EFFECT OF MANNITOL ON *IN VITRO* CONSERVATION OF LOCAL AND EXOTIC TARO-GENOTYPES (*COLOCASIA ESCULENTA VAR ESCULENTA*)

BENJAMIN BONSU BRUCE[1*], MARIAN DORCAS QUAIN[2], GERTRUDE OSEI-DIKO[2], RICHARD ADU AMOAH[1], KINGSLEY ORCHAR[1]

Taro is cultivated as a major food crop for its nourishing leaves and plump corms. The diversity of its germplasm must be protected; thus, the conserved field gene bank is vulnerable to loss caused by biotic and abiotic factors and therefore requires *in vitro* conservation. *In vitro* slow growth is one of the most promising techniques to be utilized for conservation. Finding a medium-term *in vitro* conservation procedure for local and exotic taro genotypes was the aim of this investigation. The medium-term conservation study was conducted using actively growing shoot obtained from *in vitro* cultures. Explants were grown in full-strength Murashige and Skoog medium that was supplemented with mannitol at various concentrations (0 g/l, 20 g/l, 25 g/l, and 30 g/l). Another medium which was tested as an additional treatment is the White's medium. Survival rate was high, and explants remained almost green and healthy on all mannitol supplemented media. However, on the White's medium, growth decreased with time. High number of shoots (6.33) and leaves (22.67) occurred on medium containing 20 and 25 g/l mannitol in genotype SAO 006, whereas 30 g/l mannitol was the best to restrict growth for the entire 6 months period in terms of shoot height (22.50 cm). The study shows that culture media with mannitol supplements can inhibit *Colocasia* plantlet growth, particularly in terms of stem height. Culture growth after six months of conservation revealed that after six months of conservation in media containing 20 gl^{-1} and 25 gl^{-1} mannitol, healthy Taro shoot cultures could be produced.

Keywords: complete Murashige and Skoog medium, culture conditions, mannitol, slow growth conservation, *Colocasia esculenta var esculenta*.

INTRODUCTION

Taro (*Colocasia esculenta* (L) Schott) is an important staple food crop grown throughout many parts of the world for its fleshy corms and nutritious leaves. Nigeria, China, Ghana, and Cameroon are the top taro growers globally (FAOSTAT, 2013). Located just below the soil's surface, the plant's core is a

[1] CSIR-Plant Genetic Resources Research Institute P.O. Box 7, Bunso, Eastern Region, Ghana.
[2] CSIR-Crops Research Institute P.O. Box 3785, Kumasi.
* Corresponding author: benjaminbonsubruce@ymail.com

corm. The corm's apical bud at its top produces roots, while the lower portion of the corm produces leaves. Each type of corm develops laterally: runners, daughter corms, and cormels. The fibrous root system includes peltate leaves and is primarily found in the top meter of soil. For many communities in Africa, this crop serves as a major source of staple food because it is primarily grown for its edible leaves and corm (Darkwa and Darkwa, 2013; Boampong *et al*., 2020).

In addition to their 1.4% secondary metabolites and 13–19% carbohydrate, taro corms also include antioxidant, anti-inflammatory, anticancer, and anti-metastatic properties (FAOSTAT, 2013). Taro's bioactive substances are also employed in the creation of anti-cancer medications (FAO, 2012). Only a small number of growers cultivate it extensively for both domestic and international markets. Eight distinct variants of the Colocasia esculenta species have been found, of which two are regularly grown as food (Purseglove, 1972).

Lebot and Aradhya (1991) used isozyme analysis to show that there are two gene pools for farmed taro, one in Asia and the other in the Pacific. Researchers have utilized AFLP markers (Kreike *et al*., 2004) and SSR (Simple Sequence Repeats) markers to discriminate between these two different gene pools. Taro can be regarded as a native plant of the region because of this evidence that it was domesticated in both Asia and the Pacific.

As suckers are the primary planting materials utilized in West Africa, they have historically been preserved in field collections, exposing the plants to harmful biotic and abiotic stressors, and limiting the availability of high-quality planting material. Farmers typically recycle taro suckers from their own farms to build new fields since they have limited availability to high-quality planting material. Owing to prior exposure to virus vectors such as aphids and whiteflies in the field, the practice of recycling tubers promotes the accumulation of systemic pathogens. Prior exposure to virus vectors and subsequent expression of disease symptoms can also be a huge constraint to sustainable conservation and utilization of taro genetic resources. In view of this, there is the need for the development of taro propagating strategies that provide protection from pathogens and increase access to planting material for conservation and direct use by farmers.

One *in vitro* technique that has recently gained importance for plant growth, the eradication of disease, and the synthesis of secondary metabolites is tissue culture (Shatnawi *et al*., 2007). Tissue culture or micropropagation techniques provide a sustainable solution to the problems associated with the conventional propagation of sweet potato (IAEA, 2004). The main plant tissue culture (PTC) methods for growing healthy (pathogen tested/disease-free) clones of planting materials today include micropropagation techniques like shoot meristem or nodal tip culture, along with thermotherapy or cryotherapy (Quain, 2001). Alternative but

less common methods include changing culture media by reducing the nutritional concentration of the basal medium, adding an osmoticum, removing growth regulators from the medium, or adding growth retardants to the medium. On a medium devoid of sucrose, carrot embryogenic cultures were kept for two years, and when a sucrose solution was added, they overgrew (Jones, 1974). Slow growth culture for medium-term preservation and cryopreservation procedures for medium- to long-term preservation are two types of in vitro germplasm conservation technologies that could provide useful ways to save plant species' germplasm (Tandon and Kumaria, 2005).

There is therefore the need for slow growth methods to conserve from short to medium term period to save time and reduce cost of maintaining cultures *in vitro*.

Slow growth techniques are currently successfully and often used to a variety of species and across a variety of genotypes within species, according to a report on the application of in vitro techniques for the conservation and utilization of plant genetic resources (Ahmed *et al*., 2011). The objective of the study therefore was to find out a medium-term *in vitro* conservation protocol of local and exotic taro genotypes (*Colocasia escunlenta var esculenta*). The performance of cultures following conservation was also investigated.

MATERIALS AND METHODS

The study was conducted in the Plant Biotechnology Section's Tissue Culture laboratory at the CSIR-Crops Research Institute in Fumesua, Kumasi.

***In vitro* medium-term conservation:** *In vitro* established cultures of Taro genotypes SAO 002, SAO 006, CE/MAL/32, and CE/MAL/14 with proliferating shoot were used to set up medium-term conservation experiment. Individual shoots were separated, and leaves and root were trimmed off. The length of the resultant explant used was approximately 0.8 cm, as shown in Plate 1. Isolated explants were cultured on complete MS basal salt with vitamins supplemented with 30 g/l sucrose and different concentrations (0, 20, 25, and 30 g/l) of mannitol, and White medium for medium term conservation plus 0.8% purified (phytoblend) agar. Culture vessels used were sigma ware test tubes with diameter of 25 mm × 150 mm height covered with plastic closure and incubated at 20±1°C, 12 hours photoperiod and light intensity of 3000 lux.

Plate 1. Conservation shoots explant.

***In vitro* recovery:** The conservation experiments were conducted over a six-month period, at the end of which surviving shoots were excised and cultured on complete MS basal salt with vitamins supplemented with 30 g/l sucrose, 0.5 µM NAA, 5.0 µM BAP and 80 mg/l $AdSO_4$ plus 0.8% purified (phytoblend) agar for growth *in vitro.* Cultures vessels used were sigma ware test tube with diameter of 25 mm × 150 mm Height covered with plastic closure and incubated at 25±1°C, 16 hours photoperiod and 5000 lux.

Data collection and statistical analysis: Genotypes SAO 002, SAO 006, CE/MAL/32, and CE/MAL/14 were used for medium term conservation and SAO 006 for recovery experiment. Number of shoots, Number of leaves and Shoot height of individual *in vitro* plantlet were measured at week 4, 8, 12, 16, 20 and 24 using a ruler. Weekly monitoring to identify contaminated cultures was conducted. Data were analyzed using one-way ANOVA tests and comparison of treatment means by LSD (Least Significant Difference) using GENSTAT 9. Level of Significance was determined at the 5% probaility (Gomez and Gomez, 1976) and the data presented in tables and figures are mean values of three replicates ± SD.

RESULTS AND DISCUSSIONS

Slow growth conservation has the benefit of not requiring regular cultures. Another benefit of slow growth conservation is that the culture may be easily recovered after a lengthy time of conservation for the purpose of producing new plants with improved genetic integrity (Ahmad and Anjum, 2010). After twenty-four weeks of *in vitro* conservation, well developed shoots were obtained in most cultures. However, no contamination was observed in all cultures conserved.

Shoot Development: After twenty-four weeks conservation, SAO 006 genotype gave the highest number of shoots (6.33) which occurred on 30 g/l mannitol, followed by same SAO 006 genotype (5.67) on Control treatment as compared to CE/MAL/32 of average number of shoot (2.43), SAO 002 (2.00). However, the same SAO genotype performed poorly in shoot development (0.833) on White media as shown in Fig. 1 below.

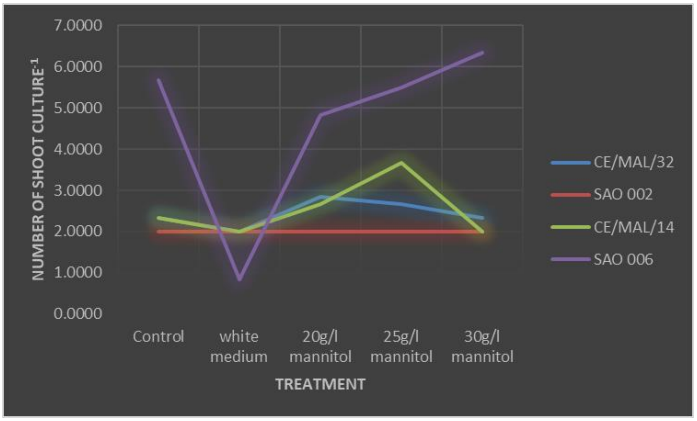

Fig. 1. Effect of mannitol concentrations and White medium on shoot development at 6 months in culture.

Leaf development: SAO 006 genotype had the highest number of leaves (21.33) on 30 g/l mannitol medium after *in vitro* conservation. Same SAO 006 genotype gave a higher number of leaves (19.00) which occurred on Control medium followed by SAO 006 genotype (18.67) on 25 g/l mannitol medium, SAO 006 (16.67) ON 20 g/l Mannitol, SAO 002 (16.33) on Control and CE/MAL/32 (13.67) on Control medium. SAO genotype had as low as 1.17 leaves per shoot on White media compared to other genotypes as shown in Fig. 2 below.

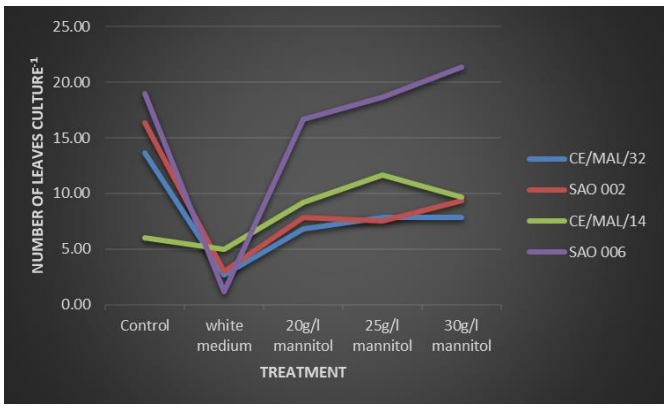

Fig. 2. Effect of mannitol concentrations and White medium on leaves development at 6 months in culture.

Shoot height: CE/MAL/14 genotype recorded the highest figure of (29.33 cm) on Control medium after *in vitro* conservation. Lower value of 26.03 cm, 25.70 cm, 25.51 cm and 25.47 cm were obtained on SAO 002, CE/MAL/32, SAO 006 on Control and SAO 002 genotype which occurred on 20 g/l mannitol medium respectively. SAO 006 genotype with 1.07 cm on White medium was the lowest growth in height after twenty-four weeks of conservation compared with SAO 002, CE/MAL/14, CE/MAL/32 as shown in Fig. 3 below.

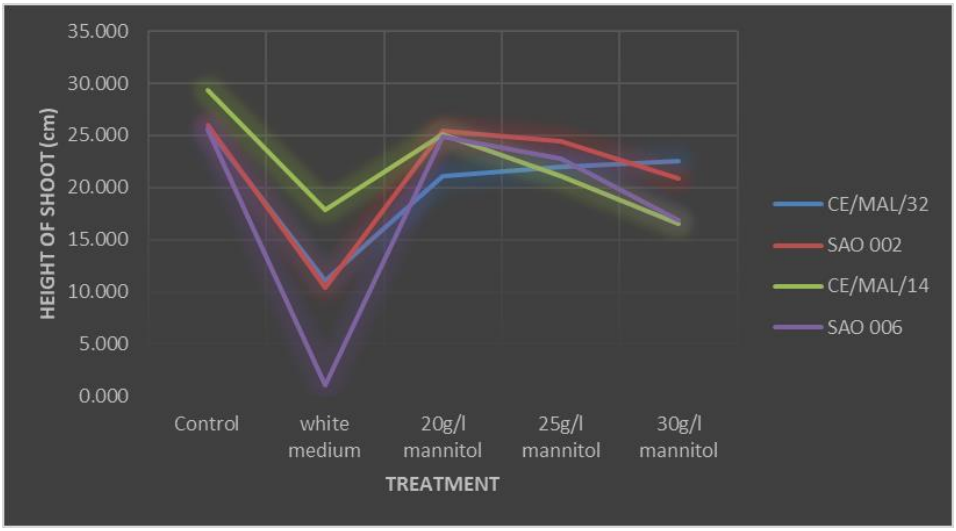

Fig. 3. Effect of mannitol concentrations and White's medium on height of shoot at 6 months in culture.

Regarding the effect of different mannitol concentrations, results in Fig. 1, Plate (2.0–5.0) showed that medium supplemented with 30 g/l mannitol resulted in the lowest mean value of shoot height followed by 25 g/l mannitol, Fig. 3, Plates 2–5. In this respect, Staritsky *et al*. (1986) found that low concentrations of MS medium and the lower concentration of mannitol (2.5%) successfully increased subculture duration of pear genotypes.

Almost all SAO 006 genotype died on White medium at the end of the six month period of conservation because of decline in growth and development of cultures – results in Figs. 1–3, Plates 2e–5e. Reduced plant height is critical in culture conservation as plantlets do not easily get overgrown. Similar outcomes were also seen for Artemisia herba-alba plantlets that were preserved using slow growth culture (Sharaf *et al*., 2012). Osmotic agents inhibited *in vitro* grown cultures' growth, according to certain other findings (Bajaj, 1995; Montalvo-

Peniche, 2007; Du *et al*., 2012). Similar results considering the effect of mannitol on growth limitation were reported in *Ensete ventricosum* (Sarkar and Naik, 1998), in the rare taxon *Veronica multifida* ssp. *Capselli carpa* (Negash *et al*., 2001), (Holobiuc *et al*., 2006).

By variations in the osmotic pressures of the cells, macro, micro, and vitamin mineral components dissolved in water are delivered into the cells. The addition of sugar alcohol to a medium raises its osmotic potential, which inhibits cells from absorbing the medium's minerals and nutrients. Plant growth is thus postponed (Holobiuc *et al*., 2008). A high mannitol concentration applied to the media had an osmotic impact that slowed the development of the taro plantlets. As shown in Fig. 3 and Plates 2d–5d of different taro genotypes, the amount of mannitol given to the medium affected the height of the shoots. 30 g/l mannitol was the most effective, followed by 25 g/l mannitol treatment (2.c–5.c). During the six-month period of culture, shoots growing on 0 g/l and 20 g/l mannitol supplemented media grew to fill the culture test tube, however, the White's medium could not adequately sustain growth as shown in Fig. 3 and Plates 2–5.

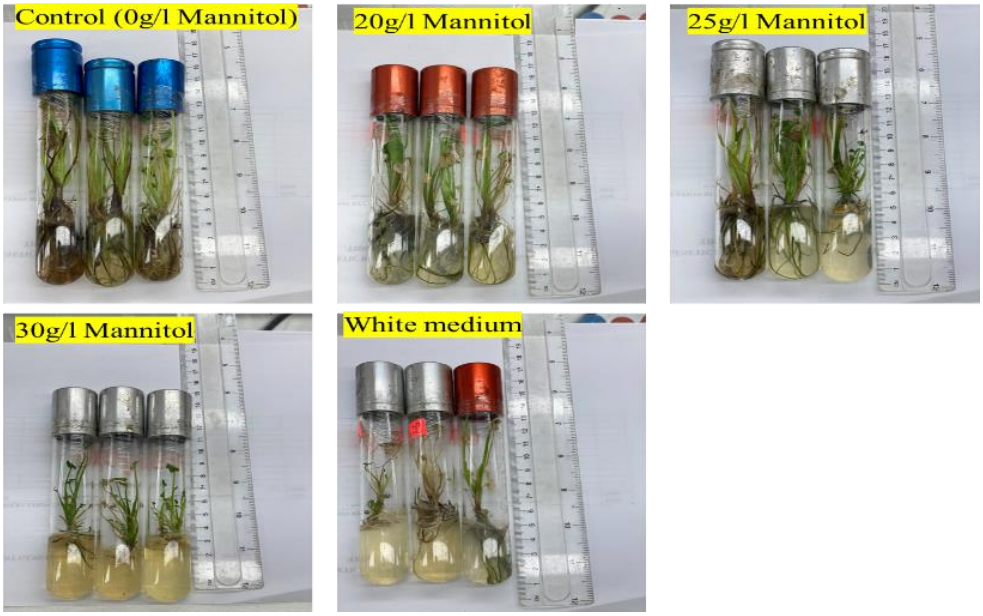

Plate 2. Genotype SAO 002 development on complete MS medium supplemented with Control (0 g/l), 20 g/l, 25 g/l, 30 g/l mannitol and on White medium at 6 months in culture.

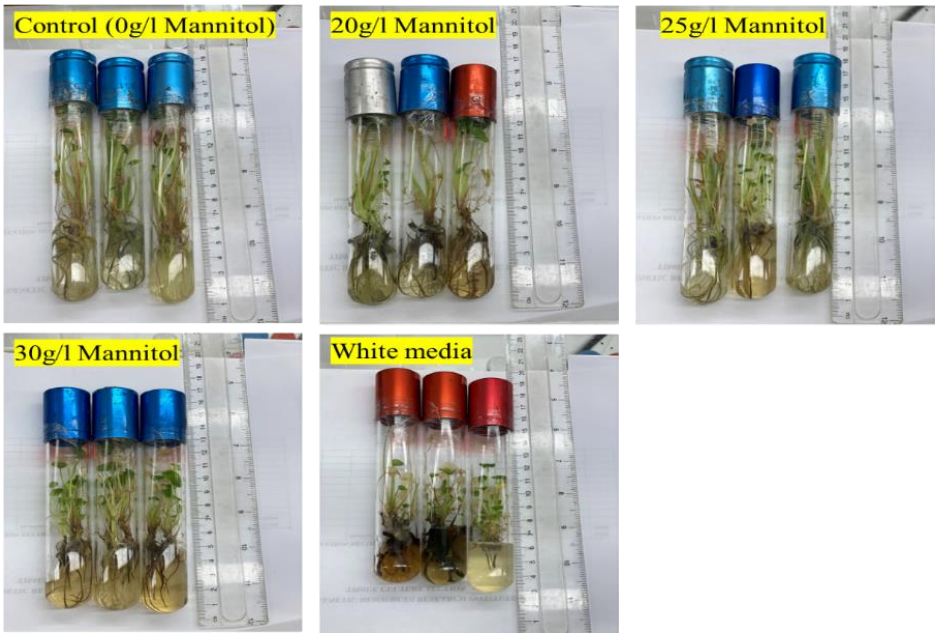

Plate 3. Genotype CE/MAL/32 development on complete MS medium supplemented with Control (0 g/l), 20 g/l, 25 g/l, 30 g/l mannitol and on White medium at 6 months in culture.

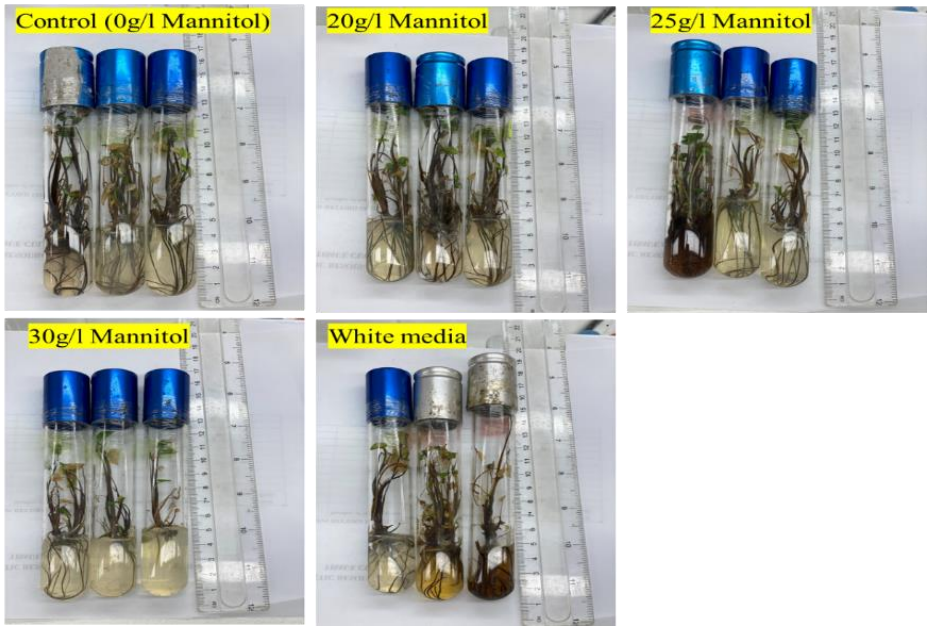

Plate 4. Genotype CE/MAL/14 development on complete MS medium supplemented with Control (0 g/l), 20 g/l, 25 g/l, 30 g/l mannitol and on White medium at 6 months in culture.

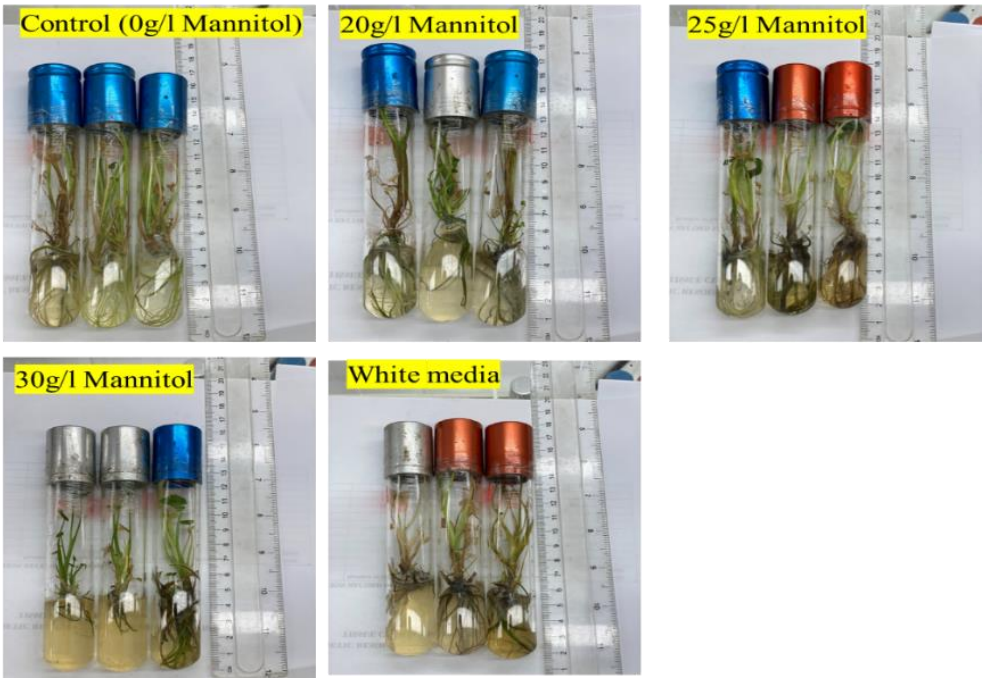

Plate 5. Genotype SAO 006 development on complete MS medium supplemented with Control (0 g/l), 20 g/l, 25 g/l, 30 g/l mannitol and on White medium at 6 months in culture.

***In vitro* recovery:** Regarding the effect of different mannitol concentrations and White medium, results clearly revealed that conservation medium with 20 g/l and 25 g/l mannitol resulted in the highest regeneration percentage (91.67%) after culturing on recovery medium and incubation under normal conditions for 4 weeks. Also, cultures from medium containing 25 g/l mannitol performed better than cultures from other treatment media in the number of new shoots (1.75 shoots/ explant), Fig. 4. White's medium generated cultures had the highest shoot length (6.46 cm), Fig. 6, Plate 6, while conservation medium with 30 g/l mannitol resulted in the lowest regeneration percentage (66.67%). These results agree with those of Thompson *et al*. (1986) who demonstrated that the use of mannitol as an osmotic agent can be metabolized by the plantlets after few months of storage and exhibition an incremental growth rate. In the same line, Bekheet *et al*. (2001) showed that healthy shoot cultures of date palm were obtained after 6 months of storage in medium containing 40 gl^{-1} mannitol.

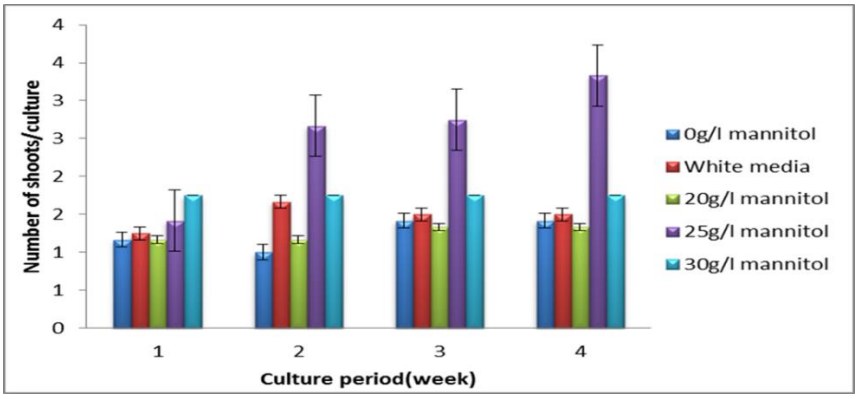

Fig. 4. Culture growth following 6-month conservation on shoots development of genotype SAO 006.

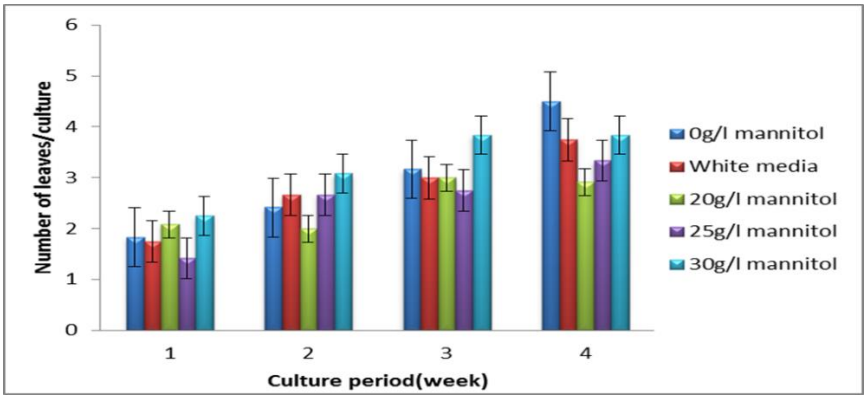

Fig. 5. Culture growth following 6-month conservation on leaves development of genotype SAO 006.

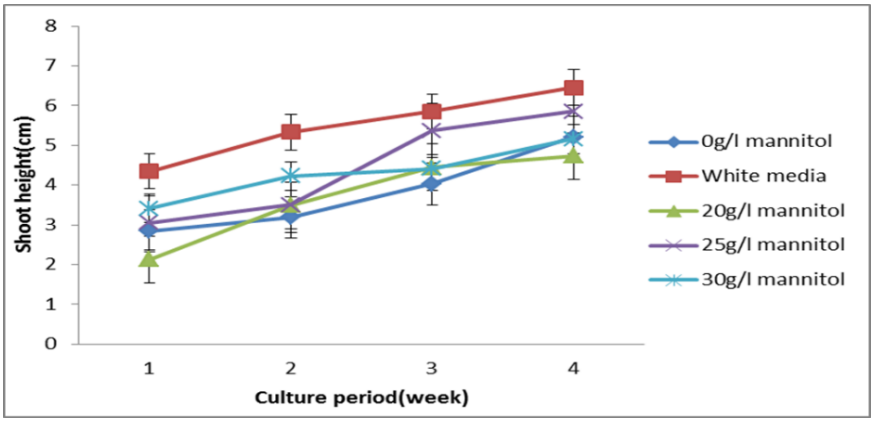

Fig. 6. Growth of cultures on medium for four weeks after the genotype SAO 006 has been retrieved from a 6-month conservation culture on various treatments.

Plate 6. Plantlets developing (4-week old) following 6-month conservation.

CONCLUSION

A growth retardation technique based on mannitol as a limiting factor can effectively be used to achieve medium-term conservation for a Colocasia genotypes *in vitro* establishment. The study shows that culture media with mannitol supplements can inhibit *Colocasia* plantlet growth, particularly in terms of stem height. Culture growth after six months of conservation revealed that after six months of preservation in media containing 20 gl^{-1} and 25 gl^{-1} mannitol, healthy Taro shoot cultures could be produced. This study provides vital information to facilitate germplasm conservation using limited resources to ensure the various varieties are preserved for posterity.

Conflict of interest disclosure: The authors declare no conflict of interest.

REFERENCES

1. Ahmad, M.; M.A.; Anjum, 2010. *In vitro* storage of some pear genotypes with the minimal growth technique. *Turk J. Agric*., 34: 25–32.
2. Ahmed, M.; Anjum, M.A.; Ahmed, M.J.; Sajid, G.M.; Yoqub, A.; Shafqat, M., 2011. Role of plant growth regulators in preservation of *Pyrus* germplasm *In vitro*. *Afr. J. Biotechnol*., 10, 14029–14037.
3. Ashun, M.D., 1996. *In vitro studies on micropropagation of various yam species* (*Dioscorea* species).
4. Bajaj, Y.P.S., 1995. Cryopreservation of plant cell, tissue, and organ culture for the conservation of germplasm and biodiversity. In: Y. BAJAJ (Ed.): *Biotechnology in agriculture and forestry*, 3–28. Springer-Verlag Press, New York, USA.
5. Bekheet, S. A.; H. S. Taha.; M. M. Saker, 2001. *In vitro* long-term storage of date palm (Phoenix dactylifera). The second Introduction Conference on Date Palm in Al-Ain (United Arab Emirates).

6. Boampong, R., Boateng, S.K., Adu Amoah, R., Adu Gyamfi, B., Aboagye, L.M., & Ansah, E.O., 2020. Growth and Yield of Taro (Colocasia esculenta (L) Schott.) as Affected by Planting Distance. *International Journal of Agronomy*, 1–8.
7. Bridgen, M.P.; Staby, G.L., 1981. Low pressure and low oxygen storage of Nicotiana tabacum and Chrysanthemum folium tissue cultures. *Plant Sci. Lett*, 22: 177–186.
8. Darkwa, S.; and Darkwa, A.A., 2013. "TARO 'Colocasia Esculenta': Its Utilization in Food Products in Ghana." *Journal of Food Processing & Technology* 04, no. 05.
9. Du, Y.P.; Li, W.Y.; Zhang, M.F.; He, H.B.; Jia, *G. X*., 2012. The establishment of a slow-growth conservation system *In vitro* for two wild Lily species. *Afr. J. Biotechnol.* 11.
10. FAO, FAOSTAT, 2012. FAO Statistics Division.
11. FAOSTAT, 2013. *Worldwide, the top producers of taro are Nigeria, China, Ghana, and Cameroon*.
12. FAOSTAT, 2013. Food and Agriculture Organization, statistical yearbooks.
13. Golmirzaie, A.; J. Toledo, 1997. Non-cryogenic, long-term germplasm storage, pp. 95–101. In R.D. Hall (ed.). Methods in Molecular Biology, Vol. 111: *Plant Cell Culture Protocols*. Humana Press Inc., Totowa.
14. Golmirzaie, A.; J. Toledo, 1998. *In vitro* conservation of potato and sweet potato germplasm. CIP programme Report, CIP. Lima, Peru, pp: 351–356.
15. Golmirzaie, A.; J. Toledo, 1999. Non-cryogenic, long-term germplasm storage, pp. 95–101. In R.D. Hall (ed.). Methods in Molecular Biology, Vol. 111: *Plant Cell Culture Protocols*. Humana Press Inc., Totowa.
16. Gomez, K.A.; Gomez, A.A., 1976. *Statistical procedures for agricultural research, with emphasis on rice*. International Rice Research Institute, Los Banos, Philipines, 294 p.
17. International Atomic Energy Agency (IAEA), 2004. Low-cost options for tissue culture technology in developing countries. Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, 26–30, Vienna. IAEA-TECDOC-1384,.102p
18. Jones, L.H., 1974. Long term survival of embryoids of carrot *(Daucus carota* L.). *Plant Sci. Lett*., 2: 221–224.
19. Holobiuc, I.; Păunescu, A.; Blindu, R., 2006. *Ex situ* conservation using *in vitro* methods in some Caryophyllaceae plant species from the Red List of the vascular plants in Romania, *Rev. Roum. Biol. – Biol. Veget*., 49–50: 3–16.
20. Holobiuc, I.; Rodica B.; Carasan, M.; Helepciuc, F.; Voichiţă, C.; Negrean, G., 2008. *In vitro* conservation strategy in *Veronica multifida* ssp. *capsellicarpa* (Dubovik) a. Jelen. *Romanian Journal of Biology – Plant Biology*, 53 (2): 71–81.
21. Kreike, C.M.; Van Eck, H.J.; Lebot, V. 2004. Genetic diversity of taro, *Colocasia esculenta* (L.) Schott, in Southeast Asia and the Pacific. *Theoretical and Applied Genetics*, 9: 761–768.
22. Lebot, V.; Aradhya, K.M., 1991. Isozyme variation in taro (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. *Euphytica*, 56: 55–66.
23. Montalvo-Peniche, M.C., 2007. *In vitro* germplasm conservation of habanero pepper (*Capsicum chinense Jacq.*). *Hort. Sci*, 42, 1247–1252.
24. Mullin, R.H.; D.E., 1976. Schlegel. Cold storage maintenance of strawberry meristem plantlets. *HortScience*, 11:100–101.
25. Negash, A.; Krens, F.; Schaart J.; Visser, B., 2001. *In vitro* conservation of enset under slow-growth condition. *Plant Cell Tiss. Org. Cult*, 66: 107–111.
26. Noyer, J.L.; Billot, C.; Weber, A.; Brottier, P.; Quero-Garcia, J.; Lebot, V., 2003. Genetic diversity of taro (*Colocasia esculenta* (L.) Schott) assessed by SSR markers. Paper presented at the Third Taro Symposium, SPC-TPGRI-FAO-CIRAD, Nadi, Fiji, 22–24.
27. O'Sullivan, J.N.; Asher, C.J.; Blamey, F.P.C., 1996. *Nutritional disorders of taro*, Australian Centre for International Research.
28. Purseglove, J.W., 1972. *Tropical crops, monocotyledons*, London.
29. Quain, M.D., 2001. Propagation of root and tuber crops by tissue culture in Ghana. *West Africa Seed and Planting Material*, 8: IITA.

30. Ruredzo, T.J.; Hanson, J., 1991. *In vitro* conservation. In: F. Attere, E. Zedan, NQ Ng, P. Perrino, eds. *Crop genetic resources of Africa*, Vol. 1, Trinity Press, U.K.
31. Sarkar, D.; Naik P.S., 1998. Factors affecting minimal growth conservation in potato microplants *In vitro*, *Euphytica*, 102(2):275–280.
32. Sharaf, S.A.; Shibli, R.A.; Kasrawi, M.A.; Baghdadi, S.H., 2012. Slow-growth preservation of wild shih (*Artemisia herba-alba* Asso.) microshoots from Jordan. *J. Food Agric. Environ*., 10, 1359–1364.
33. Shatnawi, M.; Shibli, R.; Qrunfleh, I.; Bataeinhe, K.; Obeidat, M., 2007. *In vitro* propagation and cryopreservation of *Prunus avium* using verification and encapsulation dehydration methods. *J Food Agr Environ Sci*, 5, 204–208.
34. Staritsky, G.; Dekkers, A.J.; Louwaars, N.P.; Zandvort, E.A., 1986. *In-vitro* conservation at reduced temperature and under osmotic stress. In: Withers L.A., Anderson P.G. (eds.) Butherwords, London, *Plant Tissues Culture and its Agricultural Applications*, pp. 277–284.
35. Tandon, P.; Kumaria, S., 2005. Prospects of Plant Conservation Biotechnology in India with Special Reference to Northeastern Region. In *Biodiversity: Status and Prospects*; Tandon, P., Kumaria, S., Eds.; Norasa Publishing House: New Delhi, India, pp. 79–91.
36. Thompson, M.R.; Douglas T.J.; Obata-Sasamoto, H.; Thorpe, T.A, 1986. Mannitol metabolism in cultured plant cells. *Physiol. Plant*, 67:365–369.