Journal of Plant Sciences
2018; 6(3): 93-100
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20180603.13
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

SciencePG
Science Publishing Group

The Use of Encapsulation-Dehydration Technique for Short-Term Preservation of Endangered Sweet Potato Landraces (*Ipomoea batatas* Lam) from Benin

Arsène Mahoutondji Doussoh[1], Justine Sossou Dangou[2], Arnaud Agbidinoukoun[1, *], Serge Sètondji Houedjissin[1], Corneille Ahanhanzo[1]

[1]Department of Genetic and Biotechnology, Faculty of Science and Technology, University of Abomey-Calavi, Central Laboratory of Plant Biotechnology and Plant Breeding, Abomey-Calavi, Benin

[2]Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Laboratory of Research in Applied Biology, Abomey-Calavi, Benin

Email address:

dousserne@yahoo.fr (A. M. Doussoh), dangouj@yahoo.fr (J. S. Dangou), arnaudag2002@yahoo.fr (A. Agbidinoukoun), sergesth01@yahoo.fr (S. S. Houedjissin), corneillea@yahoo.com (C. Ahanhanzo)

[*]Corresponding author

To cite this article:

Arsène Mahoutondji Doussoh, Justine Sossou Dangou, Arnaud Agbidinoukoun, Serge Sètondji Houedjissin, Corneille Ahanhanzo. The Use of Encapsulation-Dehydration Technique for Short-Term Preservation of Endangered Sweet Potato Landraces (*Ipomoea batatas* Lam) from Benin. *Journal of Plant Sciences*. Vol. 6, No. 3, 2018, pp. 93-100. doi: 10.11648/j.jps.20180603.13

Received: June 25, 2018; **Accepted**: July 11, 2018; **Published**: August 6, 2018

Abstract: *Ex situ* conservation of genetic resources remains a challenge for preservation of vegetatively propagated species such as sweet potato. The objective of this study was to establish the protocol based on the encapsulation-dehydration technique for short term preservation of endangered sweet potato landraces produced in Benin. Thus, the evaporative dehydration duration on silicagel was previously determinated on the empty beads which were made using alginate sodium (3%) and calcium chloride (1.32M). Then, the young shoots of two sweet potato landraces (Koïdokpon and Dokoui carotte) growing in the screen house were cutted aseptically and desinfected with 10% sodium hypochlorite. The apices were excised on stereoscope and were encapsulated prior their dehydration on silicagel during 5 and 6h. The encapsulated apices were finally conserved in eppendorf tubes at 2°C in batches for 15 days and 90 days. The encapsulated apices were cultured in MS medium supplemented with 0.15 mg/l BAP, 0.2 mg/l NAA, 0.08 mg/l GA3 and 80 mg/l adenine sulfate. The survival and the regeneration rates were then evaluated. At our experimentation condition, the dehydration duration which allowed around 20% water content of the beads was 6h. For the encapsulated apices, the highest survival rates (59.26% and 37.04%) and the highest regeneration rate (37.04% and 11.11%) were recorded respectivelly with the landraces "Koïdokpon" and "Dokoui carotte" when the apices were dehydrated by 6h and stored for 15 days. The regeneration rates decreased according to the stored duration. Significant difference was noted on the regeneration of apices for the landraces tested. This method can be used to preserve the endangered sweet potato landraces and other species during at least three months without subcultures. It also reduce the cost of conservation in terms of consumables and permit better genotype stability during the storage.

Keywords: Sweet Potato, *Ex Situ* Preservation, Artificial Seeds, Shoot Apices, Benin

1. Introduction

Sweet potato (*Ipomoea batatas*) is the third tuber crop which contribute to food security near to cassava and yams in Benin. Many landraces have been cultivated based on the specific criteria such as earliness, high yield and market value [1, 2]. However, many constraints limit their agronomic performance and the quality of the planting material such as pathogen attack, poor storage of the tubers, preference of farmers for high yield varieties leading to the progressive abandonment followed by the disappearance of some landraces. Therefore, the conservation of the diversity is a challenge for future program of breeding and varietal improvement. At this end, tissue culture techniques are reliable tools for the production and propagation of virus-free plantlets, conservation, germplasm exchange for

breeding and research purposes [3, 4]. Besides, ex situ conservation by *in vitro* culture techniques is one of the most widely used pathways in the world to preserve genetic resources, especially those with vegetative propagation [5]. The constitution of *in vitro* collection through micropropagation has been reported by several studies in preservation using short and medium term way [6, 7]. It is based on slowdown in growth of vitroplants by modification of the composition of the culture medium or by changes in culture conditions of which temperature and illumination [8, 9]. However, this technique remains limited by the appearance of somaclonal variations, as the number of subcultures increases. It presents risks of contamination during transplanting and remains very expensive in terms of consumables for poor countries [10]. Many plant species have been stored *in vitro* under minimal growth conditions such as low temperature, low irradiance, reduced carbon or nutrient supply, or in the presence of growth retardants [11, 12]. Another strategy for *in vitro* preservation is the application of alginate encapsulation technology to produce capsules and synthetic seed [13]. The alginate coat of encapsulated explants protects plant tissues against physical and environmental damage, minimises dehydration, and provides mechanical pressure to hold explants within the gel matrix during long-term storage [14]. Alginate encapsulation can be simpler and more cost-effective than cryopreservation and has been used successfully for storage of a wide range of woody species [15, 16]. Critical factors often associated with successful encapsulation in species include plant hormones, genotype, capsule or medium nutrient composition, planting substrate, storage temperature and storage medium [17-19]. Thus the present work aims to establish alginate encapsulation technique for storage of two sweet potato landraces from Benin by (i) to determine the duration of dehydration of the alginate beads with 20% water content of fresh weight and (ii) to evaluate the survival and the regeneration rates of the apices preserved at 2°C after encapsulation-dehydration on a culture medium.

2. Material and Methods

2.1. Plant Materials

The plant material constituted of two sweet potato landraces, "Dokoui carotte" and "Koïdokpon" identified to be extinct in Southern Benin [1] and which colored flesh are an indicator of vitamin A richness (Figure 1).

“Dokoui carotte” with skin and flesh orange

“Koïdokpon” with red skin and yellow flesh

Figure 1. *Extinct sweet potato landraces in Southern Benin.*

2.2. Determination of the Optimal Dehydration Duration of the Empty Beads

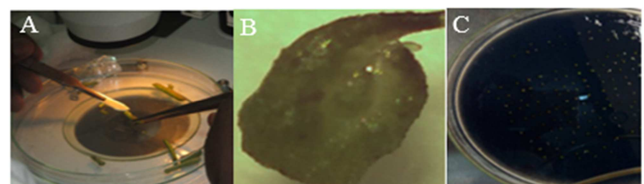
The protocol used to produce the alginate beads and to determine the times of dehydration is already used by Gonzalez-Arnao and Engelmann with some modifications [5]. Sodium alginate solution (3%) is poured dropping using a sterile pipette into calcium chloride solution (polymerization solution) under laminar flow. These solutions were previously prepared and sterilized. After 20 minutes, the alginate beads formed were dried on filter paper. The beads were transferred into a 0.75M sucrose solution contained in an Erlenmeyer flask covered with aluminum foil. The solution was shacked for 24 hours for osmotic dehydration. The beads were removed and drying, after placed in boxes containing 15 g of silica gel coated with aluminum foil previously sterilized at 103°C in an oven and cooled in a hood. By interval times (0h, 2h, 4h, 5h, 6h, 7h and 8h), each batch of beads was removed from the silica gel boxes and put in the oven for 24 hours.

2.3. Disinfection of Explants and Stem Meristem Dissection

Stem fragments of three to five nodes (axillary buds) were cutted from the mother plants of two sweet potato landraces previously cultivated in greenhouse. They were subsequently disinfected in 70° alcohol for 1 minute and 10% of sodium hypochlorite for 15 minutes followed by three rinses under horizontal laminar flow. The stem meristems (apices) were taken under a binocular stereoscope using scalpels mounted with lamina No. 10.

2.4. Pre-culture of Apices

The apices taken from each sweet potato landraces were deposited on a preculture medium M1 (MS + 2g /l of activated charcoal + 102.6 g /l of sucrose + 5.6 μM of ascorbic acid) in petri dishes. These boxes were wrapped in aluminum foil and put in the culture room for 24 hours (Figure 2).

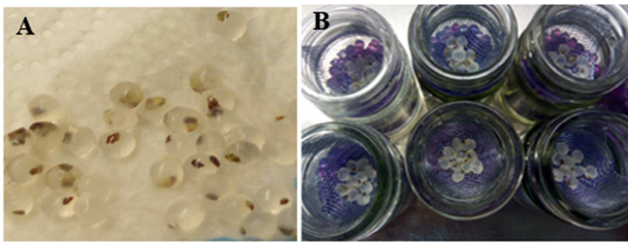


A: Dissection; B: Apices; C: Apices on M1 medium.

Figure 2. *Apices excided on stereoscope and cultured on M1 medium.*

2.5. Encapsulation-Dehydration

A part of apices pretreated on M1 medium (360 apices with 180 apices per landrace) were taken up in sodium alginate solution at 3%. To coat the apices in the beads, the alginate solution containing the apices is poured drop by drop using a sterile pipette in calcium chloride solution was used as solution of polymerization. The apices encapsulated were dried on sterile filter paper and were then immersed in 0.75 M sucrose pre-culture solution and stirred for 24 hours. They are again dried on filter paper and then divided into five lots of 36 per variety. The first batch is the control batch which has not undergone dehydration and directly cultured on M2 medium (MS + 2g /l of activated charcoal + 30g /l of sucrose + 0.15mg /l of BAP + 0.2 mg /l NAA + 0.08 mg /l GA3 + 80 mg /l adenine sulfate). The second and third batches were dehydrated on silica gel contained in boxes for 5 hours and 6 hours respectively (Figure 3) and they were placed in a refrigerator at 2 ° C for two weeks (15 days). The fourth and fifth batches were dehydrated on silica gel respectively for 5 hours and 6 hours and placed in a refrigerator at 2° C for three months (90 days). They were then taken again on a medium of culture M2, put in the dark during 7 days then transferred to the light in the culture room.



A: Apices encapsulated; B: Evaporative dehydration of the apexes on silica gel

Figure 3. *Apices encapsulated and dehydrated on silica gel.*

2.6. Analytic Parameters and data Analysis

The experimental design is a completely randomized block. The determination of optimal dehydration time of alginate beads, at each time interval, 10 beads were used per silica gel box with three repetitions. Following the time intervals, the beads removed from each silica gel box were weighed for getting fresh water of beads (FWt). These beads were transferred to the oven at 103°C for 24 hours. After oven, the beads were weighted again for its dried water (DWt). The water content (WC) of the dehydrated beads at each time interval was then determined by the formula:

$$WC\ (\%) = \frac{(FWt - DWt)}{FWt} X\ 100$$

Descriptive statistics were used for data analysis. The average of the water contents of beads dehydrated for each time interval were calculated and were used to construct the curve of dehydration.

The evaluation of the survival apices on M2 medium after encapsulation-dehydration, 12 apices per variety were used with three repetitions and constituted each apex batch. The parameters evaluated in this experiment were as follows:

a) Apices survival averages by treatment from encapsulation-dehydration. They were determined two weeks after culturing on the M2 medium by counting the number of viable apices. The viable apices are those on which the green color has been observed.
b) Apices regeneration averages rates per treatment resulting from encapsulation-dehydration. They were determined six weeks after culturing on the M2 medium. Regenerated apices are those that have formed at least one leafy shoot in relation to the total number of apices.

An analysis of variance preceded by the tests of Ryan-Joiner and Levene were carried out to verify the normality and equality of the variance. When the analysis of variance indicates a significant difference, Student-Newmann-Keuls test at the 5% threshold was performed to compare the averages. The different statistical analyzes were carried out with the Minitab 16 software.

3. Results

3.1. Optimal Time of Dehydration of Beads

The data collect was used to determine the water content of the empty beads per time interval. Table 1 shows the weight of beads and their water content. The average of water content of the beads after pre-culturing in sucrose solution is 69.74%. It decreases rapidly to reach 49.99% and 29.46% respectively after 2h and 4h of dehydration on silica gel. This content decreases gradually to 20.96% after 6 hours of dehydration (Figure 4). Thus, the immersion duration of 6h represents the time of dehydration of encapsulated apices on silica gel.

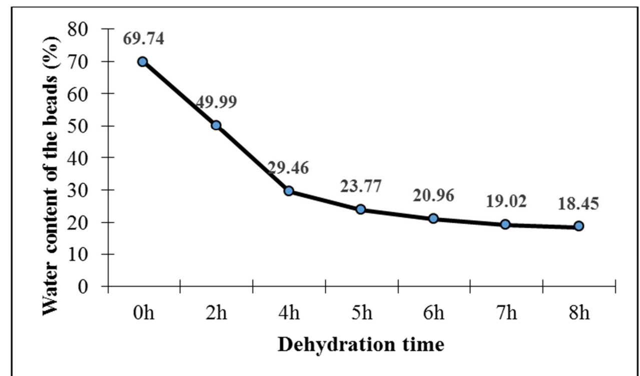


Figure 4. *Dehydration curve of beads.*

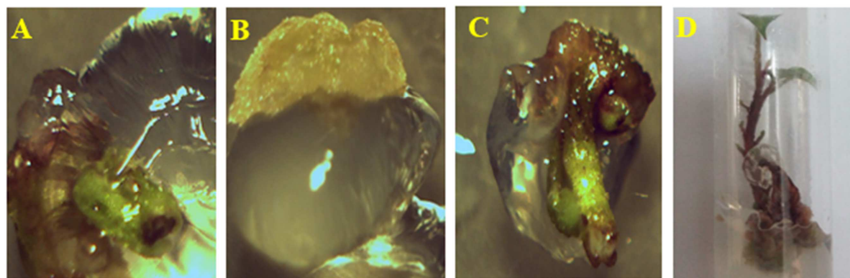
Table 1. Weight of fresh and dried beads per time interval.

Dehydration		After osmotic dehydration			After dehydration in oven.				
Hours	Replicate	P_vC (g)	P_vC + (g)	FW (g)	P_vC + B (g)	DW (g)	FWt – DWt	$\frac{(FWt - DWt)}{FWt}$ X 100	Average water content (WC)
	0a	4.03	4.59	0.55	4.20	0.16	0.38	69.55	
0	0b	4.00	4.57	0.56	4.17	0.16	0.39	70.06	69.73
	0c	4.07	4.61	0.53	4.24	0.16	0.37	69.59	
	2a	4.00	4.32	0.31	4.14	0.13	0.17	56.30	
2	2b	3.99	4.33	0.33	4.17	0.17	0.16	48.49	49.99
	2c	3.98	4.28	0.30	4.15	0.16	0.13	45.18	
	4a	3.98	4.23	0.25	4.15	0.16	0.08	33.84	
4	4b	4.01	4.25	0.23	4.18	0.17	0.06	27.15	29.46
	4c	4.04	4.27	0.23	4.21	0.17	0.06	27.39	
	5a	4.07	4.29	0.21	4.24	0.16	0.05	23.45	
5	5b	3.96	4.18	0.21	4.13	0.16	0.04	21.89	23.77
	5c	3.98	4.22	0.24	4.16	0.18	0.06	25.98	
	6a	4.07	4.30	0.22	4.24	0.17	0.05	22.88	
6	6b	3.99	4.20	0.21	4.16	0.16	0.04	20.59	20.96
	6c	4.04	4.27	0.22	4.22	0.18	0.04	19.41	
	7a	4.05	4.26	0.20	4.22	0.16	0.03	19.10	
7	7b	4.05	4.25	0.20	4.22	0.16	0.03	18.44	19.02
	7c	4.03	4.24	0.21	4.20	0.17	0.04	19.52	
	8a	4.02	4.23	0.21	4.20	0.18	0.03	16.07	
8	8b	4.04	4.24	0.20	4.20	0.15	0.04	22.81	18.45
	8c	4.02	4.22	0.20	4.18	0.16	0.03	16.48	

3.2. Response of Apices on M2 Medium After Encaspulation-Dehydration

Apices presented various aspects on M2 culture medium (Figure 5). It was noted:

a) A necrosis characterized by a browning of the apices corresponding to death of the cellular tissues.
b) Apex survival characterized by swelling of apices which remained green after two weeks.
c) Callus formation characterized by the presence of cell clusters without formation of shoots
d) Regeneration of apices characterized by the formation of leafy shoots.



A: Inflated apex showing survival; B: callus formation after 3 weeks of culture; C: Regenerated apex; D: Shoot regenerated after 6 weeks of culture.

***Figure 5.** Response of the encapsulated apices on M2 culture medium.*

3.3. Effect of Dehydration Time and Stored Duration on Encapsulated Apices Survival for Two Landraces Tested

The results of the analysis of variance of the survival of the encapsulated apices (Table 2) showed that there is a significant difference respectively for the factors "landraces " and " treatments " and their interaction.

***Table 2.** Analysis of variance on survival of encapsulated apices based on different treatments.*

Source of variation	Degree of freedom	Sum of squares	Average of squares	Variance	Probability
Landraces	1	4.80	4.80	7.58	0.012
Treatments	4	59.80	14.95	23.61	< 0.001
Landraces*Treatments	4	7.53	1.88	2.97	0.044
Error	20	12.66	0.63		
Total	29	84.80			

Figure 6 shows the evolution of apices survival rates with treatments of genotypes. For landrace “Koïdokpon’’, it is found that the encapsulated apices undergone 6h of dehydration and stored at 2°C for 15 days (T3) have a higher survival rates (59.26%) compared to T2 and T5 treatments (25.92% for each) and T4 (18.51%). The test of ranking of averages of Student, Newman and Keuls at 5% threshold showed that there is a significant difference between T2

treatment and T3, T4 and T5 treatments. As for landrace ‘’Dokoui carotte’’, we note that T3 treatment (6h of dehydration + 15 days of storage at 2°C) also recorded high survival rate (37.03%) compared to T2 treatment (5h of dehydration + 15 days of storage at 2°C) and T4 and T5 treatments (respectively at 5h and 6h of dehydration for 90 days of storage at 2°C). The test of ranking of averages of Student, Newman and Keuls at 5% threshold showed that there is a significant difference between T3 and T4 treatments, but the difference is no significant between T3, T2 and T5 treatments.

Moreover, " Koidokpon " showed high survival rates (70.37%; 59.26%; 18.51%) respectively for T1 treatment (without dehydration), T3 and T4 treatments compared to " Dokoui carotte " (48.14%; 37.04%; 14.81%). However, for the T2 treatment, the survival rate of “Dokoui carotte” (29.62%) is higher than ''Koïdokpon'' (25.93%).

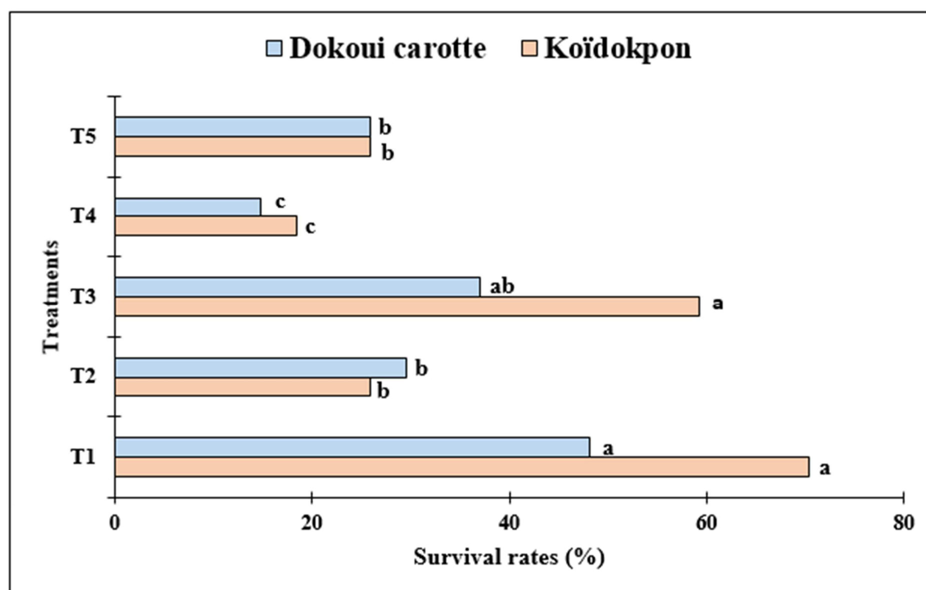


Figure 6. *Variation of the survival rates after 5h and 6h dehydration of encapsulated apices stored during 15 and 90 days at 2°C.*

T1 = Encapsulated apices directly cultivated on M2 medium; T2 = Encapsulated apices dried 5h on silicagel and stored 15 days at 2°C; T3 = Encapsulated apices dried 6h on silicagel and stored 15 days at 2°C; T4 = Encapsulated apices dried 5h on silicagel and stored 90 days at 2°C; T5 = Encapsulated apices dried 6h on silicagel and stored 90 days at 2°C.

3.4. Effect of Dehydration Time and Stored Duration on Regeneration of Encapsulated Apices for Two Landraces Tested

The results showed that the regeneration of encapsulated apices (Table 3) is significantly different for the factors "landraces" and " treatments " as well as their interaction.

Table 3. *Analysis of variance on regeneration of encapsulated and dehydrated apices.*

Source of variation	Degree of freedom	Sum of squares	Average of squares	Variance	Probability
Landraces	1	7.50	7.50	22.50	<0.001
Treatments	4	31.80	7.95	23.85	<0.001
Landraces*Treatments	4	6.33	1.58	4.75	<0.007
Error	20	6.66	0.33		
Total	29	52.30			

Figure 7 shows the evolution of the regeneration rates of dehydrated apices after encapsulation as a function of treatments and genotype. It is found that the encapsulated apices of the two landraces "Koïdokpon" and "Dokoui carotte" undergone T3 treatment (6h of dehydration + 15 days of storage at 2°C) gave the highest regeneration rates (37.04% for Koïdokpon and 11.11% for Dokoui carotte) compared with apices undergone T2, T4 treatments. However, these rates are lower than those of the control (T1) of the both cultivars (40.74% for "Koïdokpon" and 24.92% for "Dokoui carotte"). The ranking test of Student, Newman and Keuls at the 5% threshold of averages showed that, for “Koïdokpon”, T3 treatment does not differ from T1 control treatment but has a significant difference with the T2, T4 and T5 treatments. For "Dokoui carotte", there is a significant difference between the treatments T1 and T3. Moreover, the regeneration rate of "Koïdokpon" is higher than "Dokoui carrotte". For example, the dehydration time of 6 hours (T3 treatment) gave a survival rate of 37.04% for "Koïdokpon" against 11.11% for "Dokoui carotte".

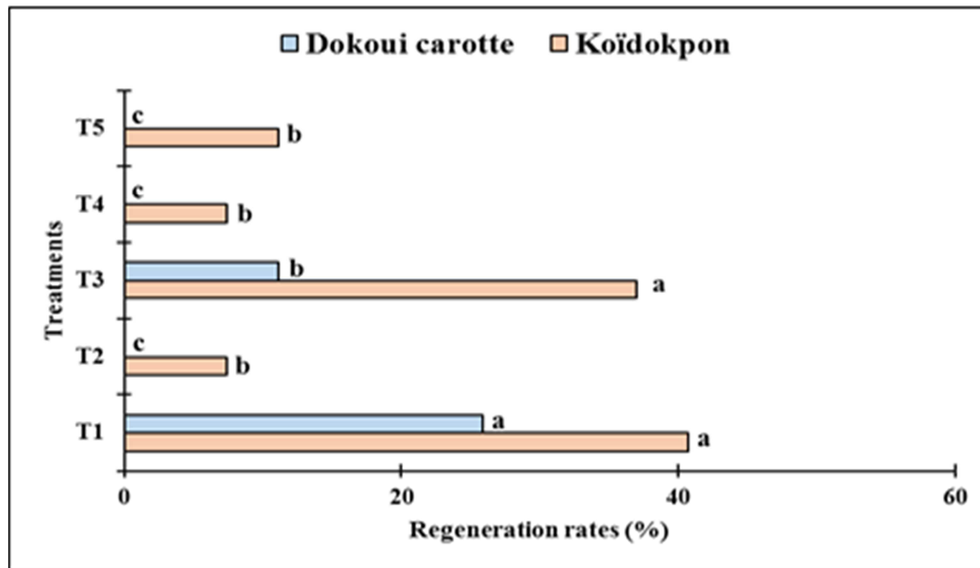


Figure 7. *Variation of the regeneration rates after 5h and 6h dehydration of encapsulated apices stored during 15 and 90 days at 2°C.*

T1 = Encapsulated apices directly cultivated on M2 medium; T2 = Encapsulated apices dried 5h on silicagel and stored 15 days at 2°C; T3 = Encapsulated apices dried 6h on silicagel and stored 15 days at 2°C; T4 = Encapsulated apices dried 5h on silicagel and stored 90 days at 2°C; T5 = Encapsulated apices dried 6h on silicagel and stored 90 days at 2°C.

4. Discussion

The success of encapsulation-dehydration technique required in this work the determination of dehydration modality of alginate beads. For this purpose, empty alginate beads were used and their water contents at different dehydration duration on silica gel were evaluated. The results obtained during the present work showed that the best duration of dehydration to obtain beads content approximately 20% (20.96%) water is 6h. The water content of beads which ensures the highest survival after cooling especially in liquid nitrogen at -196°C is about 20% for most vegetative propagated species including sweet potato and corresponds to the amount of non-frozen water [5]. According to the same author, dehydration of empty alginate beads with silica gel provides conditions for reproducing the experiment with encapsulated apices. Similarly, it reduces the risk of contamination of the plant material (apex) because of many necessary manipulations that are dangerous for the sterility of the explants. This determined duration of 6 hours which leads to 20% of water content of the beads to be used for evaporative dehydration of encapsulated apices of sweet potato is different from that of other authors [5, 18, 20]. Gallard obtained beads with a water content of approximately 20% using 20 beads per dish on 40g of silica gel for 3h for dehydration of *pelargonium* apex [20]. Similarly, Mubbarakh have used 50 g of silica gel for 3 hours with 10 beads to obtain water contents of 20% for dehydration of the apices of rose (*Rosa hybrida*) [18]. In contrast, Gonzalez-Arnao and Engelmann used 8 g of silica gel for 5 hours with 5 beads to obtain the same water content of the beads for sugar cane [5]. From these observations, our results can be explained by the fact that the mass of silica gel and the number of beads per box have an influence on the duration of dehydration. Indeed, the mass of silica gel used in our experiments is 15g and the number of beads is ten (10).

For apices survival and regeneration after encapsulation-dehydration, the results showed that at 2°C, apices survival rates stored for 90 days (T4 and T5 treatments) were lower compared to apices stored for 15 days (T2 and T3). These results would be explained by fact that at 2°C, the apices are viable only for a short storage time. Indeed, other reports have shown that after encapsulation dehydration, sugarcane apices stored at a temperature close to 0°C (4°C, - 12°C and - 25°C) rapidly lose viability as their storage time increases [18]. It occurs at these temperatures devitrification followed by recrystallization of free radicals which are detrimental to the cell integrity and which cause damage. According to the same authors, at lower storage temperatures (-70 ° C to -80 ° C) somatic embryogenic in oil palm, no change was observed in the survival rates as the duration of conservation increases. This was not the case in citrus fruit where there was a decrease in survival rates depending on the shelf life. Thus, the ultra-low temperatures between -135°C and -196°C in liquid nitrogen are those indicated for long-term preservation of the encapsulated apices of different plant species and which do not do not influence their survival and regeneration [21]. The work presented on the short-term conservation by other authors of medicinal species, including *Ocimum kilimandscharicum* apex, has shown that it is possible to maintain the viability of the encapsulated shoot tips up to 90 days storage at an ambient 25°C, hence the use of this technique for short-term preservation [22].

Apart from the storage times, the duration of dehydration of encapsulated apices of two sweet potato landraces also influenced apices survival and regeneration rates. The results also showed that the lowest survival rates after storage (2°C)

for 15 days were observed for 5h of dehydration (T2) compared to 6h of dehydration. This treatment T2 corresponds to 23.77% water content of the beads. On the other hand, the treatment corresponding to approximately 20% of water content (T3 = 6 h of dehydration), high survival rates were obtained. These results can be explained by the fact that a very important water content (23.77%) would have caused a crystallization of the water in the tissues during the freezing, and consequently the death of the apices which was translated by necrosis as reported Shibi et al on Wild fennel [23]. Gallard has shown that for encapsulation-dehydration technique, the difference in water content of pelargonium apex encapsulated explains the different survival rates obtained [22]. The analyze of variance of the survival of the encapsulated apices in our study also showed that there is a significant difference for the " varieties " factor (p = 0.012). Thus, genotype is a factor that influences the survival of encapsulated apices. Clavero-Ramirez obtained survival rates varied between 23% and 63% on apices of seven strawberry genotypes after encapsulation-dehydration [24]. With respect to apex regeneration after encapsulation-dehydration, it resulted in formation of leafy shoots in both of sweet potato landraces. The best rates were observed with the apex undergone 6 hours of dehydration on silica gel (37.04% for "Koïdokpon" and 11.11% for "Dokoui carotte"). The variance analysis of regeneration has also shown that there is significant difference for the "treatment" and "Landraces" factors. This shows that the genotype influences the regeneration of encapsulated and dehydrated apices as pointed out by Gallard on four varieties of *pelargonium* and Dumet on cassava [20, 25].

5. Conclusion

Through this study it is clear that the alginate encapsulation can be used successfully for storage of sweet potato landraces. At our experimental condition, the results show that the best duration of dehydration which can allow less water content (approximately 20%) of the complex beads-apex is 6h. The survival and regeneration of encapsulated apices are highly dependent on the genotype and the storage duration. The further studies are needed to optimize the level of those parameters. This method can be used to preserve the endangered sweet potato landraces and other species during at least three months without subcultures. It also reduce the cost of conservation in terms of consumables and permit better genotype stability during the storage.

Acknowledgments

The authors thank HOUNGUE Jérôme, ASSOGBA Armel for the corrections brought to the English version of the manuscript and AISSI Jacques, KOUKE Jaurès, Technicians at the Central Laboratory of Plant Biotechnology and Plant Breeding of the Faculty of Sciences and Techniques of the University of Abomey-Calavi.

References

[1] Doussoh, A. M., Dangou, J. S., Houedjissin, S. S., Assogba, A. K. and Ahanhanzo, C. (2016) Analyse des connaissances endogènes et des déterminants de la production de la patate douce [*Ipomoea batatas* (L.)], une culture à haute valeur socioculturelle et économique au Bénin. *International Journal of Biological and Chemical Sciences* 10(6): 2596-2616.

[2] Sanoussi, A., Adjatin, A., Dansi, A., Adebowale, A., Sanni, L. and Sanni, A. (2016) Mineral Composition of Ten Elites Sweet Potato (*Ipomoea Batatas* [L.] Lam.) Landraces of Benin. *International Journal of Current Microbiology and Applied Sciences* 5(1): 103-115.

[3] Doussoh, A., Dangou-Sossou, J., Houédjissin, S., Cacaï, G., Assogba, A. and Ahanhanzo, C. (2017) Influence of mercuric chloride on survival and suitability for in vitro regeneration of three sweet potato landraces (Ipomoea batatas L.) produced in Benin. *International Journal of Current Research in Biosciences and Plant Bioogy.* 4(1): 56-64.

[4] González-Arnao, M. T., Dolce, N., González-Benito, M. E., Martínez, C. R. C. and Cruz-Cruz, C. A. (2017). Approaches for In Vitro Conservation of Woody Plants Germplasm. In *Biodiversity and Conservation of Woody Plants*. *Springer* 355-419.

[5] Gonzalez-Arnao, M. T. and Engelmann, F. (2006) Cryopreservation of plant germplasm using the encapsulation-dehydration technique: review and case study on sugarcane. *CryoLetters* 27(3): 155-168.

[6] Agbidinoukoun, A., Ahanhanzo, C., Adoukonou-Sagbadja, H., Adjassa, M., Djikpo-Tchibozo, M. A. and Agbangla, C. (2013) Impact of osmotic dehydration on the encapsulated apices survival of two yams (*Dioscorea spp*.) genotypes from Benin. *Journal of Applied Biosciences* 65: 4999-5007

[7] Alam, I., Sharmin, S. A., Naher, K., Alam, J., Anisuzzaman, M. and Alam, M. F. (2010) Effect of Growth Regulators on Meristem Culture and Plantlet Establishment in Sweet Potato [*'Ipomoea Batatas'*(L.) Lam.]. *Plant Omics* 3(2): 35-39.

[8] Arrigoni-Blank, M. D. F., Tavares, F. F., Blank, A. F., Santos, M. C. D., Menezes, T. S. A. and Santana, A. D. D. D. (2014) *In vitro* conservation of sweet potato genotypes. *The Scientific World Journal* 2014:1-7

[9] Bekheet, S., Matter, M. & El-Ashry, A. (2016). In vitro Conservation of Jojoba (Simmondsia chinensis) Shootlet Cultures Using Osmotic Stress and Low Temperature. *Middle East Journal* 5(4): 396-402.

[10] Rafique, T., Yamamoto, S.-i., Fukui, K., Tanaka, D., Arizaga, M. V., Abbas, M., Matsumoto, T. & Niino, T. (2016). Cryopreservation of shoot-tips from different sugarcane varieties using D cryo-plate technique. *Pakistan Journal of Agricultural Sciences* 53(1): 151-158.

[11] Capuana, M. and Ponti, F. (2008) *In vitro* medium-term conservation of *Myrtus communis* L. *Propagation of Ornamental Plants* 8(2): 111-113.

[12] Gonçalves, S. and Romano, A. (2007) *In vitro* minimum growth for conservation of *Drosophyllum lusitanicum. Biologia Plantarum* 51(4): 795-798.

[13] Hegde, V., Makeshkumar, T., Sheela, M., Chandra, C. V., Koundinya, A., Anil, S. R., Muthuraj, R. and Darshan, S. (2017) Production of Synthetic Seed in Cassava (*Manihot esculenta* Crantz). *Journal of Root Crops* 42(2): 5-9.

[14] Ghanbarali, S., Abdollahi, M. R., Zolnorian, H., Moosavi, S. S. & Seguí-Simarro, J. M. (2016) Optimization of the conditions for production of synthetic seeds by encapsulation of axillary buds derived from minituber sprouts in potato (*Solanum tuberosum*). *Plant Cell, Tissue and Organ Culture* 126(3): 449-458.

[15] Benson, E., Harding, K., Ryan, M., Petrenko, A., Petrenko, Y. and Fuller, B. (2018) Alginate encapsulation to enhance biopreservation scope and success: a multidisciplinary review of current ideas and applications in cryopreservation and non-freezing storage. CryoLetters 39(1): 14-38.

[16] Germana, M. A., Micheli, M., Chiancone, B., Macaluso, L. and Standardi, A. (2011) Organogenesis and encapsulation of *in vitro*-derived propagules of Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirius trifoliata* (L.) Raf]. *Plant Cell, Tissue and Organ Culture* 106(2): 299-307.

[17] Gantait, S., Kundu, S., Ali, N. and Sahu, N. C. (2015) Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. *Acta physiologiae plantarum* 37(5): 98.

[18] Mubbarakh, S. A., Izhar, N. A., Rajasegar, A. and Subramaniam, S. (2014) Establishment of encapsulation-dehydration technique for *in vitro* fragmented explants of *Rosa hybrida* L. cv. Helmut Schmidt. *Emirates Journal of Food and Agriculture* 26(6): 565-576.

[19] Verma, S., Khosla, S., Choudhary, D. K. and Lal, A. K. M. (2016) Plant regeneration of A. Lakoocha from encapsulated nodal explants. *Eurpean Journal of Botany, Plant Sciences and Phytology* 2(3): 17-26.

[20] Gallard, A., Panis, B., Dorion, N., Swennen, R. and Grapin, A. (2008) Cryopreservation of Pelargonium apices by droplet-vitrification. *CryoLetters* 29(3): 243-251.

[21] Engelmann, F. (2011) Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cellular & Developmental Biology-Plant* 47(1): 5-16.

[22] Saha, S., Sengupta, C. and Ghosh, P. (2015) Encapsulation, short-term storage, conservation and molecular analysis to assess genetic stability in alginate-encapsulated microshoots of *Ocimum kilimandscharicum* Guerke. *Plant Cell, Tissue and Organ Culture* 120(2): 519-530.

[23] Shibli, R. A., Hawmdeh, F. A., Duwayri, M., Hadidi, N., Al-Qudah, T. S., Tahtamouni, R. W., Younes, L. S. and Zateemeh, A. (2016) Experimenting Two Cryopreservation Techniques (Vitrification and Encapsulation-Dehydration) as Approaches for Long-term Conservation of in vitro Grown Shoot Tips of *Wild Fennel*. *Jordan Journal of Biological Sciences* 9(3): 147-154.

[24] Clavero-Ramirez, I., Galvez-Farfan, J., Lopez-Aranda, J. and Gonzalez-Benito, M. (2005) Apex cryopreservation of several strawberry genotypes by two encapsulation-dehydration methods. CryoLetters 26(1): 17-24. Engelmann, F. (2011) Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cellular & Developmental Biology-Plant 47(1): 5-16.

[25] Dumet, D., Korie, S. and Adeyemi, A. (2009) Cryobanking cassava germplasm at IITA. *In International Symposium on Cryopreservation in Horticultural Species* 908, 439-446.