

## Development of *in vitro* technologies for virus free plant materials through meristem or bud culture for sweet potato (*Ipomoea batatas*)

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### Introduction

virus has commonly infested Sweet potato, this has result in loss of production and income for farmers. Virus free plant materials can result in two-fold yield increase. Hagnin 1998, reported reduction of 20% of root yield due to virus in China. Sweet Potato Research Workshop has been conducted at MARDI on the 8th of August 1988, at the workshop virus threat to sweet potato in Malaysia has been highlighted and the need for clean planting materials has been stressed. Zamora, Paef, and Altoverous (1994) indicated that culturing of isolated meristem *in vitro* could eliminate virus infections in potato. The virus free plants materials can then be increased by culturing node or shoot through *in vitro* technique. The materials can then be provided to farmers to ensure them with high production and income. The specific objective of this research is to develop *in vitro* techniques for producing virus free plant materials for sweet potato. Therefore this research was conducted as follows:

- a. To develop *in vitro* protocol for sweet potato.
- b. To identify plant organs such as meristem, axial bud etc that are virus free.
- c. To study heat therapy treatment for source plant, followed by serological indexing-ELISA (serum development).
- d. To establish *in vitro* technique and protocol such as meristem culture or bud culture for virus free plant production.

### Materials and Methods

The initial step of this study was to develop an *in vitro* protocol for sweet potato, this step was divided into two parts. Part 1 is to develop sterilization technique for sweet potato. Ethanol at the concentration levels of 70-96% and Clorox at the concentration levels of 10-100% and combination of both were used at different exposure time from 2 second to 20 minutes. The second part of this step was to test two different explants types that were the meristem and axillary buds. Plant regeneration system from callus was also developed using different levels of auxins and cytokinins. The auxins used were Naphthaleneacetic acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D), the concentration levels used were 0 (control), 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mgL<sup>-1</sup>. For regeneration from callus cytokinins such as 6-Benzylaminopurine (BAP) and kinetin were used at the concentration levels of 0 (control), 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mgL<sup>-1</sup>.

### Results and Discussion

From this study it was observed that the best sterilization technique was by exposing explants to 96% ethanol solution for 2 seconds followed by 15 minutes exposure in 50% Clorox solution this result in 52% contamination free, however with only 8% survival rate. Two types of explants were used for this study, it was found that the 8% survived explants were mainly axillary bud explants. Meristem explant due to it smaller in size and at the very juvenile stage were unable to survive when exposed to high Clorox solution of 50%. Callus induction from stem explants were achieved by culturing the stem at various concentration of NAA and 2,4-D. The best auxin for callus induction was 2,4-D at the concentration level of 0.25 mgL<sup>-1</sup>. Plant regeneration from callus were also carried out by culturing callus in media containing various concentration of cytokinins such as BAP and kinetin, however, to date no regeneration were observed.

**Conclusions**

From the research conducted sterilization procedure for meristem and bud culture was successfully developed. Callus induction and production from stem explants has been achieved however, regeneration from callus was not successful at the present moment. Future research will focus more on regeneration from callus, and also virus elimination from field explants.

**Benefits from the study**

This study will provide procedures in overcoming virus infections and providing virus free planting materials for sweet potatoes and hence keep the industry viable in the future.

Patent(s), if applicable:none

Stage of Commercialization, if applicable: none

***Graduate Research***

<b>Name of Graduate</b>	<b>Research Topic</b>	<b>Field of Expertise</b>	<b>Degree Awarded</b>	<b>Graduation Year</b>
Md. Rozaidi Md Yusof	Shoot regeneration from callus	Bioindustry	<b>B.S</b>	2002
Juliana Munang	Callus induction	Bioindustry	B.S	2001
Saripah Rehad	Sterilization technique	Agriculture	B.S	1999
Ritah Konolon	Sterilization technique	Agriculture	B.S	1999

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