Journal and Proceedings of the Royal Society of New South Wales, vol. 144, nos. 3&4, pp. 91-101.
ISSN 0035-9173/11/020091-11"

Effects of coconut water on callus initiation and plant regeneration potentials of sweetpotato

Patrick S. Michael

Department of Agriculture, PNG University of Technology, Lae, Papua New Guinea

E-mail: patrick.michael@adelaide.edu.au

Abstract

Embryogenic callus initiation and plant regeneration in sweetpotato *in vitro* have been accomplished through various amendments with supplements. Such amendments include use of appropriate growth regulator combinations or inclusion of other supplements that have the potentials to enhance callus initiation and shoot proliferation. Coconut water has been reported to enhance callus induction, shoot development and multiplication in tissue culture of plants but has never been tried in sweetpotato, which is still recalcitrant to most *in vitro* treatments reported. The objective of this study was to evaluate callus initiation; shoot proliferation and plant regeneration potentials of four different quantities of coconut water levels ($0mlL^{-1}$, $25mlL^{-1}$, $50mlL^{-1}$, $75mlL^{-1}$ and $100mlL^{-1}$) on three sweetpotato cultivars of Papua New Guinea *in vitro*, on a modified Murashidge and Skoog (MS) medium. The modified medium was supplemented with $3mgL^{-1}$ 2, 4-dichlorophenoxyacetic acid and $0.5mgL^{-1}$ 6-benzyl amino purine. The control medium was set without any of the coconut water levels. At coconut water levels lower than $75mlL^{-}$, callus initiation and plant regeneration potentials of all the sweetpotato cultivars were relatively low. At coconut water levels of $75mlL^{-1}$or higher, more than 85% of SK010, 75% of WHCH005 and 50% of PRAP496 initiated callus that were capable of proliferating into shoots. Shoot proliferation was also poor at lower coconut water levels. Shoot isolates that proliferated from calli at higher coconut water levels were able to grow to maturity.

Keywords: Coconut water, callus initiation, plant regeneration, sweetpotato

Introduction

Sweetpotato (*Ipomoea batatas L.*) genetic resources are an important component of the biological diversity in Papua New Guinea (PNG) where cultural and environmental diversity exist. PNG has a rich array of cultivars, semi cultivars or landraces of more than 1500 sweetpotato (SP) that are adapted to different environmental conditions. The richness in diversity and the existence in the light of growing shift from mixed cultivation to monoculture in the farmers' fields, continuous degradation of soil fertility, rapid population increases, lack of proper farm management practices, introduction of pests and diseases and unpredictable weather patterns coupled with natural disasters have indicated growing need for SP genetic diversity conservation, use and improvement. As a result of these factors, there is an irreplaceable erosion of SP genetic resources once found and face the serious risks of losses if no proper efforts towards sustainable utilization and management of the richness in diversity are addressed (Michael, 2004).

Traditionally, SP is conserved by subsistence farmers because of the mixed cropping systems practiced, and it is not surprising to find thirty to fifty different cultivars in the farmers' fields. The presence of the diversity shows how

important this crop is and the wide spread acceptance of the crop by the people, especially in the highlands where the population density is often high and SP plays an important role in the lives of the people socio-economically (Michael, 2004). Not only that, it is also reported to be an important food security crop in many other developing countries (Burden, 2005), especially in the tropical, subtropical and warm temperate regions of the world (Sihachakr et al., 1997).

Despite the importance of SP, the crop is often subjected to disease causing pathogens, prone to pest and disease infestations (Aritua et al., 1998) and there is a need to improve the crop genetically. The genetic improvement of the SP however, is limited due to male sterility (Sihachakr et al., 1997; Otani and Shimada, 2002; Song et al., 2004), incompatibility and the hexaploid nature of the SP genome (Dhir et al., 1998; Michael, 2005; 2009). These limitations have prompted SP researchers to use alternative techniques such as genetic manipulation (transformation) and several plant tissue culture techniques (Michael, 2005; 2009; 2010). Plant tissue culture underpins many of the *in vitro* techniques in genetic manipulation and plays an important role in the manipulation of plants for micopropagation of planting materials, elimination of tissue borne disease causing pathogens such as virus, isolation and development of genetic variants and conservation of plant genetic resources *in vitro* (Michael, 2005; 2007).

There are several reports of embryogenesis and organogenesis in SP tissue culture and plants have been reported to be regenerated through somatic embryogenesis through roots (Jarret et al., 1984) and root disc (Carswell and Locy, 1984). Despite the advances made in SP tissue culture, the crop is still recalcitrant to different *in vitro* treatments in terms of plant regeneration from callus (Michael 2005; 2009) and many amendments have to be made to the mineral composition or the growth regulator combinations used. Such amendments include the use of correct combination of growth hormones and inclusion of other *in vitro* callus induction and proliferation factors, such as coconut (*Cocos nucifera L.*) extracts (Michael, 2007).

The report of Agampodi and Jayawardena (2009) shows that coconut contains plant growth hormones that are normally used in tissue culture. Similarly, other research reports show that supplementation of coconut water in tissue culture media has enhanced callus initiation (Namdeo et al., 2006), shoot development (Tefera and Wannakrairoj, 2004) and multiplication in combination with synthetic auxins (Loc et al., 2005) in plants. Despite these works, the applications have been limited due to low reproducibility (Saranga and Cameron, 2007; Agampodi and Jayawardena, 2009) and the uses of coconut water in SP tissue culture have never been reported. This study was conducted to test the effects of five different coconut water levels (CWL) on callus initiation, shoot proliferation and plantlet regeneration potentials of three sweetpotato cultivars *in vitro*.

Materials and methods

Field practices and sources of plant materials

The cuttings of three SP cultivars used in the present study were supplied by PNG National Agriculture Research Institute (NARI). The cultivars were high yielding, drought tolerant and widely cultivated SP cultivars (SK010, WHCH005 and PRAP496) in the PNG highlands. The respective cuttings of the cultivars were potted out in a compost mixture of farm yard manure and sand in the proportion 3:1 and grown to maturity under glasshouse conditions at the Department of Agriculture, PNG University of Technology, as sources of explants. The plants were watered daily and a slow release, granular fertilizer (NPK, 15-15-15) was applied to support normal plant growth. Cuttings from these plants were used as source of explants throughout the study.

Surface sterilization and explant preparation

For laboratory use, the top shoot tips on the youngest nodes containing 2-3 leaf primodia of 2 months old plants were sampled, placed in several vials containing 20ml of reverse osmosis water to prevent explants dehydration and taken to the lab for immediate use. For culture initiation, the nodes between the first and the second most youngest leaves of the shoot tips of all cultivars were cut into required sizes (~3mm) and surface sterilized by placing them in pre-labeled 100ml beakers containing 20% sodium hypochlorite (NaOCl) for 5 minutes followed by rinsing them in sterile reverse osmosis water under a sterile cabinet for 3 minutes. The sterile stocks were further excised (~2mm in length), cut transversely into sections and again in half along the axis and used as explants.

Coconut water and medium preparation

The CWL were prepared using young nuts (~9 months old), collected from the Agriculture Department Farm, PNG University of Technology. The nuts were cracked opened and the water was sieved through a double-folded muslin cloth, 2-3 times into several 100ml plastic vials with screw caps and kept frozen in a freezer at -4^{0}C prior to use.

The modified MS-based medium consisted 3mgL^{-1} 2, 4-dichlorophenoxyacetic acid (2, 4-D), 0.5gL^{-1} polyvinylpyrrolidone (PVP) as an antioxidant, 0.5mlL^{-1} 6-benzylaminopurine (BAP) and 30gL^{-1} sucrose as energy source. Agar at 8gL^{-1} was used as the gelling agent (MS0.8 medium). The auxin-cytokinin combinations used was based on previous studies on sugarcane tissue culture research (Michael, 2007) in our lab at the University of Technology Biotechnology Centre (UBC). To identify the optimum CWL, a range of CWL (0ml, 25ml, 50ml, 75ml and 100ml) were supplemented in a litre of MS0.8-based medium. A litre each of CWL was prepared (a total of 8L (4L for callus initiation and 4L for direct plant regeneration respectively)). The control medium contained all the supplements except the CWL (MSCo.8 medium). Two litres of MSCo.8 medium was prepared.

The callus initiation medium contained all the supplements together with the different CWL (MSCi.8 medium) and the medium for plant regeneration contained the same supplements and CWL except 2, 4-D (MSCr.8 medium). The pH of the media was adjusted to 5.6 with 1N sodium hydroxide (NaOH) and heated on a hotplate with continuous stirring until all the supplements have dissolved. A 25ml of the media were dispensed using a manual dispenser into 25ml plastic vials, followed by sterilizing in an autoclave at 15psi (121^{0}C) for 15-21 minutes. A total of 40 vials containing 25ml of the medium per litre each of the CWL and control were prepared. This procedure was followed subsequently to prepare all the media required throughout the study. A total of 28L of both the control and treatment media were prepared.

Culture initiation and plant regeneration

All the explants were handled aseptically. The meristematic tips for callus initiation were further excised into segments (0.5-1.0mm), wounded throughout and cultured on MSCi.8 medium. Five explants were cultured in a vial of each CWL and replicated 5 times. A total of 20 vials each were allocated to each SP cultivar (5 vials per CWL), and cultured with 100 explants. There were 60 vials cultured with a total of 300 explants of all the cultivars; and set up using a complete randomized design. The cultures were incubated inside dark cupboards and routinely sub-cultured at 3 weeks intervals for further callus initiation, proliferation and multiplication until adequate amounts of calli were obtained (Table 1).

For plantlet regeneration, 28 day old embryogenic calli (EC) were transferred onto fresh MSCr.8 medium and incubated directly

under a 16 hour photoperiod of 30μmol m^{-2} s^{-2} photosynthetic photon flux using cool white fluorescent tubes in an incubation room as per Michael (2007) at a room temperature of 24 ± 2^{0}C. The miniature shoots of about 3-5cm long with small roots obtained from callus were singled out and transferred onto fresh MSCr.8 medium for rooting. Plantlets were further rooted on an MSCr.8-based medium supplemented with 0.5mgL^{-1} cytokinin (BAP), prepared separately for this purpose and grown to maturity. These cultures were kept under reduced *in vitro* conditions at half the original strength of MSCr.8 medium and conserved. The conserved materials were routinely sub-cultured every 6 months and maintained throughout. This procedure developed was adapted and is routinely used in our laboratory at University of Technology Biotechnology Center (UBC).

Data collection and statistical analysis

Callus growth was assessed by taking the differences between the final and initial calli weights. The initial calli weights were obtained by weighing all the calli during the first subculture and the final weights by weighing the same cultures during the second subculture respectively; within 28 days at an interval of two weeks, until transferred to the MSCr.8 medium for further calli proliferation, shoot initiation and plant regeneration. The data collected were entered on Microsoft Excel 2007 and stored prior to analysis. The callus growth data collected were analyzed using Statistix 9.1 (Statistical Software). One-way ANOVA was also done to test the significant differences between different treatments (CWL) at $p<0.05$. The analyzed data presented in Table 2.

Results

Callus initiation potential

The callus initiation potentials of the explants (meristem tips) cultured on the modified MS-based media containing the different CWL gave different results, showing strong cultivar responses to the *in vitro* treatments provided. Compared to MSCr.8 medium, the explants and callus cultured on MSCo.8 medium were comparatively low in terms of callus and shoot initiation, proliferation, and development. The callus initiation potentials of the cultivars in the order of responsiveness were SK010, WHCH005 and PRAP496 (Table 1).

Cultivar	**Coconut water levels (mlL^{-1})**				
	0	25	50	75	100
SK010	10	13	20	56	86
WHCH005	6	8	15	43	77
PRAP496	3	4	11	23	63
The percentages were calculated based on the total number of explants that induced callus raised over the total number of explants (125) cultured per CWL on MSCi.8 medium. The data under zero (0) CWL are the performances of explants cultured on the control medium (MSCo.8).					

Table 1. Callus initiation potentials as percentage (5) of meristem tips of the three sweetpotato cultivars.

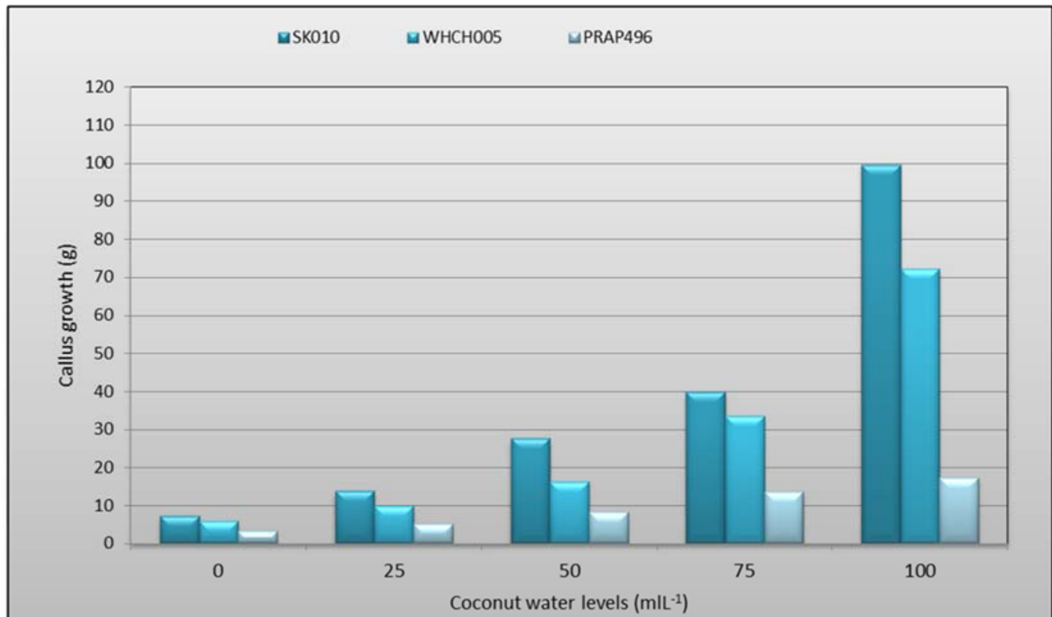

Figure 1. Callus proliferation of the three sweetpotato cultivars on CWL. The data used (mean weights) are the differences between the callus weights obtained during the first subculture and at the second subculture respectively.

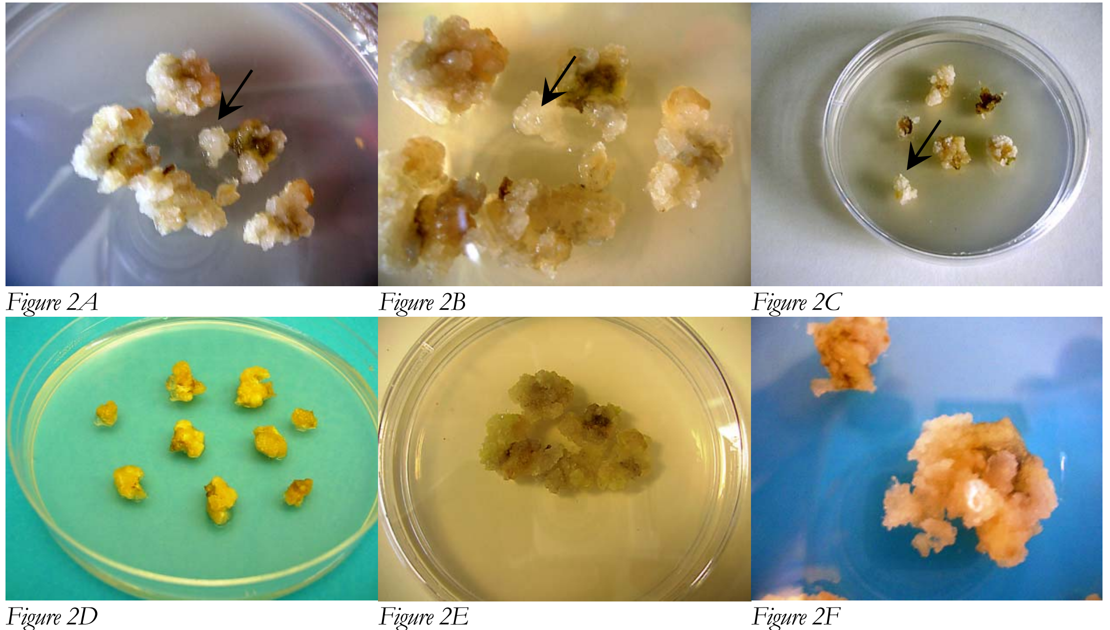

Figure 2. Callus initiation on MSCi.8 medium. Callus developing on SK010 (A), WHCH005 (B) and PRAP496 (C) at 100mlL-1, and PRAP496 (D), SK010 (E) and WHCH005 (F) on 75mlL-1 21 days after culture respectively. The arrowheads show white and friable embryogenic (embryo-like) calli.

Embryogenic callus initiation and proliferation

There was a huge difference in terms of callus proliferation (growth) of the entire test SP cultivars used. In terms of callus initiation, all the cultivars performed poorly at lower CWL (25-50mlL^{-1}) on MSCi.8 medium. At CWL of 75mlL^{-1} or higher, most of the explants responded well and varying amounts of calli were obtained (Fig. 1).

Most of the calli produced at lower CWL were however, hard, friable and non-embryogenic in nature (Fig. 2D (full data not shown)). At 75mlL^{-1}, all the cultivars were able to induce and produce calli that were somewhat EC in nature but slightly at lower rates. Interestingly, at CWL of 100mlL^{-1}, all the explants of all the SP cultivars responded well and huge amount of calli were induced (Fig. 2). These calli were embryogenic in nature and had high regenerative potentials (Fig. 3). When the SP cultivars were compared in terms of callus initiation at 100mlL^{-1}, more than 85% (106/125) of cultivar SK010 (Fig. 2A), 75% (94/125) of WHCH005 (Fig. 2B) and 50% (63/125) of PRAP496 (Fig.2C) meristem tips induced callus on MSCi.8 medium (Table 1). One-way analysis of variance showed that all the higher CWL used for inducing callus were significantly different with p-values of the CWL 75mlL^{-1} [0.00 (WHCH005), 0.03 (PRAP496), 0.25 (SK010)] and 100mlL^{-1} [0.01 (WHCH005), 0.05 (PRAP496), 0.24 (SK010)) respectively.

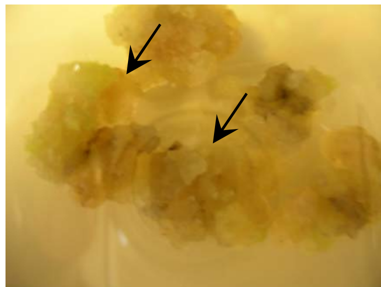

Figure 3A

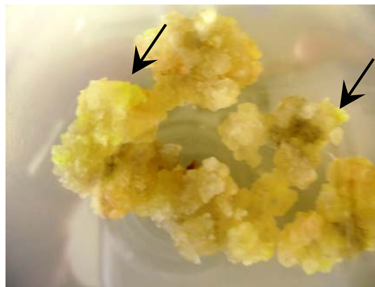

Figure 3B

Figure 3C

Figure 3D

Figure 3E

Figure 3F

Figure 3. Plantlet regeneration on MSCr.8 medium. Proliferating EC of SK010 (A), WHCH005 (B), miniature shoot isolates from calli rooting (C), a single plant isolate grown to maturity (D), meristem tips generating shoots (E), and a shoot isolate grown to maturity (F). The arrowheads show calli proliferation (greenish) into miniature shoots.

Plantlet regeneration.

When selected EC were isolated and transferred onto MSCr.8 medium (Fig. 2), more than 90 percent proliferated into miniature shoots within 28 days after transfer (data not shown). Callus proliferation was high on CWL higher than 75mlL-1. The cultivar SK010 had a higher regeneration potential (Fig. 3A) followed by WHCH005 (Fig. 3B).

The cultivar PRAP496 was recalcitrant to any of the *in vitro* treatments used in this study in terms of plant regeneration. Comparatively, calli proliferation and shoot initiation of PRAP496 was also low, when transferred onto MSCi.8 medium (Table 1).

Coconut water levels (CWL)	Sweetpotato cultivars SK010	WHCH005	PRAP496
0mlL-1			
Mean	7.20	5.80	3.20
Standard deviation	2.17	0.84	0.84
Variance	4.70	0.70	0.70
Standard error means	0.97	0.37	0.37
Coefficient of variation	30.11	14.43	26.15
25mlL-1			
Mean	13.80	9.80	5.20
Standard deviation	1.48	2.86	0.84
Variance	2.2	8.20	0.70
Standard error means	0.66	1.28	0.37
Coefficient of variation	10.75	29.22	16.09
50mlL-1			
Mean	27.60	16.20	8.20
Standard deviation	7.83	3.11	0.84
Variance	61.3	9.70	0.70
Standard error means	3.50	1.39	0.37
Coefficient of variation	28.37	19.23	10.20
75mlL-1			
Mean	27.60	33.60	13.60
Standard deviation	7.83	9.48	3.05
Variance	61.30	89.80	9.30
Standard error means	3.50	4.24	1.36
Coefficient of variation	28.37	28.20	22.42
100mlL-1			
Mean	99.60	72.20	17.20
Standard deviation	21.10	11.19	4.44
Variance	445.30	125.20	19.70
Standard error means	9.44	5.00	1.98
Coefficient of variation	21.19	15.50	25.81

The mean weights (g) were calculated by dividing the total callus weights of each CWL by the total number of culture vials. Standard deviation, coefficient of variation and standard error means of each CWL are also shown.

Table 2. Assessment of callus proliferation (growth) by coconut water levels.

Root initiation in all the plantlets began immediately after the first pair of leaves have emerged and got fully established during the 2nd pair of leaf development (Fig. 3C). When the meristem tips of such plants were isolated, excised into single nodal segments and transferred to MSCr.8 medium (Fig. 3E) for shoot multiplication, the mini explants responded to the medium well at CWL higher than 50mlL^{-1} and somewhat got hardened, followed by mini root development.

When the roots were fully established in the medium, small shoots started protruding from the dormant buds on the nodes (Fig. 3E). Most of the plants derived from both callus and nodal segments rooted well and such plants were successfully isolated and grown to maturity under glasshouse conditions (Fig. 3F). Comparatively, callus initiation, proliferation and regeneration of plants on the MSCo.8 medium were poor. Callus initiation rate was slow and explants got dehydrated and wilted within 4 weeks of culture with little or no response, which were later discarded.

Discussion

The report of Mamaril et al. (1986) shows that growth factors have been successfully isolated from coconut water, however; their (growth factors) uses in SP tissue culture have not been tested. The observations made in this study showed that more than 50 percent of the explants cultured on the different CWL were potentially capable of inducing callus with strong cultivar differences at higher CWL (Table 1). Comparatively, more callus was produced by SK010 than WHCH005 and least by PRAP496. When performances of callus were compared among the different CWL, callus initiation and proliferation rates were concentration dependent. At lower CWL, callus proliferation was low whereas it was high at higher CWL (Table 1). Similarly, plant regeneration was low comparatively although the cultivars SK010 and WHCH005 showed some favorable responses. The latter two varieties also produced huge amount of calli at higher CWL (100mlL^{-1}).

When selected EC was transferred onto the MSCr.8 medium containing higher CWL (100mlL^{-1}), more than 80 percent of the calli produced miniature shoots (Fig. 3C) within 28 days, which later developed into whole plants (Fig. 3D). It was seen that the surface of the white EC, such as those shown in Figure 2A, B, C, turned slightly brown within 14 days after transfer (Fig. 3A, B), followed by greenish appearances on proliferating calli surfaces (Fig. 3A, B). Such surfaces, produced multiples of shoots which were later isolated and transferred onto fresh MSCr.8 medium for further growth and development (Fig. 3D). As shown in Figure 2, the calli induced on cultivars SK010 and WHCH005 were white in nature whereas Triqui et al. (2008) however, reported producing reddish calli which underwent various stages of callus growth. These workers observed that the calli were milky and mucilaginous. Likewise, the calli induced in this study were also milky and mucilaginous in appearance with high regenerative potentials (Fig. 2A, B).

Sihachakr et al. (1997) have shown that somatic embryos at cotyledonous stage turned green and gave rise to 3-5 plantlets per cluster of embryos cultured. In this study, embryo-like clusters of a small amount of greenish callus isolated and transferred onto fresh MSCr.8 medium also produced similar number of plantlets (Fig. 3C). It was also seen that plantlets rooted well when transferred onto the MSCr.8 medium containing cytokinin (BAP) at 0.5mgL^{-1} alone, whereas results of similar experiments elsewhere needed an auxin-cytokinin combinations at lower concentrations (Triqui et al., 2008; Sihachakr et al., 1997. In earlier researches in sugarcane tissue culture in our lab at UBC, it was observed that 2mgL^{-1} of BAP together with 100mlL^{-1} of coconut water without an auxin source resulted

in well rooted sugarcane plantlets (Michael, 2007).

The differences in cultivar performances observed in this study are consistent with the results of other workers in SP tissue culture (Sihachakr et al., 1997; Otani et al., 1998; Song et al., 2004; Michael, 2005; Oggema et al., 2007; Triqui et al., 2008; Michael, 2009; 2010). The latter studies conclude that SP is still recalcitrant to *in vitro* treatments and every genotype needs specific amendments for successful plant regeneration. The observations made, to a large extent show that inclusion of CWL in the culture medium can be useful in SP tissue culture research although the role performed by the unknown constituents of the extracts at this stage is not yet clear and warrants further research.

Studies using coconut water elsewhere show that coconut water is rich in lysine, cystine, histidine, methionine and other essential amino acids (Thio, 1982), has a high concentration of vitamins and minerals together with potassium, calcium and magnesium (Gopikrishna et al., 2008) and contains a lot of sugars (Jackson et al., 2004). Because the performances of explants or the callus cultured on MSCo.8 medium or at lower CWL were poor in terms of callus initiation, proliferation and plantlet regeneration, it is safe for this study to conclude that the higher callus initiation and proliferation, including regeneration potentials observed at higher CWL may be due to presence of growth promoting factors in the higher CWL as reported by other researchers (Thio, 1982; Gopikrishna et al., 2008; Jackson et al., 2004). The findings reported here have been tested, applied and used in tissue culture researches within our lab using other plants such as taro (*Colocasia esculenta*), yam (*Discorea esculenta*), licorice (*Licorice* sp.), noni (*Morinda citrifolia*) and Irish potato (*Solanum tuberosum*) and worked quite well. The MS-based medium (MSCr.8) has been adapted in our lab for conservation of *in vitro* micropropagated crop plants at reduced strengths also.

Acknowledgements

The study would not have been possible without the SP materials being supplied by NARI. The preliminary study was undertaken initially as part of an industrial training program under the supervision of Mr. Humphrey Saese. Special thanks to Mrs. Totave Kamen and the 2003 project students at the UBC, Department of Agriculture, PNG University of Technology, PNG, for technical assistances and research material preparations provided during the study.

References

Agampodi, V. A. and Jayawardena, B. (2009) Effects of coconut (*Cocos nucifera* L.) water extracts on adventitious root development in vegetative propagation of *Dracaena purplecompacta* L.; *Acta Plant Physiology*; **31**, 279-284.

Aritua, V., Adipala, E., Carey, E. E. and Gibson, R. W. (1998) Incidence of sweetpotato virus disease: Virus resistance of sweetpotato grown in Uganda; Annual of Applied Biology; **132**, 399-411.

Carswell, G. K. and Locy, R. D. (1984) Root and shoot initiation by stem, leaf and storage root explants of sweetpotato; *Plant Cell Tissue Organ Culture;* **3**, 229-236.

Dhir, S. K., Oglesby, J. and Bhagsari, A. S. (1998) Plant regeneration via somatic embryogenesis and transient gene expression in sweetpotato protoplast; *Plant Cell Reports;* **17**, 665-669.

Gopikrishna, V., Baweja, P. S., Venkateshbabu, N., Thomas, T. and Kandaswamy, D. (2008) Comparison of coconut water, propolis, HBSS, and milk on PDL cell survival; *Journal of Endodontics*; **34**, 587-589.

Jackson, J. C., Gordon, A., Wizzard, G., McCook, K. and Rolle, R. (2004) Changes in chemical composition of coconut; *Journal of Science of Food and Agriculture;* **84**, 1049-1052.

Jarret, R. L., Salaza, F. and Fernandez, Z. R. (1984) Somatic embryogenesis in sweetpotato; Horticultural Science; **19**, 397-398.

Loc, B. H., Duc, D. T. and Kwon, T. H. (2005) Micropropagation of Zedoary (Curcuma zeloaria, Roscoe)-valuable medicinal plants; Plant Cell Tissue Organ Culture; **81**, 119-122.

Mamaril, J. C., Trinidad, L. C. and Paner, E. T. (1986) Methods of extraction of plant growth hormones in coconut water: UV characterization; *Transactions National Academy of Science and Technology;* **8**, 225-238.

Michael, P. S. (2004) PNG sweetpotato improvement project. Pacific Agriculture Resource Network (PAPGREN); Plant Genetic Resources News from the Pacific (available online).

Michael, P. S. (2005) Tissue culture and genetic transformation of sweetpotato for crop genetic improvement. MSc Thesis, The University of Nottingham, United Kingdom.

Michael, P. S. (2007) Micropropagation of elite sugarcane planting materials from callus culture *in vitro*; The Journal and the Proceedings of the Royal Society of New South Wales; **140** (3&4), 79-86.

Michael, P. S. (2009) Embryogenic callus induction on different explants of sweetpotato. Proceedings of the TWAS Regional Young Scientist Conference on Food, Health and Fuel: Plants for the Future. 2nd – 5th November 2009, Armada Hotel, Selango, Malaysia (published in CD ROM).

Michael, P. S. (2009) Sweetpotato genetic transformation. Proceedings of the TWAS Regional Young Scientist Conference on Food, Health and Fuel: Plants for the Future. 2nd – 5th November 2009, Armada Hotel, Selango, Malaysia (published in CD ROM).

Michael, P. S. (2010) Sweetpotato genetic transformation for crop genetic improvement. Proceedings of the 3rd Annual Science and Technology Conference. 10th – 20th August 2010, Divine Word University, Madang, Papua New Guinea (published by the Office of Higher Education and Divine Word University).

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures; *Physiology of Plants;* **15**, 473-497.

Namdeo, A. G., Mahadik, R. R. and Kadam, S. S. (2006) Cost effective method for callus initiation from Catharanthus roseus leaves; Pharmacology Magazine; **2**, 227-231.

Oggema, J. N., Kinyua, M. G. and Ouma, P. (2007) Optimum 2,4-D concentration suitable for embryogenic callus induction in local Kenyan sweetpotato cultivars; Asian Journal of Plant Sciences; **6**, 484-489.

Otani, M. and Shimada, T. (2002) "Transgenic sweet potato with agronomically important genes"; in Khachatourian, G. G., McHughen, A., Scorza, R., Wai-K. Nip & Hui, Y. H. (Eds.); *Transgenic Plants and Crops,* 699-716.

Otani, M., Shimada, T. and Saito, A. (1998) Transgenic plant production from embryogenic callus of sweetpotato (*Ipomoea batatas* (L.) Lam.) using *Agrobacterium tumefaciens; Plant Biotechnology*; **15**, 11-16.

Saranga, J. and Cameron, R. (2007); Adventitious root formation in Anacardium occidentale L. in response to phytohormones and removal of roots; Horticultural Science; **111**, 164-172.

Sihachakr, D., Haicour, R., Cavalcante, J. M., Umboh, I., Nzoghe, D., Servaes, A. and Ducreux, A. (1997) Plant regeneration in sweetpotato (*Ipomoea batatas* L., Convolvulaceae); *Euphytica*; **96**, 143-152.

Song, Guo-Q., Honda, H. and Yamaguchi, Ken-I. (2004) Efficient *Agrobacterium tumefaciens*-mediated transformation of sweetpotato (*Ipomoea batatas* (L.) from stem explants using a two-step kanamycin-hygromycin selection method; *In Vitro Cellular and Developmental Biology;* **40**, 359-365.

Tefera, W. and Wannakrairoj, S. (2004) Micropropagation of Karwan; Science Asia; **30**, 9-15.

Thio, G. L. (1982). Small-scale and home processing of fresh coconut (Oil Manufacture) and utilization of by-products. Department of Agriculture, Royal Research Institute Bulletin No. 209.

Triqui, Z. El A., Guedira, A., Chlyah, A., Chlyah, H., Souvannavong, V., Haicour, R. and Sihachakr, D. (2008) Effect of genotype, gelling agent, and auxin on the induction of somatic embryogenesis in sweetpotato (Ipomoea batatas Lam.); Plant Biology and Pathology; **331**, 198-205.

Patrick S. Michael

(Manuscript received 22 August 2011; accepted 17 November 2011.)

Patrick S. Michael has an MSc in plant genetic manipulation from the University of Nottingham, UK and Bachelor of Science in Agriculture from PNG University of Technology, Papua New Guinea. Currently, he is a member of the academic staff and teaches a range of subjects at the Department of Agriculture, PNG University of Technology, Papua New Guinea in plant biotechnology, genetics and breeding, biochemistry. His on-going research interest is in plant genetic manipulation, underpinned by plant tissue culture. His previous research includes investigations into oil palm and sugarcane agronomy, sugarcane breeding and crop variety improvement.