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Effect of Solid and Liquid Media on *in vitro* Propagation of *Plectranthus edulis* (Vatke) (Agnew)

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Abstract
Plectranthus edulis (Vatke), Agnew is one of the four economically important tuber bearing species of Plectranthus. It originated in Ethiopia and has been grown in mid and high altitude areas. The plant is propagated vegetatively by tuber. During cultivation of P. edulis farmers face shortage of disease free planting materials. In vitro propagation of P. edulis can be used for production of disease free planting material. The objective of the present study was to investigate the effect of solid and liquid media on in vitro axillary shoot proliferation of P. edulis. Shoots were multiplied using MS solid and liquid media containing different concentrations of BAP or TDZ either alone or in combinations. Rooting experiment was done using half strength MS medium containing different concentrations of IAA and IBA. Among the different treatment combinations used for multiplication,5.85±2.17and 6.07±2.30 mean numbers of axillary shoots were obtained on 1.5 mg/l BAP + 0.5 mg/l TDZ, on solid and liquid media respectively. In vitro developed shoots were rooted best with mean root number of 10.55±5.47 on solid medium and 13.11±7.15 on liquid medium in 0.1 mg/l IAA. The survival rate of the acclimatized plantlets in the glasshouse was 100% and 82.85% from solid and liquid media, respectively. Moreover the overall effect of solid and liquid media was observed to be best of shoot multiplication and rooting, respectively.
Keywords: Acclimatization, Oromo Dinich, Shoot multiplication, Tuber

INTRODUCTION

Plectranthus edulis (Vatke) is a species of annual plants belonging to the family *Lamiaceae (Labateae*) and order *Lamiales* (Ryding, 2006). It is believed to be originated in Ethiopia like Tef (*Eragrostis tef)*, noug (*Guizotia abyssinica*), Ethiopian mustard (*Brassica carinata*), Enset (*Ensete ventricosum*), Anchote (*Coccinia abyssinica*) and Coffee (*Coffea arabica*) (Dandena Gelmesa, 2010). And the plant also cultivated in many countries in Africa, Asia and Australia (Yeshitila Mekbib, 2007).The plant has different name by Ethiopian farmers Depending where it is cultivated, such as, Dinicha Oromo, Wolaita dono, Gamo Dinich, Gurage Dinich, Agaw Dinich, Ethiopian potato etc (Weyessa Garedew *et al*., 2009).

Like other root and tuber crops, *P. edulis* has a long history of local use and is important to the cultural, social and economic life of the households in Ethiopia (Ababe Demissie 1988). *P. edulis* has different uses such as the boiled tuber for consumption and the leaf also cooked and eaten as a vegetable in some western and southwestern part of Ethiopia, particularly, in Kefa area (Zemede Asfaw and Zerihun Woldu, 1997). The people also use the leaf as a traditional medicine to treat different diseases (Mulugeta Taye, 2008).

However, the farmers reported that *P. edulis* production has been declining due to shortage of seed tuber, land, water and poor storage ability, poor market opportunity as well as late maturity of the crop (Yeshitila Mekbib, 2007). All these factors resulted in replacement of *P. edulis* by different plants such as maize, sweet potato and potato.

Storage of seed tuber is a major and other problem of this plant and it may be overcome by designing multiplication system and methods with higher rate of multiplication. Like other tuber crops, for example, sweet potato, to overcome shortage of seed tuber, different experiments were carried out using *in vitro* produced planting materials (Lommen *et al.,* 1999). So this work may contribute to overcome shortage of seed tuber by using micropropagation in liquid and solid medium.

P. edulis is conventionally propagated either from storage roots or by stem cutting. According to the study by Mulugeta Taye (2008) majority of farmers in southern Ethiopia reported that disease reduced the number and/or size of the progeny tubers which resulted in reducing *P. edulis* which result in shortage of tuber for planting. However, micropropagation is the most efficient method of plant propagation. Therefore propagating *P. edulis* by *in vitro* micropropagation using meristem culture is identified as good strategy to get disease free plant material. However, the protocol is too expensive to use for rooting and multiplication. Hence, this study was designed to reduce the cost of agar and sub culturing time.

MATERIALS AND METHODS

All the laboratory activities and experiments were conducted in Plant Propagation and Tissue Culture Laboratory at Addis Ababa University.

Stock plant preparation

In vitro propagated stock plants (cultivar Holeta) were obtained from Addis Ababa University, Plant

Biotechnology Laboratory. *In vitro* cultivated stock plant were maintained by sub culturing shoot at one month interval on MS basal medium without PGRs and 30 g/l sucrose using GA-7 Magenta and sealed with parafilm. The medium was gelled with 8 g/l agar and the pH was adjusted to 5.8 before autoclaving at 120^0C for 15 minute. Then 30 days old *in vitro* propagated shoots were used for the study as explants.

Preparation of nutrient medium

All the experiment were done by preparing MS stock solution, therefore, the amounts of macro- and micro nutrients, organic salts, and vitamins recommended by Murashige and Skoog (1962) were prepared [using double distilled water]. Also the growth regulators used in this experiment were prepared by dissolving the powder in 2-3 drops of 1 N NaOH and / or 1 N HCl based on the PGRs (i.e. NaOH for auxin and HCl for cytokinin. Finally, the solution of each PGRs was poured into labeled 50 ml plastic bottles and filled with double distilled water to the required volume and stirred using magnetic stirrer and stored at +4°C for short term use (a week).

Full strength MS culture media were prepared from the stock solution, liquid media [without gelling agent, (agar)] and solid medium (with agar). The two medium also contained 3% sucrose, and different concentration and combination of plant growth regulators. After all those mixed using magnetic stirrer the pH was adjusted to 5.8 using 1 N HCl and/or 1 N NaOH after adding 8.0 g/l agar (for solid media). After adjusting the pH, the gently mixed medium was heated in microwave oven until the agar was melted (solid media). Then 40 ml and 20 ml of the prepared medium ware dispensed in to Magenta GA-7 culture vessels and test tubes for solid and liquid medium respectively. The culture vessels and test tubes were covered with caps immediately after dispensing the medium and autoclaved by at a temperature of 121°C and 105 KPa pressure for 15 minutes.

Shoot multiplication and rooting

Young and healthy micro-shoots were cultured on shoot multiplication medium containing different concentration of thidiazuron (TDZ), and 6-Benzyl Amino Purine (BAP) alone and combination in (table 1). Nine shoots per culture vessel with three replicas and one shoot per test tube with 27 replicas for each treatment were used for solid and liquid medium, respectively. The initiated cultures were aseptically cutoff and cultured on multiplication medium, the culture vessels and test tubes were sealed, labeled and randomly placed on the growth room chambers with 16 hours photoperiod and light intensity of 2000 µmol/m^2/s at 29 ± 2°C and subculture was carried out every four weeks. PGRs free medium were used as a control.

Table1. Combination of BAP and TDZ for shoot multiplication

PGRs (mg/l)	Concentration															
BAP	0	0.1	0.5	1	1.5	0	0	0	0	0.1	0.5	0.5	1	1	1.5	2
TDZ	0	0	0	0	0	0.1	0.5	1	1.5	0.1	0.1	1.5	0.5	1	0.5	0.5

Rooting of already multiplied shoots in both solid and liquid medium was conducted on half strength MS basal medium containing different concentration of indol-3- butyric acid (0.1, 0.3, 0.5 mg/l) and indol-3-cetic acid (0.1, 0.3, 0.5 mg/l) alone and Growth regulators free medium was used as a control. Three replications with nine explants per GA-7 Magenta and one explant per test tube were used for solid and liquid medium, respectively. Finally, the cultures were placed randomly on the growth room.

Acclimatization

After the plantlets were well grown, they were considered to be ready for acclimatization *in vivo*. Therefore, individual plantlets were removed from the culture vessels and test tubes and washed carefully under tap water to facilitate the removal of agar from their roots. Then, they were transferred to pots containing a mixture of red soil, compost and sand in their respective ratio of 2:1:1. Each plastic pot was labeled and covered with polythene bags to ensure high humidity and kept in the glasshouse. The plastic bags were removed after two weeks. Thirty five plantlets from each medium (solid and liquid medium) were acclimatized.

Data analyses

Statistical analysis of quantitative data was carried out by SPSS computer software of version 14. A difference at probability level of $p \leq 0.05$ was considered significant for analyses. Data were subjected to analysis of variance and variables that showed significant difference were compared by the LSD at 5 % probability.

Result

Shoot multiplication and rooting

The shoots on a medium containing different concentration of BAP and TDZ alone and in combination gave different response on both solid and liquid medium. The best shoot multiplication medium from the treatments used and the best media type that provide higher number of axillary bud was obtained.

The maximum mean number of axillary shoot obtained on solid medium was 5.85±2.17 (fig.1 A and B) and

on liquid medium was 6.07±2.30 (fig.1 C and D) on MS multiplication medium containing 1.5 mg/l BAP + 0.5 mg/l TDZ. The multiplication rate of *P. edulis* on a medium containing BAP or TDZ alone was relatively less than that of the combination of the two PGRs.

The maximum mean shoot length was observed on growth regulator free media. Using solid medium, 6.25±2.01 cm long shoots were obtained whereas the liquid medium produce 5.03±2.72 cm long shoots. But the combination of BAP and TDZ showed medium shoot length.

The overall multiplication for the two types of media was not much difference but solid medium exhibited maximum number of axillary bud proliferation and shoot length as compared to liquid media.

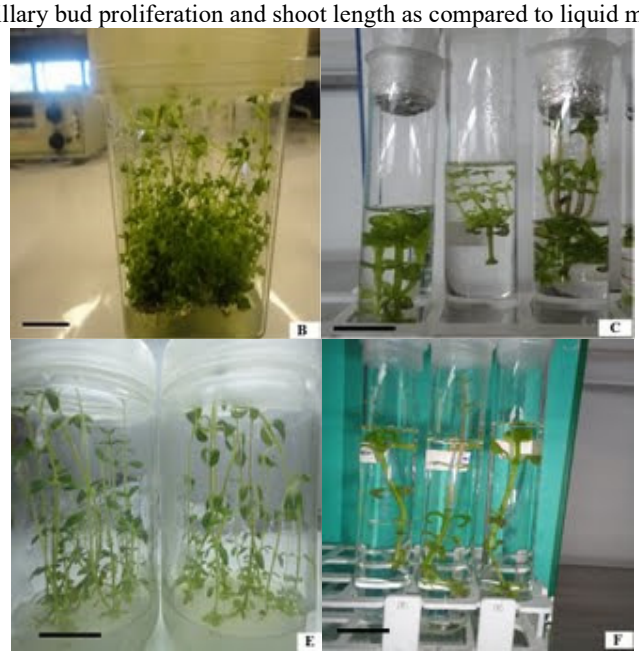

Figure 1 Multiple shoots of *P. edulis* on different shoot multiplication media.**(**B) 1.5 mg/l BAP + 0.5 mg/l TDZ on solid medium**, (C**) on liquid medium, PGRs free (E) solid medium **(F**) liquid medium**.**

The shoots cultured in half strength MS basal media supplemented with different concentrations IBA and/or IAA showed different rooting responses except the control (PGRs free). Liquid media showed higher mean number of roots and root length than solid media.

The highest mean numbers of roots per shoot was 10.55±5.47 and 13.11±7.15 was obtained in medium containing 0.1 mg/l IAA in both solid and liquid medium, respectively and the highest mean root length was observed on 0.1 mg/l IAA (4.97±0.94) in liquid medium and 4.79±0.99 on solid media containing 0.5 mg/l IBA. However, no roots were observed on both growth regulators free solid and liquid media.

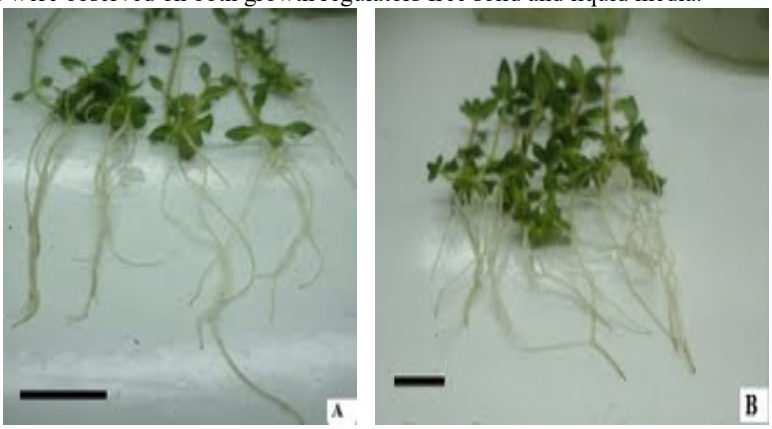

Figure 2 Rooted shoots of *P. edulis* on different rooting conditions (A, B) Rooted shoots on ½ MS + 0.1 mg/l IAA from liquid and solid medium, respectively.

Acclimatization

In vitro plantlets planted on plastic pot and transferred to a greenhouse condition grew actively during the acclimatization process. After month of growth, the survival percentage was found to be 100% and 82.85% for

solid media and liquid media, respectively.

Figure3 Acclimatization of *in vitro* rooted shoots of *P. edulis* in glasshouse. (A) Plantlets covered by polythene plastic bags (B).

Discussion

Shoot multiplication and Rooting

From this study it was observed that the cultured shoot tips gave varying response to all of the culture media composition. This result is observed due to combined effect of different factors especially media type and growth regulators composition of the media. According to Dokhaniyeh *et al*. (2011), combination of TDZ with other growth regulators strongly induce plantlet regeneration in highly efficient manner on different cultivar of potato including Agria. This is quite similar to the results of this study which showed an excellent axillary shoot multiplication. It was also reported that cocoyam has been successfully micropropagated *in vitro* using combination of BAP and TDZ which produced significantly more axillary shoot per explants (Sama *et al*., 2012; Wattanawikkit *et al.*, 2011). Mohamed *et al.* (2007) reported cytokinin (BA) and cytokinin-like compound (TDZ) break apical dormancy which affects cell division, showing that existence of BA and TDZ would enhance both the survival of the explants and growth of the proliferated axillary shoot on sweet potato.

The result of this study revealed number of axillary shoot proliferation is higher on solid media than the liquid media in different concentrations of BAP and TDZ alone or in combination. This result disagrees with different researches which reported that liquid media had significantly more nodes than the solid media due to the close contact of the tissue with the media may stimulate and facilitate the uptake of nutrients and phytohormones leading to better shoot response (Mbiyu *et al.,* 2012; Nkere *et al*., 2011; Sama *et al*., 2012; Kuria *et al*., 2008; Alam *et al.*, 2010). Rooting response of *P. edulis* on half strength medium containing IAA or IBA at different concentrations was used and gave different response. Half strength MS medium is recommended by different authors for *in vitro* rooting (Dalal *et al*., 2006; Hartman *et al*., 2004). Ahmad *et al.* (2003) also reported that efficiency of rooting was improved when salt concentration of the medium was reduced by half.

P. edulis responded differently to different concentrations of IAA or IBA. As Dalal *et al*. (2006) reported that IBA is to be most effective for root induction *in vitro*. In the present study, however, from the comparison made between the rooting hormones, IAA and IBA, IAA was most effective to increase root number whereas IBA increased the root length.

Growth regulators free medium in the present study didn't show any root production which is in agreement with the work of Zulfiqar *et al*. (2009) on avocado plant, Abd-Elaleem *et al*. (2009) on potato who reported that growth regulator free medium are much weaker and the number of roots produced is lower than the other treatments.

However, the overall best result was observed in liquid medium as compared to solid medium. Our result was also in agreement with the results of Mbiyu *et al*. (2012) who reported that liquid medium showed more efficient for root proliferation than the solid medium in three Kenyan potatoes. Shibli *et al*. (2002) also reported *in vitro* rooting on solid medium showed low number of root per plantlets on spunta potato cultivar.

Acclimatization

The plantlets produced *in vitro* are highly susceptible to the *ex vitro* condition until they develop adaptation mechanism to best cope the stress up. The observation made for the two medium types after four weeks of growth in the glasshouse revealed higher survival for plantlets obtained from solid media than the liquid media. The result is consistent with the work of Mesfin Tsegaw (2005) on *P. edulis* who reported 100% survival from solid medium. As Puchooa (2004), reported, hardened plantlets showed significantly high survival rate (84%)

after eight weeks for *Dendrobium* plants. This was also supported by Mazri (2012), who reported that shoots elongated in liquid media showed some difficulties during the acclimatization and a pre-acclimatization stage in a solid medium for date palm (*Phoenix dactylifera* L.) cv. Najda.

Conclusion

With regard to the media type, solid medium has better response in axillary shoot proliferation than liquid media. But Liquid medium is best for obtaining maximum number of root than the solid which excludes the cost of agar for rooting. It was therefore concluded that it is more economical to use liquid media for rooting because it minimizes the cost of agar.

Reference

Ababe Demissie (1988). Potentially valuable crop plants in a Vavilovian center of diversity: Ethiopia. pp. 89-98 in *Crop Genetic Resources of Africa.*

Abd-Elaleem, G. K., Modawi, S. R. and Khalafalla, M. M. (2009). Effect of cultivar and growth regulator on *in vitro* micropropagation of potato (*Solanum tuberosum* L). *Am. Eurasian J. Sustain. Agric.* **3**(3)**:** 487-492.

Ahmad, T., Ur-Rahman, H., Ahmad, C. M. and Laghari, M. H. (2003). Effect of culture media and growth regulators on micropropagation of peach rootstock GF 677. *Pak. J. Bot*. **35**(3)**:** 331-338

Dalal, A. M., Das, B., Sharma, K. A., Mir, A. M., Sounduri, S. A. (2006). *In vitro* cloning of apple (*Malus domestica* Borkh) employing forced shoot tip cultures on M9 rootstock. *Indian J. Biotechnol.* **5:** 543-550.

Dandena Gelmesa (2010). Shifting to alternative food source: potential to overcome Ethiopia's malnutrition and poverty problems. Agriculture and Food. Montpellier, France.

Dokhaniyeh, Y. A., Kohnehrouz, B. B., Mousavi, A., Gholizadeh, A. and Khalighi, A. (2011). Rapid and high efficiency regeneration from potato (*Solanum tuberosum* L.) using thidiazuron as cytokinins source. *J. Food Agric. Environ*. **9**(3&4)**:** 613-617.

Hartman, H. T., Kaster, D. E., Davies, F. T. and Geneve, R. L. (2004). *Plant Propagation Principle and Practices*. 6th ed. Prentice Hall of India Private Limited, New Delhi, India, pp.770.

Kuria, P., Demo, P., Nyende, A. B., and kahangi, E. M. (2008). Cassava starch as an alternative cheep gelling agent for the *in vitro* micropropagation of potato. *(Solanum tuberosum*). *Afr. J. Biotechnol.* **7**(3)**:** 301-307.

Lommen, W. J. M., (1999). Causes for low tuber yields of transplants from *in vitro* potato plantlets from early cultivars after field planting. *J. Agr. Sci. Cambridge.* **133:** 275−284.

Mazri, A. M. (2012). Effect of liquid media and *in Vitro* pre-acclimatization stage on shoot elongation and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Najda. *J. Ornamental and Hortic. Plants.* **2** (4)**:** 225-231.

Mbiyu, M., Muthoni, J., Kabira, J., Muchira, C., Pwaipwai, P., Ngaruiya, J., Onditi, J. and Otieno, S. (2012). Comparing liquid and solid media on the growth of plantlets from three Kenyan potato cultivars. *Am. J. Exp Agr.* **2**(1)**:** 81-89.

Mesfin Tsegaw (2012). Micropropagation of *Plectranthus edulis* (Vatke) Agnew from Meristem culture. M. Sc. Thesis. Addis Ababa University, Addis Ababa.

Mohamed, F. M., Abdalla, M. A. M. and Damarany, A. M. A. (2007). Differential axillary-bud proliferation responses of two sweet potato cultivars to benzyladenine and thidiazuron. *Ass. Univ. Bull. Environ. Res.***10** (2)**:** 21-30.

Mulugeta Taye (2008). Studies on agronomy and crop physiology of *Plectranthus edulis* (Vatke) Agnew. Ph.D. Thesis. Wageningen University, Netherlands.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco cell cultures. *Physiol. Plant*. **15**: 473-497.

Nkere, C. K., Ihezie, **C. I. and** Mbanaso, E. N. A. (2011**). Micropropagation of Yam (*Dioscorea rotundata)*: Assessment of performance in cassava starch-gelled medium. *Biotechnol.* 10(6): 551-553.**

Puchooa, D. (2004). Comparison of different culture media for the *in Vitro* culture of *Dendrobium* (Orchidaceae). *Int. J. Agri. Biol*. **6** (5)**:** 884–888

Ryding, O. (2006). *Lamiaceae.* **In:** flora of Ethiopia and Eritrea, vol. 5, *Gentiaceae* to *cyclochelaceae* , Inga Hedberg, Ensermu Kelbessa, Sue Edward, Sebsebe Demissew and Person, E.(eds), the national herbarium, AAU, Ethiopia and Uppsala Sweden.

Sama, E. A., Hughes, G. H., Abbas, S. M. and Shahba, A. M. (2012). An efficient *in vitro* propagation protocol of cocoyam (*Xanthosoma sagittifolium* (L) Schott). *The Scientific World Journal.*

Shibli, A. R., Ab-Ein, M. A. and Ajlouni, M. M. (2002). *In vitro* and *in vivo* multiplication of virus free spunta potato clone. *Pak. J. Agric. Res*. **17**(1)**:** 71-75.

Wattanawikkit, P., Bunn, E., Chayanarit, K., and Tantiwiwat, S. (2011). Effect of cytokinins (BAP and TDZ) and Auxin (2, 4-D) on growth and development of *Paphiopedilum. Nat. Sci.* **45**(1)**:** 12-19.

Weyessa Garedew, Admasu Tsegaye, Bezuayehu Tesfaye and Hussein Mohammed. (2009). Variability and

association of quantitative traits in *Plectranthus edulis* (Vatke) Agnew. *East Afr. J. Sci*. **3**(1)**:** 61-69.

Yeshitila Mekbib **(**2007). Phenotypic variation and local customary use of Ethiopian potato (*Plectranthus edulis* (Vatke) (Agnew**)**. M. Sc. Thesis Uppsala University, Swedish.

Zemede Asfaw, Zerihun Woldu (1997). Crop associations of home-gardens in Welayta and Gurage in southern Ethiopia. *SINET: Ethiop. J. Sci*. **20:** 73-90.

Zulfiqar, B., Abbasi, A. N., Ahmad, T. and Hafiz, I. A. (2009). Effect of explants sources and different concentrations of plant growth regulators on *in vitro* shoot proliferation and rooting of avocado (*Persea americana*mill.) cv. "fuerte". *Pak. J. Bot*. **41**(5)**:** 2333-2346.