Gezira j. of agric. sci. 10(2): 118-124 (2012)

SHORT NOTE

Establishment of an encapsulation technique for *in vitro* propagated plantlets of banana (*Musa* AAA) cv. Albeely

Mohammed H. A. Othman[1], Mohamed A. Ali[2] and Igbal A. Abdellatif[1]

1 Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan. 2.Plant Tissue Culture, Agricultural Research Corporation, Wad Medani, Sudan.

Edible triploid banana are vegetatively propagated by suckers because viable seeds are not usually produced in these cultivars. New and effective means of propagating bananas would be advantageous, over the conventional use of sucker material, for germplasm maintenance, exchange and transportation. *In vitro* culture of floral apices or vegetative meristem is the most promising propagation method available to date (Ganapathi *et al*., 1992). Synthetic seeds are defined as artificially encapsulated somatic embryoes, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed, or other *in vitro*-derived tissue capable of conversion into plantlets under *in vitro* or *in vivo* conditions and that retain this potential after storage (Ganapathi *et al.*, 1992). Nevertheless, to reach such objective, the encapsulation technique must be firstly improved regarding its composition and conditions for short term storage. In the case of banana (*Musa* AAA), the use of synthetic seed technique may improve the quality of the plantlets and reduce the production costs. This technique was successfully employed in the cvs. 'Basrai' by Ganapathi *et al.* (1992). The successful demonstration of encapsulation of tissue culture derived propagules in a nutrient gel has initiated a new line of research on synthetic seeds. The use of artificial seeds makes the tissue culture techniques more advantageous due to rapid multiplication, maintained genetic uniformity of plants, direct

delivery propagules to the field, thus eliminating transplant and omitting the acclimatization steps, reduction of storage space, reduction in costs of vegetatively propagated plants and reduction in the breeding cycle (Maruyama *et al*., 1996). Therefore, the objectives of this study were to establish a technology for synthetic seeds production using tissue culture plantlets and test the viability of the developed synthetic seeds of the banana cv. “Albeely"

This study was conducted in the plant tissue culture laboratory at the Agricultural Research Corporation (ARC), Wad Medani, Sudan, during the year 2011. The banana cultivar used in this study was “Albeely”. Suckers 5 to 7 months old of banana plants were obtained from the greenhouse of the Agricultural Research Corporation, Wad Medani,Sudan.

The first experiment was conducted to establish an encapsulation technique for the banana cv. "Albeely and test the viability of synthetic seeds. Sodium alginate was added at a concentration of 4% (w/v) to liquid MS medium for shoot proliferation or sterilized distilled water. For complexation, 100 mM $CaCl_2 2H_2O$ solution was prepared using liquid medium for shoot proliferation**.** Both the gel matrix and complexing agent were autoclaved at 120 °C for 20 min and stored at room temperature. Encapsulation was accomplished by dipping the shoot tips in gel matrix, followed by dropping the shoot tips into the complexation agent for 30 min. Alginate beads containing shoot tips were collected and rinsed twice for 10 min each in sterilized water to wash away calcium chloride residues. Encapsulated shoot tips were placed in either empty glass jars or in glass jars containing 40 ml basal medium (MS) and stored at 4 °C and in a culture room at 25 °C. Viability of shoot tips was tested weekly for four weeks. The shoot tips were considered alive if they were still green, with no necrosis. Each treatment was replicated 5 times and 4 shoot tips represented an experimental unit.

The second experiment was conducted to test the viability of encapsulated shoot tips of the banana cv. "Albeely", stored at 4 °C on different MS strengths (full MS, half MS and quarter

MS). All cultures were maintained at 25 °C under 16 h lights with an intensity of 1500 lux. Data on survival of encapsulated shoot tips were scored after four weeks in culture. The third experiment was conducted to test the viability of encapsulated shoot tips of the banana cv. "Albeely", stored at 25 °C on different MS strengths (full MS, half MS and quarter MS). All cultures were maintained at 25 °C under 16 h light with an intensity of 1500 lux. Data on survival of encapsulated shoot tips were scored after four weeks. All Experiments were conducted using a completely randomized design with six replicates in micropropagation experiments and five replicates for storage of encapsulated shoot tips. Data were collected after 4 weeks. Analysis of variances was carried out using MSTATC computer program and Duncan's Multiple Range Test was used for means separation (MSU, 1993).

Effect of storage temperature on viability of encapsulated shoot tip explants of the banana cv. "Albeely" after 4 weeks

The viability of encapsulated shoot tips decreased significantly with increased storage time at both temperatures (5 °C and 25 °C). However, the reduction in viability was more at 25 °C in comparison with 5 °C (Table 1). The percentage of viable encapsulated shoot tips of the banana, cv. "Albeely, decreased significantly after three and four weeks at both temperatures. The percentages of viable encapsulated shoots at both temperatures were comparable in the first and second weeks. The viability at 25 °C was comparable after three and four weeks, while significant differences were observed in shoot viability after three and four weeks at 5 °C. In agreement with these results, Redenbaugh *et al*. (1987) found a decrease in the viability of encapsulated shoot tips with an increase in the storage period at similar temperatures (25 °C and 5 °C). Better survival of encapsulated shoot tips was probably due to the slow desiccation rate of synthetic seed that might have occurred under low temperature (5 °C). Kitto and Janick (1985) reported that asexual carrot embryoes survived for as long as 16 days at 4 °C storage temperature compared to 4 days at 26 °C. Ravindra and Staden (2005)

reported that 4 °C is ideal for long term storage, whereas at other temperatures, beads failed to regenerate. In addition, storage of alginate- encapsulated shoot tips at low temperature (4 °C) was found to be an acceptable method for conservation of germplasm cultured *in vitro* (Maruyama *et al*., 1997). Brodelius *et al*. (1982) reported that the alginate bead effect on growth might be attributed to a reduction in the respiration process in encapsulated tissue.

Table 1. Effect of storage temperature on viability of encapsulated shoot tip explants of the banana cv. "Albeely" after 4 weeks.

Storage period (weeks)	Percent viability of encapsulated shoot tips				
	Storage temperature (°C)				
	5		25		
One	(100)	10.0 a	100	10.0 a	
Two	(100)	10.0 a	90	9.5 ab	
Three	(85)	9.2 b	80	8.9 bc	
Four	(75)	8.7 c	70	8.4 c	
Mean	(90)	9.5	(85)	9.2	
SE±	0.15	0.15		0.19	
C.V (%)	3.9	6.5			6.5
Significance level	***	**		**	

Means in columns followed by different letter(s) are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.
Data between brackets are original.
** and *** = Significant at $P \leq 0.01$ and $P \leq 0.001$, respectively.

Effect of storage temperature on viability of encapsulated shoot tip explants of banana cv. "Albeely" on different dilutions of MS medium after 4 weeks

The percentages of viability of encapsulated shoot tip explants of the banana cv. "Albeely" decreased significantly with the

decrease in the strength of MS basal salts at both storage temperatures (Table 2). However, the decrease in viability was more at 25 °C compared with 5 °C. The percentage of viable encapsulated shoot tips for full MS at 5 °C and 25 °C storage temperature was 100% and 95%, respectively. For half MS the viability of encapsulated shoot tips was 95% and 85% at 5 °C and 25 °C, respectively. For quarter MS the viability of encapsulated shoot tips was 85% and 75%, respectively. Viability of encapsulated shoot tips was better at 5 °C than at 25 °C. At 5 °C, the encapsulated shoot tip viability on dry (empty gar) was 70% whereas at 25 °C, the viability of encapsulated shoot tips was 60%. In a similar study, encapsulated microshoots of the banana cv. "Grand Nain" showed 100% shoot emergence in response to treatments with MS solely or in combination with PEG (10% and 20%) after a short-term storage of 10 days at 4 ºC (Sandoval-Yugar *et al*., 2008). After 4 weeks, the percentage of viable synthetic seeds was higher when they were conserved on MS medium (96%) compared to those conserved in empty gars (35%). Synthetic seeds storage at 4 °C for a longer duration reduced the viability of the encapsulated shoot tips.

Table 2. Effect of storage temperature on viability of encapsulated shoot tip explants of the banana cv. "Albeely" on different strengths of MS after 4 weeks.

MS medium dilution	Percent viability of encapsulated shoot tips			
	Storage temperature (°C)			
	5		25	
Full MS medium	(100.0)	10.0 a	(95.0)	9.7 a
Half MS medium	(95.0)	9.7 ab	(85.0)	9.2 ab
Quarter MS medium	(85.0)	9.2 b	(75.0)	8.7 b
No MS medium	(70.0)	8.4 c	(60.0)	7.7 c
Mean	(87.5)	9.3	(78.8)	8.8
SE±		0.2		0.2
CV. (%)		6.4		7.3
Significance level		***		***

Means in columns followed by different letter(s) are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.
Data between brackets are original.
MS = Murashige and Skoog (1962).
*** = Significant at $P \leq 0.001$

These results are in agreement with the findings of Sarmah *et al*. (2010). It is thought that the reduction in the germination percentages observed with encapsulated propagules stored for 3 months might have been caused by inhibition of respiration by the alginate in the capsule (Bajaj, 1995). Future work is needed to determine the best storage conditions for maximization of viability and regeneration of encapsulated explants.

REFERENCES

Bajaj, Y.P.S. 1995. Somatic Embryogenesis and Synthetic Seed. In: Biotechnology in Agriculture and Forestry. Vol. 30. Springer-Verlag, Berlin, Germany.

Brodelius, P., L. Linse and K. Nilsson. 1982. Viability and biosynthetic capacity of immobilized plant cells, pp 371. In: A. Fujiwara (ed.) Plant Tissue Culture. Proceedings of

the 5th International Congress of Plant Tissue Cell Culture, Maruzen, Tokyo, Japan.

Ganapathi, T. R, P. Suprasanna, V. A. Bapat and P.S. Rao. 1992. Propagation of banana through encapsulated shoot tips. Plant Cell Report 11: 571-575.

Kitto, S. L. and J. Janick. 1985. Production of synthetic seeds by encapsulating asexual embryoes of carrot. Journal of American Society for Horticultural Science 110: 277-282.

Maruyama E., I. Kinoshita, K . Ishii, k. Ohba and A. Satio. 1996. Germplasm conservation of the tropical forest trees. *Cedrela Odorata* L., *Guazuma Criinita* and *Jacaranda mimosaefolio* D. Don, by shoot tip encapsulation in calcium alginate and storage at 12-25 ºC. Plant Cell Report 16: 393-396.

MSU. 1993. MSTAT-C Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State University, East Lansing, Michigan. USA.

Ravindra, B. and V. Staden. 2005. Storability and germination of sodium alginate encapsulated somatic embryoes derived from the vegetative shoot apices of mature *Pinus patula* trees. Plant Cell, Tissue and Organ Culture 82: 259-265.

Redenbaugh, K., D. Slade, P. Viss and J. A. Fujii. 1987. Encapsulation of somatic embryoes in synthetic seed coats. Hortcultural Science 22: 803-809.

Sandoval-Yugar, E. W., L. Vesco, D. A. Steinmacher, E. C. Stolf and M. P. Guerra. 2008. Microshoots encapsulation and plant conversion of *Musa* sp. cv. Grand Naine. Ciência Rural, Santa Maria 39 (4): 998-1004.

Sarmah, D.K., M. Borthakur and P. K Borua. 2010. Artificial seed production from PLBs regenerated from leaf base of *Vanda Coerulea* Grifft. Ex. Lindl. An endangered orchid. Current Science 98: 686-690.

تأسيس تقنية الكبسلة للنباتات المكاثرة نسيجيا من الموز (*Musa* AAA) صنف البيلي

محمد حمود علي عثمان[1]، محمد احمد علي[2] و اقبال عبد القادر عبد اللطيف[1]

1 كلية العلوم الزراعية، جامعة الجزيرة، ود- مدني- السودان.

2 هيئة البحوث الزراعية – واد مدني - السودان

الخلاصة

أجريت هذه الدراسة في معمل زراعة اللأنسجة النباتية في هيئة البحوث الزراعية 'واد مدني'السودان'خلال العام 2011. كان الهدف من هذه الدراسة هو تأسيس تقنية الكبسلة وانتاج البذور اللاصطناعية باستخدام شتلات مكاثرة نسيجيا للموز صنف البيلي واختبار حيويتها. تمت عملية الكبسلة للنموات الطرفية بحجم 0.5 سم للموز باستخدام الجينات الصوديوم بتركيز 4% مع كلوريد الكالسيوم بتركيز 100 ملي مول خلال 30 دقيقة من التبادل اللايوني تمت عملية الكبسلة. اجري اختبار الحيوية للبذور الصناعية بعد خزنها تحت درجتي حرارة 5 و25 مئوية علي الوسط الغذائي لموراشي و سكوج (1962) لمدة 4 اسابيع. تناقصت الحيوية تناقصا معنويا مع زمن الخزن في كلتا الدرجتين. كما تم اختيار الحيوية للبذور اللاصطناعية بالخزن علي وسط غذائي (جافة) تناقصت تناقصا معنويا مقارنة بتلك المخزنة علي مختلف اللأوساط الغذائية اللأخري خلال 4 أسابيع من الخزن للبذور اللاصطناعية لصنف الموز البيلي. الوسط المغذي الصناعي لموراشيجي و سكوج (1962) غير المخفف كان اللأفضل لخزن البذور اللاصطناعية لصنف الموز البيلي.