of sucrose or sorbitol were able to still alive as the survival percentage (100%) except the explants conserved on half strength MS medium with 10 gl^{-1} sorbitol (95.83%) without significant differences among them and the explants conserved on full strength MS medium with 30 gl^{-1} sucrose (control) as the survival percentage 58.33 % with significant percentage among all treatments.

After 8 months of storage, all explants conserved on half strength MS media with 10, 20 and 40 gl^{-1} sucrose and half strength MS media with 40 or 50 gl^{-1} sorbitol were able to still alive as the survival percentage (100%), this percentage decreased without significant differences with conservation on half strength MS medium with 50 gl^{-1} sucrose and half strength MS medium with 30 gl^{-1} sorbitol (95.83 %), the same survival percentage (87.50 %) was noticed when explants conserved on half strength MS medium with 10 or 20 gl^{-1} sorbitol. Explants conserved on full strength MS medium with 30 gl^{-1} sucrose for 8 months showed the lowest survival percentage (50.00 %).

The same trend was noticed after 10 months of storage, the highest survival percentage (95.83%) were appeared when explants conserved on half strength MS media with 10, 20 and 40 gl^{-1} sucrose and half strength MS media with 40 or 50 gl^{-1} sorbitol without significant differences among them, the survival percentage decrease to (83.33 %) when explants conserved on half strength MS medium with 30 gl^{-1} sucrose or 20 gl^{-1} sorbitol and (66.66 %) when explants conserved on half strength MS medium with 10 gl^{-1} sorbitol with significant differences between them. While, the lowest survival percentage (37.50 %) was noticed when explants conserved on full strength MS medium with 30 gl^{-1} sucrose (control).

Most shoot tips conserved on different media for 10 months were developed successfully when cultured on multiplication medium and rooted medium at 25± 2°C under a 16-h photoperiod and transferred to acclimatization conditions (Figure 2).

All shoot tip explants treated with sorbitol appeared some growth during storage for 10 months and some cultured shoot tip explants remained quiescent when treated with sucrose (Figure 3). These results were agreement with Ko *et al*. [13] and Edirisinghe *et al*. [15] who observed this behavior in the conserved banana cultures.

Our study, in close agreement to George *et al*. [18] who indicated that the use of osmotica like sucrose, sorbitol or manitol delayed growth and development of banana shoot tip explants and it resulted to increase the storage duration of the cultures and reported that banana cv. Dwarf Cavendish germplasm could be conserved for 8 months under 15°C temperature regime on half strength MS medium + 30 gl^{-1} sucrose and was the best minimum growth conditions for short term *in vitro* preservation of banana shoot tip cultures. Also, Edirisinghe *et al*. [15] reported that, salt concentration, sucrose and sorbitol on MS medium can slow the explants growth and

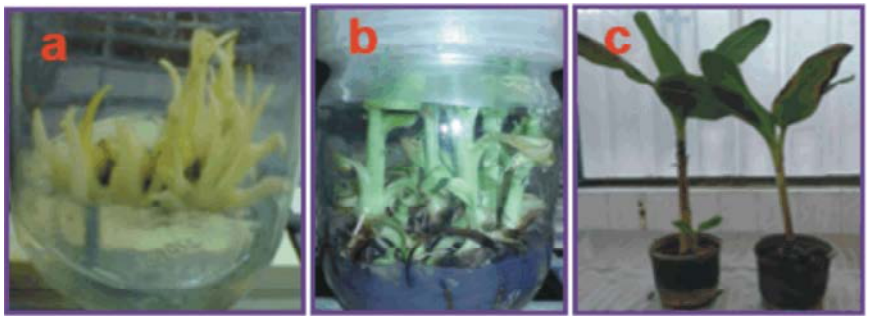

Fig. 1: Micropropagation of Grand Nain banana cultivar
a- Start stage on MS medium containing 5.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA
b- Multiplication stage on MS medium + 2.0 mgl^{-1} BAP + 2.0 mgl^{-1} Kin and 0.1 mgl^{-1} NAA
c- Acclimatization stage on medium containing Peatmoss: Sand (1:1) after one year

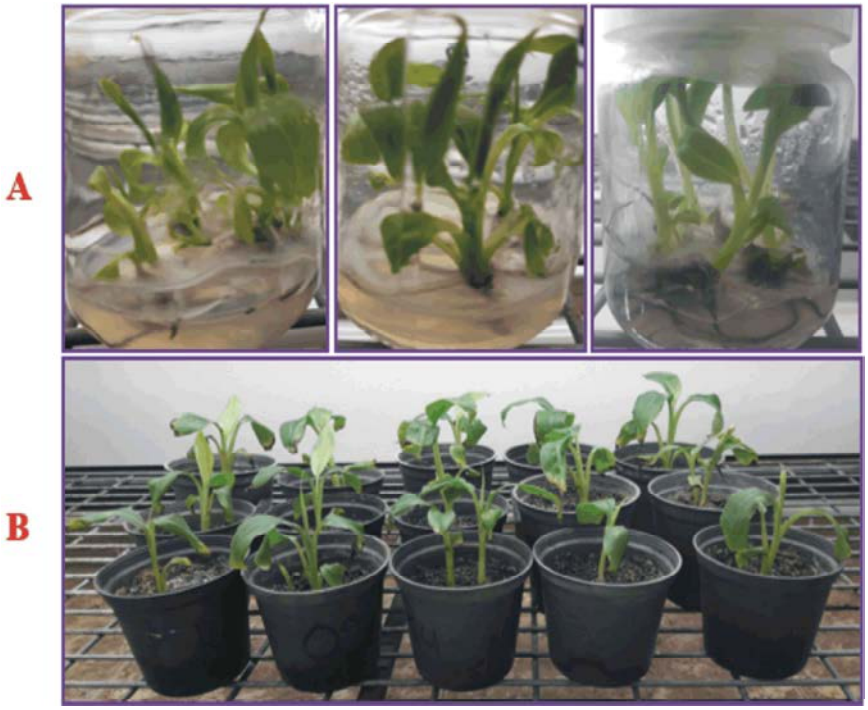

Fig. 2: Proliferation shoot tip explants conserved 10 months after cultured on mltiplication medium MS + 2.0 mg1^{-1} BAP + 2.0 mg1^{-1} Kin and 0.1 mg1^{-1} NAA (A) and acclimatization medium containing Peatmoss : Sand (1:1) after one month (B)

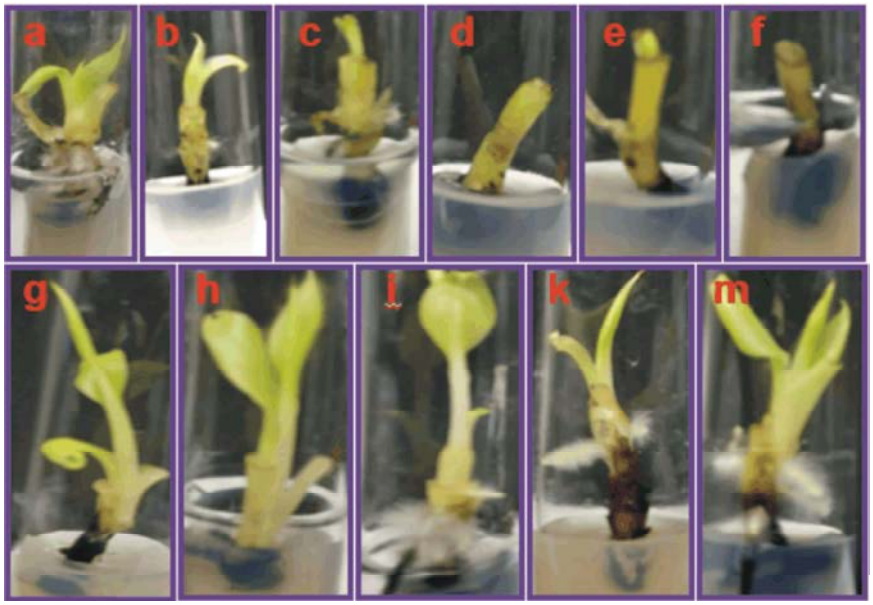

Fig. 3: Conserved Grand Nain" banana shoot tip explants at the end of 10 months on different media
a- On Full strength MS medium + 30 g/l sucrose (control)
b- On half strength MS medium + 10 gl^{-1} sucrose
c- On half strength MS medium + 20 gl^{-1} sucrose
d- On half strength MS medium + 30 gl^{-1} sucrose
e- On half strength MS medium + 40 gl^{-1} sucrose
f- On half strength MS medium + 50 gl^{-1} sucrose
g- On half strength MS medium + 10 gl^{-1} sorbitol
h- On half strength MS medium + 20 gl^{-1} sorbitol
i- On half strength MS medium + 30 gl^{-1} sorbitol
k- On half strength MS medium + 40 gl^{-1} sorbitol
m- On half strength MS medium + 50 gl^{-1} sorbitol

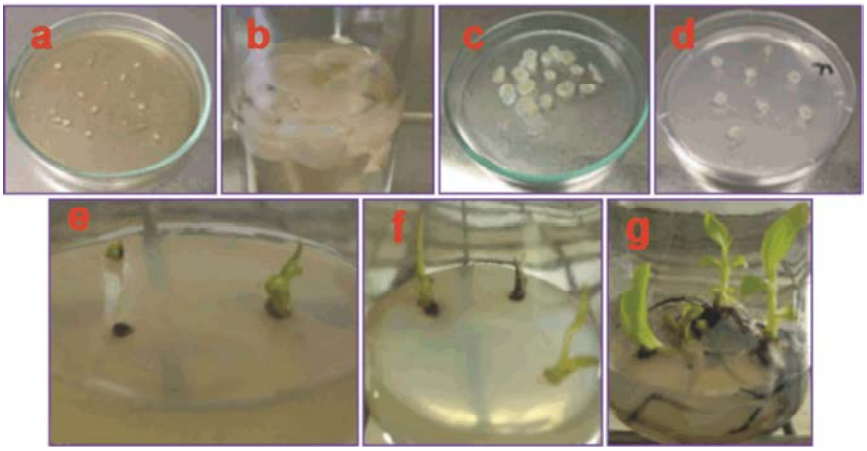

Fig. 4: Encapsulation microshoots of Grand Nain banana cultivar and regrowth percentage
a- Microshoots in sodium alginate b- Formation of capsules in calcium chloride
c- Encapsulated microshoots after rinsed with sterilized distilled water
d- Encapsulated microshoots on semi solid half strength MS medium free growth regulators
e- Regrowth percentage of encapsulated microshoots on multiplication medium after 15 days of culture, f- after 30 days of culture and g- after 60 days of culture

Table 4: Effect of conservation periods (days) on regrowth percentage of encapsulated microshoots Grand Nain banana cultivar at 23°C under complete darkness.

Treatment	Regrowth %
Non encapsulated microshoots (control)	100 A
Encapsulated microshoots for 15 days	100 A
Encapsulated microshoots for 30 days	75 B
Encapsulated microshoots for 45 days	66.66 B

Means followed by the same letter (s) in each column are not significantly different from each other at 5 % level.

appropriate for the purposes of banana germplasm storage. The medium combination half strength MS + 15 gl^{-1} Sucrose + 1% Sorbitol was best for *in vitro* storage banana explants of Ambon, Rath Kesel and Pulathisi varieties with nine months period at 25°C than the other tested conservation media combinations.

Results in Table (4) clear the effect of different conservation periods on regrowth percentage of encapsulated microshoots Grand Nain banana cultivar conserved at 23°C under complete darkness after cultured on multiplication medium.

Results about the effect of different conserved period revealed that different conservation periods affect significantly the regrowth of microshoots. The highest regrowth percentage of microshoots showed with non encapsulated microshoots (control) and encapsulated microshoots after conserved for 15 days (100%) without significant difference between them. Meanwhile, increasing the conservation period from 15, 30 to 45 days showed the decrease regrowth percentage (100, 75 and 66.66%) respectively, with significant differences among regrowth percentage of encapsulated microshoots conserved for 15 days and 30 or 45 days and non significant between regrowth percentage of encapsulated microshoots conserved for 30 and 45 days.

Studies on *in vitro* germplasm conservation using alginate bead techniques have been reported for many plant species, on the encapsulation of banana shoot tips Ganapathi *et al*. [19], *Morus* spp.; Pattnaik *et al*. [20]; *Plumbago zeylanica* L. Rout *et al*. [21] and *Ananas comosus* L. Merr. Soneji *et al*. [22].

These similar observations have been made with Kamnoon and Nararatn [23] who reported that microshoots for conservation of M. balbisiana 'Kluai Hin' were encapsulated in 3% sodium alginate prepared in MS medium and complexed with 50 mM calcium chloride. Maximum conversion frequency of 73% was noted from encapsulated microshoots cultured on MS medium supplemented with 22 μM BA. Plantlets developed from encapsulation were successfully transferred to field conditions and all the resultant plants were shown to be morphologically normal.

In conclusion, an effective micropropagation system for Grand Nain banana cultivar has been worked out utilizing shoot tip explants. Our studies also provide an efficient protocol for storage of Grand Nain banana cultivar microshoots under slow growth conditions at 23°C under photoperiod of 2 h light for 10 months without maintenance. In addition, germplasm conservation utilizing encapsulation technique using 3%

sodium alginate dissolved in liquid MS medium containing 1.0 $g1^{-1}$ Calcium chloride ($CaCl_2$) and 10 $g1^{-1}$ sucrose for complexation. Recovery plants were rooted and acclimatization successfully.

REFERENCES

1. Rowe, P. and F.E. Rosales, 1996. Bananas and plantains. Fruit Breed., 1: 167-211.
2. Vuylsteke, D., R. Ortiz, R.S.B. Ferris and J.H. Crouch, 1997. Plantain improvement. Plant Breed. Rev., 14: 267-320.
3. Hwan, S.C., C.L. Chen, J.C. Lin and H.L. Lin, 1984. Cultivation of Banana Using Plantlets from Meristem Culture. HortScience, 19: 231-232.
4. Ashmore, S.E., 1997. Status report on the development and application of *in vitro* techniques for the conservation and use of plant genetic resources. International Plant Genetic Resources Institute, Rome. pp: 67.
5. INIBAP, 2006. Global conservation strategy for Musa (Banana and Plantain). International Network for Improvement of Banana and Plantain, Montpellier, pp: 27.
6. Engelmann, F., 1997. *In vitro* conservation methods. In: Ford-Lloyd B.V., Newbury H.J., Callow J.A. (Eds.), Biotechnology and plant genetic resources: conservation and use. CABI, UK, pp: 119-162.
7. Daniells, J., C. Jenny, D. Karamura and K. Tomekpe, 2001. Musalogue: a catalogue of Musa germplasm. Diversity in the genus Musa. International Network for the Improvement of Banana and Plantain, INIBAP, pp: 213.
8. Buitink, J., F.A. Hoekstra and O. Leprince, 2002. Biochemistry and biophysics of tolerance systems. In: Black M., Pritchard H.W. (Eds.), Desiccation and survival in plants: Drying without dying. CABI Publishing, pp: 293-318.
9. Engelmann, F., 2004. Plant cryopreservation: progress and prospects. *In vitro* Cellular & Developmental Biology Plant, 40: 427-433.
10. Benson, E.E., 2008. Cryopreservation theory. In: Reed B.M. (Ed.), Plant cryopreservation: A practical guide. Springer, pp: 15-32.
11. Reed, B.M., 2008. Plant cryopreservation: A practical guide. Springer, USA, pp: 514.
12. Tyagi, R.K., A. Agrawal, R. Sanayaima and R. Tandon, 2010. Cost effective *in vitro* conservation of banana using alternatives of gelling agent (isabgol) and carbon source (market sugar). Acta Physiologiae Plantarum, 32(4): 703-711.
13. Ko, W.H., S.C. Hawng and F.M. Ku, 1991. A new technique for storage of meristem tip cultures of Cavendish banana. Plant Cell, Tissue and Organ Culture, 25: 179-183.
14. Sunyoto, M.D.N., 2013. Conservation of banana cv. Ambon Kuning on various media *in vitro* formula. ARPN Journal of Agricultural and Biological Science, 8(4): 322-328.
15. Edirisinghe, E.S.C., C.H. Denagamage and W.L.G. Samarasinghe, 2017. Effect of nutrient medium compositions in *in vitro* conservation of *Musa spp*. Annals of Sri Lanka Department of Agriculture, 19(2): 60-70.
16. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant, 15: 473-497.
17. Snedecor, C.W. and W.G. Cochran, 1982. Statistical Methods. 7 Ed. The Iowa State University in Ames, Iowa, USA.
18. George, L.T., A.A. Elhassan and M.A. Ali1, 2013. Effect of nutrient medium concentration and temperature on short-term *in vitro* conservation of shoot tip explants of banana. JONARES. 1: 37-40.
19. Ganapathi, T.R., P. Suprasanna and V.A. Bapat, 1992. Rao PS Propagation of banana through encapsulated shoot tips. Plant Cell. Rep., 11: 571575.
20. Pattnaik, S.K., Y. Sahoo and P.K. Chand, 1995. Efficient plant retrieval from alginate encapsulated vegetative buds of mature mulberry trees. Sci. Hort., 6: 227-239.
21. Rout, G.R., G. Das, S. Samantaray and P. Das, 2001. Micropropagation of *Plumbago zeylanica* L. by encapsulated nodal explants, J. Hort. Sci. Biotechnol., 76(1): 24-29.
22. Soneji, J.R., P.S. Rao and M. Mhatre, 2003. Germination of synthetic seeds of pineapple (*Ananas comosus* L. Merr.). Plant Cell. Rep., 20: 891-894.
23. Kamnoon, K. and P. Nararatn, 2012. Micropropagation and *in vitro* germplasm conservation of endangered *Musa balbisiana* 'Kluai Hin' (BBB group). African Journal of Biotechnology, 11(24): 6464-6469.