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Shoot tip splitting for rapid micropropagation of Philippine taro [*Colocasia esculenta* (L.) Schott]

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ABSTRACT

This study was conducted to establish a micropropagation system for Philippine taro cultivars by shoot tip culture and explore the use of shoot tip splitting in increasing propagation rate. Four recommended cultivars (VG1, VG2, NG9 and NG10) were used. One centimeter shoot tip explants were aseptically prepared, cut longitudinally to produce half and quarter shoot tips, and cultured on modified Murashige and Skoog (MS) medium supplemented with 1-5 mg/L benzylaminopurine (BAP) or 0.5-1.0 mg/L indoleacetic acid (IAA) or their combination. The micropropagation protocol developed was simple and straight forward as the modified MS medium sufficed. Shoot tip splitting remarkably promoted shoot formation, with the quarter shoot tips producing the highest number of shoot-forming explants (80-100%) as compared to half shoot tips (50-60%) and whole shoot tips (20-40%) in all cultivars except NG10 where shoot-forming explants did not significantly vary with explant size. Rooting response of half and quarter shoot tip explants was either comparable to or higher than that of whole shoot tips but the time period to root formation was delayed in split shoot tips by 2-10 days relative to that of whole shoot tips. Because of more explants per shoot tip and the promotion of shoot and plantlet development, micropropagation ratio increased with the use of half and quarter shoot tips by 3 and 8 fold in VG1, 10 and 32 fold in VG2, 7 and 10 fold in NG9 and 4 and 7.6 fold in NG10, respectively. After 3-4 weeks, plantlets were potted out and following the *ex vitro* protocol, all plantlets survived with no visible morphological variation.

INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott] is an important food crop with global production of about 12 million tons per year from over 2 million hectares (FAOSTAT, 2014). Major producers are Africa, Asia and the Pacific, with the Philippines ranking tenth. Taro is a highly nutritious plant and is considered as the healthiest among the health foods (Lee, 1999). It is especially useful to people allergic to cereals, and can be consumed by children sensitive to milk; as such taro flour is used in infant food formula and canned baby foods. The corm is an excellent source of carbohydrate, mainly starch (FAO, 1990). It contains greater amounts of vitamin B-complex than whole milk. It is low in fat and protein, but the protein content of taro is higher than that of other root crops like yam (*Dioscorea alata* L.), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.). In the Philippines, all parts of the taro plant (corm, leaves, stems and runners) are consumed. Several value-added products from taro are available in markets, including dried chips, powder and ice cream. With market demand steadily increasing, it is necessary to increase production area and yield and this requires sufficient volume of planting materials.

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Taro is conventionally propagated by vegetative means using suckers and corms. This method of producing planting materials is slow and seasonal, and shortage of planting materials is a big problem for larger planting and for expanding production. The planting materials can also carryover disease pathogens, particularly viruses, from one planting season to another resulting in reduced growth and yield potential of next generation crops. Tissue culture or micropropagation provides solution to the problems of conventional propagation. The primary advantage of micropropagation is the rapid production of high quality, disease-free and uniform planting materials (IAEA, 2004). The plants can be multiplied under a controlled environment, anywhere, irrespective of the season and weather, on a year-round basis. Tissue culture has been applied for the propagation of over 1000 different plant species (Fay, 1992; Villalobos and Engelmann, 1995; Jackson et al., 2001; Sarasan et al., 2006). Furthermore, tissue culture can be applied in germplasm conservation to address the problems of field management. In the lab, a single staff can manage the whole collection, whereas in the field handy labour is required and the collection is exposed to risks of damage or loss due to biotic (for example, insect pest and disease damage) and abiotic (for example, floods and droughts) factors.

In the Philippines, taro micropropagation has not been established. Previous works on taro micropropagation in other countries have developed different protocols (Yam et al., 1990; Tuia, 1997; Minas, 2002; Hossain, 2012), including a low cost protocol using locally available nutrients to substitute for the Murashige and Skoog (MS) medium (Ngetich et al., 2015). Nath et al. (2012) developed anotherprotocol for local Indian taro cultivarusing one-cm shoot tip. These different protocols could be due to the influence of genotype, culture condition and location. The culture of apical meristem has been used to eliminate viruses in many species and produce disease-free planting materials which usually yield better than conventional planting materials (Wang and Valkonen, 2008, 2009). There are concerns in the culture of apical meristem. One concern is that bud growth could be limited due to apical dominance. In regeneration systems with callus intermediate, somaclonal variation could happen, compromising the genetic fidelity of the resulting plants. Skirvin et al. (1994) reviewed somaclonal variation and the factors in tissue culture that can induce it, including growth regulators, explant source, and length of time *in vitro* or continuous sub-culturing. Thus, direct regeneration system is recommended to produce true-to-type plants.

Splitting of shoot tip or apical bud has the potential to promote bud proliferation by breaking apical dominance, increase the rate of propagation by increasing the number of explants, and cut on the costs of sub-culturing and time, which have been demonstrated in banana (*Msa spp*.) shoot tip cultures (Ngomuo et al., 2014a, b). The technique also minimizes physiological barriers that may require additional media formulations and the rate of somaclonal variation that could result from continuous sub-culturing. In the application of the technique, longitudinal splitting of the shoot tip or apical bud into halves or quarters is done after a certain period of time during incubation of cultures *in vitro* as in banana (Hussein, 2012; Ngomuo et al., 2014a) and canna (*Canna edulis* Ker.) (Hosoki and Sasaki, 1991). In the present study, we have shown that half or quarter shoot tips are feasible to use as starting explants in the micropropagation of taro. This study also established a simple micropropagation method for recommended varieties of Philippine taro.

MATERIALS AND METHODS

Explant source

Four recommended cultivars of taro (locally known as ‘Gabi’) approved for commercial release by the Philippine National Seed Industry Council (NSIC) were used, namely; ViSCAGabi-1 (VG1), ViSCAGabi-2 (VG2), NSIC Gabi-9 (NG9) and NSIC Gabi-10 (NG10). Three month-old suckers of these cultivarswere used for *in vitro* stock plant establishment. These were collected from field-grown plants, trimmed of leaves and roots, and brought to the Tissue Culture Laboratory of the Philippine Root Crops Research and Training Center, Visayas State University (VSU), Baybay, Leyte, Philippines.

Shoot tip preparation

The suckers were thoroughly washed with tap water and outer leaf sheaths removed until the inner cleaner section appeared with about 5 cm of shoot and 2 cm of corm. In a laminar flow cabinet, the tissues were sterilized with 5% sodium hypochlorite for 20 min followed by rinsing with sterile distilled water; this surface sterilization method was found in earlier experiments as the best treatment with less than 10% culture contamination. Outer leaf sheaths were then aseptically removed in a circularfashion using a sterile scalpel and forceps. The 1 cm shoot tips were then inoculated onto test tube (20 mm in diameter × 150 mm in height) containing the culture medium. From the initial stock plants, sufficient number of plantlets was produced for the micropropagation experiments. Different sizes of shoot tip explants (wholes, halves and quarters) were tested. The whole shoot tip was longitudinally cut with a sterile scalpel to produce the half and quarter shoot tips. Four explants were inoculated onto each 3 × 9 cm glass flask (250 mL) with 8 mL culture medium.

Culture conditions

The modified Murashige and Skoog (1962) medium was used. It consisted of macro- and micronutrients, vitamins (2 mg/L glycine, 0.1 mg/L thiamine-HCl, 0.5 mg/L nicotinic acid and 0.5 mg/L pyridoxine-HCl), 50 g/L sugar and 100 mg/L myoinositol, with 4 g/L agar (Conda Pronadisacat. no. 1816) as gelling agent. In the micropropagation experiments, the culture medium was supplemented with 1-5 mg/L BAP or 0.5-1.0 mg/L IAA or their combination. These different culture media served as sub-treatments while the three sizes of shoot tip explants as main treatments. The pH of the culture medium was adjusted to 5.8 using 1 N KOH or HCl. The culture medium was dispensed onto the culture vessel at 8 mL each and then sterilized in a pressure cooker at 15 psi and 121°C for 20 min. The explants were inoculated onto the culture mediumin the laminar flow cabinet. Fifteen cultures were prepared per repetition and each treatment repeated three times. The cultures were incubated on shelves illuminated with 40-watt white fluorescent lamps (Philips) and at a room temperature of 25±2°C. The growing shoots were excised, sectioned into quarter explants, and sub-cultured in plant growth regulator (PGR)-free modified MS medium to produce complete plantlets for potting out.

***In vitro* growth measurement**

Shoot growth was determined in terms of number of explants with shoot growth expressed in percentage of the total number of explants, number of days to shoot initiation, and increase in shoot length with incubation period. The number of shoots produced per explant was counted. Bud eye growth (percentage and days to initiation) was also determined. Root growth was measured in terms of percentage explants with root growth and number of days to root initiation. Callus formation and morphological variations in regenerated plantlets were noted.

***Ex vitro* conditions**

The growing plantlets after 3-4 weeks from sub-culturing were taken out from the culture vessels and washed in running tap water to remove adhering medium. After washing, the plantlets were dipped in benomyl solution (Benomyl 50 W; Agway Chemicals Corporation, Philippines) which was prepared at manufacturer's recommended rate (10 g/16L water). The plantlets were then individually planted on 3 × 5 cm plastic cups (150 mL capacity) containing sterilized medium consisting of a1:1:1 ratio of ordinary garden soil (clay loam type), carbonized rice hull and vermicompost. Each cup was covered with polypropylene plastic bag to avoid desiccation. The plants in cups were maintained in a plastic-covered nursery supplemented with black netting to provide about 70% shade. After two weeks, the plants were transferred to 8 × 15 cm plastic bags with the same sterilized medium as above. The plants were maintained in the nursery without the black netting for about three months (April-June; with temperature and relative humidity ranging from 25-33°C and 65-88%, respectively) following recommended nursery management practices and were observed for survival rate and variations in morphological traits.

Experimental design

The different experiments were conducted in completely randomized design (CRD) with three replications per treatment. The results were analysed by performing analysis of variance (ANOVA) and comparison of means by the least significance test (LSD) at 5% level using the MSTAT statistical package version 2.0.0 (Michigan State University, USA).

RESULTS AND DISCUSSION

The results presented here are the responses of the three types of explants (whole, half and quarter shoot tips) in PGR-free modified MS medium. Culture medium supplementation with different PGR combinations had no added benefit in micropropagating taro relative to that without PGR.

Shoot and root growth

Shoot tip splitting promoted shoot formation (Figure 1). This effect was pronounced in VG1 and VG2 cultivars which had increasing percentage of explants showing shoot growth within a shorter period of time as the size of explant decreased from whole shoot tips to quarter shoot tips. Only 20-40% of the whole shoot tip explants formed shoots in more than 1-2 weeks of incubation. In half shoot tips, 50-60% of the explants formed shoots in a week time while in quarter shoot tips 80-100% formed shoots in less than one week. In NG9 cultivar, similar trend was obtained in terms of percentage shoot-forming explants but the number of days to shoot initiation did not differ much between half and quarter shoot tips (6-7 days) while the whole shoot tips still took the longest time to initiate shoot. In NG10 cultivar, shoot-forming explants varied only from 60-70% regardless of shoot tip size and the whole shoot tips formed shoots in about 10 days or twice longer than the half and quarter shoot tips. Bud eye growth was also favoured by shoot tip splitting (Figure 2).

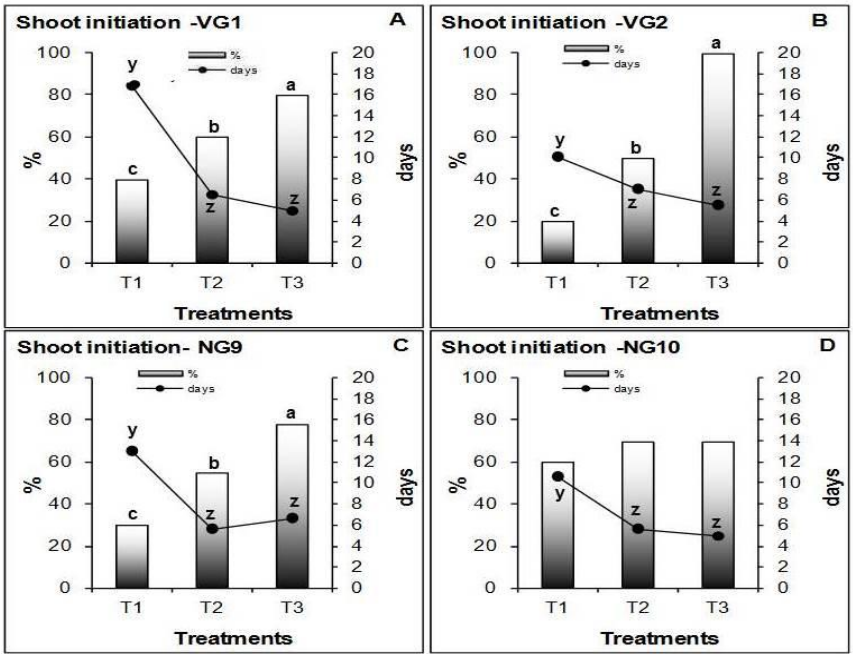

Figure 1. Shoot-forming explants (percent of total number of explants) and days to first sign of shoot growth in whole (T1), half (T2) and quarter (T3) shoot tip explants of four taro [*C. esculenta* (L.) Schott] cultivars in modified MS medium. Means in bars (%; a-c) and as line point (days; x-z) with same letter are not significantly different based on LSD, 5%. No letter assignment indicates no significant treatment differences based on ANOVA.

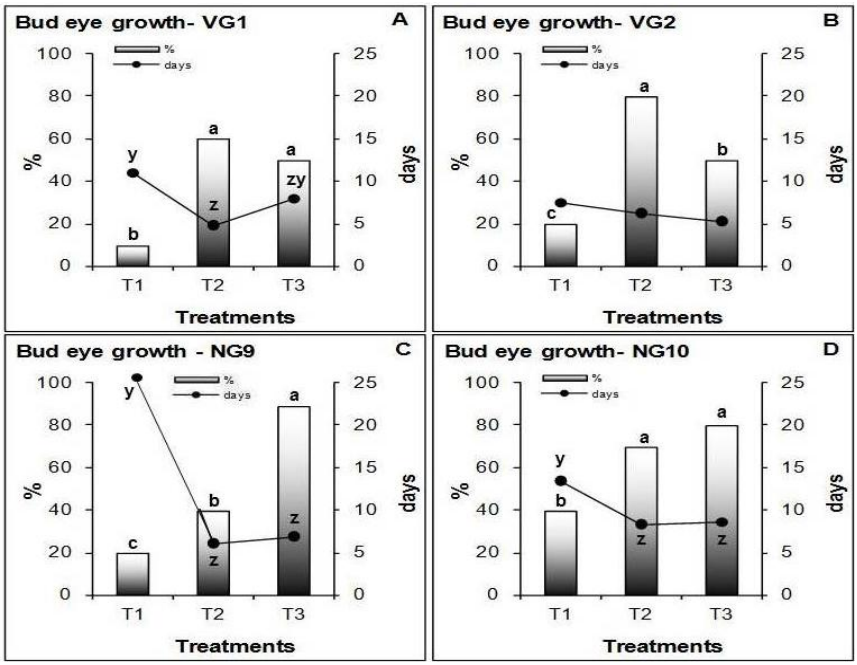

Figure 2. Bud eye growth in whole (T1), half (T2) and quarter (T3) shoot tip explants of four taro [*C. esculenta* (L.) Schott] cultivars in modified MS medium. Means in bars (%; a-c) and as line point (days; x-z) with same letter are not significantly different based on LSD, 5%. No letter assignment indicates no significant treatment differences based on ANOVA.

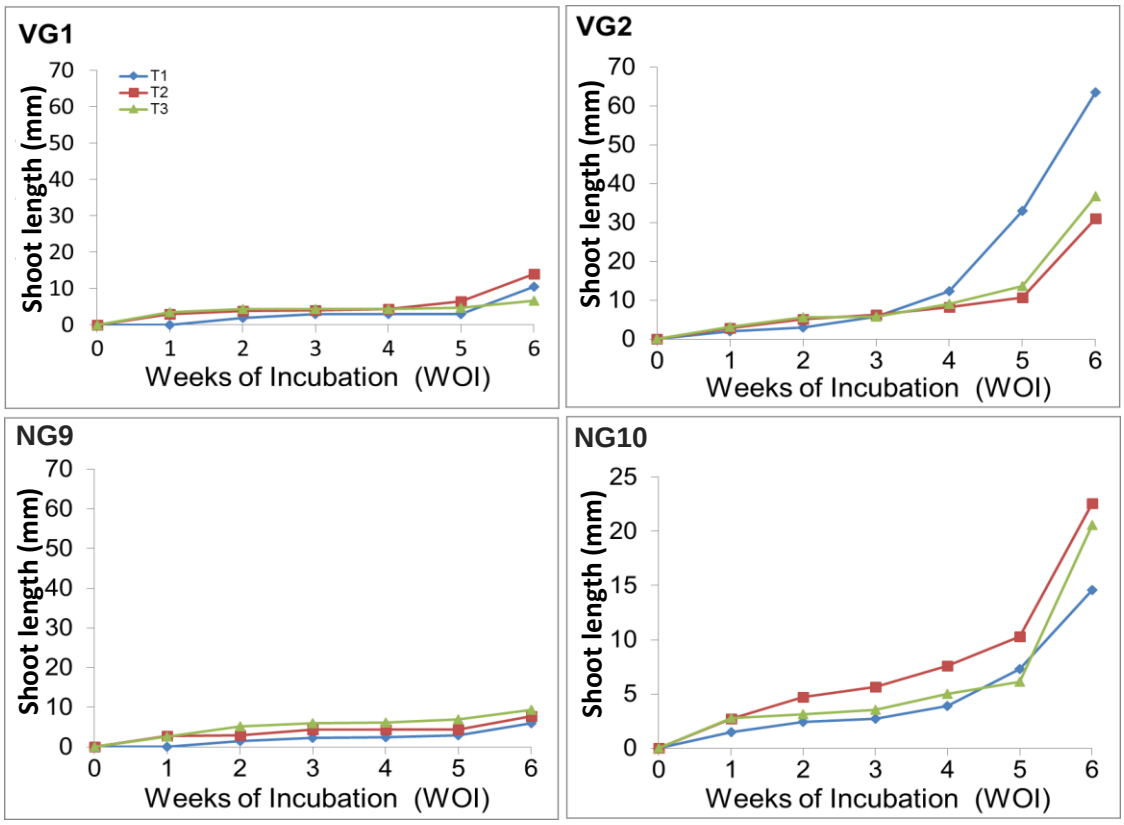

Figure 3. Growth increase of shoots from whole (T1), half (T2) and quarter (T3) shoot tip explants of four taro [*C. esculenta*(L.) Schott] cultivars in modified MS medium.

Table 1. Number of shoots produced per whole, half and quarter shoot tip explant of four taro [*C. esculenta* (L.) Schott] cultivars in modified MS medium.

Cultivar	Whole shoot tip	Half shoot tip	Quarter shoot tip
VG1	1.0	1.0	1.0
VG2	1.0	1.1	1.5
NG9	1.0	1.1	1.2
NG10	1.0	1.0	1.1

No significant differences among treatments were obtained based on ANOVA.

Cultivar response differed from that of shoot initiation as NG9 and NG10 had more explants with bud growth as shoot tip size decreased while in VG1 and VG2, more half shoot tip explants showed bud growth than quarter shoot tips. Bud growth set in earlier in half and quarter shoot tips, particularly in NG9 which took only 6-7 days in contrast to 25 days for whole shoot tips. Only one shoot per explant developed in VG1 cultivar regardless of shoot tip size while in the three other cultivars, multiple shoots (>1 shoot per explant) were formed, particularly in quarter shoot tips (Table 1). However, differences were not significant. The shoots grew and increased in length with increasing period of incubation (Figure 3). The effect of shoot tip size was evident only in the cultures of VG2 and NG10 cultivars with the whole and half shoot tips, respectively, resulting in the highest rate of growth increase. For complete regeneration, root development is important but it did not occur in all shoot-forming explants (Figure 4). Regardless of cultivar, shoots from half and quarter shoot tip explants took longer time to develop roots but the number of root-forming shoots was either equal or higher in number compared to that of whole

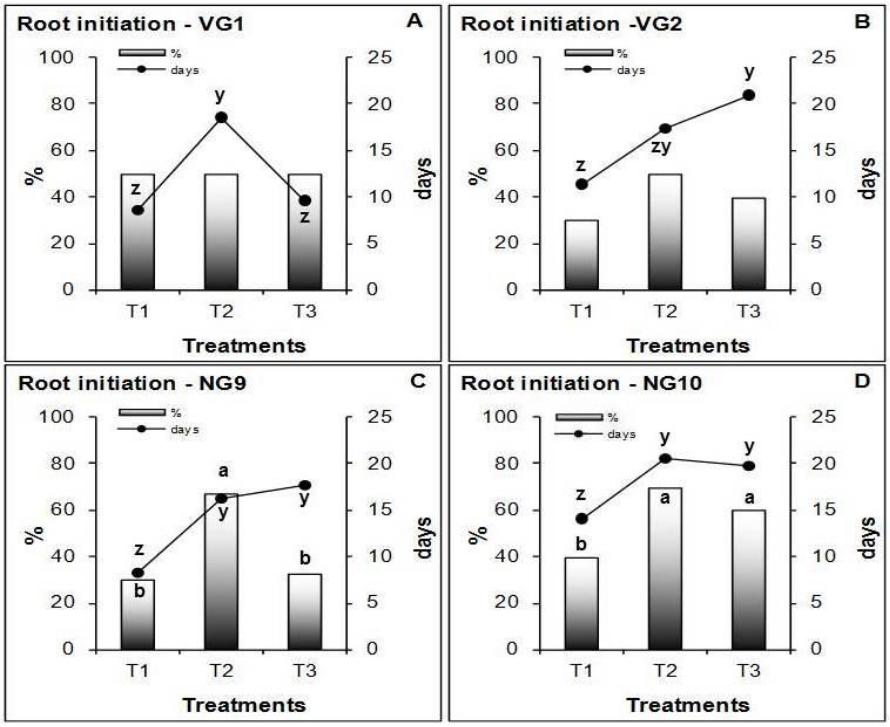

Figure 4. Root initiation in whole (T1), half (T2) and quarter (T3) shoot tip explants of four taro [*C. esculenta* (L.) Schott] cultivars in modified MS medium. Means in bars (%; a-c) and as line point (days; x-z) with same letter are not significantly different based on LSD, 5%. No letter assignment indicates no significant treatment differences based on ANOVA.

shoot tip explants. Half shoot tip explants of VG2, NG9and NG10 had the highest number of root-forming shoots. Considering that there were more half and quarter shoot tip explants that formed shoot, the number of complete plantlets increased relative to that of whole shoot tip explants (Table 2).

This study demonstrates that micropropagation of Philippine taro cultivars can be done simply by using a modified MS medium without PGR. PGRs are expensive and risky to use as they could induce genetic variation which is not desirable in multiplying true-to-type plants (Griesbach et al., 1988; Shoemaker et al., 1991). In addition, the protocol developed in this study resulted in direct organogenesis without callus formation. Other protocols used a culture medium added with PGRs or separate shoot and root regeneration medium with different PGR combinations. Yam et al. (1990) micropropagated taro using bud explants in modified MS medium with 1 mg/L 1-naphthaleneacetic acid (NAA) and taro corm extract; the explants died if NAA levels were lower than 0.1 mg/L. Tuia (1997) developeda taro multiplication protocol using MS medium with PGR applied in three variants: (I) 0.5 mg/L thidiazuron (TDZ) for four weeks; (II) 0.8 mg/L BAP for three weeks; and (III) 0.005 mg/L TDZ for three weeks. The protocol developed by Minas (2002) for taro apical meristem tips used a modified MS medium supplemented with 4.5 mg/L BAP, 0.175 mg/L indolebutyric acid (IBA), 2 mg/L glycine, 0.4 mg/L thiamine-HCl, 55.7 mg/L chelate iron, 10 mg/L ascorbic acid, 30 g/L w/v sucrose and solidified with 2.5 g/L phytagel. Hossain (2012) developed a different protocol for taro meristem using MS medium supplemented with NAA (0.5-3.0 mg/L) and BAP (0.5- 2.0 mg/L) while the protocol of Nath et al. (2012) for taro apical meristems (~1 cm) used MS medium supplemented with 5 mg/L benzyladenine (BA) and 1 mg/L NAA for shoot proliferation and hormone-free MS medium for root formation.

The present study also showed that shoot tip splitting promoted shoot formation and plantlet development. To our knowledge, there is no report on the use of shoot tip splitting in the micropropagation of taro. In banana, apical bud splitting to increase the number of explants encouraged bud proliferation particularly on MS medium with 7 mg/L BA (Hussein, 2012). Also, Ngomuo et al. (2014a) showed that splitting of banana buds containing the shoot tips into halves and quarters 14 days after culture initiation increased shoot production on MS

Table 2. Complete plantlet development and potential propagation rate of using whole, half and quarter shot tip explants in four taro [*C. esculenta* (L.) Schott] cultivars.

	A: Initial no. of explants	B: % Shoot formation (from Figure 1)	C: % Root formation (from Figure 5)	D: No. of complete plantlets (AxB/100×C/100)	E: No. of explants per shoot tip	No. of plantlets at equal no. of shoot tips (D×E)	Increase in propagation rate over whole shoot tip explant (times)
VG1							
Whole	40	40	50	8	1	8	
Half	40	60	50	12	2	24	3
Quarter	40	80	50	16	4	64	8
VG2							
Whole	40	20	30	2	1	2	
Half	40	50	50	10	2	20	10
Quarter	40	100	40	16	4	64	32
NG9							
Whole	40	30	30	4	1	4	
Half	40	50	70	14	2	28	7
Quarter	40	80	30	10	4	40	10
NG10							
Whole	40	60	40	10	1	10	
Half	40	70	70	20	2	40	4
Quarter	40	70	60	19	4	76	7.6

medium supplemented with 5 mg/L of BAP. Highest number of shoots was obtained from quarter split buds. The present study has a different approach as the taro shoot tips were split into halves and quarters for inoculation and culture *in vitro* but the results seemed to conform to that in banana as the quarter shoot tip explants had the highest number of shoots, particularly in VG1, VG2 and NG9 varieties.

Shoots proliferation in split apical buds has been attributed to the large surface area to volume ratio in contact with the culture medium which increases nutrient uptake resulting in rapid growth of shoots (Ngomuoet al., 2014a). In addition, shoot tip splitting could have arrested apical dominance which otherwise inhibits axillary meristem growth (Hussein, 2012; Ngomuo et al., 2014b). Auxin has a primary function in apical dominance (Vernoux et al., 2010). When the apical shoots are damaged or injured, such as by splitting, auxin metabolism and function undergo changes, resulting in the release of apical dominance and promotion of bud and shoot formation. Auxin is also the primary plant hormone that induces root initiation (Gaspar et al., 1996). When the apical tissues are damaged, auxin supply is altered resulting in delay or inhibition of root formation. As obtained in the present study, half and quarter shoot tips of taro had delayed root formation relative to that of whole shoot tips. In general, cultivars differ in the degree of shoot proliferation tendency and apical dominance (Ngomuo et al., 2014b).

Mircropropagation ratio

The use of half and quarter shoot tips increased the micropropagation ratio not only because of more explants that can be used per shoot tip but also the promotion of shoot and plantlet production compared to whole shoot tips (Table 2). Assuming all other factors equal, the use of half shoot tips should increase the micropropagation rate by two fold while the use of quarter shoot tips, by four fold relative to the use of whole shoot tips. But because of increased number of shoot-forming explants some of which produced roots to complete plantlet development, micropropagation ratio increased by 3, 10, 7 and 4 fold with the use of half shoot tips and further by 8, 32, 10 and 7.6 fold with the use of quarter shoot tips in VG1, VG2, NG9 and NG10, respectively. From this result, VG2 appeared to be the most responsive to shoot tip splitting. Furthermore, the remaining shoots that did not develop into complete plantlets (shoots with roots) are potential sources of shoot tips for micropropagation. This will further increase the micropropagation ratio.

Ex vitro growth

Figure 5 shows the development of quarter shoot tips up

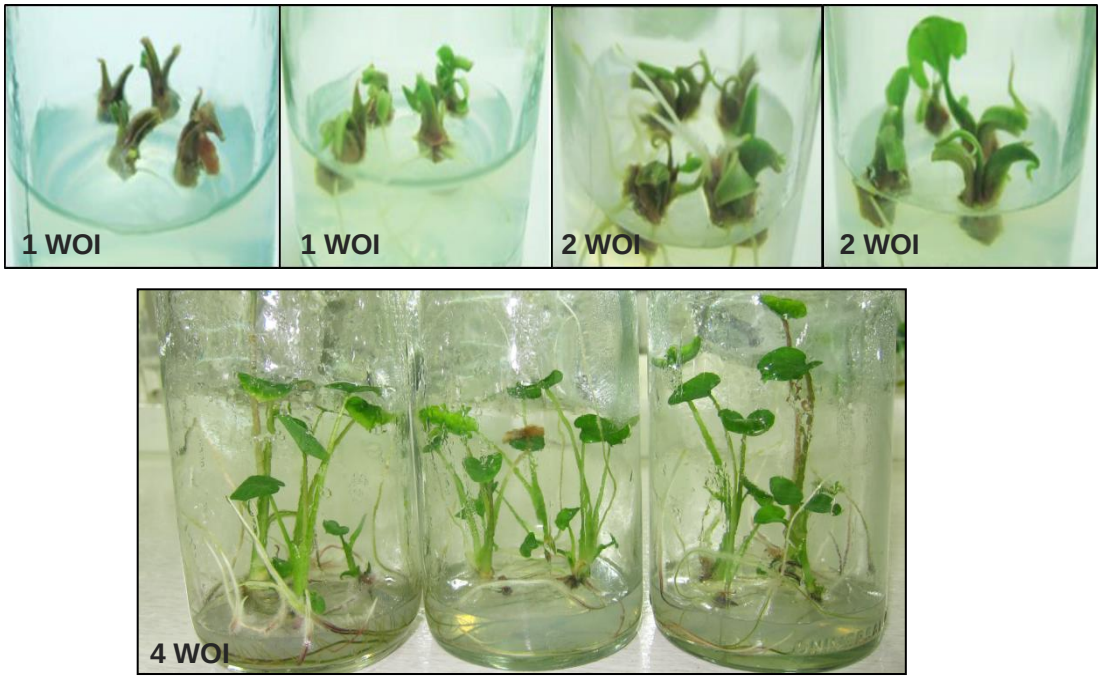

Figure 5. Plantlet development of the quarter shoot tip explants of VG2 taro [*C. esculenta* (L.)Schott] cultivar after 1-4 weeks of incubation (WOI).

Figure 6. Nursery maintenance of shoot tip-derived plants of VG2 taro [*C. esculenta* (L.) Schott].

to 4 weeks of incubation *in vitro* when the plantlets were ready for potting out. Following the *ex vitro* protocol, all potted-out plantlets of the four cultivars survived. No variation in morphological traits of tissue culture-derived plants was observed from the four cultivars. Figure 6 shows the potted plants after 4 weeks and 3 months in

the nursery. These *ex vitro* responses reinforce the feasibility of the micropropagation system and the use of the micropropagated plants in taro production.

Conclusion

A simple micropropagation protocol for four major cultivars of Philippine taro (VG1, VG2, NG9 and NG10) was developed using a PGR-free modified MS medium which did not induce callus formation. Using split shoot tips increased micropropagation ratio by increasing the number of explants and promoting shoot and plantlet development but the responses differed with cultivar. Quarter shoot tips produced the highest number of shoot-forming explants in all cultivars except NG10 where half and quarter shoot tips had comparable response. Root formation was delayed in split shoot tips. After 3-4 weeks, plantlets were potted out and following the *ex vitro* protocol, all plantlets survived and developed into normal plants with no visible morphological variation.

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