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A procedure for *in vitro* evaluation of potato varieties using general plant growth parameters

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Abstract

Presented in this paper is a procedure in which meristem tips (0.02 mm) of seventeen recently introduced potato varieties from the International Potato Center (IPC) and a local variety (Sequoia) from PNG were micropropagated and evaluated in vitro *based on general Plant Growth Parameters (PGP; shoots, roots, leaves, nodes and height). Using this procedure, the study was able to place the tested varieties into three potentially diverse genetic groups. LSD mean separations also showed highly significant differences ($p < 0.05$) among the evaluated parameters; showing the differential genetic variations that exist among these varieties. Regression analysis further indicated that shoot has strong influence on general pattern of plant growth, and coincides with observations that shoot was the first PGP to proliferate on the explants cultured*.

Keywords*: Potato varieties, Mircopropagation, Plant growth parameters, Evaluation,* In vitro

1. Introduction

Cultivated potato (*Solanum tuberosum* L.) is the world's third most important food crop (Knutson *et al.*, 1967) and is increasingly used for food, feed and raw materials for industrial uses (e.g. starch and alcohol). In Papua New Guinea (PNG), potato is an important staple food, especially in the highlands where population densities are often high, arable land is limited and food security is a continuous issue. Production of this crop, except for commercial production, is on smaller-scale and mixed cropping dominants subsistence gardens. Potato is also a popular dish in shops, restaurants and food courts in PNG. In the light of climate change, increase in population pressure on limited arable land and food security issues, potato has recently become an important strategic food crop.

Despite the importance of the crop, shortage of quality and improved planting material is the single most important factor limiting potato production in PNG, coupled with pests, and diseases outbreaks. Additionally, the conventional method of supplying inadequate planting materials and lack of storage facilities limit the success of the potato industry in this country. These problems in the conventional methods of seed potato production require new techniques to improve planting materials that are clean, disease-free and in adequate supply. One of such techniques that can cater for planting materials that are disease-free and in large numbers, often from limited available plant stock (e.g. meristems), is micropropagation *in vitro* through plant tissue culture (Michael, 2007; Michael, 2009a; Michael, 2010).

Potato has been subjected to plant tissue culture techniques (Lam, 1975; Westcott *et al.*, 1977; Ahloowalia, 1982; Xingzhi and Han, 1984; Estrada *et al.*, 1986; Karp *et al.*, 1989; Nasiruddin *et al.*, 2013) since the early 1950s (Steward and Caplin, 1951; Chapman, 1955) serving rapid micropropagation (Roca *et al.*, 1978), breeding for disease, viruses, nematodes, and pathogen resistances (Kassanis, 1957; Wenzel and Uhrig, 1981; McMorran and Allen, 1983; Bulk, 1991). Similarly, responses to salinity (Naik and Widholm, 1993), genetic analysis (Henry *et al.*, 1994), commercial potato production (Uyen and Zaag, 1985), trans-boundary movement (Roca *et al.*, 1979), breeding and genetic improvement (Carputo *et al.*, 1995; Jain, 2001) and germplasm storage (Westcott, 1981) have been additionally studied through tissue culture techniques (Lam, 1975; Wang and Hu, 1982; Xingzhi and Han, 1984; Lenttini and Earle, 1998).

The findings on certain varieties, however, cannot be extrapolated to other varieties due to genetic differences, type of explants used or the tissue culture conditions under which micropropagation was carried out. It is also evident based on the literature that no study has been conducted to establish a procedure in which *in vitro* regenerated plants can be evaluated based on general PGP, identify possible potential genetic groups and establish the relationships between the PGP evaluated. Therefore, the aim of the current study was to (i) evaluate the regenerative potentials of seventeen newly-bred potato varieties released by the IPC together with a local cultivar when micropropagated *in vitro* and (ii) to analyze the relationship between shoot and other PGP to assess whether shoots have an influence on general plant growth.

2. Materials and Method

2.1 Source of Explants

The seeds of the seventeen potato varieties (E1, E2, E4, E9, E10, E11, E20, E38, E41, E45, E46, E48, E52,

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E53, E54, , E55, E57) certified by IPC and a local cultivar (*Sequoia*) were supplied by PNG National Agriculture Research Institute (NARI) for the study. The seeds were initially treated with a solution (50 mg L^{-1}) of a systemic fungicide (dimethomorph, Agro-care Chemical) to protect them from late blight and germinated in a sterilized media containing top soil and sand (1:1, w/w) in pots to establish whole plants in a greenhouse. Upon establishment, the plants were watered daily and maintained by applying 2 kg of NPK per pot once every month throughout the study for four months.

2.2 Explants (Tissue) Preparation

Four weeks old shoot tips (0.5 cm) containing the first rolled leaf primordia of the greenhouse-grown plants were sampled by chopping off at the base of the first visible node using a sterile blade. These explants were then washed using deionized water and surface sterilized in 15% sodium hypochlorite (NaClO) for 15 min, and rinsed in sterile deionized water by dipping for 2 min. These explants were blot dried for another 3 min on sterile, double-folded tissue papers under aseptic conditions.

The sterile explants were aseptically excised further to obtain the meristem tips (0.02 mm), and meristem culture established as per (Hussey and Stacey, 1981) on a modified Murashidge and Skoog (Murashige and Skoog, 1962) media, supplemented with 1.0 mg L^{-1} benzyl adenine (BA), 0.05 mg L^{-1} naphthalene acetic acid (NAA), 0.5 mg L^{-1} nicotinic acid, 0.5 mg L^{-1}, pyridoxine- HCl, 0.1 mg L^{-1} inositol, 30 g L^{-1} sugar, 100 mL L^{-1} coconut water and 8 g L^{-1} agar (Sigma Products) as a gelling agent. The final pH of the media was adjusted to 5.8 prior to autoclaving and a 10ml of the media was dispensed into 25 ml vials and sterilized in an autoclave at 15 psi (121 ^{o}C) for 15 min (Michael, 2007).

A single meristem tip of each variety was then, aseptically cultured in a vial and replicated 10 times. These cultures were incubated at 24 ± 2 ^{o}C under a 16h photoperiod in a tissue culture room in a randomized complete design manner (RCD). Upon culture establishments, contaminated cultures (e.g. Figure 1**d**) were routinely sub-cultured on fresh media for the first 2 weeks by soaking contaminated explants in 70% ethanol for 15 min following by flaming for 30 sec, cool down for another 2 min and the contaminated ends excised and removed. These processes were continued until clean cultures for all the tested varieties were established.

All the clean cultures were sub-cultured in the first month by aseptically excising at the base of the nodes, with the youngest node (0.5 mm) containing a single bud selected for sub-culture. This was carried out to ensure that there was uniformity in the growth among the tested varieties prior to collecting the data. Thereafter, data collection was started after the first two leaves of a shoot has emerged (e.g. Figure 1**a**), and shoot and root developments have become visible (e.g. Figure 1**b**, **c**).

2.3 Plant Growth Parameter Assessments

The growth performances of the plantlets *in vitro* were monitored by assessing and scoring the number of shoots, roots, leaves and nodes developed and the increase in plant height (mm) as per (Michael, 2007). Data were collected at an interval of two weeks for 8 weeks using 5 replicate cultures (vials). Roots, leaves, shoots and nodes were physically counted whilst plant height (mm) was measured on possible shortest and the tallest shoot (their differences were calculated

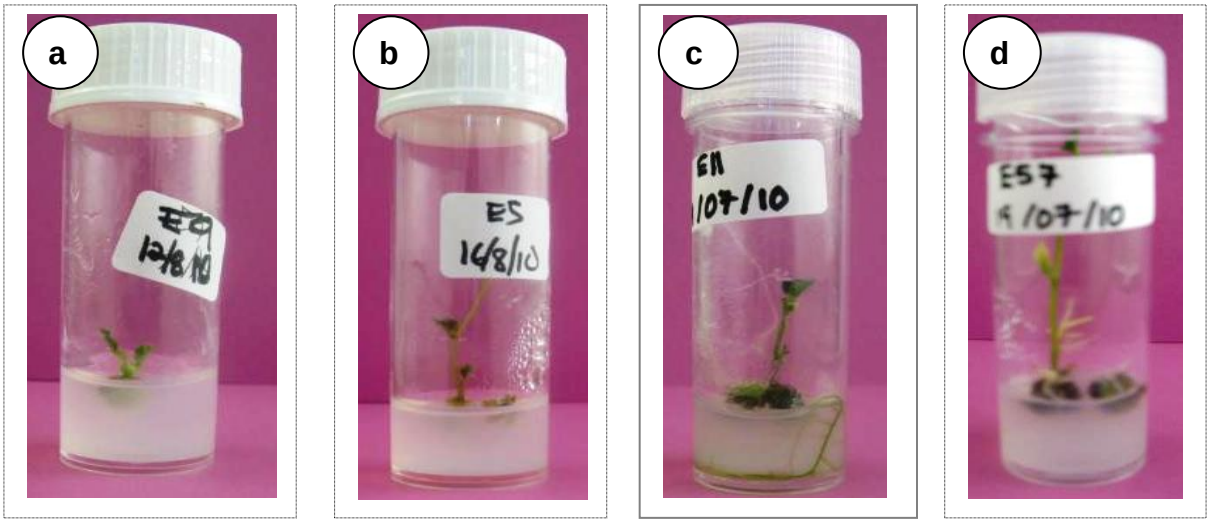

Figure 1. Micropropagation of potato plants *in vitro*. A shoot regenerate (a), fully grown shoot (b), rooting shoot (c) and a typical contaminated culture requiring sub-culture (d).

and kept). Only new roots were counted following the previous counts to avoid repetition, and secondary roots were not considered. All data collections were under taken whilst the plantlets were still in their culture vials to avoid contamination and to ensure continuity until the cultures were sub-cultured.

2.4 Data Analysis

All the data collected were summarized and analyzed using Statistix 10 Statistical Software, Tallahassee FL, USA (Tables 1 and 2). Significant mean differences were further analyzed using LSD ($p<0.05$) in order to compare the treatment means. Exponential regression analysis was further performed to predict the relationships between shoot and rest of the PGP as per the second aim (Figures 2 and 3).

3. Results and Discussion

3.1 Plant Growth and Development

Observations made on the cultures have shown that explants initially started developing shoots, followed by leaf development and internode elongation or increase in plant height. Root formation in most varieties started after the shoots have sprouted and the first four to six leaves have developed (2 - 4 weeks after culture initiation).The PGP evaluated of the plantlets regenerated have shown that differential regenerative potentials exist among the varieties as indicated by distinctive shoot, leaf and node developments, root formation, and height increase.

Based on the second aim of the study, the shoot data was used to group the eighteen varieties into three groups with shoot numbers ≥ 5 in group one, 3-4 in group two (Table 1), and ≤ 2 in group three (Table 2); E10 and E55 have been excluded due to poor shoot development. Generally, all the varieties in group one produced adequate number of shoots, roots, leaves and nodes; as a result, most of them were fast growing (tall) plants (Table 1). The varieties in group two were relatively similar to the group one varieties except that rooting was poor (Table 1). The group three varieties had poor regenerative potential (shoot), low rooting ability and hence, slow general plant growth (Table 2).

Comparatively, the performances of all the varieties in response to the *in vitro* treatments were significantly different ($p < 0.05$), indicating that performances were genotype-specific and independent. Similar results were reported in sweet potato micropropagated *in vitro* (Michael, 2007; Michael, 2011). The LSD test performed indicated that the mean differences of some evaluated varieties were not significantly ($p > 0.05$) different. This shows that the PGP development of most tested varieties was similar and most importantly, *in vitro* treatment conditions provided were conducive for independent expression of their genetic potentials with less or minimal influence from the culture conditions (media composition, growth regulators combinations or supplements). This finding agrees with that of (Yang, 2010) in sweet potato meristem tip culture. Regression analyses of shoot number against other PGP are given in Figures 2 and 3. The data presented show that shoot has strong influence on leaf ($r^2 = 0.80$), root ($r^2 = 0.79$), node ($r^2 = 0.66$) and height ($r^2 = 0.62$); hence general plant growth. This analysis shows a perfect indication of the order in which the PGP developed, as discussed above.

3.2 Micropropagation and Genetic Variations

The plantlets micropropagated have shown great variations in their potentials to regenerate shoots, develop leaves, form roots and influence the general plant growth (increase in height). Since the media used was formulated to induce single shoots, multiple shoots were initially not obtained from the single cultured meristem tips but a lot of plantlets were micropropagated throughout the study period, ranging from over 200 plantlets a month per variety from the best performing varieties. This could equate to thousands of plantlets from a single available stock (Michael, 2007) if sub-cultured frequently.

The LSD mean separation also showed performances of the varieties were significantly different to one another, indicating the genetic variations observed existed in different potato varieties. Such differential variations were reported by other potato researchers (Juned *et al.*, 1991), similar to the findings in the present investigation that the observed differences are due to the genetic differences of the potato varieties. The phenotypic variations observed are good indications of the genetic potentials these varieties possess, although the *in vitro* treatment conditions (e.g. growth regulator combinations) appear to be capable of inducing *in vitro* variability on the regenerates as shown by one study (Pina-Escutia *et al*., 2010). The latter study agrees with the established understanding that any genotype will interact with a given environmental condition during normal plant growth and development.

Moreover, the results of similar studies conducted using other plant genotypes (e.g. sugarcane, sweet potato, taro etc.) in our laboratory to identify the genetic potentials of plants based on *in vitro* phenotypic observations as in this study have shown that *in vitro* regenerated plants show less variations when grown and evaluated under greenhouse conditions, compared to the parent stocks (Michael, 2007; Michael, 2009b; Michael, 2011). Such results indicate that the outcome of a study such as this has the potential to identify best performing varieties, not only potato but other crops as well.

Table 1. Evaluation of potato genetic potentials based on *in vitro* phenotypic observations using PGP. The descriptive statistics: standard deviation (SD), variance and coefficient of variation (C.V.) are calculated based on the average data of each PGP obtained. The shoot, root, leave and node data are in number. The superscripts "[a]" means the given value x 10^3, * group one and two ** varieties.

PGP	Potato varieties								
	E1*	E38*	E52*	E9*	E57*	E54**	E2**	E20**	E4**
Shoot									
Mean	7	6	6	5	5	4	3	3	3
SD	0.08	0.20	0.06	0.40	0.52	0.58	0.15	0.19	0.24
Variances	5.75[a]	0.04	3.25[a]	0.16	0.27	0.34	0.02	0.04	0.06
C. V.	1.09	3.28	1.00	7.43	10.03	14.56	4.87	6.83	9.10
Roots									
Mean	8	7	5	4	4	5	4	4	3
SD	0.15	0.28	0.18	0.79	0.28	0.14	0.59	0.12	0.12
Variances	0.02	0.08	0.03	0.62	0.08	0.02	0.35	0.02	0.02
C.V.	1.94	3.95	3.46	22.38	7.94	2.51	15.16	3.46	4.43
Leaves									
Mean	18	13	11	6	7	7	7	7	5
SD	0.56	0.61	0.26	0.65	0.25	0.67	0.26	0.32	0.08
Variances	0.31	0.38	0.07	0.42	0.06	0.44	0.07	0.10	5.75[a]
C.V.	3.14	4.72	2.40	10.85	3.35	9.16	3.66	4.72	1.41
Nodes									
Mean	7	6	6	5	5	4	6	3	3
SD	0.16	0.08	01.0	0.08	0.08	0.26	0.11	0.23	0.26
Variances	0.02	6.75[a]	9.25[a]	5.75[a]	5.75[a]	0.07	0.01	0.05	0.07
C.V.	2.24	1.32	1.68	1.40	1.34	6.56	1.83	7.37	9.71
Height (mm)									
Mean	36	31	29	29	23	26	17	22	12
SD	0.04	0.20	0.59	1.40	0.47	0.22	0.24	1.63	0.33
Variances	0.16	0.04	0.35	1.96	0.22	0.05	0.06	2.64	0.11
C.V.	1.11	0.63	2.07	4.88	2.03	0.88	1.39	7.35	2.80

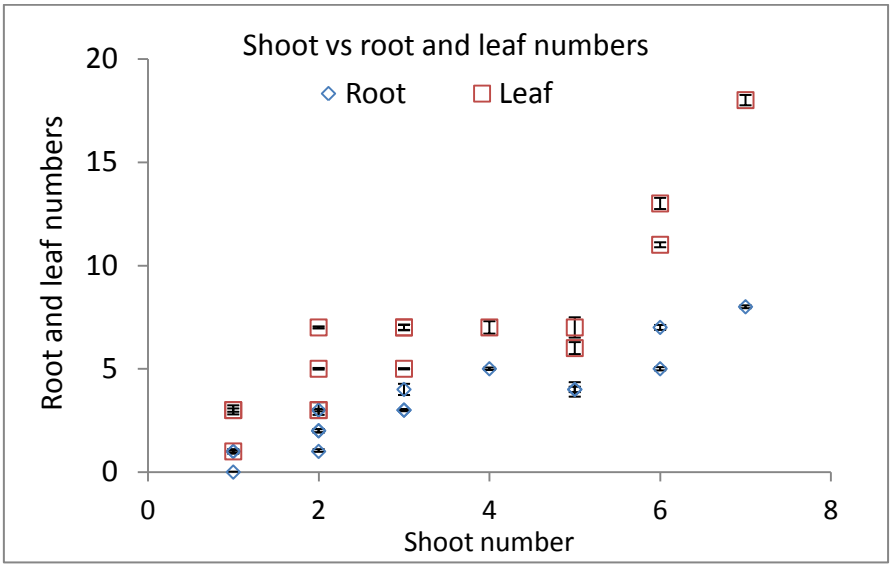

Figure 2. Comparison of leaf and root development against shoot number. The PGP values are means of five replicates. Each point is the mean ± the s.e. of five replicates.

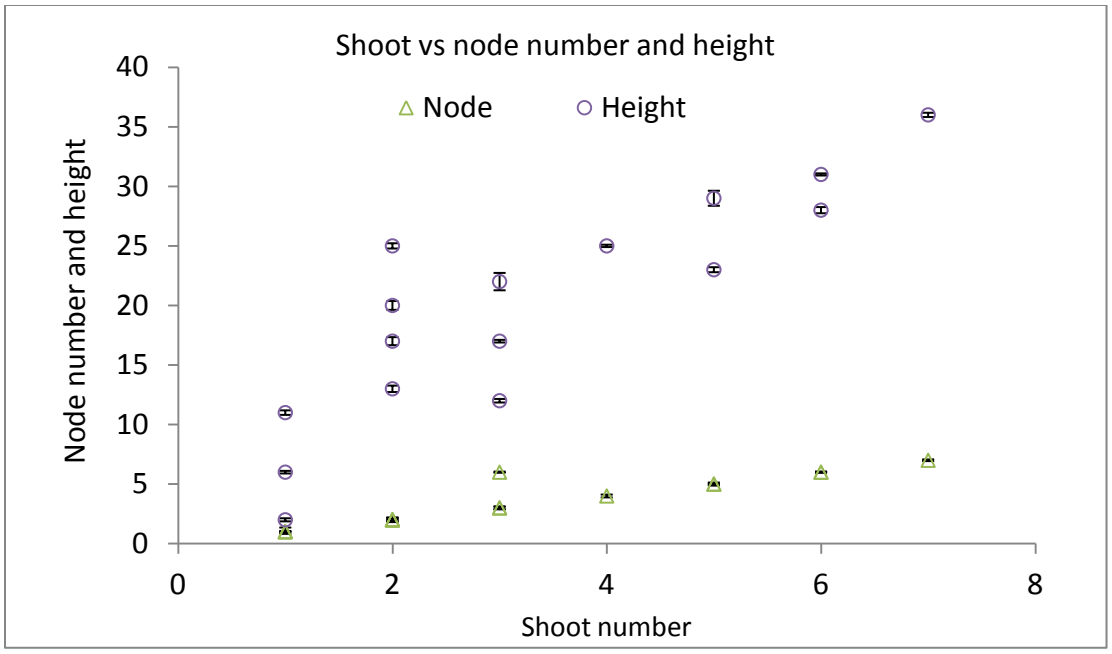

Figure 3. Comparison of node number and height against shoot number. The PGP values are means of five replicates. Each point is the mean ± the s.e. of five replicates.

Table 2. Evaluation of potato genetic potentials based on *in vitro* phenotypic observations using PGP of group three varieties. Descriptions of the parameters are given in Table 1. The notation (-) means no PGP development, and hence no statistical data.

PGP	Potato varieties						
	E48	E41	E53	E45	Sequoia	E46	E11
Shoot							
Mean	2	2	2	1	1	2	1
SD	0.12	0.08	0.18	0.20	0.24	0.17	0.10
Variances	0.01	5.75[a]	0.03	0.04	0.06	0.03	0.01
C. V.	5.40	3.93	9.78	13.68	19.62	10.12	19.56
Roots							
Mean	2	3	2	2	1	1	-
SD	0.17	0.14	0.19	0.14	0.24	0.24	-
Variances	0.03	0.02	0.04	0.02	0.06	0.06	-
C.V.	9.14	4.77	11.69	9.19	19.83	17.95	-
Leaves							
Mean	7	5	3	3	3	3	1
SD	0.10	0.09	0.85	0.18	0.46	0.52	0.12
Variances	0.01	8.25[a]	0.72	0.03	0.21	0.27	0.01
C.V.	1.56	1.74	25.44	6.12	18.39	20.74	8.72
Nodes							
Mean	2	2	2	1	3	2	1
SD	0.43	0.34	0.14	0.09	0.79	0.40	0.12
Variances	0.19	0.12	0.02	8.25[a]	0.63	0.16	0.02
C.V.	23.55	17.27	6.34	6.35	31.62	23.61	22.27
Height							
Mean	25	13	20	11	6	17	2
SD	0.49	0.60	0.83	0.43	0.24	0.76	0.30
Variances	0.24	0.36	0.69	0.18	0.06	0.58	0.09
C.V.	1.95	4.63	4.08	3.83	3.78	4.59	16.86

4. Conclusions

The results obtained in this study are important from an economical, time and research point of view compared to the conventional field evaluations which are relatively expensive, time consuming and labor intensive. This study showed that within a short period of time at a minimal cost, the genetic potential of plant varieties can easily be identified and possible potential varieties further field evaluations.

In this study, standard *in vitro* conditions (with slight modifications) were provided to evaluate the eighteen genotypes, therefore it is safe to conclude that group one varieties are genetically more promising, followed by group two and group three varieties. It is also fair to conclude that the data presented do not necessarily indicate how well the potato varieties can perform under field conditions however; based on the results the eighteen varieties evaluated were categorically grouped into potentially three diverse genetic groups.

There seems to be no potato tissue culture study that reported the observations that shoot is the first PGP that proliferates from an explant well before the other PGP.

Therefore, this study is the first to report the procedure described to evaluate the genetic potential of potato varieties based on *in vitro* phenotypic observations. In addition, the evaluation procedure described is a good step forward for future potato research *in vitro*.

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