The Production of Virus-Free Sweet Potato Mother Plants in Malaysia Through Meristem Culture

.

ABSTRACT

|  |
| --- |
| **Aims:** Sweet potato is a significant root crop in Malaysia due to its nutritional and economic importance. This research represents the first report on the virus elimination technique for sweet potatoes in Malaysia. There is a growing demand for virus-free propagation materials of sweet potato varieties, specifically VitAto and Lembayung, to meet market requirements. This study aims to investigate the effect of plant growth regulators on meristem culture, which includes shoot and root regeneration, to validate the presence of viruses in meristem-derived plantlets, and to observe the development of planting material (cuttings) from virus-free sweet potato plants used as mother plants. **Methodology:** Young shoots that originated from sweet potato tubers were used as explants in this study. The shoots were sterilised and then examined under a stereomicroscope to extract the meristematic tissue. These meristems were cultured on Murashige and Skoog (MS) medium supplemented with various plant growth regulators (PGRs). Additionally, the MS medium with different PGR combinations was assessed for its effectiveness in promoting shoot and root regeneration. To validate the presence of viruses in the meristem-derived plantlets, a PCR method was employed. **Results:** The findings indicated that the MS medium enhanced with 1 mg/L BAP (Benzylaminopurine) and 1 mg/L GA3 (Gibberellic Acid) was the most efficient for Lembayung meristem culture. In contrast, the ideal medium for VitAto comprised MS medium enhanced with only 0.5 mg/L BAP. Consequently, both Lembayung and VitAto exhibited optimum plantlet regeneration when cultivated in MS media free of plant growth regulators. The Lembayung plantlets were found to be 97.2 % virus-free through meristem culture, while the VitAto plantlets were assessed to be 84.7 % virus-free. None of the meristem-derived plantlets were infected by multiple viruses. The SPVG virus was the only virus found in the meristem-derived plantlets, affecting approximately 2.8 % of Lembayung and 15.3 % of VitAto. These plantlets were acclimatised and developed into virus-free mother plants under a netting structure. After two months of transplanting, Lembayung yielded a maximum of 108 cuttings per plant, while VitAto generated 98 cuttings per plant. **Conclusion:** Meristem culture facilitates the production of disease-free, genetically stable, and high-quality planting materials. The capacity to rapidly propagate superior cultivars provides a steady supply of healthy plantlets, enhancing agricultural productivity and bolstering food security in Malaysia. |

*Keywords: Organogenesis, Multiplication, 6-Benzylaminopurine, Naphthalene acetic acid, gibberelic acid, keledek*

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) has become an important crop in agriculture due to its adaptability, nutritional value, and potential contribution to food security. As a rich source of carbohydrates, vitamins, and minerals, sweet potato plays a crucial role in addressing malnutrition, especially in developing countries. Its ability to thrive in various ecological conditions makes it an appealing option for farmers dealing with climate variability (Alam et al., 2012). However, challenges such as viral diseases pose a threat to sweet potato production. In Ethiopia, infections have increased significantly due to inadequate quarantine measures (Buko, 2019). Similarly, in Malaysia, challenges in sweet potato cultivation include insufficient farm maintenance and the control of insect vectors that transmit viral infections (Razean Haireen et al., 2023a). Viral infections can lead to yield losses ranging from 50% to 98%, significantly hindering production (Alam et al., 2012; Loebenstein, 2015). In regions reliant on this crop, these viruses not only degrade tuber quality but also diminish overall yield, posing a serious threat to food security (Alam et al., 2012). The genetic development of viral resistance is hampered by the lack of flowering in the plants (Krishna et al., 2022).

The Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Virus G (SPVG) are the most commonly detected viruses, with high incidence rates observed in both farmers' fields and research stations in Malaysia. These viruses often cause single infections as well as synergistic infections (Buko, 2019). Additionally, the continued spread of these viruses is facilitated by the use of infected planting materials, as there is a scarcity of virus-free resources in Malaysia. To combat these challenges, meristem culture has emerged as an effective technique for producing virus-free planting materials.

This method involves the in vitro cultivation of the plant's meristematic tissues, which are typically free from viral infections due to their rapid cell division and lack of vascular connections. Several studies have demonstrated the effectiveness of meristem culture in eliminating viruses from sweet potatoes. For example, Alam et al. (2012) found that over 85% of mericlones derived from 0.3–0.5 mm meristems showed no evidence of viral infection. Similarly, Wondimu et al. (2012) achieved a 99% success rate in producing virus-free plantlets using meristem culture techniques. The success of meristem culture largely depends on the composition of the culture media and the use of appropriate plant growth regulators. In the case of sweet potato meristem cultures, the combination of 6-benzylaminopurine (BAP) and gibberellic acid (GA₃) has been shown to effectively stimulate shoot initiation and proliferation (Alam et al., 2010).

Meristem culture is a promising method for producing virus-free planting materials for sweet potato. By optimizing the culture conditions and combinations of plant growth regulators (PGRs), it's possible to regenerate healthy and high-yielding plants. This approach can help reduce the negative effects of viral infections on sweet potato production. This study aims to investigate the effect of plant growth regulators on meristem culture, including shoot and root regeneration, to validate the presence of viruses in meristem-derived plantlets, and to observe the development of planting material (cuttings) from virus-free sweet potato plants used as mother plants.

2. material and methods

2.1 Planting Materials

Both varieties of local sweet potato tubers, VitAto and Lembayung, were obtained from the producers or farmers. Planting material for cuttings was also acquired from MARDI Bachok, which was virus positive. The young shoots that have emerged from the sweet potato tubers were excised and utilised for meristem culture. The experiment was conducted at the MyGeneBank Complex, MARDI Serdang, Selangor (coordinates:2°58’42.3”N, 101°41’17.4”E)

**2.2 Meristem cultures**

The shoots were cleaned with running tap water for few minutes and brought to the laminar air flow. Disinfection was carried out using 70% concentrations of ethanol and 2.5 % of Sodium hypochlorite combined with a few drops of Tween 20, followed by rinses with double-distilled water. The cleaned explants were dissected for the meristem part under a stereomicroscope. Then, the excised meristems were cultured on the Murashige and Skoog (MS) (1962) media with various combination of PGRs treatment such as 6-Benzylaminopurine (BAP), Naphthalene acetic acid (NAA) and Gibberellic acid (GA3). For the first two weeks, the cultures were maintained in dark condition. After that, the cultures were maintained at 25 ± 2°C under an 18/6-hour light/dark photoperiod. After four weeks of cultivation, data on shoot initiation and shoot length were recorded. Each experimental condition included four replications, with five units.

The media were then optimised for the VitAto variety. All the PGRs employed in the previous examination were utilised at half concentration for this study, except for NAA, which remained unchanged, and an extra treatment comprising the use of GA3 as a single PGR (0.5mg/L). The study adhered to the same methodology as the recent study. The reason this study was conducted was that VitAto meristems showed PGR overdose symptoms such as excessive callus induction and decline in shoot height that caused concern.

**2.3 Shoot and Root Regeneration**

After four weeks of culture on shoot initiation media, the meristem that initiated the shoot was moved to the regeneration medium, which consisted of MS media containing various concentrations and combination of PGRs such as BAP and Kinetin (Kn) for Lembayung and Kn and GA3 for VitAto. This investigation was conducted without BAP in the VitAto's culture media due to its sensitivity to BAP, which negatively affects plant growth morphology. The cultures were maintained at 25 ± 2°C under an 18/6-hour light/dark photoperiod. Data on shoot number, shoot height (cm), root number and root length (cm) were recorded. Each experimental condition included four replications, with three units.

**2.4 Validation of Virus**

Validation of virus-free sweet potato plant material was achieved using Polymerase Chain Reaction (PCR). This involved two sources: a) leaves from the mother plant before meristem culture and b) leaves from the plantlet after the meristem culture during the culture or acclimatisation phase. Juvenile leaves were collected and transported to the laboratory for DNA extraction.

For the post-meristem culture samples, these were divided into several groups, each containing 3 to 5 individual plants. Disease symptoms were documented both quantitatively and visually. The extraction of sweet potato leaves was conducted using an RNA extraction kit (NucleoSpin RNA Plus, Macherey-Nagel). cDNA was synthesized from 1 μg of total RNA template.

Reverse Transcription PCR (RT-PCR) was performed to detect the presence of Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Virus G (SPVG) in the samples, utilising specified primers (Table 1). The examination of the DNA sequences matching SPFMV and SPVG viruses will subsequently be performed using GeneBank data and the BLAST program (www.ncbi.nlm.nih.gov).

**Table 1: Primers for RT-PCR analysis for the detection of sweet potato virus**

|  |  |  |  |
| --- | --- | --- | --- |
| Virus type | Oligonucleotide sequence of specified primers | Fragment size (bp) | Annealing temperature (°C) |
| SPFMV | (F) 5'AAGTGTATGCCCAGACAAGG 3' (R) 5' TCTTTCCACTGTACATCGCG 3' | 356 | 52 |
| SPVG | (F) 5' CAATGCCAAATGGAAGAATAG 3' (R) 5' GCATGATCCAATAGAGGTTTTA 3' | 286 | 58 |

**2.5 Acclimatisation and Development of Virus-Free Mother Plant**

Sweet potato plantlets measuring 4 to 5 cm in height and exhibiting root development may commence the acclimatisation process. Plantlets were rinsed under running tap water to remove the agar medium. Seedling trays containing Holland peatmoss were utilised for planting the plantlets and covered with transparent plastic to preserve moisture levels for one week. The plants were maintained under a netted or enclosed structure (to avoid insect vectors) at a temperature of 33±3°C, exposed to sunlight ranging from 15000 to 17000 Lux for a minimum of three weeks, depending upon the variety. Seedlings were subsequently transplanted onto a 16x16 inch polybags and equipped with fertigation system.

**2.6 Statistical Analysis**

An analysis of variance (ANOVA) was conducted using the Statistical Analysis System (SAS) software version 9.4. The experiment was conducted using a completely randomized design (CRD). Mean differences in PGR concentrations in meristem culture media were examined for significance using Duncan's Multiple Range Test (DMRT), with statistical significance set at P < 0.05.

3. results and discussion

**3.1 Meristem Culture**

The study aimed to evaluate the procedure of meristem culture by assessing the effects of single and combined applications of plant growth regulators (PGRs) on shoot initiation and shoot elongation in meristem-derived shoots of two sweet potato varieties, Lembayung and VitAto. (Fig. 1). No contamination was observed in the meristems used for this investigation. In the absence of growth regulators (0 mg/L BAP, GA3, and NAA), no shoot initiation occurred in either variety (Fig. 2). However, a combination of 1 mg/L BAP and 1 mg/L GA3 significantly enhanced shoot initiation, achieving 100% in Lembayung and 70% in VitAto. Notably, there was no significant difference among all media with an additional 1 mg/L BAP for the VitAto variety. For the treatments containing 1 mg/L BAP and 1 mg/L GA3, Lembayung exhibited an average shoot length of 1.88 cm, while VitAto showed slower growth with an average shoot length of only 0.21 cm. These results suggest that the combination of BAP and GA3 promotes shoot initiation and elongation in Lembayung, while its effect on VitAto is minimal.

Additionally, the results indicated that callus formation was significantly stimulated in the meristem of VitAto when grown on media supplemented with plant growth regulators. Alam et al. (2010) reported that the application of TDZ or BAP tends to promote callus formation instead of shoot development, suggesting that these substances are not suitable for meristem culture. In contrast, Masekesa et al. (2016) found that the presence of BAP in the media enhanced shoot production from meristems. The varying responses to the treatments in this study are likely due to the genotypic variation present in sweet potatoes (Alam et al., 2010). The mortality rate of isolated meristems from VitAto (27 to 50%) is significantly higher than that of Lembayung (0 to 40%), regardless of the media used. The reasons for the mortality of isolated meristems could be attributed to injuries sustained during dissection or the toxicity of BAP due to excessive hormone supplementation, which varies with genotype response (Alam et al., 2010; Nur Azimi, 2017).

**A collage of plants in a plastic container

AI-generated content may be incorrect.**

**Fig. 1. The procedure for the production of plantlets derived from meristem culture. (a) Sweet potato tubers were planted to generate sprouts; (b) Young shoots were collected for explant preparation; (c) A close-up view of the meristem region used as the explant; (d) Dissection of the explant under sterile conditions; (e) Initiation of shoot growth on culture media; (f) Development of plantlets in vitro.**

A group of round objects with different shapes

AI-generated content may be incorrect.

**Fig. 2. Responses of meristem cultures from two varieties of sweet potato. Lembayung (1a-1d) and VitAto (1e-1h) were observed after four weeks cultured on MS media supplemented with various PGRs. a & e) Control, b & f) 1 mg/L BAP, c & g) 1 mg/L BAP + 0.01 mg/L NAA, and d & h) 1 mg/L BAP + 1 mg/L GA3.**

**Table 2. The effect of a single or combination of BAP with NAA or GA3 in MS medium on the meristem development of two sweet potato varieties.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Concentration of PGRs (mg/L)** | | | **Shoot Initiation (%)** | | **Shoot Length (cm)** | |
| **BAP** | **GA3** | **NAA** | **Lembayung** | **VitAto** | **Lembayung** | **VitAto** |
| 0 | 0 | 0 | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a | 0 ± 0 a |
| 1 | 0 | 0 | 55 ± 11.41 b | 55 ± 11.41 b | 0.44 ± 0.11 b | 0.14 ± 0.05 b |
| 1 | 0 | 0.01 | 47 ± 10.51 b | 50 ± 11.47 b | 0.48 ± 0.09 b | 0.16 ± 0.05 b |
| 1 | 1 | 0 | 100 ± 0 c | 70 ± 10.51 b | 1.88 ± 0.11 c | 0.21 ± 0.07 b |
| Probability  level | | | \*\* | \*\* | \*\* | \* |

*\** *Note: Values are represented as means ± SE; values represented with the same letter in the same column were not significantly different (P > 0.05), as determined by Duncan’s multiple range test.*

Due to the negative effect of BAP on VitAto, the study was repeated using half the concentration from the previous experiment (Fig. 3). The highest percentage of shoot initiation (100%) was observed in media supplemented with either 0.5 mg/L BAP, 0.5 mg/L GA₃ alone, or a combination of 0.5 mg/L BAP and 0.5 mg/L GA₃. However, there was no significant difference compared to media containing 0.5 mg/L BAP and 0.01 mg/L NAA, which showed a 90% shoot initiation rate in VitAto (Table 3). In contrast, media without any plant growth regulators (PGRs) exhibited a significantly lower initiation rate of only 20%, indicating that external hormonal stimulation is essential (Table 3).

The effect of different plant growth regulator (PGR) combinations on shoot length showed a trend similar to that of shoot initiation. As the concentration of BAP decreased, the shoot length significantly increased, reaching a maximum of 0.56 cm in MS medium supplemented with 0.5 mg/L BAP. This was followed by a length of 0.52 cm in the medium containing 0.5 mg/L BAP and 0.01 mg/L NAA, and 0.48 cm in the medium with 0.5 mg/L BAP and 0.5 mg/L GA3 (Table 3). The lowest shoot length, measuring just 0.04 cm, was observed in the control treatments that did not include any PGRs, highlighting the critical role of PGRs in shoot development within the meristems (Table 3). Additionally, the medium with only GA3 produced the second lowest shoot length at 0.33 cm (Table 3). These results indicate that BAP is the primary factor driving shoot proliferation, while GA3 promotes elongation without further enhancing shoot induction beyond the effects of BAP alone (Sharma, 2018).

Additionally, the mortality rate of isolated meristems of VitAto cultivated on the optimised medium was 0%. In contrast, the medium that was free of PGRs showed approximately 25% mortality. Furthermore, the optimisation results indicated a minimal callus induction of about 17% for VitAto meristems cultured in MS media supplemented with 0.5 mg/L BAP and 0.01 mg/L NAA, as well as in media with 0.5 mg/L BAP and 0.5 mg/L GA3.

Several previous studies have determined optimal combinations of different concentrations of PGRs, such as BAP, NAA and GA3 that significantly enhance shoot induction and multiplication rates in various sweet potato cultivars. Wondimu et al. (2012) discovered that various sweet potato cultivars require varied levels of BAP. The study indicated that meristem from the cultivars Bellela (100%) and Temesgan (100%) exhibited optimal shoot initiation when cultured on MS media augmented with 5 mg/L BAP, 0.01 mg/L NAA, and 1 mg/L GA3, whereas the cultivars LO-323 ( 90%) and Zapallo (80%) demonstrated superior shoot initiation on MS media supplemented with 2 mg/L BAP, 0.01 mg/L NAA, and 1 mg/L GA3 (Wondimu et al., 2012). For the cultivars Awassa-83 (66%) and Guntute (66%), optimal shoot initiation occurred on MS media enriched with 1 mg/L BAP, 0.01 mg/L NAA, and 1 mg/L GA3. In contrast, for the Awassa local cultivar (63%), the most effective shoot initiation media consisted of the same BAP and NAA concentrations, but with an increased GA3 concentration of 2 mg/L (Dugassa and Feyissa, 2011). While Jemal and Feyissa (2020) reported that among the four sweet potato cultivars studied, Beletech exhibited superior performance, achieving 90% shoot initiation followed by Ogensegen (56.7%) and Koka-12 (70%) when cultured on MS medium supplemented with 1.0 mg/L BAP, 0.01 mg/L NAA and 2.0 mg/L GA3.

A group of petri dishes with labels

AI-generated content may be incorrect.

**Fig. 3. Responses of optimised meristem cultures of VitAto after four weeks of cultivation on MS media supplemented with various PGRs. a) Control, b) 0.5 mg/L BAP, c) 0.5 mg/L BAP + 0.01 mg/L NAA, d) 0.5 mg/L BAP + 0.5 mg/L GA3, e) 0.5 mg/L GA3.**

**Table 3. Effect of single or combination of BAP with NAA or GA3 in MS medium on the shoot development of VitAto’s meristem.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration of PGRs (mg/L)** | | | **Shoot Initiation (%)** | **Shoot Length (cm)** |
| **BAP** | **GA3** | **NAA** |
| 0 | 0 | 0 | 20 ± 9.18 a | 0.04±0.2 a |
| 0.5 | 0 | 0 | 100 ± 0 b | 0.56±0.05 c |
| 0.5 | 0 | 0.01 | 90 ± 6.88 b | 0.52±0.06 c |
| 0.5 | 0.5 | 0 | 100 ± 0 b | 0.48±0.05 c |
| 0 | 0.5 | 0 | 100 ± 0 b | 0.33±0.03 b |
| Probability  level | | | \*\* | \*\* |

*\** *Note: Values are represented as means ± SE; values represented with the same letter in the same column were not significantly different (P > 0.05), as determined by Duncan’s multiple range test.*

**3.2 Shoot and Root Regeneration**

After four weeks of culturing meristems on a shoot initiation medium, the shoots were moved to regeneration media based on MS medium. For Lembayung, there were three treatment types: a control medium (free of PGRs), a medium containing 0.5 mg/L BAP, and a medium with a combination of 0.5 mg/L BAP and 0.5 mg/L Kn (Table 4). While for VitAto, four treatment types were used: a control medium (free of PGRs) and media with various combinations of Kn and GA3 (Table 5). BAP was not utilised for VitAto due to the sensitivity observed in previous studies.

The supplementation of BAP and Kinetin had a minimal effect on the shoot length of Lembayung. However, there was a significant increase in the number of shoots (Table 4). Despite this, no meaningful differences were observed between the various treatments for either parameter. Additionally, the application of PGRs in the regeneration media significantly inhibited root formation (Table 4). The best root development for Lembayung occurred when cultured in a medium that was free of PGRs.

**Table 4. Effect of single or combination of BAP with Kn in MS medium on shoot and root development of meristem-derived shoots in Lembayung.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Concentration of PGRs (mg/L)** | | **Shoot Number** | **Shoot Length (cm)** | **Root Number** | **Root Length (cm)** |
| **BAP** | **Kn** |
| 0 | 0 | 1.42 ± 0.15 a | 1.88 ± 0.22 a | 1.5 ± 0.29 b | 6.51 ± 1.25 b |
| 0.5 | 0 | 1.83 ± 0.27 a | 1.57 ± 0.29 a | 0 ± 0 a | 0 ± 0 a |
| 0.5 | 0.5 | 2.08 ± 0.26 a | 1.7 ± 0.22 a | 0 ± 0 a | 0 ± 0 a |
| Probability  level | | ns | ns | \*\* | \*\* |

*\** *Note: Values are represented as means ± SE; values represented with the same letter in the same column were not significantly different (P > 0.05), as determined by Duncan’s multiple range test.*

The application of Kinetin and GA3 drastically reduced shoot length in VitAto compared to the medium free of PGRs (Table 5). There was no significant effect on the shoot number within the applied treatments. Similar to Lembayung, application of cytokinin (BAP or Kinetin) suppressed the VitAto root development (Table 5). This result suggests that employing a medium free of PGRs to stimulate the shoot and root development of VitAto. An application of auxin either Indole-3-acetic acid (IAA) or NAA may be necessary to facilitate root development.

Feng et al. (2000) suggested that BAP (Benzylaminopurine) is a crucial external PGR for in vitro cultivation of sweet potato. However, they emphasized that higher concentrations could lead to excessive callus formation. It is recommended to use a low dose of BAP, between 0.5 to 1.0 mg/L, to minimize callus development. In this study, however, both Lembayung and VitAto cultures grown on media treated with PGR resulted in callus induction during the processes of shoot and root regeneration.

**Table 5. Effect of single or combination of Kn with GA3 in MS medium on shoot and root development of meristem-derived shoots in VitAto.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Concentration of PGRs (mg/L)** | | **Shoot Number** | **Shoot Length (cm)** | **Root Number** | **Root Length (cm)** |
| **Kn** | **GA3** |
| 0 | 0 | 1.67 ±0.26 a | 1.72 ±0.23 a | 0.83±0.30 b | 1.98±0.96 b |
| 1 | 0.25 | 1.92± 0.29 a | 1.03±0.22 a | 0.33±0.26 ab | 0.25±0.20 a |
| 2 | 0.375 | 1.5±0.26 a | 0.58±0.08 a | 0±0 a | 0±0 a |
| 3 | 0.5 | 1.75±0.30 a | 0.83 ±0.15 a | 0±0 a | 0±0 a |
| Probability level | | ns | \*\* | \* | \* |

*\** *Note: Values are represented as means ± SE; values represented with the same letter in the same column were not significantly different (P > 0.05), as determined by Duncan’s multiple range test.*

**3.3 Validation of Virus**

Virus screening was performed on two varieties of Malaysian sweet potatoes mother plants derived from tubers. Electrophoresis of PCR for virus detection was analysed based on the presence of bands at 365bp for SPFMV (Fig. 4) and 286bp for SPVG (Fig. 5) in both mother plant and meristem-derived plantlets. VitAto mother plant demonstrated approximately 8.3% single viral infection and 91.7% multiple viral infections, while Lembayung exhibited 75% single infection and 16.7% multiple infection (Table 6). Lembayung plantlets was determined to be 97.2% virus-free through meristem culture, whereas VitAto was assessed to be 84.7% virus-free after the same procedure (Table 6). Not one meristem-generated plantlet tested positive for multiple viruses. The results were comparable to those of Wondimu et al. (2012), who examined the meristem culture of four Ethiopian sweet potato cultivars. They discovered that 75 to 87.5% of the multiple virus infections in these cultivars and only 4% of the plantlets derived from meristem culture were infected by multiple viruses. Daurov et al. (2018) discovered that meristem culture plants of sweet potato conducted in Kazakhstan are entirely free from SPFMV and Sweet Potato Latent Virus (SPLV). Jemal and Feyissa (2020) also found no virus infection in meristem-derived plantlets of different cultivars (Beletech and Koka-12).

Alternative methods for virus elimination in plants include thermotherapy, chemotherapy, and cryotherapy. These approaches are frequently employed alongside other techniques in conjunction to enhance the efficacy of virus elimination (Szabó et al., 2024). A frequently employed technique is thermotherapy (heat treatment). Kiemo et al. (2021) investigated the combination of heat treatment and meristem tip cultivation. Only 50% of virus-infected plants survived the heat treatments, resulting in a complete absence of viruses.

**Table 6. Virus indexing of mother plants and meristem-derived plantlets of two sweet potato cultivars using Polymerase Chain Reaction (PCR).**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variety** | **No. of positive Mother Plants** | **No. of meristem culture group** | **Mother Plants** | | | **Meristem Culture Plantlets** | | | |
| **Single Virus Infection (%)** | | **Multiple Viruses Infection (SPFMV+SPVG)**  **(%)** | **Positive with Single Virus (%)** | | **Positive with Multiple Viruses (SPFMV+SPVG) (%)** | **Free Virus** |
| **SPFMV** | **SPVG** | **SPFMV** | **SPVG** |
| Lembayung | 12 | 35 | 0 | 75 | 16.7 | 0 | 2.8 | 0 | 97.2 |
| VitAto | 12 | 13 | 0 | 8.3 | 91.7 | 0 | 15.3 | 0 | 84.7 |

A screenshot of a computer

AI-generated content may be incorrect.

**Fig. 4. Bands of DNA amplified by PCR of sweet potato feathery mottle virus (SPFMV) (356 bp). Left: L1-L4, Lembayung mother plant; Right: L1-L4, Lembayung meristem culture plantlets; Left: V1-V4, VitAto mother plant; Right: V1-V4, VitAto meristem culture plantlets. +ve, positive control; -ve, negative control; 100bp, DNA marker.**

A screenshot of a computer

AI-generated content may be incorrect.

**Fig. 5. Bands of DNA amplified by PCR of sweet virus G (SPVG) (286 bp). Left: L1-L4, Lembayung mother plant; Right: L1-L4, Lembayung meristem culture plantlets; Left: V1-V4, VitAto mother plant; Right: V1-V4, VitAto meristem culture plantlets. +ve: positive control, -ve: negative control, 100bp: DNA marker.**

Previous investigations have shown that asymptomatic infection of sweet potato plants by viruses is relatively common (Gibson et al., 1997; Tugume et al., 2008; Buko et al., 2024). This phenomenon was also observed in the present experiment, where plants produced from meristem culture that tested positive for Sweet Potato Virus G (SPVG) exhibited asymptomatic characteristics. Such asymptomatic infections may contribute to the prevalence of these viruses in sweet potato crops (Kreuze et al., 2020).

In Malaysia, there are two major sweet potato viruses: Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Virus G (SPVG). While SPVG is a member of the genus Potyvirus, it is distinct from SPFMV (Almeyda et al., 2013). SPFMV is the most prevalent virus affecting sweet potatoes worldwide (Flamarique et al., 2020). It is primarily transmitted by aphids, specifically *Myzus persicae* and *Aphis gossypii*, in a nonpersistent manner (Muimba-Kankolongo, 2018). Infected crop leaves may show chlorotic spots with purplish borders. The visibility of these symptoms on foliage is influenced by cultivar susceptibility, stress levels, growth stages, and strain pathogenicity (Muimba-Kankolongo, 2018). Milgram et al. (1996) reported that infection with SPFMV alone did not significantly reduce yields compared to virus-free controls. However, when sweet potatoes were co-infected with both SPFMV and Sweet Potato Chlorotic Stunt Virus (SPCSV), there was a yield reduction of 50% or more.

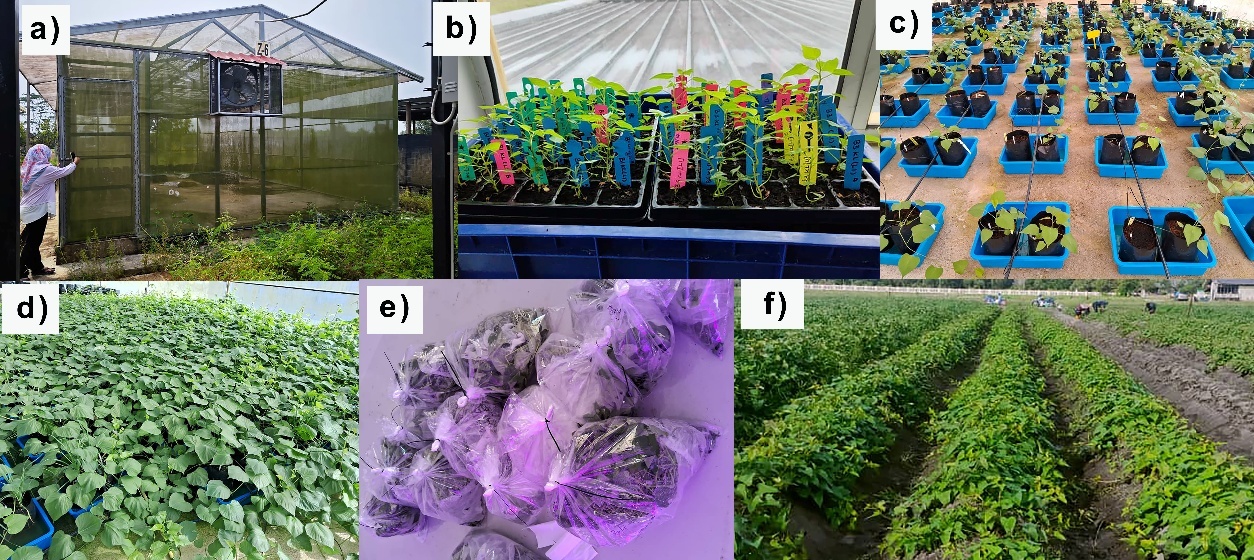
SPVG is the second most common virus affecting sweet potatoes, and it spreads more quickly than SPFMV (National Clean Plant Network, 2023). Initially documented in China (Colinet et al., 1993; Colinet et al., 1994; Loebenstein et al., 2003), SPVG has also been found in the United States, Africa, Egypt, Argentina, and Peru (Colinet et al., 1994; Ishak et al., 2003; Souto et al., 2003; Rännäli et al., 2008; Untiveros et al., 2010). Pardina et al. (2012) described the first complete genomic sequence of SPVG isolated from South America. This virus spreads mechanically and through aphids, *M. persicae* and *A. gossypii*, also in a nonpersistent manner (Souto et al., 2003). Plants that are infected typically display mottled patterns and yellowish spots on their leaves (Loebenstein, 2012). Additionally, SPVG may contribute to a synergistic effect, resulting in increased symptom intensity (Ishak et al., 2003).

The use of insecticides to control virus spreads can lead to acute poisoning and leave toxic residues in food. It is essential to integrate pest and vector management with biological control strategies to help farmers improve their agricultural practices while minimizing health-related environmental risks. Razean Haireen et al. (2023) discovered that growing sweet potatoes alongside chives resulted in a lower average population of aphids and whiteflies compared to sweet potatoes grown without chives. Furthermore, the incidence of viral infections in sweet potatoes was significantly reduced when chives were present, with rates of 27.5% for the chive-treated group compared to 41.2% for the control group. These results strongly support the strategic integration of chives in sweet potato agriculture to improve plant health and resilience.

**3.4** **Acclimatisation and Development of Virus-Free Mother Plant**

The fact that sweet potato viruses are mostly transmitted by insect vectors, meristem-derived plantlets were acclimatized and cultivated inside a netting structure (Fig. 6). The survival rate of acclimatised plantlets is 82% for VitAto and 85% for Lembayung. It is crucial to address the significant challenges that tissue-cultured plants face after transplantation, particularly from soil-borne diseases such as damping-off and Fusarium root rot. Taking preventive measures against these diseases is essential for ensuring the continued success of plant growth and development. Based on our experience, sweet potato seedlings exhibit a higher susceptibility to infection by this disease than other tuber crops such as taro. Notably, taro in vitro seedlings achieved a remarkable 100% success rate when acclimatized with 100% Holland peat moss (Noor Camellia et al., 2022). This may be attributed to the more delicate structure of sweet potato plantlets compared to those of taro. To enhance seedling survival, autoclaved soilless media such as peat moss, sand, and perlite were utilized during the acclimatization process. This approach ensures optimal growth conditions, promoting robust and healthy seedlings.

Plantlets that survived were transferred to polybags filled with cocopeat. Fertigation systems were employed to provide the necessary water and nutrients for the plants (Fig. 6). After two months of planting, these plants were ready to be used as mother plants for cuttings (Fig. 6). In this study, the number of primary branches for the Lembayung variety varied from 3 to 6, while for VitAto, the primary branches ranged from 2 to 8. The lengths of Lembayung branches ranged from 1772 to 3145 cm, while VitAto produced longer branches, measuring between 1021 and 4457 cm, indicating a superior ability for branch growth. Regarding branch diameter, both sweet potato varieties showed no significant difference, with diameters ranging from 0.5 to 0.65 cm. The maximum number of cuttings produced per plant was 108 for Lembayung, while VitAto produced 98 cuttings per plant.



**Fig. 6. Production system for virus-free planting materials of sweet potato. a) A netted structure for planting virus-free plantlets, b) Acclimatized seedlings, c) Plantlets transferred to polybags equipped with a fertigation system, d) Two months old virus-free mother plant, e) Cuttings from the mother plant packed for transportation to the field, f) One month old sweet potato plants grown from virus-free cuttings.**

4. Conclusion

Meristem culture is an effective and reliable technique for propagating and improving sweet potatoes. This method utilizes the apical meristem, allowing for the production of disease-free, genetically stable, and high-quality planting materials. It addresses viral infections, which are one of the primary obstacles to sweet potato cultivation. Additionally, the ability to rapidly multiply elite varieties ensure a consistent supply of healthy plantlets, increasing agricultural output and contributing to food security in Malaysia.

References

[1] Alam, I., Sharmin, S. A., Naher, M. K., Alam, M. J., Anisuzzaman, M., & Alam, M. F. (2012). Elimination and detection of viruses in meristem-derived plantlets of sweetpotato as a low-cost option toward commercialization. 3 Biotech, 3(2): 153–164.

[2] Buko, D. H. (2019). Sweet Potato Virus in Ethiopia - Detection, Characterization, Elimination and Management. Philosophiae Doctor (PhD) Thesis, Norwegian University of Life Sciences:1-190

[3] Razean Haireen M. R., Siti Noor Aishikin A. H., Nur Zainih J. J., Rawaida R., Norma H., Nurul Afza K., Faizah S. A. R., Mohd Nazri B., Anuar A., Mohd Aziz R., Izyani R., & Nurul Ain A. (2023). Management of Sweet Potato Virus Disease Using Prophylactic Measure Strategy. Research Advances in Microbiology and Biotechnology, 7: 121–132.

[4] Loebenstein, G. (2015). Control of sweet potato virus diseases. Advances in Virus Research, 91:33–45.

[5] Krishna, R., Ansari, W. A., Khandagale, K., Benke, A. P., Soumia, P. S., Manjunathagowda, D. C., Gawande, S. J., Ade, A. B., Mokat, D. N., Singh, M. (2022). Meristem culture: a potential technique for in vitro virus-free plants production in vegetatively propagated crops. Advances in Plant Tissue Culture. Elsevier: 325–343.

[6] Wondimu, T., Feyissa, T., Bedadav, G. (2012). Meristem culture of selected sweet potato (*Ipomoea batatas* L.Lam.) cultivars to produce virus-free planting material, Journal of Horticultural Science and Biotechnology, 87(3): 255-260.

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

[8] Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology, 15: 473-497

[9] Masekesa, R. T., Gasura, E., Matikiti, A., Kujeke, G., Ngadze, E., Icishahayo, D., Robertson, A. (2016). Effect of BAP, NAA and GA3, either alone or in combination, on meristem culture and plantlet establishment in sweet potato (CV BRONDAL). African Journal of Food, Agriculture, Nutrition and Development, 16(1): 10653–10669.

[10] Nur Azimi, M. A. (2017). The Effect of BAP and NAA Treatment on Micropropagation of *Cucumis sativus*.L International Journal of Science and Research, 6(11): 170-176.

[11] Sharma,H. (2018). Use of BAP in tissue culture of medicinal plants - a review. Annals of Biology, 34(3): 270–274.

[12] Dugassa, G. and Feyissa, T. (2011). In vitro production of virus-free sweet potato [*Ipomoea batatas* (L.) Lam] by meristem culture and thermotherapy. SINET Ethiop J. Sci., 34 (1):17-28.

[13] Jemal, N. and Feyissa, T. (2020). Production of Virus Free Sweet Potato (*Ipomoea batatas* (L.) Lam.) through Meristem Culture and Chemotherapy. 17 June 2020, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-35805/v1]

[14] Feng, G., Yifu, G., Pinbo, Z. (2000). Production and deployment of virus-free sweet potato in China. Crop Protection, 19: 105-111.

[15] Daurov, D., Zhapar, K., Daurova, A., Volkov, D., Bakbergenova, M., Tolegenova, D., Shamekova, M., Zhambakin, K. (2018). Production of virus-free sweet potato planting material for the southeast of Kazakhstan. International Journal of Agriculture and Biology, 20: 851–856.

[16] Szabó, L.K., Desiderio, F., Kirilla, Z., Hegedűs, A., Várallyay, E., Preininger, E. (2024). A mini-review on in vitro methods for virus elimination from Prunus sp. fruit trees. Plant Cell Tissue and Organ Culture, 156 (42): 1-11.

[17] Kiemo, F. W., Salamon, P., Jewehan, A., Toth, Z., Szabo, Z. (2021). Detection and elimination of viruses infecting sweet potatoes in Hungary. Plant Pathology, 71: 1001–1009.

[18] Gibson, R., Mwanga, R., Kasule, S., Mpembe, I., Carey, E. (1997). Apparent absence of viruses in most symptomless field‐grown sweet potato in Uganda. Annals of Applied Biology. 130(3): 481490.

[19] Tugume, A., Mukasa, S., Valkonen, J. (2008). Natural wild hosts of Sweet potato feathery mottle virus show spatial differences in virus incidence and virus-like diseases in Uganda. Phytopathology. 98(6): 640-652.

[20] Buko, D. H., Spetz, C., Hvoslef-Eide, T. (2024). Detection of Viruses and Elimination of Sweet Potato Feathery Mottle Virus in High-Yielding Varieties of Sweet Potato (*Ipomoea batatas*) from Ethiopia. Agriculture, 14(11): 19-29.

[21] Kreuze, J. F., Perez, A., Gargurevich, M. G., Cuellar, W. J. (2020). Badnaviruses of sweet potato: symptomless coinhabitants on a global scale. Frontiers in Plant Science, 11(313): 1-13.

[22] Almeyda, C. V., Abad, J. A., Pesic-VanEsbroeck, Z. (2013). First Report of Sweet potato virus G and Sweet potato virus 2 infecting Sweet potato in North Carolina. Plant Disease, 97(11): 1516-1516.

[23] Flamarique, S. S., Perez, A. V., Malavera, A. P., Martino, J., Di Feo, L. (2020). Interaction between potyvirus and crinivirus in sweet potato. Pesquisa Agropecuária Tropical, 50(4): 1-9.

[24] Muimba-Kankolongo, A. (2018). Root and Tuber Crops. In: Food Crop Production by Smallholder Farmers in Southern Africa: Challenges and Opportunities for Improvement. Chapter 9: 123–172. Amsterdam: Elsevier.

[25] Milgram, M., Cohen, J., and Loebenstein, G. (1996). Effects of sweet potato feathery mottle virus and sweet potato sunken vein virus on sweet potato yields and rate of reinfection on virus-free planting material in Israel. Phytoparasitica, 24: 189–193.

[26] National Clean Plant Network. (2023). Sweet Potato Virus. https://www.nationalcleanplantnetwork.org/sweetpotatovirus.

[27] Colinet, D., Kummert, J., Lepoivre, P., Semal, J. (1993). Identification of distinct potyviruses rn mixedly-infected sweetpotato by the polymerase chain reaction with degenerate primers. Phytopathology, 84: 65 69.

[28] Colinet, D., Kummert, J., Lepoivre, P. (1994). The complete nucleotide sequences of the coat protein cistron and the 3’ non-coding region of a newly-identified potyvirus infecting sweetpotato, as compared to those of sweetpotato feathery mottle virus. Archives of Virology, 139: 327-336.

[29] Loebenstein, G., Fuentes, S., Cohen, J., Salazar, L.F. (2003). Sweet Potato. In: Loebenstein, G., Thottappilly, G. (eds) Virus and Virus-like Diseases of Major Crops in Developing Countries. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0791-7\_9.

[30] Ishak, J. A., Kreuze, J. F., Johansson, A., Mukasa, S. B., Tairo, F., Abo El-Abbas, F. M. and Valkonen, J. P. T. (2003). Some molecular characteristics of three viruses in SPVD-affected sweet potato plants in Egypt. Archives of Virology, 148: 2449-2460.

[31] Souto, E. R., Sim J., Chen J., Valverde R. A., Clark C. A. (2003). Properties of strains of sweet potato feathery mottle virus and two newly recognized potyviruses infecting sweet potato in the United States. Plant Disease, 87: 1226–1232.

[32] Rännäli, M., Czekaj, V., Jones, R., Fletcher, J., Davis, R., Mu, L., Dwyer, G., Coutts, B., Valkonen, J. P. T. (2008). Molecular genetic characterization of Sweet potato virus G (SPVG) isolates from areas of the Pacific Ocean and southern Africa. Plant Disease, 92: 1313-1320.

[33] Untiveros, M., Quispe, D., Kreuze, J. (2010). Analysis of complete genomic sequences of isolates of the Sweet potato feathery mottle virus strains C and EA: molecular evidence for two distinct potyvirus species and two P1 protein domains. Archives of Virology, 155: 2059-2063.

[34] Pardina, P. E., Bejerman, N., Luque, A. V., Di Feo, L. (2012). Complete nucleotide sequence of an Argentinean isolate of sweet potato virus G. Virus Genes, 45(3): 593-595.

[35] Loebenstein, G. (2012). Viruses in Sweetpotato. In: Loebenstein, G., Lecoq, H. (eds) Advances in Virus Research, 84:325-343. Springer, Dordrecht.

[36] Razean Haireen M. R., Siti Noor Aishikin A. H., Nur Zainih J. J., Rawaida R., Norma H., Nurul Afza K., Faizah S. A. R., Mohd Nazri B., Anuar A., Mohd Aziz R., Izyani R., & Nurul Ain A. (2023). Management of Sweet Potato Virus Disease Using Prophylactic Measure Strategy. Research Advances in Microbiology and Biotechnology, 7: 121–132.

[37] Noor Camellia, N. A. and Abdul Muhaimin A. K. (2022). In vitro micropropagation of two local taro cultivars for large-scale cultivation. Journal of Plant Biotechnology, 49: 124-130.