Effects of Priming with Orange Juice and Coconut Water on Proliferation and Growth of Plantlets from Tubers of Sweet Potato (*Ipomoea batatas* (L.)

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Abstract- **This experiment was conducted to investigate the effects of priming on regeneration of plantlets and early vegetative growth of a local variety of sweet potato (*Ipomoea batatas* L). The local variety, Ex-Igbariam, was primed by soaking medium sized cut tubers (12cm x10cm or 100g) in 10% coconut water, 10% orange juice, and in 10% coconut water and 10% orange juice mixture with water as control for 12 hours before planting. Each treatment was replicated three times. The time taken to regenerate plantlets was recorded. Three weeks after planting, single regenerated plantlets were transplanted into perforated plastic containers and "hardened" under sunlight. Six weeks after planting, the number of plantlets, leaves, nodes and vine length were recorded. Data were analysed by analysis of variance (ANOVA) in a completely randomised design (CRD) at P = 0.05 and means were compared by use of the least significant difference (LSD) at P = 0.05. There was no significant difference in time taken for new plantlets to regenerate. Coconut water had significantly higher number of plantlets than other treatments. There were no significant differences in number of leaves, nodes and vine length of plantlets primed with coconut water, orange juice, and the mixture of coconut water and orange Juice. Water (control) produced significantly fewer leaves, nodes and shorter vines than other treatments. This study has shown that cut medium sized tubers primed with 10% coconut water, could double the number of healthy plantlets and thus provide more planting materials for farmers.**

Keywords: **- Priming, regeneration, plantlets, sweet potato**

I. INTRODUCTION

The markets for sweet potato (*Ipomoea batatas* L) as a value-added processed food and source of bio-based industrial products are growing [1] Apart from its use as food and animal feed [2], [3], sweet potato can also produce large yields of biomass suitable for conversion to industrial products [4]. The starch can be converted to simple sugars and then used to produce plastics or fuels, such as ethanol and butanol [5]. Propagation of sweet potato is mainly by vine cuttings [6], which is gotten from established farms [1], [7] or feral plants from abandoned fields [8]. The greatest challenge in the propagation of sweet potatoes is shortage of vines for planting at the beginning of the farming season, due to the long period between harvest and the new planting season at the onset of rains [9]. Another major challenge is that with the use of vine cuttings, disease and insect infestation can be continuously spread from season to season and to other locations [10]. The alternative has been the use of pathogen free planting materials usually through use of costly procedures such as tissue culture. However, high production cost and lack of facilities have been an impediment to tissue culture adoption especially in sub-Saharan Africa [10]. An alternative to propagating sweet potato by use of vine cuttings or tissue culture is to plant whole roots in soil under shelter to allow for early sprouting [11], [12]; use small roots or, more commonly, pieces of roots [1] or mini-tubers [13]. The sprouts produced from storage roots prove to be excellent planting material for commercial farmers worldwide, but this method is slow and prone to losses due to decay of the roots [14]. One advantage of storage roots over vine cuttings is that they help to maintain genetic variability [6]. Thus more studies are crucial to progress root piece planting or mini-tubers as a viable alternative to vines in sweet potato production. Researchers [1] outlined five areas for further studies with specific regard to storage roots to include (a) examination of the nature of heritability and the relationship between traits; (b) investigation of plant hormones or other chemical treatments to induce sprouting and rooting (c) understanding the developmental physiology and morphology of storage roots in sweet potato in relation to cultural management and breeding studies of root piece planting (d) investigations of the practical issues surrounding the use of root piece planting in commercial sweet potato production systems and (e) estimating the potential time and economic savings root piece planting may provide over conventional sweet potato production systems in terms of labour inputs, crop and seed increase potential and ultimately, profitability. This study therefore seeks to address, one of the areas of challenges identified, not by use of chemicals but by exploiting natural substances produced by plants. Plant growth regulators are natural substances produced by plant tissues in small quantities, and include auxins, cytokinins, abscisic acid, gibberellins and ethylene [15], especially at the apical points and transported to other regions acropetally and basipetally where they perform various physiological activities in the plant. They are normally active at very low concentrations in plants and play an important role in several physiological and developmental processes. These include control of cell cycle, apical dominance, lateral root initiation, stem elongation, leaf and cotyledon expansion [16]. Also for regulation of many plant processes and the differentiation of

cells into specific plant parts, a variety of ratios and concentrations of these plant hormones are required rather than a single hormone acting alone [17]. They may act as both stimulators and inhibitors of growth, and could cause different plant parts (shoots, buds and roots) to respond differently, although the proportion of auxins (NAA) to cytokinins (BAP) determines the type and extent of organogenesis in plant cell cultures [18]. With the renewed interest in "no chemical" farming or organic farming, techniques and technologies are being developed based on the use of natural products as growth hormones, carbon and vitamin sources for *in vitro* and *in vivo* regeneration and propagation of plantlets [13], [19]. Earlier, [20] had pointed out the benefits of natural plant products such as fruit juices which could enhance the usefulness of plants as renewable resources of valuable materials. Such products can be optimized to play an increasingly significant role in commercial development of new products for regulating plant growth. Such substances should contain growth hormones or their precursors or have exhibited some potential to accelerate plant growth or induce sprouting and rooting of mini-tubers of sweet potato. Orange juice is a complex organic extract that contains carbohydrates, protein, several vitamins, lower levels of some amino acids and organic acids [21]. It is composed of vitamin C, thiamin, folate, vitamin B6, vitamin A, niacin, potassium, magnesium, calcium, iron, sodium and myo-inositol, [15], [22]. A study [23] found that the highest mean number of shoots from Radish (*Raphanus sativus* L.) Var. Beeralu observed in MS basal medium was with 10% orange juice whereas the 2nd highest shoots were obtained with 20% coconut water. Plant products such as coconut milk, banana extract and tomato juice can be very effective in providing organic nutrients and as growth factors [24], [25]. Coconut water contains mainly water (94%) and growth promoting substances that can influence *in vitro* cultures including inorganic ions, amino acids, organic acids, vitamins, sugars, alcohols, lipids, nitrogenous compounds and phytohormones [26]. Coconut water is a natural source of phytohormones such as auxin and cytokinin and they happen to be the most active phytohormones in coconut water [26]. The ability of coconut water to support plant growth is due to its capability to stimulate cell division and morphogenesis. Coconut water, a liquid endosperm, is reported to have high levels of zeatin a naturally occurring growth promoter in its composition and is frequently used in micro-propagation protocols of economically important crops [26],[27],[28]-[29]. Coconut water is also reported to contain indole-3-acetic acid (IAA) [26]; the primary auxin in plants and gibberellins [30, 31]. Other components found in coconut water include sugar, alcohol, lipids, amino acids, nitrogenous compounds, organic acids, and enzymes [32] and they play different functional roles in plants due to their distinct chemical properties. One advantage of coconut water is that it results in considerable plant cell proliferation without increasing the number of undesirable mutations [33]. This study was conducted therefore to investigate how priming with orange juice and coconut water would affect the proliferation of plantlets and shoot growth from mini-tubers of sweet potato.

II. MATERIALS AND METHODS

Preparing of primers

The primers in this study, comprised: fresh orange juice, coconut water from mature coconut fruit, a mixture of coconut water and orange juice and water (control). Coconut water and orange juice primers were prepared by adding 50ml of each to separate 500ml of water (1:10 ratio of natural substance to water) while the mixture had 50ml of orange juice and 50ml of coconut water to 500ml of water.

Preparation of sweet potato tubers and treatment applications

The sweet potato variety used for this study was the Ex-Igbariam a local white skinned, white fleshed variety obtained from the Rivers State University farm, Port Harcourt. Tubers of the sweet potato were washed under running water after which the tubers were cut into medium sized pieces of approximately 12cm x 10cm size or 100g weight to ensure that the primers could easily penetrate the tubers through the cut surfaces. Each medium sized tuber was soaked in the prepared natural substance primers for 12hours. At the end of priming, the cut medium sized tubers were removed and planted in perforated disposable transparent rectangular bowls (46cm length x 17cm width) containing 500gm of loamy soil with watering carried out as required for multiplication of plantlets. Three weeks after priming, each regenerated plantlet was transplanted into 60cl transparent plastic containers containing 200g of loamy soil and kept outside in direct sunlight to harden the plantlets preparatory to transplanting to the field.

Experimental design, data collection and statistical analysis

Treatments were the four natural substance primers described earlier with water as control in a completely randomized design with 3 replications. The data collected were the initial number of days taken to sprouting of the cut medium sized tubers. At 6weeks after priming/planting, number of plantlets produced, number of leaves, number of nodes, and vine length were recorded. All data were analyzed for significant differences using analysis of variance (ANOVA) in a completely randomized design (CRD) at ($P \geq 0.05$). Mean separation was carried out using the Least significance difference LSD at ($P \geq 0.05$).

III. RESULTS AND DISCUSSION

Effects of priming on time taken to first sprouting of plantlets from tubers

The effect of natural substances on the time taken to first sprouting is shown in Fig. 1. There was no significant difference ($P < 0.05$) in the time taken to produce the first sprouts of plantlets of cut medium sized tubers in all priming treatments.

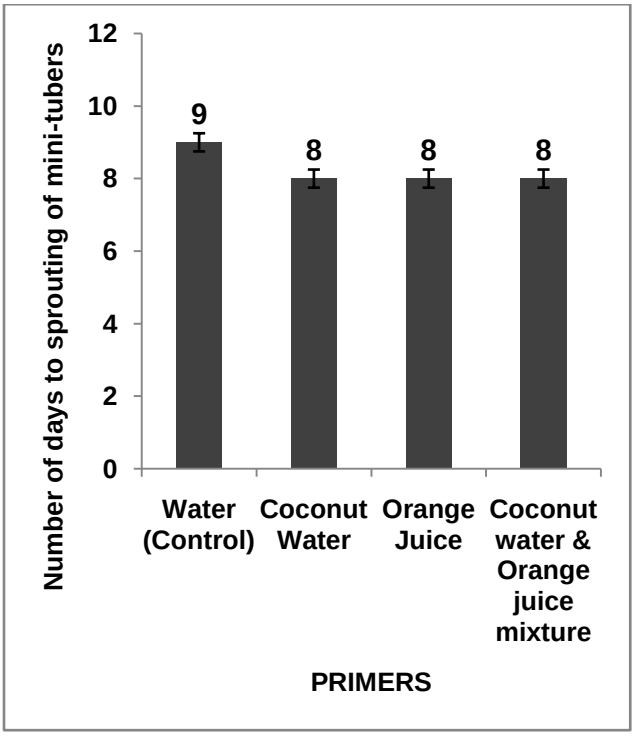

Fig. 1. Effects of priming on time taken to first sprouting of plantlets tubers of Ex-Igbariam variety of sweet potato (*Ipomoea batatas* L)

Effect of priming on number of plantlets

The cut medium sized tubers primed with coconut water had significantly higher (P >0.05) number of plantlets than those of other priming treatments (Fig. 2). It has been reported that when added to a medium containing auxin, coconut water can induce plant cells to divide and grow rapidly [27]. The growth of spinach tissue on a medium supplemented with 10% to 15% (v/v) mature coconut water increased the weight of spinach callus after 5 weeks and accelerated shoot regeneration [34]. Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) was also improved by coconut water [35]. Reference [36] reported that coconut water at a level of 75ml/L or higher initiated callus that was capable of proliferating into shoots. Coconut water was also used successfully for *in vitro* olive micro-propagation [37], for shoot induction of tissue culture of orchid [38] and for *in vitro* shoot regeneration of *Celosia* sp. [39]. Supplementation of coconut water to the culture medium enhanced sweet potato shoot regeneration and growth, with 20% coconut water showing significantly highest value [40] The number of plantlets obtained from the cut medium sized tubers primed with water, though higher was not significantly different (P <0.05) from those obtained from cut medium sized tubers primed with orange juice and those primed with the mixture of orange juice and coconut water. Although natural plant products such as fruit juices have been found to contain hormones that increase regeneration of plantlets from plant tissue during *in vitro* propagation [41], in this study there was no significant advantage in number of plantlets produced by orange juice or mixture of coconut and orange juice compared to other treatments.

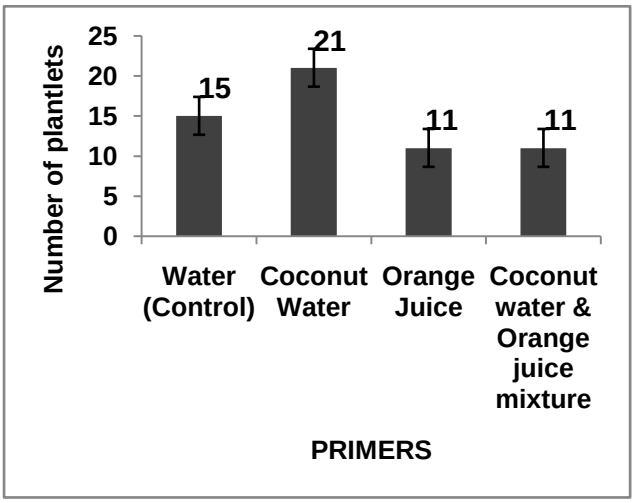

Fig. 2. Effects of priming on the number of plantlets from tubers of Ex-Igbariam variety of sweet potato (*Ipomoea batatas* L)

Effects of priming on the number of leaves

The effect of priming on the number of leaves is shown in Figure 3. Plantlets from medium sized tubers primed with water (control) produced significantly (P >0.05) fewer number of leaves compared to all other treatments. There were no significant (P <0.05) differences in number of leaves among plantlets primed with orange juice, coconut water and those of the mixture of orange juice and coconut water. Reference [42] attributed the robustness and high survival rate of plants cultured in coconut water to the high carbohydrate content which could be used to meet the respiratory demands while surviving the physiological shocks of ex-vitro procedures. It had been reported [43] that coconut water only had positive effect on number of leaves of *in vitro* plants, without affecting shoot length and the other parameters.

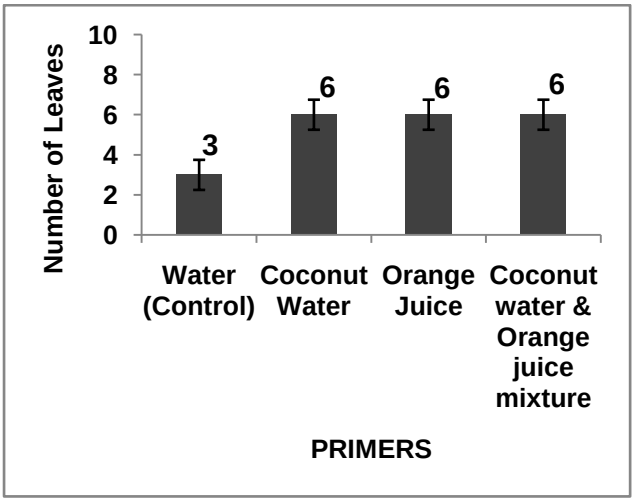

Fig. 3. Effects of priming on number of leaves of plantlets from tubers of Ex-Igbariam variety of sweet potato (*Ipomoea batatas* L)

Effects of priming on the number of nodes

Medium sized tubers primed with coconut water, orange juice and coconut water and orange juice mixture did not differ significantly (P <0.05) in the number of nodes produced by their regenerated plantlets, but the number of nodes they produced was significantly higher (P >0.05) than those primed with water (control). Reference [44] found that maximum shoot length, number of shoots and number of nodes were achieved on MS medium containing 20% (v/v) coconut water with 2.0mg/L of BAP. Medium supplemented with NAA and coconut water yielded fastest emergence and highest number of buds in orchid [38]. It was observed [43] that the general trend indicating the increase in coconut water dose from 0 to 100ml L-1 is positively associated with the increase in shoot length, number of leaves and number of internodes had indirectly led them to conclude about the positive effect of coconut water in the seedling development and growth of olive embryos.

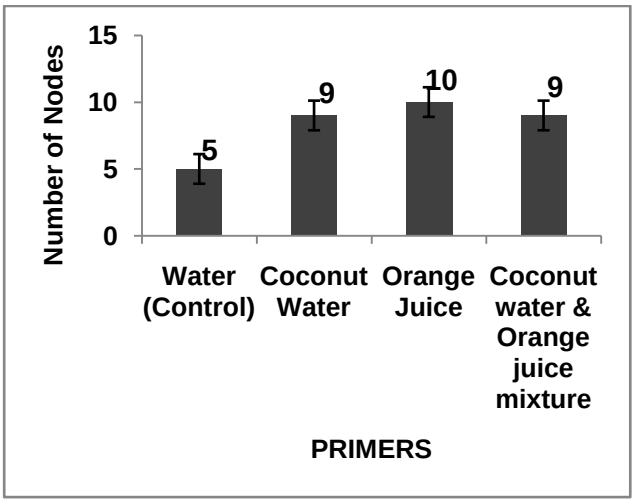

Fig. 4. Effects of priming on number of nodes of plantlets from tubers of Ex-Igbariam variety of sweet potato (*Ipomoea batatas* L)

Effects of priming on vine length

Priming with coconut water, orange juice and the mixture of coconut water and orange juice increased the length of sweet potato vines significantly (P >0.05) compared to tubers primed with water (control). Reference [25] had shown that the highest shoot length of banana was observed in the medium containing 100ml L^{-1} of coconut water. Also addition of 100ml L-1 of coconut water and 50mg L^{-1} ascorbic acid, respectively in culture medium increased the rate of shoot regeneration/ multiplication per explants and shoot length as well as rooting ability of banana plantlets. Coconut water is a source of main growth hormones and vitamins *viz.*, zeatin (-alyl aminopurine), inositol and reduced nitrogen compounds. [45] also demonstrated the advantage of coconut water for stem elongation and plant development in passion fruit. The researchers [46] working with the olive cultivar 'Ascolano 315' reported that 25ml L^{-1} of coconut water associated with 500µg L^{-1} of BAP generated plantlets with higher shoot length and heavier fresh biomass than in plantlets cultivated without these compounds. Orange juice, a source of ascorbic acid could also have improved vine length as reported [25] that average shoot length was increased significantly when 50L-ascorbic acid was applied in the medium.

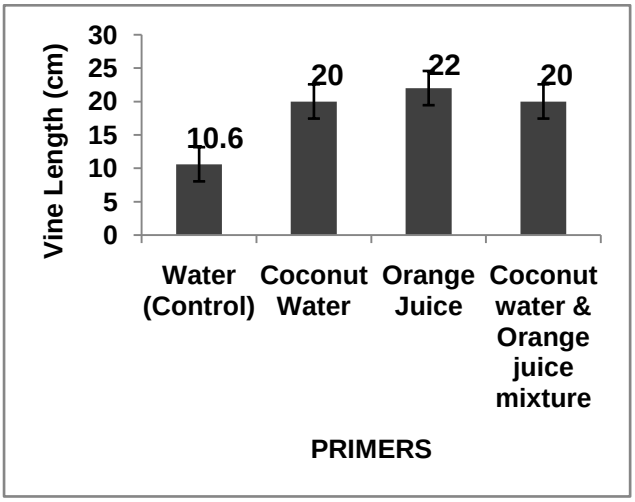

Fig. 5. Effects of priming on vine length of plantlets tubers of Ex-Igbariam variety of sweet potato (*Ipomoea batatas* L)

These results are in agreement with the findings of other researchers who have reported the efficacy of natural substances in regeneration of plantlets using *in vitro* techniques and macro-propagation methods in the regeneration of plantlets [47], [48], [49], [50], [44], [51], [52] [13].

IV. CONCLUSION

This study has shown that using cut medium sized tubers primed with 10% coconut water, macro-propagation of healthy plantlets could be doubled in nurseries.

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REFERENCES

[1]. George, N. A., Pecota, K. V., Bowen, B. D., Schultheis, J. K. and Yencho, G. C., (2011). 'Root piece planting in sweet potato - a synthesis of previous research and directions for the future', *Horttechnology*. 21(6):703-711.
[2]. Lebot V., (2009). Tropical root and tuber crops: Cassava, sweet potato, yams and aroids Oxfordshire: CABI. 91-274.
[3]. Loeenstein G., and Thottappilly G., (2009). The sweet potato. Springer Verlag, Dordrecht.
[4]. Ziska,L.H., Runion, G.B., Tomecek, M., Prior, S.A., Torbet, H.A., and Sicher. R. (2009). An evaluation of cassava, sweet potato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland. *Biomass Bioenergy*. 33:1503–1508.
[5]. Klass D.L, (1998). Biomass for renewable energy, fuels and chemicals. Elsevier, Sydney, Australia.
[6]. Stenvall, N., Haapala, T., and Pulkkinen, P., (2006). The role of a root cutting's diameter and location on the regeneration ability of

hybrid aspen', *Forest Ecology and Management*, 237(1-3): 150-155.

[7]. Far, M. M. M. E. L., and Ashoub, A., (2009). "Utility of thermotherapy and meristem tip for freeing sweet potato from viral infection." *Australian Journal of Basic and Applied Sciences.* 3:153–159

[8]. Tjintokohadi, K., Atu, L., Laranang, L., Velasco, V,, Joe, J,, Timothy, M., Francis Siuromea, F., Tuhuna, M,, Jackson, G., and Ezeta, F ., (2007), 'A report of sweet potato survey held in Guadalcanal, Solomon Islands', ACIAR sweet potato project.

[9]. Namanda, S., and Gibson, W. R., (2013). Shortage of sweet potato planting material caused by pro-longed dry seasons in Africa: Strategies to increase its availability in Uganda. Book of Abstracts, 9th Triennial African Potato Association Conference Naivasha, Kenya; 30th June-4th July. 51.

[10]. Ogero, K.O., Mburugu, G. N., Mwangi, M., Ngugi, M. M., and Ombori, O., (, 2012). "Low-cost tissue culture technology in the regeneration of sweet potato [*Ipomoea batatas* (L) Lam." *Research Journal of Biology*. 2:51–58.

[11]. Okwuowulu, P. A., (2003). 'Effect of harvest age on the storability of four sweet potato varieties in an indoor barn', *Tropical Science*, 43(1):35-38.

[12]. Gibson, R. W., Namanda, S. & Sindi. K. "sweetpotato seed systems in East Africa." *Journal of crop Improvement* 2011;10: 449–451

[13]. Wilson, V., (2019). An Alternative Low Cost Multiplication Technique: Natural Substances for Regenerating Plantlets from Mini Tubers of Sweet Potato (*Ipomoea batatas*, L.) *Asian Journal of Research in Crop Science.* 4(1):1-9

[14]. Khan, V. A., Stevens, C., Fyffe, A. E,, Rhoden, E. G., Bartlett, J. R,, Kittrell, D. M., and Peek, W., (2008). The use of plasticulture technology in sweetpotato production, Recent advances in agriculture.

[15]. Chiwocha, S. D. S., Abrams, S. R., Ambrose, S. J.; Cutler, A. J., (2003). Loewen, M.; Ross, A.R.S.; Kermode, A.R. A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: An analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. *Plant Journal*, 35: 405–417.

[16]. Al-Khassawneh, N. M., Karam, N. S., and Shibli, R. A., (2006). Growth and flowering of black iris (*Iris nigricans*) following treatment with plant growth regulators. *Scientia Horticulturae* 107:187–193.

[17]. Ubalua, A. O., Ikpeama, A. I., Okeagu, O. D. (2015). Effect of Different Concentrations of Orange Juice for in Vitro Regeneration and Multiplication of Cocoyam (Taro), *American Journal of Plant Sciences*, 6:2569-2575.

[18]. Nair, R. R., Kavitha, M., Thilaga, S., and Ganesh, D., (, 2012). Conservation and in Vitro Multiplication of Highly Endangered Indian Traditional Medicinal Plant (*Morinda reticulata* Gamble) through Nodal Explants. *Plant Knowledge Journal*. 1:46-51

[19]. Ghazzawy, H. S., El-Sharabasy, S. F., (2019). Effect of natural additives as coconut milk on the shooting and rooting media of *in v*itro barhi date palm (*Phoenix dactylifera L.*). Materials Research Proceedings. 11:186-192. Available:https://doi.org/10.21741/9781644 900178-13

[20]. Ali, F. M., Ahsan, M., Saeed, N. A., Ahmed, M., Ali, Q., Kanwal, N., Tehseen, M. M., Ijaz, U., Bibi, I., and Niazi, N. K. (2014). Establishment and Optimization of Callus-to-Plant Regeneration System Using Mature Andimmature Embryos of Maize (Zea mays). *International Journal of Agricultural Biology*. 16:111-117.

[21]. Rahman, A., Islam, M. O., Prodhan, A., and Ichihashi, S. (2004). Effects of Complex Organic Extracts on Plantlet Regeneration from PLBs and Plantlet Growth in the Doritaenopsis Orchid. Japan Agricultural Research Quarterly, 38:55-59.

[22]. Clements R. S. Jr., (1980). Darnell B. Myo-inositol content of common foods: Development of a high-myo-inositol diet. The American Journal of Clinical Nutrition. 33:1954-1967.

[23]. Manawadu, I., Dahanayake, P; N., and Senanayake, S. G. N., (2014). Effects of Different Natural Organic Additives on *in Vitro* Shoot Regeneration of *Raphanus sativus* L. Var. Beeralu. *Journal of Agricultural Science and Technology.* A4:219-223.

[24]. Boase, M. R., Wright, S., and McLeay, P. L., (). Coconut milk enhancement of axillary shoot growth in vitro of kiwifruit, New Zealand. *Journal of Crop and Horticultural Science.* 21(2)"171-176, DOI: 10.1080/01140671.1993.9513764

[25]. Mondal, S, Ahirwar, M. K, Singh, M. K, Singh, P and Singh, R. P., (2012). Effect of coconut water and ascorbic acid on shoot regeneration in banana variety Dwarf Cavendish, *Asian J. Hort.*, 7(2):416-419.

[26]. Yong, J. W. H., Ge, L., Ong, Y. F., Tan, S. N. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, *Molecules*, 2009;14: 5144-5164.

[27]. Molnar, Z., E. Virag, V. Ordog (2011). Natural substances in tissue culture media of higher plants. Acta Biologica Szegediensis, 55(1): 123 - 127.

[28]. Vigliar, R., Sdepanian, V. L., and Fagundes-Neto, U., (2006). [Biochemical profile of coconut water from coconut palms planted in an inland region]. J. Pediatr., 82: 308-312

[29]. Beshir, I., S.E. Sharbasy, G. Safwat and A. Diab, 2012. The effect of some natural material in the development of shoot and root of banana (Musa sp.) using tissue culture technology. N. Y. Sci. J., 5: 132-138

[30]. Ma, Z., Ge, L., Lee, A. S. Y., Yong, J. W. H., Tan, S. N., Ong, E. S., (2008). Simultaneous analysis of different classes of phytohormones in coconut (*Cocos nucifera* L.) water using high performance liquid chromatography and liquid chromatography tandem mass spectrometry after solid-phase extraction. *Anal. Chim. Acta*. 610:274–280.

[31]. Ge, L., Yong, J. W. H., Goh, N. K., Chia, L. S., Tan, S. N. and Ong, E. S., (2005). Identification of kinetin and kinetin riboside in coconut (Cocos nucifera L.) water using a combined approach of liquid chromatography-tandem mass spectrometry, high performance liquid chromatography and capillary electrophoresis, *Journal of Chromatography*, 829: 26–34

[32]. Arditti J., (2008). Micropropagation of orchids, 2nd edn. Blackwell, Cambridge

[33]. Matsumoto', T. K., (2006). Gibberellic Acid and Benzyladenine Promote Early Flowering and Vegetative Growth of Milton iopsis Orchid Hybrids. HORT Scienc. 41(1).

[34]. Al-Khayri J., Huang F., Morelock T., Busharar T. (1992). Spinach tissue culture improved with coconut water. HortScience, 27: 357 - 358.

[35]. Al-Khayri, J.M., (2010). Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. Biotechnology, 9: 477-484

[36]. Michael, P. S. (2011). Effects of coconut water on callus initiation and plant regeneration potentials of sweet potato. Journal and Proceedings of the Royal Society of New Soth Wales, 144: 91 - 101.

[37]. Peixe, A., A. Raposo, R. Lourenco, E. Macedo (2007). Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. Scientia Horticulturae, 113(1): 1 - 7. doi: 10.1016/j.scienta.2007.01.011

[38]. Maukarlina, A. Listiawati, S. Mulyani (2010). The effect of coconut water and naphthalene acetic acid (NAA) application on the *in vitro* growth of Paraphaleaeonopsis serpentilingua from West Kalimantan. Bioscience, 2(2): 62 - 68.

[39]. Daud, N., R. M. Taha, N. N. Noor, H. Alimon (2011). Effects of different organic additives on *in vitro* shoot regeneration of Celosia sp.. Pak. J. Biol. Sci., 14(9): 546 – 551

[40]. Hang Vu Thi Thuy, Ha Thi Anh Thu, Vu Dinh Hoa (2016). Developing an efficient regeneration protocol for sweetpotato, ipomoea batatas (l.) lam., using nodal explants. Vietnam J. Agri. Sci. 2016, Vol. 14, No. 10: 1491 -1501

[41]. Ali, S., Charles, T. C., Glick, B. R., (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase**.** *Plant Physiol. Biochem*., 80:160-167. https://doi.org/10.1016/j.plaphy.2014.04.003

[42]. George, E.F., 1993. Plant Propagation by Tissue Culture. 2nd Edn., Exegetics Ltd., London, UK., pp: 457

[43]. Souza, R.A.V., F.T. Braga, T.A. Setotaw, J.V. Neto, P.H. de Azevedo, V.H. de Azevedo, G.M. de Almeida Cancado, 2013. [Effect of coconut water on growth of olive embryos cultured in vitro]. Ciencia Rural, 43: 290-296, 10.1590/S0100-204X2011000300012 (In Portuguese).

[44]. Nasib, A., Ali, K. and Khan, S. (2008). An optimized and improved method for the in vitro propagation of kiwifruit (*Actinidia deliciosa*) using coconut water, *Pakistan Journal of Botany*, 40: 2355-2360.

[45]. Trevisan, F., and Mendes, B. M. J., (2005). Optimization of *in vitro* organogenesis in passion fruit (*Passiflora edulis* f. Flavicarpa). Scientia Agricola, v.62, n.4, p.346-350, 2005. doi: 10.1590/S0103-90162005000400007

[46]. Villa, F., Pasqual, M., and de Faria Freitas, G., (2010). Optimization of a protocol for the micropropagation of olive tree cv]. Revista Ceres, 57: 530-534, doi: 10.1590/S0034-737X2010000400015 (In Portuguese)

[47]. Murthy, H. N. and Pyati, A. N., (2001). Micropropagation of *Aerides maculosum* Lindl. (Orchidaceae), *In Vitro Cellular & Developmental Biology* – Plant, 37: 223-226

[48]. Mandal, A. B., Aparna, M. and Elanchezhian, R., (2002). *In vitro* micropropagation of *Ananas comosus* L. (Merr.) var Queen. *J. Applied Hortic*., 4: 107-112

[49]. Pyati, A. N., Murthy, H. N., Hahn, E. J. and Paek, K. Y., (2002). *In vitro* propagation of *Dendrobium macrostachyum* Lindl.: A threatened orchid, *Indian Journal of Experimental Biology*, 40: 620-623

[50]. Aktar, S., Nasiruddin, K. M. and Hossain, K., (2008). Effects of different media and organic additives interaction on in vitro regeneration of Dendrobium orchid, *Journal of Agricultural Research and Development*, 6: 69-74.

[51]. Baskaran, P., Velayutham, P. and Jayabalan, N. (2009). In vitro regeneration of *Melothria maderaspatana* via indirect organogenesis, *In Vitro Cellular & Developmental Biology – Plant*, 45: 407-413

[52]. Cheong, E. J., Mock, R. and Li, R., (2009). Optimizing culture medium for meristem tissue culture of several Saccharum species and commercial hybrids, *Journal of American Society of Sugar Cane Technologists*, 29: 149-165.