In vitro PROPAGATION OF SWEET POTATO VARIETIES VC6, VC7 (Ipomoea batatas (L.) LAM) THROUGH LATERNAL BUD CULTURE

Le Thi Thuy1*, Tran Thi Giang1, Tong Thi Thu Hoai1, Tran Thi Hai2

Abstract. This research aimed to optimize the protocol for in vitro propagation of VC6 and VC7 sweet potato varieties, using sodium hypochlorite (NaOCl/Javel), 6-benzyl amino purine (BAP), and indole-3-butyric acid (IBA) phytohormones for sterilization, shoot multiplication, and root formation, respectively, along with determining the optimal substrate for explants during the acclimatization stage. The results demonstrated that the most effective disinfection formula for both varieties was Javel at a 3% concentration for 10 minutes, resulting in the lowest contamination rates (20% in VC6 and 0% in VC7). In the shoot formation experiment, the best shootting was all observed at 1.0 mg/L BAP, with 93.33% and 86.67% of bud-forming explants and approximately 1.9 and 2.13 shoots per explant in varieties VC6 and VC7, respectively. Additionally, a significant rooting rate (100%) was observed at the concentration of IBA (1.0 mg/L), as each explant had more than two primary roots and numerous secondary roots. Furthermore, after one month of planting in the substrate, a mixture of sand and perlite at a 1:1 ratio exhibited a 100% survival plantlet rate, the highest in the number of leaves (2.2 leaves/explant in VC6 and 2.13 leaves/explant in VC7), and the highest in height (2.4 cm/explant in VC6 and 1.87 cm/explant in VC7).

Keywords: Lateral bud, micropropagation, substrates, sweet potato.

1. INTRODUCTION

Sweet potato varieties VC6 and VC7 were new varieties that were bred and selected by the Root Crop Research and Development Center under the Fields Crops Research Institute in 2012 (Hoai et al., 2020).

This tuberous root vegetable is grown predominantly in developing nations during warm seasons and serves as a vital source of sustenance for people, feed for animals, and material for industrial use (Marcos et al., 2019). The sweet potato is regarded as the seventh most crucial crop globally and the fifth most significant in developing nations (Loebenstein et al., 2009). In Vietnam, sweet potatoes are a significant food crop, ranking just below rice and corn in importance (Vu Thi Lan and Chu Hoang Ha, 2017). In industry, this vegetable crop is utilized not only in home cooking but also as a raw material for producing sweets, flour, flakes, and starch. Furthermore, it plays a vital role in generating biomass for biofuel (Tumwegamire et al., 2011).

Sweet potato propagation relies heavily on stem cuttings which is a traditional method and has a number of disadvantages. This approach can lead to the accumulation of

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diseases in the cuttings over generations, resulting in decreased root yield and the loss of superior genetic strains. Additionally, this propagation strategy is time-consuming, and requires a large geographical area (Beyene et al., 2020). Sweet potato micropropagation has several benefits, including the ability to produce large numbers of disease-free plants quickly, the ability to eradicate bacterial, fungal, and viral infections, the production of uniform, high-quality plantlets, increased propagation rates, the maintenance of germplasm in small spaces under controlled conditions, and a reduction in labor requirements (Chukwunalu et al., 2023).

2. RESEARCH METHODS

2.1. Materials

Sweet potato varieties VC6 and VC7 were obtained from the Root Crop Research and Development Center. They were then grown by stem cuttings in the Experimental Garden. Tubers of two sweet potato varieties were harvested and grown in greenhouses to limit exposure to pathogens. After about 2 months, plants growing from tubers were used to provide samples (lateral bud) for the micropropagation.

2.2. Methods

The experiments were carried out at Experimental Garden of Faculty of Biology and Laboratory of Plant tissue culture, Faculty of Biology – Hanoi National University of Education, from April 2023 to March 2024.

2.2.1. Experiment 1: Determine the most effective surface sterilization protocols

The procedure involved cutting the veins from mother plants into sections with approximately 20 cm in length, each containing approximately 7-10 nodes. Subsequently, all leaves were removed, and the sections were rinsed under running tap water for approximately 3 minutes. Following this, the sections were cut into smaller segments (3-4 cm), with each segment containing a single lateral bud. These segments were then immersed in 70 °C alcohol for 3 minutes before being rinsed with distilled water to remove any residual alcohol. The surface sterilization of these stem segments was then conducted according to the following procedure, which was carried out entirely within a biological safety cabinet.

Step 1: Transfer the stem segments to a sterilized Erlenmeyer flask.

Step 2: Add varying concentrations of Javel (1%, 3%, and 6%) to the flask and shake well for different durations (5 minutes, and 10 minutes).

Step 3: Pour out Javel, then rinse the segments with sterilized distilled water twice to remove all NaOCl.

Step 4: Blot the segments dry with sterile absorbent paper.

Step 5: Transfer the sterilized segments to MS medium supplied with BAP 1.0 mg/L.
The samples were then placed under daily lighting conditions of 16 light hours and 8 hours of darkness, at 24 ± 2 °C, and relative humidity of 70%. This condition was applied to all experiments of this study.

The experimental results were evaluated through the following criteria:

Percentage of contaminated samples = (Total number of contaminated samples/Total number of cultured samples) x 100.

Percentage of purified samples = (Total number of purified samples/Total number of cultured samples) x 100.

Among purified explants, percentage of shoot-forming explants = (Total numbers of shoot-forming explants/Total number of purified explants) x percentage of purified explants.

Among purified explants, percentage of died explants = (Total numbers of died explants/Total number of purified explants) x percentage of purified explants.

2.2.2. Experiment 2: Effect of BAP concentration on the shoot regeneration

Sterilized nodal segments were cultivated in MS medium flask supplemented with varying concentrations of BAP, specifically 0.5, 1.0, 1.5, and 2.0 mg/L. Each flask contained five samples, and each formulation was observed in three flasks. The results were evaluated after a three-week cultivation period by analyzing the following criteria:

Percentage of shoot-forming explants (%) = (The number of shoot-producing explants/Total number of explants) x 100.

Average number of shoots per explant = (Total number of shoots/Total number of shoot-producing explants)

2.2.3. Experiment 3: Effect of IBA on root induction from the shoot explant

The differentiated shoot explants of two sweet potato varieties VC6, VC7 were transplanted to MS medium supplemented with different concentrations of IBA, specifically 0.5, 1.0, 1.5, and 2.0 mg/L. After 30 days of culture, some parameters related to root induction were recorded:

Rooting rate (%) = (Total number of explants with roots/Total number of explants) × 100.

Parameters related to root quality were evaluated at 3 levels:

+ + +: Explants had a high number of roots (5-6 roots/explant), with long and well-branched structures (main root length >10 cm).

+ + : Explants had fewer roots (3-4 roots/explant); roots were short, and less branched, the average main root length < 10 cm).

+ : Explant had 1-2 roots; roots were notably short and weak.
2.2.4. *Experiment 4: Effect of substrate on planlet’s quality at the greenhouse stage*

Once the sweet potato explants reached a height of approximately 3 cm, with 4-5 leaves, they were selected for acclimatization stage. Before planting, the roots of the sweet potato plantlets were carefully cleaned to remove any residual culture media, ensuring the roots remained undamaged. The selected plantlets were grown in pots containing one of five types of substrates (100% sand, 100% Tribat, 1 sand : 1 pearlite, 1 sand : 1 Tribat, 1 pearlite : 1 Tribat, in volume). Each plantlet is planted in a pot measuring 17 x 15 cm. Watering was done daily to maintain moisture in the substrate, the amount of water was the same in all treatments. The pots were placed in a greenhouse environment with optimal moisture and lighting conditions to facilitate growth.

The experimental results were evaluated after a month by analyzing the following criteria:

*Percentage of survival explants (%) = (the total number of survival explants/the total number of explants) x 100.*

*Plantlet increased height (cm) = (plant height after one month grown on substrate) – (initial in vitro plant height).* The height was measured from the stem above the ground to the shoot tip of the plantlet.

*Number of new formed leaves: Count the number of new leaves formed from in vitro plants after 1 month of growing on the substrate.*

*Parameters related to plantlet quality were evaluated at 3 levels:*

+++: Explants grew approximately 2 or more leaves after a month, height increased by 1.5 cm or more, and leaves were green.

++: Explants grew about 1-2 more leaves, height increased from 1 cm to less than 1.5 cm, and some leaves turned yellow.

+: Explants didn’t appear any new leaves or only 1 new leaf appears, height increased less than 1 cm tall, the leaves turned yellow and felt off.

2.2.5. *Data processing methods*

Experimental data were analyzed using biostatistics methods with the Excel program.

3. RESULT AND DISCUSSIONS

3.1. *Determine the optimal surface sterilization protocols*

The effectiveness of sterilization significantly impacts the efficiency of the in vitro sterilization procedure because it directly influences the establishment and maintenance of plants in vitro cultures. Obtaining sterile plant material is difficult because living materials cannot be exposed to extreme heat while still retaining their biological capabilities,
therefore plant organs and tissues are generally sterilized by treatment with a disinfecting solution. Javel, one of the most popular bleach, was used in this experiment to sterilize sweet potato explants. Mengs et al used 10% Javel to sterilize lateral bud explants, while Beyene et al demonstrated that Javel 1% combined with Tween-20 had high disinfection efficiency to sweet potato shoot tips. Therefore through testing, in this study we selected 3 concentrations of Javel to sterilize the surface of sweet potato samples: 1%, 3% and 6%. The results were presented in Table 1 below.

**Table 1. Effect of Javel concentration and disinfection time on explant quality**

<table>
<thead>
<tr>
<th>Time</th>
<th>Javel concentration</th>
<th>Total number of explants</th>
<th>Percentage of contaminated explants (%)</th>
<th>Percentage of purified explants (%)</th>
<th>Among purified explants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
</tr>
<tr>
<td>5 minutes</td>
<td>1%</td>
<td>30</td>
<td>53.33</td>
<td>26.67</td>
<td>46.67</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>30</td>
<td>33.33</td>
<td>13.33</td>
<td>66.67</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>30</td>
<td>33.33</td>
<td>6.67</td>
<td>66.67</td>
</tr>
<tr>
<td>10 minutes</td>
<td>1%</td>
<td>30</td>
<td>40.00</td>
<td>6.67</td>
<td>60.00</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>30</td>
<td>20.00</td>
<td>0</td>
<td>80.00</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>30</td>
<td>13.33</td>
<td>0</td>
<td>86.67</td>
</tr>
</tbody>
</table>

The results in Table 1 showed that, when remaining the same period of exposed time and increasing the concentration of Javel (from 1% to 6%), there was a steady reduction in the number of contaminated explants, and eventually resulted in complete cleanliness (0% contaminated explants) in two formulations. Specifically, treatment of sweet potatoes with a 1% Javel solution (the lowest concentration) for 5 minutes (the shortest duration) resulted in the highest infection rates observed, reaching 53.33% in VC6 and 26.67% in VC7. When expanding the time to 10 minutes, the percentage of contaminated explants decreased significantly, to 20% and 13.33% in VC6 with 3% and 6% Javel concentration, respectively, and reached 0% in VC7. Despite achieving greater disinfection efficacy, the highest budding rates (73.33% in VC6 and 86.67% in VC7) were observed when using the 3% Javel concentration for 10 minutes, whereas these rates decreased to 20% in VC6 and 53.33% in VC7 with the use of 6% concentration for the same duration. Therefore, it is evident that increasing the concentration of Javel during the disinfection process has enhanced its efficacy in eradicating fungi and bacteria present on the surface of explants due to its potent oxidizing properties. However, a high concentration of Javel has adversely impacted the germination capacity of sweet potato explants. Based on that, the most optimal formula for disinfecting VC6 and VC7 sweet potatoes appears to be Javel at concentrations of 3% for 10 minutes.

### 3.2. Effect of BAP concentration on the shoot regeneration

Table 2 provided information about the effect of different concentrations of BAP on the shoot development of sweet potato explants.
Table 2. Effect of BAP concentrations on the shoot regeneration

<table>
<thead>
<tr>
<th>BAP concentration (mg/l)</th>
<th>Total number of explants</th>
<th>Percentage of shoot-forming explants (%)</th>
<th>Average number of shoots/explant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>60.00</td>
<td>60.00</td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
<td>93.33</td>
<td>86.67</td>
</tr>
<tr>
<td>1.5</td>
<td>15</td>
<td>66.67</td>
<td>66.67</td>
</tr>
<tr>
<td>2.0</td>
<td>15</td>
<td>46.67</td>
<td>53.33</td>
</tr>
</tbody>
</table>

The data in Table 2 illustrated that as the BAP concentration increased from 0.5 mg/L to 1.0 mg/L, there was a corresponding increase in the total number of explants that developed shoots, from 60% to 93.33% in VC6 and from 60% to 86.67% in VC7, and in the average number of shoots per explant, from 1.1 to 1.9 in VC6 and from 1.53 to 2.13 in VC7. However, further increase in BAP concentration to 1.5 mg/L and 2.0 mg/L resulted in a decrease in both the percentage of bud-forming explants (46.67% and 53.33% in VC6 and VC7 respectively when cultivating them on MS medium supplemented with 2.0 mg/L BAP) and the average number of shoots per explant was 1 in VC6 and 1.07 in VC7 varieties. This results suggested that an optimal concentration of BAP for leaf development in sweet potato explants was MS media supplemented with 1.0 mg/L BAP.

Mengs' research revealed the same results in two varieties (Kullufo and Tulla), at which the effectiveness of BAP concentrations of 0.5 and 1 mg/L was notably higher compared to concentrations of 1.5 and 2 mg/L in promoting bud formation, shoot length, shoot fresh weight, and shoot dry weight (Mengs et al., 2018). Low concentrations of BAP were found to be effective in rapidly initiating shoot growth due to their role in activating cellular processes. Conversely, high concentrations (2 mg/L) of BAP resulted in significantly lower shoot initiation rates due to their inhibitory effect on metabolism and shoot elongation (Beyene et al., 2020).

3.3. Effects of IBA on root inducement from the shoot explant

The differentiated shoot explants of sweet potato varieties VC6 and VC7 were transplanted to MS medium supplemented with different concentration of IBA. After 30 days culture, we obtained rooted plantlets and the effects of IBA on root induction from the shoot explants were listed in Table 3.

Table 3. Effect of IBA on root inducement from the shoot explants

<table>
<thead>
<tr>
<th>IBA concentration (mg/L)</th>
<th>Total number of explants</th>
<th>Number of explants that have roots</th>
<th>Rooting rate (%)</th>
<th>Root quality</th>
<th>Average Root length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>+++</td>
</tr>
<tr>
<td>1.5</td>
<td>15</td>
<td>9</td>
<td>6</td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No root</td>
</tr>
</tbody>
</table>
The results in Table 3 showed that at lower concentrations of 0.5 mg/L and 1.0 mg/L IBA, a rooting rate of 100% was observed in both varieties. Specifically, explants cultured in MS medium supplemented with 1.0 mg/L IBA demonstrated the highest root count, averaging about 5-6 roots per explant, and these roots predominantly exhibited elongated, well-branched structures, exceeding 10 cm in length. While a smaller number of explants cultivated in 0.5 mg/L IBA have roots compared to those in 1.0 mg/L IBA, the roots were fewer in number (3-4 roots per explant), shorter, and less branched (with an average root length below 10 cm). Moreover, as the concentration of IBA increased to 1.5 mg/L, the rooting rate decreased to 60% in VC6 and 40% in VC7, accompanied by a decrease in the average number of roots and shorter root lengths. Notably, at the highest concentration of 2.0 mg/L IBA, no roots were formed in both varieties, indicating a complete inhibition of root development. These findings suggested that while lower concentrations of IBA promote successful rooting in sweet potato explants, higher concentrations can lead to a significant reduction or complete inhibition of root formation. Therefore, MS medium supplemented with 1.0 mg/L IBA proved to be the most suitable medium for root inducement stage in sweet potatoes varieties VC6 and VC7.

However, research conducted by Berihu Mengs has revealed considerable differences when cultivating samples at lower concentrations. Specifically, Kullufo and Tulla explants cultured in a MS medium supplemented with 0.25 mg/L and 0.5 mg/L IBA consistently displayed a significantly higher percentage of root formation and a greater number of roots per explant compared to those grown in media supplemented with 0.75 mg/L and 1 mg/L IBA (Mengs et al., 2018). An even lower concentration of IBA (at 0.1 mg/L) proved to be the most suitable for the Awassa-83 variety, resulting in the highest number of roots per shoot (6.34) (Abdissa et al., 2011). This difference in results may be related to the characteristics of sweet potato varieties.

3.4. Effect of substrate on plantlet quality at the greenhouse stage

*In vitro* rooted plants with a height ranging from approximately 3 centimeters and bearing about 4 to 5 leaves are chosen after cultivation in an IBA medium. These selected explants are then transferred to a greenhouse environment where they are grown in five different substrates. The results were recorded after one month and were shown in Table 4.

The data in Table 4 indicated that the explants grown in substrate including sand and perlite (ratio of 1:1) had the highest number of new formed leaves per explant (2.2 in VC6 and 2.13 in VC7), and plantlet increased height (2.4 cm in VC6 and 1.87 cm in VC7). Conversely, only 20% of explants in both varieties grown in 100% Tribat medium survived, these plantlets had the lowest result about number of new formed leaves (0.9 in VC6 and 0.87 in VC7), and plant increased height (0.3 cm in VC6 and 0.73 cm in VC7).

Though rich in nutrients, the data indicates that as the proportion of Tribat in the soil increases, the plant death rate rises, which can be explained by several reasons. Firstly, its smooth texture increases water retention while reducing substrate aeration. Secondly, its high nutrient content can elevate osmotic pressure, reducing the ability of *in vitro* plant
roots to absorb water. Lastly, *in vitro* roots are typically delicate and adapted to artificial culture conditions, so they easily lose vitality in unsuitable substrate conditions.

**Table 4. Effect of substrates on plantlet quality at the greenhouse stage**

<table>
<thead>
<tr>
<th>Types of culture substrates</th>
<th>Total No. of explants</th>
<th>Percentage of survival explants (%)</th>
<th>Number of new formed leaves</th>
<th>Plantlet increased height (cm)</th>
<th>Plantlet quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
<td>VC7</td>
<td>VC6</td>
<td>VC7</td>
</tr>
<tr>
<td>100% sand</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>1.8 ± 0.12</td>
<td>1.93 ± 0.14</td>
</tr>
<tr>
<td>10% Tribat</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>0.9 ± 0.09</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>1 sand : 1 perlite</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>2.2 ± 0.21</td>
<td>2.13 ± 0.13</td>
</tr>
<tr>
<td>1 sand : 1 Tribat</td>
<td>15</td>
<td>86.67</td>
<td>80</td>
<td>1.4 ± 0.18</td>
<td>1.47 ± 0.22</td>
</tr>
<tr>
<td>1 perlite : 1 Tribat</td>
<td>15</td>
<td>46.67</td>
<td>73.33</td>
<td>1.2 ± 0.11</td>
<td>1.67 ± 0.19</td>
</tr>
</tbody>
</table>

Furthermore, due to the characteristics of substrates containing perlite, which are well-drained and aerated, it becomes easier for explants to absorb water from the soil. Therefore, the combination of sand and perlite resulted in a significantly accelerated growth rate compared to 100% sand.

4. CONCLUSIONS

Optimal disinfection of VC6 and VC7 sweet potatoes is achieved by using a 3% Javel solution for 10 minutes (80% purified explants in VC6 and 100% purified explants in VC7 varieties).

The most suitable medium for shoot regeneration of sweet potato varieties VC6 and VC7 is MS medium supplemented with 1.0 mg/L of BAP (93.33% shoot-forming explants in VC6 and 86.67% in VC7).

The MS medium supplemented with 1.0 mg/L IBA is the most effective for root inducement (100% rooting rate, 3-4 roots/explants with long and well-branched structures).

When transitioning the explants to the garden, *in vitro* plants should be grown in a mixture of sand and perlite with a 1:1 ratio, as it ensures the highest survival rate (100%), the highest plantlet growth height (2.4 cm/explant in VC6 and 1.87 cm/explant in VC7), and the highest number of new leaves formed (2.2 leaves/explant in VC6 and 2.13 leaves/explant in VC7).

REFERENCES


NHÂN GIỘNG in vitro GIÓNG KHOAI LANG VC6 VÀ VC7 (Ipomoea batatas (L.) Lam) TỪ CHỒI NÁCH

Lê Thị Thuỷ1*, Trần Thị Giang1, Tống Thị Thu Hoài1, Trần Thị Hải2

Tóm tắt: Nghiên cứu này được thực hiện nhằm xây dựng quy trình nhân giống in vitro từ choi nách đối với 2 giống khoai lang VC6 và VC7. Trong đó, dung dịch natri hypochlorit (NaOCl/Javel) được sử dụng làm chất khử trùng, các chất điều hòa sinh trưởng gồm benzylaminopurin (BAP) và axit indole-3-butyric (IBA) được sử dụng để nhân chồi và tạo rễ; cây giống in vitro được huấn luyện trên một số loại gia thể chứa cát, perlite, và gia thể Tribat ở vườn ươm. Kết quả nghiên cứu cho thấy, công thức khử trùng cho hiệu quả tối ưu ở cả hai giống là sử dụng Javel 3% trong 10 phút với tỉ lệ mâu nhiễm ở 2 giống VC6 và VC7 lần lượt là 20% và 0%. Trong giai đoạn tạo chồi, môi trường chứa 1,0 mg/L BAP cho hệ số nhân chồi cao nhất (93,33% mâu VC6 bắt chồi với trung bình 1,9 chồi/mầu, các kết quả này ở giống VC7 là 86,67% và 2,13 chồi/mầu). Ở giai đoạn tạo cây hoàn chỉnh, 100% mầu chồi tạo rễ, rễ dài và phân nhánh tốt khi nuôi cây chồi in vitro của 2 giống trong môi trường chứa 1,0 mg/L IBA. Ở giai đoạn vườn ươm, trong 5 loại gia thể nghiên cứu (cát; Tribat; perlite; cát: perlite (1:1) và cát: Tribat (1:1)) giá thể trồng thích hợp nhất cho cây giống in vitro của 2 giống là hổp cát và perlite tỉ lệ 1:1, đạt tỉ lệ 100% cây sống, sau 1 tháng trồng, cây giống VC6 tăng 2,4 cm chiều cao và trung bình tạo mỗi 1,3 lá/cây, trong khi giống VC7 cao thêm khoảng 1,87 cm/cây và tăng trung bình 2,13 lá/cây.

Từ khóa: Chồi nách, vi nhân giống, gia thể, khoai lang.

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