Effects of Acclimatization on the Ethylene Production of Tissue-Cultured Yam (*Dioscorea alata L.*)

**ABSTRACT**

Acclimatization is an indispensable process for the production of healthy plantlets before their transport, distribution and planting in the field. However, unfavorable conditions caused by biotic or abiotic factors during acclimatization may induce the production of ethylene by the tissue cultured plantlets. To further explore this process, this study was conducted to investigate the influence of different durations of acclimatization in a natural environment on the production of ethylene of tissue-cultured yam (*Dioscorea alata* L.). The study was set out in a Randomized Complete Block Design (RCBD) where one-month-old tissue cultured VU2 yam plantlets in glass bottles were used as test plants and exposed to acclimatization for one day, two days, three days, and four days before transport, distribution, and field transfer. Yam plantlets acclimatized for one day exhibited the lowest ethylene production (8.53 nl g⁻¹h⁻¹) but is not significantly different from the amount of ethylene produced by plantlets acclimatized for two and three days. Plantlets with the lowest ethylene produced showed the highest percentage survival (80.72%) but were not significantly different from plantlets acclimatized for two and three days. Plantlets acclimatized up to four days had the lowest percentage survival (43.22%). Longer acclimatization up to four days enhanced contamination in culture bottles with pathogenic microorganisms. One day acclimatization is found sufficient to increase plant survival and improve growth response of seedlings.

**Keywords:** Yam, acclimatization, post-transport, vibration simulator, tissue culture.

**INTRODUCTION**

Yam (*Dioscorea alata* L.) is used as an ingredient in many sweet desserts as well as food coloring or dye because of its intense purple color. Yam contains reasonably substantial amounts of protein, starch and essential amino acids (Huang et al., 2007). *Dioscorea alata* or *Ubi* is commonly cultivated in the Philippines with an annual production of 26,464 metric tons for the period 2000-2005, and consumption for the same period 5.0% only (BAS, 2006).

Multiplication of yam using tubers as seeds is the most common method; however, it is inefficient and costly. The use of tubers as seeds results in high production cost of about 30% of the total yield. The tubers may also require as much as 63% of the total variable cost incurred per season of cultivation (IITA, 2011). The multiplication survival rate in the field using traditional system is also very low (1:5 to 1:10) and also seed yam tubers affected by pests like nematodes and viruses also make poor result in yield.

To sustain production at farm level, there is a need to improve the production system. Tissue culture or in vitro propagation is used...
as an alternative and efficient method for quick propagation of several plant species (Hazarika, 2006). The in vitro plantlets are characterized by abnormal morphology, anatomy and physiological features brought about by the in vitro condition of low irradiance, low temperature, high humidity and the cultivation media supplemented with carbon as energy source (Pospilova et al., 1999). The in vitro leaves of some plantlets may not develop a waxy cuticle and functional stomata as found in ex vitro plants (Seelve et al., 2003). Consequently, an abrupt transfer of the plantlets to the natural environment and direct exposure to sunlight and temperature might cause wilting of plants (Chandra et al., 2010) and therefore decrease the survival of the plantlets.

There is a need to understand the post-culture handling to increase the quality outturn of the plantlets. During the ex vitro establishment phase, it is observed high mortality rate of the in vitro propagated plantlets. After taking away from culture, plantlets become susceptible to excessive desiccation which incites the water stress and subsequent plant death (Hazarika, 2003).

Initially, plantlets need to be accustomed to the natural condition by a process of acclimatization (Lavanya et al., 2009) before field transfer. Proper acclimatization strategies are useful in developing or modifying the physiological features of the in vitro propagated plantlets making them ready to encounter stress factors during transit, distribution and transplanting in the field. One method of acclimatization is the use of the natural light (Kodym & Zapata-Arias, 1999; Talavera et al., 2005). Light allows the photostimulation of the biosynthesis of compounds necessary for growth (Larcher, 2000). In addition, light enhances structural modifications needed for better plant adaptation to the outside environment (Whatley & Whatley, 1982). The adaptation of plantlet by acclimatization method is an important method for transplantation, relating to survival percentage, growth, and development (Van Huylenbroeck et al., 1998, 2000; Kadlecěk et al., 2001; Fila et al., 2006). However, under unfavorable condition, plant tissues may produce ethylene gas. The presence of ethylene is not always beneficial (Optimal Fresh, 2000) especially when it comes to postharvest shelf life. The presence of ethylene in increased amounts and/or the plantlets' exhibited sensibility to this phytohormone pose an important limitation in in vitro propagation (Levinsh et al., 2000).

Thus, the objective of this study was to evaluate the influence of the duration of acclimatization on the ethylene production and survival of tissue cultured yam plants.

**MATERIALS AND METHODS**

**Experimental Design and Treatments**

The study was conducted at PhilRootCrops Tissue Culture Laboratory and at the Post-harvest Technology Laboratory of the Department of Horticulture, Visayas State University, Baybay, Leyte, Philippines. The experiment was laid out in a Randomized Complete Block Design (RCBD) having the following treatments:

- Acclimatization (day)
  - A0 = 1d
  - A1 = 2d
  - A2 = 3d
  - A3 = 4d

All treatments were replicated three times. Each replicate was represented by one (1) container. Each container had eight (8) plantlets which represented the number of sample plantlets per replicate.

The study was conducted from September 2014 to December 2014.
Preparation of tissue-cultured yam plantlets

The tissue-cultured plantlets of purple yam VU2 variety were obtained from the Philippine Root Crops Tissue Culture Laboratory of the Visayas State University. Plantlets were cultured in glass vessels under laboratory conditions.

Plantlet acclimatization

One-month-old and contamination-free tissue-cultured yam plantlets in glass bottles with culture medium were simultaneously withdrawn from the incubation room and placed in the outside environment for acclimatization at 1, 2, 3, and 4 days. During acclimatization, the plantlets in bottles were placed in an acclimatization chamber made of wood and wire mesh and placed in a partially shaded area. Natural light served as the source of light during the in vivo acclimatization. Each duration of acclimatization was represented by three bottles, each bottle containing eight (8) yam plantlets.

Ethylene (C2H4) gas

Gas samples from the three glass bottles (with 250 ml volume of headspace) for each duration of acclimatization were collected separately using a 5-ml syringe inserted into the top portion of each container; they were then injected and stored in 5-ml vacutainers. Each duration of acclimatization was represented by three (3) glass bottles, each bottle containing eight (8) plantlets. The mouth portion of the vacutainer was minimally soaked in a water-filled container and placed in a chiller to prevent gas seepage. A Shimadzu GC-2014 gas chromatograph with flame ionization detector was used to determine the amount of ethylene produced by plantlets. The rate of ethylene production was calculated using the following formula:

\[
\text{Ethylene Production (nl.g-1h-1)} = \frac{R1 \times Vf}{R2 \times C \times (t)(w)}
\]

Where:
- \(R1\) – ethylene reading for sample
- \(R2\) – ethylene reading for standard
- \(C\) – concentration of standard, ppm
- \(Vf\) – volume of headspace, ml
- \(T\) – time interval, hour
- \(w\) – weight of the commodity, g

Potting out the plantlets

After each analysis of the ethylene gas production, the plantlets were individually potted to plastic cups filled with sterilized growth medium mixture of soil, carbonized rice hull and compost at 1:1:1 ratio. The potted plantlets were placed in a partially shaded in the screen house. For the first two weeks, the plantlets were covered with plastic film to increase the relative humidity. After the plastic film had been removed, the potted plantlets were transferred to a greenhouse; they were subjected to a gradual to full exposure of sunlight and received watering of approximately 100 ml water/pot every other day. Spraying of insecticide was done once a week for one month to prevent the larvae and insects (grasshopper) from attacking the plantlets.

Plant survival

The survival percentage was determined by counting the number of surviving yam plantlets as well as by their developed leaves and roots after one-month acclimatization.

Statistical Analysis

Data were analyzed by using analysis of
variance (ANOVA) R version 3.1.0 statistical software. Where significant difference existed, the mean separation was subjected to Tukey’s HSD (honestly significant difference) test.

RESULTS

Ethylene production. Ethylene production of tissue cultured plantlets during acclimatization may be induced by unfavorable conditions. Plantlets acclimatized for 1 day produced the lowest amount of ethylene gas; however, the amount of ethylene gas produced was not significantly different with that of the plantlets acclimatized for 2 days and 3 days. Plantlets acclimatized for 3 and 4 days produced comparable amounts of ethylene; specifically, plantlets acclimatized for 4 days produced the highest amount of ethylene. The increased rate of ethylene on the fourth day of acclimatization was also attributed to the high contamination of the culture vessels since 45% of the glass bottles were contaminated.

<table>
<thead>
<tr>
<th>Acclimatization (day)</th>
<th>Amount of ethylene (nl g-1h-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d</td>
<td>8.53  b</td>
</tr>
<tr>
<td>2d</td>
<td>10.37 b</td>
</tr>
<tr>
<td>3d</td>
<td>14.71 ab</td>
</tr>
<tr>
<td>4d</td>
<td>17.62 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>46.48</td>
</tr>
</tbody>
</table>

Mean separation by Tukey’s HSD (honestly significant difference) test, 5%.

<table>
<thead>
<tr>
<th>Acclimatization (day)</th>
<th>Survival percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d</td>
<td>80.72 a</td>
</tr>
<tr>
<td>2d</td>
<td>76.04 a</td>
</tr>
<tr>
<td>3d</td>
<td>65.10 ab</td>
</tr>
<tr>
<td>4d</td>
<td>43.22 b</td>
</tr>
<tr>
<td>CV (%)</td>
<td>21.79</td>
</tr>
</tbody>
</table>

Mean separation by Tukey’s HSD (honestly significant difference) test, 5%.

DISCUSSION

Plant tissue culture is extensively used for large scale multiplication, and it has expanded importance in the areas of plant propagation, disease elimination, and plant improvement. In yam production, the use of tissue cultured plantlets as a replacement for the traditional use of tubers as planting materials had gained importance. Tissue culture technique makes use of minute tissue grown inside glass bottles in a laboratory under controlled environment.
During the ex vitro establishment phase, tissue-cultured plants are often associated with high mortality rate due to desiccation as a result of the immediate removal of the plantlets from the culture vessel. Eventually, desiccation increases the development of water stress and subsequent plant death (Hazarika, 2003).

Essentially, an acclimatization step is necessary to increase growth rates and possible survival of in vitro plantlets upon transfer to outside environment. However, during acclimatization, the tissue-cultured plantlets are exposed to various stress conditions which affect the quality and eventually decrease their survival rate. Unfavorable conditions during acclimatization also induce ethylene production. One strategy in plantlet acclimatization is the gradual exposure of the plantlets to light. Light is considered one of the most important factors due to its decisive influence on plant development. It favors the photostimulation of the biosynthesis of compounds necessary for growth (Larcher, 2000). Light also allows structural modifications necessary for improved plant adaptation to the external environment (Whatley & Whatley, 1982). However, the duration of light exposure of plantlets during acclimatization is an important consideration. The results of the study showed that plantlet acclimatization up to four days increased their ethylene production to 17.62 nl g-1h-1. In potato plantlets, ethylene gas at 5nL.L-1 caused severe leaf senescence symptoms (Roustan et al., 1989; Kumar et al., 1998; Wang et al., 2002) including thickened stems and leaves, axillary shoot growth, lack of growth, yellowing and epinasty (Mustafa et al., 2005). The presence of ethylene is not always beneficial, when it comes to postharvest shelf life (Optimal Fresh, 2000). Exposure of the commodity at low concentration of ethylene throughout the marketing period can cause significant harm (Wills et al., 2000). More importantly, the accumulation of ethylene in culture vessels is an important limitation in in vitro propagation (Levinsh et al., 2000). Consequently, acclimatization up to four days also increased the susceptibility of the plantlets to pathogenic microorganisms causing contamination of the cultures. This condition caused by microorganisms like viruses, bacteria, fungi, insects and nematodes also stimulates ethylene production (Abeles et al., 1992) and an increase in ethylene level is frequently observed during the interaction between a host and a pathogen.

Plantlets acclimatized for one day exhibited low ethylene production and showed the highest percentage survival. Results showed that plantlet adaptation during acclimatization is an important adapting strategy in the transplanting process of tissue-cultured plants, relating to survival, growth, and development (Van Huynlenbroeck et al., 1998; Van Huynlenbroeck et al., 2000; Kadlec et al., 2001; Fila et al., 2006).

**CONCLUSION**

Acclimatization for one to two days is sufficient to allow plantlet survival, and this is because of the low ethylene production of the acclimatized plantlets. A longer acclimatization period of four days increases microbial contamination thus also increasing the amount of ethylene in the culture vessels which eventually decreases the quality of the tissue cultured yam plantlets.

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