Vietnam J. Agri. Sci. 2016, Vol. 14, No. 10: 1491 -1501 Tạp chí KH Nông nghiệp Việt Nam 2016, tập 14, số 10: 1491 - 1501
www.vnua.edu.vn

DEVELOPING AN EFFICIENT REGENERATION PROTOCOL FOR SWEETPOTATO, *Ipomoea batatas* (L.) Lam., USING NODAL EXPLANTS

Vu Thi Thuy Hang, Ha Thi Anh Thu, Vu Dinh Hoa*

Vietnam National University of Agriculture

Email: vdhoa@vnua.edu.vn*

Received date: 06.09.2016 Accepted date: 03.11.2016

ABSTRACT

An efficient regeneration protocol of large scale micropropagation for clonal establishment of sweet potato from seedlings is an essential step for clonal selection. The objective of this study was to evaluate the potentials of young coconut water and simple aerated culture condition in glass bottles with Milliseal® for *in vitro* propagation of sweet potato using nodal explants. Shoot regeneration of sweet potato nodes bearing dormant buds was effectively promoted by aerated culture condition in full strength MS salt solidified by 8g/L agar supplemented with 3% sucrose, BAP 1.0 mg/L, 0.5 mg/L IAA and 20% coconut water and subsequent root induction was effective on full strength MS salt solidified by 8g/L agar supplemented with 3% sucrose and 0.3 mg/L α - NAA. The plantlets derived from aerated culture condition were more vigorous compared to conventional non-aerated culture condition, leading to high survival rate (~ 100%) and enhanced growth during acclimatiztion. Sand – smoked rice husk potting mix was suitable for hardening of *in vitro* - derived plantlets.

Keywords: Aeration culture, coconut water, nodal explants, shoot regeneration, sweet potato.

Xây dựng quy trình tái sinh cây khoai lang, *Ipomoea batatas (*L.) Lam., hiệu quả sử dụng đốt mang mắt ngủ làm mô cấy

TÓM TẮT

Quy trình vi nhân giống hiệu quả để thiết lập dòng vô tính ở khoai lang từ cây con thực sinh của các tổ hợp lai là bước cần thiết cho quá trình chọn lọc. Mục tiêu nghiên cứu nhằm đánh giá tiềm năng của nước dừa non và điều kiện nuôi cấy thoáng khí đơn giản bằng Milliseal® để nhân giống khoai lang *in vitro* bằng đốt mang mắt ngủ. Tái sinh chồi từ đốt mang mắt ngủ được xúc tiến hiệu quả trong điều kiện nuôi cấy thoáng khí trên môi trường MS bổ sung 8g/L agar, 3% sucrose, 1mg/L BAP, 0,5 mg/L IAA và 20% nước dừa và sự tạo rễ có hiệu quả trên môi trường MS bổ sung 8g/L agar, 3% sucrose 0,3 mg/L α - NAA. Cây con thu được từ môi trường nuôi cấy thoáng khí có sức sống khỏe hơn so với môi trường nuôi cấy kín truyền thống nên có tỉ lệ sống cao (~100%) và sinh trưởng tốt hơn trong thời kỳ thuần hóa thích nghi môi trường. Giá thể hỗn hợp cát-trấu hun thích hợp để thuần hóa thích nghi cây con từ nuôi cấy *in vitro*.

Từ khóa: Khoai lang, mô cấy mang mắt ngủ, nuôi cấy thoáng khí, nước dừa, tái sinh chồi.

1. INTRODUCTION

Sweet potato is an outbreeding, highly heterozygous polyploid and propagated vegetatively. In effect, the seeds are heterogeneous and used only for the purpose of breeding. In breeding sweet potato, clonal selection in genetically variable populations derived from crosses remains a major approach because any superior genotype selected is preserved and maintained through asexual reproduction. However, reliable selection for quantitative characters is based on observations on multiple plants. In order to accelerate breeding process, seedlings from true seeds of a cross should be multiplied to ensure sufficient

individuals of each clone for making effective selective decision. Asexual multiplication by vine cuttings, however, is time-consuming. Thus, rapid *in vitro* propagation might be an efficient alternative.

Plant tissue culture techniques, apart from the use as a tool of research, have in recent years, become a major importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. As a highly "plastic" plant, sweet potato has been possible to regenerate de novo *in vitro* plantlets from almost all plant parts when placed into culture. Although regeneration of sweet potato using various tissues from different cultivars were reported with varying levels of reproducibility and efficiency, several researchers were able to successfully regenerate plantlets of sweet potato from cultured stems, petioles, roots and leaf disks (Gosukonda *et al.*, 1995; Sivparsad and Gubba, 2012), from meristem culture (Zamora, 1993; Nguyen Thi Ly Anh and Nguyen Quang Thach, 2003; Ying *et al.*, 2004; Alam *et al.*, 2010; Pham Van Linh *et al.*, 2014), and from single node cuttings bearing dormant axillary bud (Dolinski and Olek, 2013; Onwubiko *et al.*, 2015). Among these methods, meristem culture was well established to produce virus-free/clean planting materials for germplasm conservation and for revigouration of degenerated cultivars due to viral infection. However, *in vitro* culture of meristem and plant parts without dormant buds is rather complicated, time-consuming and genotype-specific. Despite the advances made in sweet potato tissue culture, an efficient and practicable regeneration method of sweet potato is of crucial significance for effective genetic improvement of this crop. Nodal explant culture may offer more merits because this type of propagation involves the growth of an existing morphological structure, the axillary meristem, that simply requires hormone/nutrient conditions to break its dormancy and promote its growth. Moreover, *in vitro* plantlets produced from node cuttings are easily transferred to non-sterile condition for acclimatization or transplanted directly to the field.

The coconut water, a liquid endosperm, has high levels of zeatin in its composition it is frequently used in micropropagation protocols of economically important crops (Yong et al., 2009; Molnar *et al.*, 2011). Thus, coconut water might be used to replace expensive compounds like zeatin and other organic substances required for the growth and development of olive seedlings in culture medium condition (Peixe *et al.*, 2007; Souza *et al.,* 2013). Nasib *et al.* (2008) also proved the advantage of using 20% (v/v) coconut water combined with BAP during the *in vitro* propagation ò Kiwifruit. The same authors concluded that the use of coconut water prolonged the sub-culturing time and produced highly robust plants which were more able to survive in greenhouse. Despite these works, the use of coconut water for sweet potato micropagation was scare. For sweet potato, Michael (2011) reported that coconut water at a level of 75ml/L or higher initiated callus that was capable of proloferating into shoots. Thus, it is hyothesized that the use of coconut water as a component of culture medium might enhance the response of dormant buds to shoot regeneration of sweet potato.

Most culture reports were derived from closed flasks/vessels or capping system. The confinement of atmosphere during culture of explant *in vitro* in closed vessels, although preventing moisture loss but restricting gas exchange can lead to dramatic modifications of the gaseous condition and consequently to poor plant development with high mortality when relocated into greenhouse for weaning. It is long believed that the growth of *in vitro* plants depends largely on the composition of nutrients and thus efforts are mainly made to improve the composition of the growing medium. In recent times, there has been much interest on the aeration of culture vessels to minimize the difference between the gaseous atmosphere *in vitro* and the surrounding atmosphere of the vessel. The aeration of the culture vessel has proved to have many advantages over the traditional airtight system (Buddendorf-Joosten and Woltering, 1994; Zobayed *et al.*, 2000). For

instance, the growth and development of the plantlets and shoot regeneration rate from bulblets of *Lilium longiflorum* were found better in aeration culture in comparison with traditional culture system (Duong Tan Nhut *et al.*, 2004).

The present research aimed at defining optimum supplements of plant growth regulators, coconut water to full strength MS medium and culture condition to establish an efficient and praceable protocol for *in vitro* propagation of sweet potato using single nodes bearing dormant axillary bud from seedlings derived from crosses.

2. MATERIALS AND METHODS

2.1. Establishment of *in vitro* plantlets

True seeds of the sweet potato cross, KLT10 x HL6-3, were scarified and sown on sand-compost mixture. At four true leaf stage, fifty seedlings were potted in sterilized potting medium and maintained in an insect-proof nethouse at Gia Loc Field Crops Research Institute. The apical shoot segments bearing axillary buds from three month old pot-grown seedlings were harvested with the leaves removed and rinsed under running tap water for 15 minutes. The shoot segments were sliced into nodal cuttings of 2.5 - 3 cm in length. The nodal cuttings were then washed with detergent for 15 minutes, followed by rinsing twice with distilled water and dried with aseptic tissue before putting into sterile chamber.

To establish *in vitro* cultures, the nodal cuttings were immersed in 70% (v/v) ethanol for 30 seconds and rinsed three times with distilled water. The node cuttings were then surface-sterilized in 0.1% mercuric chloride with 2 drops of Tween -20 for 10 min and immediately rinsed three times with distilled water. The node segments of 2.5 - 3 cm in length were skimmed to nodal explants of 1 ± 0.2 cm in length and cultured in basal MS (Murashige and Skoog, 1962) salt medium solidified with 8 g/L agar and supplemented with 3% sucrose at pH of 5.8 as shoot inititation medium (SIM). Shoots having at least six nodes were used to obtain explants for the subsequent culture experiments.

2.2. Effect of Kinetin and BAP on shoot induction

The nodal explants of 1 ± 0,2 cm in length were isolated and cultured on SIM containing kinetin and BAP applied separately at concentration of 0 mg, 0.5mg, 1.0mg, 1.5 mg and 2mg/L in 250 ml Erlenmeyer flasks. Optimal concentration of either type of cytokinin (Kinetin or BAP) was used for next experiment in combination with IAA.

2.3. Effect of IAA on shoot induction

The nodal explants of 1 ± 0,2 cm in length were cultured on SIM supplemented with 1 mg/L BAP (optimum concentration identified from preceding experiment) combined with IAA concentration of 0 mg, 0.1 mg, 0.3 mg, 0.5 mg and 0.7 mg/L.

2.4. Shoot induction with supplementation of coconut water

The nodal explants obtained as described above were cultured on optimal shoot initiation medium (SIM supplemented with 1.0 mg/L BAP + 0.5 mg/L IAA) added with young coconut water at concentration of 10%, 20%, 30% and 40%.

2.5. Effect of α-NAA on root induction

Shoots without roots obtained from previous culture experiment were cultured on SIM supplemented with α-NAA concentration of 0 mg, 0.1 mg, 0.3 mg, 0.5 mg and 0.7 mg/L.

2.6. Effect of *in vitro* culture conditions

Nodal explants of 1 ± 0.2 cm in length were cultured in Erlenmeyer flasks aerated using microporous filter Milliseal (0.5 μm) (Millipore, Japan) on the cap with a hole of 0.5 cm in diameter. The control was traditional closed or non-aerated culture system wherein the openings of the culture flasks were tightly sealed with sterile aluminum foil. The shoot initiation used was the same as described above.

2.7. Culture media treatment and culture condition

Each culture experiement described above was repeated three times each with thirty 250 ml Erlenmeyer flasks containing 25 ml medium with four explants. The culture flasks were kept under inflorescense light intensity of 3000 lux, at 16/8 hrs light/dark period, 70 - 80% RH and 27 ± 2°C. All culture media used were adjusted to pH of 5.8 and sterilized by autoclaving at 121°C and 1.4 atm for 20 min. The culture flasks were kept under inflorescense light intensity of 3000 lux, at 16/8 hrs light/dark period and at 70 - 80% RH and 27 ± 2°C. All culture experiments were recorded and evaluated four weeks after culture.

2.8. Acclimatization and potting out

The culture flasks were brought out from culture room and left at ambient conditions with caps removed for three days. The rooted plantlets were the taken from culture flasks, transferred to container of destillied water and gently rinsed to remove culture medium off the roots. The plants were transplated with care into 7 × 5 cm pots containing rooting media: i) sterilized sand, ii) sand + smoked rice husks (1:1 by volume), and iii) smoked rice husks. The experiment was placed in a nethouse in three replicates with each replicate consisting of 30 pots according to a randomized complete block design. The plant growth was recorded after four weeks.

2.9. Data analysis

Data were subjected to analysis of variance with mean separation ($p < 0.05$) by Fisher's Least Significant Difference using Crop Stat vers. 7.2.

3. RERULTS AND DISCUSSION

3.1. Effect of kinetin and BAP at different concentrations on shoot regeneration

Both shoot initiation medium (SIM) and SIM supplemented with kinetin and BAP induced shoot generation four weeks after culture. Significantly highest value ($p \leq 0.05$) of shoot development was obtained at concentration of 1 mg/L of kinetin and BAP. However, BAP at concentration of 1 mg/L produced highest leaf number (5.7 leaves), shoot length (5.6 cm) and, particularly, number of shoots per explant (2.28 shoots) and shoot quality (Table 1, Figure 1). Addae-Frimpomaah et al. (2014) reported that BAP enhanced both callus development and shoot induction of cultured sweet potato meristems and MS medium supplemented with 0.3 or 0.6 mg/L BAP was effective for shoot development of nodal explant culture. Muhammad et. al. (2007) found that liquid MS medium containing 4.0mg/L BAP produced maximum number of shoots regenerated from a single shoot tips in banana. The results of the present study also indicated that BAP is more suitable for shoot induction of nodal explants in sweet potato.

Table 1. Effect of Kinetin (Kn) and BAP at different concentrations on shoot regeneration

Concentration of Kn or BAP (mg/L)	Leaf number		Shoot length (cm)		Number of shoots per explant	
	Kn	BAP	Kn	BAP	Kn	BAP
0	3.9	3.9	3.4	3.4	1.04d	1.06c
0.5	4.6	4.6	4.3	4.3	1.40bc	1.57bc
1.0	5.4	5.7	5.2	5.6	2.14a	2.28a
1.5	5.0	5.2	4.7	5.1	1.51b	1.65bc
2.0	4.6	4.6	4.3	4.9	1.20cd	1.26c

Note: Means in a column followed by different letters are significantly different at 5% probability.

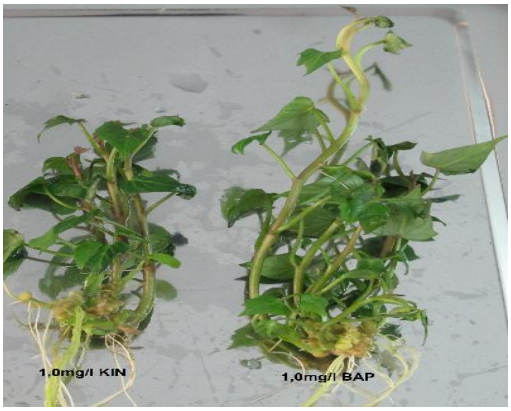

Figure 1. Shoot regeneration and growth on SIM supplemented with 1.0 mg/L Kinetin (left) and BAP (right)

Table 2. Effect of different concentrations of IAA shoot regeneration, at BAP concentration of 1.0 mg/L

IAA concentration (mg/L)	Leaf number	Shoot length (cm)	Number of shoots per explant
0	5.70	5.60	2.28d
0.1	5.70	5.70	2.54bcd
0.3	6.00	6.00	2.82b
0.5	6.20	6.30	3.26a
0.7	5.80	5.30	2.73bc

Note: Means in acolumn followed by different letters are significantly different at 5% probability.

3.2. Effect of IAA combination on shoot regeneration

At different IAA concentrations on SIM containing 1 mg/L BAP, the concentration of 0.5 mg/L IAA produced highest leaf number (6.2 leaves), shoot length (6.3 cm) and shoot number per explant (3.26 shoots) (Table 2). It is well established that cytokinins stimulate plant cell division and participate in the release of lateral bud dormancy, induction of adventitious bud formation, growth of lateral buds, while auxins exert, among others, a strong influence in initiation of cell division, meristem organization giving rise to un-organized tissue (callus) or defined organs (shoots), and root formation (Gaspar *et al.*, 1996, 2003). Beneficial effects of BAP in combination with IAA on shoot induction have been observed in melon by Kathal *et al.* (1986), Niedz *et al.* (1989) and Valdez Melara and Gatica Arias (2009). Muhammad *et al.* (2007) also showed that combination of BAP (4 mg/L) and IAA (1.0 mg/L) was best for shoot multiplication and shoot growth of banana cv. Basrai.

3.3. Effect of coconut water on shoot regeneration

The effect of coconut water was studied by culturing nodal explants on SIM supplemented with BAP 1.0 mg/L + 0.5 mg/L IAA supplenented with: 0%, 10%, 20%, 30% and 40% (v/v) coconut water. Supplementation of coconut water to the culture medium enhanced shoot regeneration and growth, with 20% coconut water showing significantly highest value (6.9 leaves/shoot, 7.1 cm in length, 4.05 shoots/explant) ($p < 0.05$) (Table 3). It is believed that coconut water, a liquid endosperm, is rich in vitamins, plant hormones (auxins and cytokinins) and various minerals and, thus, traditionally used as a growth

supplement in plant tissue culture/micropropagation (Yong *et al.*, 2009; Molnar *et al.*, 2011). Buah and Agu-Asare (2014) reported that coconut water from fresh green fruits is a suitable alternative to BAP in the in vitro culture of banana plants. The growth of spinach tissue on a medium supplemented with 10% to 15% (v/v) mature coconut water increased the weight of spinach callus after 5 weeks and accelerated shoot regeneration (Al-Khayri *et al.*, 1992).

It has been reported that when added to a medium containing auxin, coconut water can induce plant cells to divide and grow rapidly (Molnar *et. al.*, 2011). Nasib *et al.* (2008) when optimizing culture protocol of Kiwifruit reported that BAP and coconut water together showed synergistic effect on *in vitro* shoot regeneration They found that maximum shoot length, number of shoots and number of nodes were achieved on MS medium containing 20% (v/v) coconut water with 2.0 mg/L of BAP. Coconut water was also used successfully for in vitro olive micropropagation (Peixe *et al.*, 2007), for shoot induction of tissue culture of orchid, *Paraphalaenonopsis serpentilingua* (Mukarina *et al.*, 2010) and for *in vitro* shoot regeneration of *Celosia* sp. (Daud *et al.*, 2011). Medium supplemented with NAA and coconut water yielded fastest emergence and highest number of buds in orhid (Mukarina *et al.*, 2010).

Table 3. Effect of coconut water on shoot regeneration using nodal explants

Coconut water concentration (%)	Number. of leaves	Shoot length (cm)	Number of shoots per explant
0	6.1	6.3	3.35b
10	6.2	6.4	3.58b
20	6.9	7.1	4.05a
30	6,4	6.6	3.68ab
40	5.9	6.1	3.48b

Note: Means in a column followed by different letter are significant different at 5% level of probability

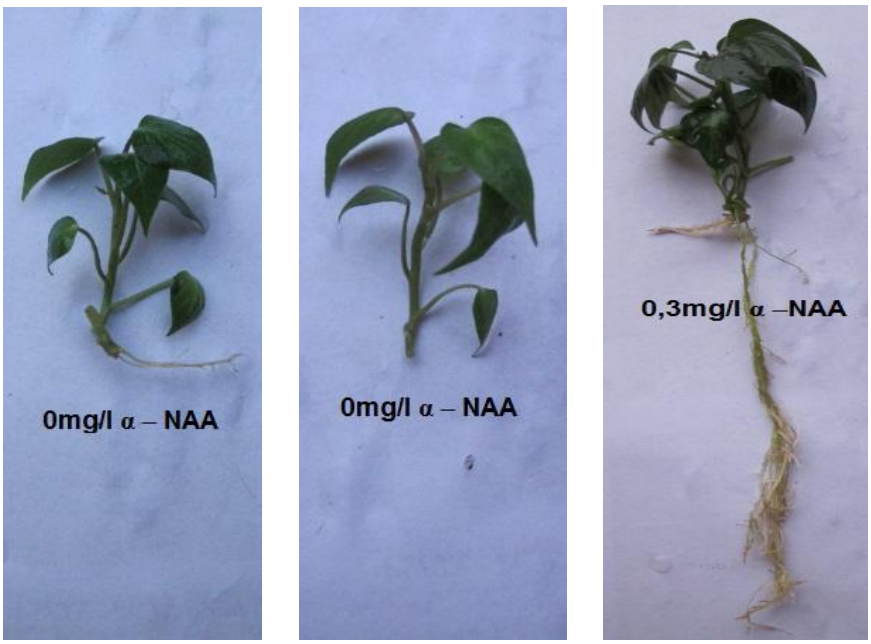

Figure 2. Effect of α-NAA on root induction and root development

Table 4. Effect of α - NAA concentrations on root induction

α-NAA concentration (mg/L)	Percentage of root induced shoots	Root number	Root length (cm)
0	43	0,45d	2,97e
0.1	100	1,58c	4,49d
0.3	100	3,04a	8,66a
0.5	100	2,45b	6,26b
0.7	100	2,14b	5,25b

Note: Means in a column followed by different letter are significant different at 5% level of probability

3.4. Effect of α-NAA on root induction

α - NAA was positive in root induction in terms percent root-induced shoots, root number per shoot and root length. Highest root number per explant (3.4 roots) and root length (8.66 cm) were obtained in the SIM supplemented with 0.3 mg/L α - NAA (Figure 2 and Table 4). Lee *et al.* (2011) reported that adventitious root induction was most effective by supplement with 0.5 mg/L α-NAA in terms of root induction rate, number of adventitious roots per explant, and root length during six weeks of culture of *Aloe vera*. NAA also had positive effect on root induction of *Hemarthria compressa* cuttings (Yan *et al.*, 2014).

3.5. Effect of aerated culture condition on shoot and root regeneration

The effect of aerated culture was assessed based on shoot regeneration, shoot growth and shoot quality in culture (*in vitro*) and post-culture (*ex vitro*). Aerated culture system increased not only number of leaves, number of shoots per explant or shoot multiplication rate, but also significantly increased the shoot length ($p < 0.05$) as well as shoot quality in terms of plant robustness (Table 5). Moreover, the combination of 1.0 mg/l BAP + 0.5 mg/l IAA + 20% coconut water under aerated culture resulted in superior plants as compared with medium containing only 1.0 mg/l BAP in traditional or non-aerated culture system (Figure 3).

Culture studies with *Lilium longiflorum* in closed and aerated system (Duong Tan Nhut *et al.*, 2004) showed that plant growth and development were much better both *in vitro* and *ex vitro* in aerated conditions compared with non-aerated conditions. Aerated micropropagation using self-adhesive gas permeable membrane (Milliseal) was found more advantageous than the conventional micropropagation with regard to the growth of *Paulownia fortunei* (Seem.) Hemsl. and *Chrysanthemum* sp. (Duong Tan Nhut *et al.*, 2005). Using plastic jars with aeration cap they obtained significantly better plant growth, i.e. higher fresh weight, better plantlet height with broad expanded, green leaves which were not produced in closed jars. They also believed that culture under aeration condition might be a good way to overcome the vitrification in the plantlets cultured in the closed vessels. Teixeira da Silva *et al.* (2005) reported that aeration culture, provided either by the presence of a Milliseal on the cap or by the use of Neoflon PFA film, enhanced the growth, rooting and subsequent acclimatization of *Anthurium andreanum.*

Although shoots derived from non-aerated culture and aerated culture formed roots within four weeks of culture in root-inducing medium (SIM containing 0.3 mg α-NAA), the plants derived from aerated culture had the root number (3.93 roots/shoot) and root length (9.91 cm) much higher ($p < 0.05$) in in comparison with non-aerated culture condition (Table 5, Figure 4). The plantlets were more healthier/vigorous and possessed stronger root system (Figure 4).

Table 4. Number of leaves, number of shoots per explant and shoot quality as affected by *in vitro* culture system, four weeks of culture

Culture system	Number of leaves	Shoot length (cm)	Number of shoots per explant	Shoot quality
Traditional	6.86b	5.09b	4.04a	Moderate
Aerated	8.66a	6.75a	4.26a	High

Note: Means in a column followed by different letter are significantly different at 5% level of probability

Figure 3. Shoots formed in SIM supplemeted with 1.0 mg/L BAP, 0.5 mg/L IAA and 20% coconut water in aerated condition (left) and SIM suplemented with 1.0 mg/L BAP in non-aerated condition (right)

Table 5. Rooting percentage, root number and root length under tradional and aerated culture conditions

Culture condition	Rooting percentage (%)	Root number	Root length (cm)
Non-aerated	100	3.04b	8,67b
Aerated	100	3,93a	9,91a

Note: Means in a column followed by different letter are significantly different at 5% level of probability

Figure 4. Effect of culture system on root formation and root length of plantlets of KLT10 x HL6-3, aeration culture (left) giving better quality plantlets than non-aerated culture condition (right)

3.6. Effect of culture system and potting mix on acclimatization of *in vitro* derived plants

Plantlets dervived from aerated culture condition resulted in significantly higher survival rate (99.75 – 100.0%) compared to non-aerated culture condition (58.64% - 77.78%) (Table 6). Similarly, aerated culture derived plantlets showed better plant growth in terms plant height (11.49 cm averaged over substrates) in comparison with those derived from non-aerated culture (6.80 cm averaged over substrates). Moreover, the aerated culture derived plantlets were more vigorous (Table 6, Figure 5).

Table 6. Survival, height and plant quality of plantlets as influenced by tissue culture condition and acclimatization substrate

Substrate	Survival rate (%)	Plant height (cm)	Plant vigor
Non-aerated condition			
Sand	58.64c	6.08f	-
Sand+ smooked rice hulls	77.78b	7.54d	+
smooked rice hulls	75.18b	6,80e	+
Aerated condition			
Sand	99.75a	10.52c	+
Sand+ smooked rice hulls	99.87a	12.47a	++
smooked rice hulls	100.0a	11.48b	++

Note: Means in a column follwed by different letters are significantly different at 5% level of probability

Plantlet derived from aerated condition (left) and non-aerated condition (right) grown in sand-smoked rice husk potting mix

Plantlet derived from aerated condition (left) and non-aerated condition (right) grown in sand

Plantlet derived from aerated condition (left) and non-aerated condition (right) grown in smoked rice husks

Figure 5. Effect of potting mix and culture condition on growth of tissue culture derived plantlets of sweet potato

Acclimatization of the plantlets was apparently high making the regeneration protocol from nodal explants of sweetpotato a success. Significant differences between areated and non-aerated culture condition during acclimatization or hardening might be attributed to the development of healthy root and leaf system. Tissue culture derived plants with well developed roots and leaves have been reported to adapt easily to natural conditions outside the growth room (Nowak and Pruski, 2002). Moreover, higher survival rate of plantlets obtained in sand-smoked rice husk potting mix might be attributed to both healthy source plants and suitable degree of aeration of the rooting medium which is favorbale for root development. It is, therefore, suggested that for acclimatization of tissue culture derived plantlets aerated rooting medium should be used.

4. CONCLUSION

The objective of this study was to establish an efficient protocol for *in vitro* clonal propagation of sweet potato using nodal explants of sweet potato seedlings to facilitate clonal selection. Aerated culture condition in full strength MS medium solidified by 0.8% agar and suplemented with 1.0 mg/L BAP, 0.5 mg/L IAA, 3% sucrose and 20% coconut water with medium adjusted pH 5.8 was optimal for shoot regeneration whereas basal MS salt solidified by 0.8% agar and supplemented with 0.3 mg/L α-NAA, 3% sucrose at pH 5.8 was suitable for root induction. Moreover, aerated culture condition was superior to non-aerated condition in terms of shoot growth and healthy plantlets. The plantlets showed higher This might be applied for large scale production of plants using nodal cuttings. Aerated potting mix of sand-smoked rice husks was favorable for acclimatization of *in vitro* derived plantlets. The study demonstrates the advantages of coconut water and aeration (achieved by Milliseal) in *in vitro* micropagation of sweet potato using nodal explants.

Acknowledgements

The authors are sincerely indebted to Gia Loc Field Crops Research Institute, Vietnam Academy of Agricultural Sciences for providing sweet potato materials and allowing the use of research facilities for the experiemnts.

REFERENCES

Abdullahil Baque, Md., Yun-Kyong Shin, Turkey Elshmari, Eun-Jung Lee, Kee-Yoeup Paek (2011). Effect of light quality, sucrose and coconut water concentration on the microporpagation of Calanthe hybrids ('Bukduseong' × 'Hyesung' and 'Chunkwang' × 'Hyesung'). Australian J. Crop Science, 5(10): 1247 - 1254.

Addae-Frimpomaah, F., J. Amponsah, T. K. Tengey (2014). Regeneration of three sweet potato (*Ipomoea batatas* (L.)) accessions in Ghana via meristem and nodal culture. International Journal of Plant Breeding and Genetics, 8: 121 - 138.

Alam, I., S. A. Sharmin, M. K. Naher, M. J. Alam, M. Anisuzzaman, M. F. Alam (2010). Effect of growth regulators on meristem culture and plantlet establishment in sweet potato [*Ipomoea batatas* (L.) Lam.]. POJ., 3(2): 35 - 39.

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

Buah, J. N. And P. Agu-Asare (2014). Coconut water from fresh and dry fruits as an alternative to BAP in the *in vitro* culture of dwarf Cavendish banana. J. Biol. Sci., 14(8): 521 - 526.

Buddendorf-Joosten, J..M.C and E. J Woltering (1994). Components of gaseous environment and their effects on plant growth and development *in vitro*. Plant Growth Regulation, 15: 1 - 16.

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

Dolinski, R., A. Olek (2013). Miropropagation of sweet potato (*Ipomoea batatas* (L.) Lam.) from node explants. Acta Sci. Pol., Hortorum Cultus, 12(4): 117 - 127.

Dương Tấn Nhựt, Nguyễn Quốc Thiện, Nguyễn Thành Hải, Đoàn Thị Quỳnh Hương, Nguyễn Thị Thúy Hằng, Nguyễn Ngọc Kim Vy, Nguyễn Văn Bình, Phan Xuân Huyên, Nguyễn Thị Diệu Hương, Đỗ Năng Vịnh (2004). Nuôi cấy lỏng và nuôi cấy thoán khí trong việc gia tăng sự tái sinh chồi và nâng cao chất lượng cây hoa lily (*Lilium longiflorum*). Tạp chí Công nghệ sinh học, 2(4): 487 - 499.

Dương Tấn Nhựt, Nguyễn Quốc Thiện, Vũ Quốc Luận (2005). Nâng cao chất lượng của cây giống hoa cúc và hông nuôi cấy *in vitro* thông qua nuôi cấy thoáng khí. Tạp chí Sinh học, 27(3): 92 - 95.

Gaspar T.H., C. Kevers, C. Penel, H. Greppin, D. M. Reid, T. A. Thorpe (1996). Plant hormones and plant growth regulators in plant tissue culture. *In Vitro* Cellular Developmental Biology-Plant, 32: 272 - 289.

Gaspar T.H., C. Kevers, O. Faivre-Rampant, M. Crevecoeur C. Penel, H. Greppin, J. Dommes (2003). Changing concepts in plant hormone action. *In vitro* Cellular Developmental Biology-Plant, 39: 85 - 106.

Gosukonda, R., C. S. Prakash, A. P. Dessai (1995). Shoot regeneration *in vitro* from diverse genotypes of sweetpotato and multiple shoot production per explant. HortScience, 30(5): 1074 - 1077.

Kathal R., S. P. Bhatnagar, S. S. Bhojwani (1986). Regeneration of shoots from hypocotyl callus of *Cucumis melo* cv. Pusa sharbati. Journal of Plant Physiology, 126: 59 - 62.

Lee Y- S., T. J. Yang, S-U. Park, J- H. Baek, S- Q. Wu, K-B. Lim (2011). Induction and proliferation of adventitious roots from *Aloe vera* leaf tissues for *in vitro* production of aloe-emodin, Plant Omics Journal 4(4):190 - 194.

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

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

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

Muhammad, A., H. Rashid, I. Hussain, S.M. Saqlan Naqvi (2007). Proliferation-rate effects of BAP and kinetin on banana (*Musa* spp. AAA Group) 'Basrai'. HortScience, 42: 1253 - 1255.

Murashige T., Skoog F.(1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol., 5: 473 - 497.

Nasib, A., K. Ali, S. Khan (2008). An optimized and improved method for *in vitro* propagation of Kiwifruit (*Actinida deliciosa*) using coconut water. Pak. J. Bot., 40(6): 2355 - 2360.

Nguyễn Thị Lý Anh, Nguyễn Quang Thạch (2003). Nghiên cứu làm sạch virus bằng nuôi cấy meristem trên một số giống khoai lang ở Bắc Việt Nam. Báo cáo khoa học hội nghị công nghệ sinh học toàn quốc, Nhà xuất bản Khoa học kỹ thuật, 735-739.

Niedz R.P., S. S. Smith, K. V. Dunbar, C. T. Stephens, H. H. Murakishi (1989). Factors affecting shoot regeneration from cotyledonary explants of *Cucumis melo*. Plant Cell, Tissue and Organ Culture 18: 313 - 319.

Nowak, J., K. Pruski (2002). Priming of tissue culture propagules: In: Low cost options for tissue culture technology in developing countries. Proceedings of a technical meeting organized by the Joint FAO/IAEA Division of Nuclear techniques in food and agriculture. 26-30 Aug. 2002, Vienna.

Onwubiko, N. C., C I. Ihezie, M. U. Mozie (2015). *In vitro* regeneration of sweet potato (*Ipomoea batatas* (L.) Lam.) from node explants. Amer. J. Experimental Agriculture, 8(2): 87 - 92.

Peixe, A., A. Raposo, R. Lourenco, E. Macedo (2007). Coconut water and BAP successfully replaced zeatin in olive (Olea europaea L.) micropropagation. Scientia Horticulturse, 113(1): 1 - 7.

Phạm Văn Linh, Nguyễn Đức Anh, Trần Thị Quỳnh Nga và cs (2014). Kết quả nhân nhanh giống khoai lang bằng phương pháp nuôi cấy *in vitro*, Tạp chí KH-CN Nghệ An, 7: 1 - 5.

Sivparsad, B. J., A. Gubba (2012). Development of an efficient plant regeneration protocol for sweet potato (*Ipomoea batatas* L.) cv Blesbok. Afri. J. Biotech, 11(84): 14982 - 14987.

Yan Y-H, Li J-L, Zhang X-Q, Yang W-Y, Wan Y, et al. (2014) Effect of nphthalene acetic acid on adventitious root development and associated physiological changes in stem cutting of *Hemarthria compressa*. PLoS ONE 9(3): e90700. doi:10.1371 /journal.pone.0090700

Yong, J. W. H. Yong, L.. Ge, Y. Fei Ng and S. Ngin Tan (2009). The Chemical Composition and Biological Properties of Coconut (*Cocos nucifera* L.). Molecules, 14: 5144 - 5164.

Teixeira da Silva, J. A., S. Nagae and M. Tanaka (2005) Effect of physical factors on micropropagation of *Anthurium andreanum*. Plant Tissue Cult., 15(1): 1 - 6.

Valdez Melara, M., A. M. Gatica Arias (2009). Effect of BAP and IAA on shoot regeneration in cotyledonary explants of Costarican melon genotypes. Agronomía Costarricense, 33(1): 125 - 131.

Ying, D. Q., Z. Ying, T. L. Davenport (2004). Meristem-tip culture boosts yield of sweet potato cv. Picadita in South Florida. Proc. Fla. State Hort. Sci., 117: 70 - 76.

Zamora, A. B. (1993). Meristem culture of sweet potato (*Ipomoea batatas*). FAO/IPBGR Plant genetic resources Newsletter, 91/92: 25 - 28.

Zobyed, S. M. A, F. Afreen, C. Kubota, T. Kozai (2000). Water control and survival of *Ipomoea batatas* grown photoautotrophically under forced ventilation and photomixotrophically under natural ventilation, Annals of Botany, 86: 603 - 610.