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The Assessment of Screen House Efficacy as a Tissue Culture Growth Chamber for *Musa* SPP

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Abstract

The increase in food insecurity in developing countries is alarming but the tissue culture technique is expensive to practice. Therefore, this work assessed the efficiency of a screen house as a growth chamber. Suckers were prepared, and cultured into the MS-basal medium which was supplemented with commercial-grade sugar at a concentration of 30 g/l and a BAP concentration of 0.004 g/L. The cultures were kept in an ideal growth chamber with a regulated temperature of 28 °C and a screen house with varied temperatures that range from 37 °C to 48 °C. Explant responses were assessed with the number of shoots, shoot height, shoot health, and number of roots for three and five weeks after initiation. The result showed high significant difference at P ≤ 0.001, in the shoot proliferation at three and five weeks, with mean shoot numbers in the growth chamber of 1.01 at three weeks, and 1.96 at 5 weeks after initiation. The variation observed in plant height, plant health, and root number was not significant. The steady temperature variation observed in the screen house hastened phonological activities, hence, reducing the life cycle of the cultures. Screen house cultures are less viable and require attention during acclimatization and field transfer.

Keywords: Tissue culture; developing country; screen house; growth chamber. *Musa* spp

Introduction

The population growth is overly alarming, with no consequent increase in food production. According to the Food and Agriculture Organization of the United Nations (2017), the world's population will most likely grow to 10 billion by 2050, thereby, boosting agricultural demand. According to the report, investments in agriculture and innovation in technology have a great potential in boosting agricultural productivity, but crop yield has slowed to rates that are too low for comfort. A larger population may lead to a shortage of food (Haiwen, 2019), which may subsequently lead to famine and war. However, satisfying these high demands on agricultural products with existing farming practices will possibly produce no significant effect. To have increased yield in staple crops like cereal, plantain, and banana, there is a need to introduce a fast propagation method that will introduce multiple disease-free plantlets for increased yield.

The tissue culture technique is a technique that can generate a whole plant and multiply it, from a living material as small as a cell or tissue. According to Abdalla et al. (2022), in plant propagation, the tissue culture technique is more finance-driven than the traditional methods of plant propagation. This has in turn increased the cost of tissue-cultured plants and makes them unavailable. However, the relevance of tissue culture technology is high as Abebaw et al. (2021) described it as the most frequently used biotechnology tool, due to its range of applications in plant science that cuts across basic to applied investigation purposes. In scientific studies, tissue culture technique has been used in chemical and hormonal control of regeneration (Skoog and Miller, 1957), basic and applied aspects of organogenesis and somatic embryogenesis

(Komamine et al., 1992), micropropagation and production of virus-free plants (Morel, 1960), production of haploid plants (Guha and Maheshwari, 1964), production of secondary metabolites (Kaul and Staba, 1965), large-scale cell culture in bioreactors (Noguchi et al., 1977), and the transfer of genes during genetic modification and gene editing of crops (Hinchee et al., 1994).

In developing countries, equipped and functional tissue culture laboratories are rare due to the high cost of machinery, consumables, reagents, and inconsistency in power supply. This has impaired the incorporation of tissue culture research into their academic and research institutions. But the population keeps increasing and this calls for the adoption of tissue culture techniques in crop propagation to increase yield. Since tissue culture laboratories are expensive to establish and maintain, there is a need to assess other possible environments like screen houses to determine their applicability as culture growth chambers, since they can be assessed and managed easily.

Therefore, this work aimed at assessing the substitution of tissue culture ideal growth chamber with screen house in tissue culturing of *Musa* spp.

Materials and Methods

Suckers of *Musa* spp. were collected from *Musa* germplasm of Ebonyi State University Abakaliki and taken to the tissue culture laboratory, National Root Crops Research Institute, Umudike. Suckers were trimmed to get the shoot tips that sized 1.0 – 1.5 cm. This block of tissue was surface sterilized by being socked in 70% ethanol for 30 sec. after which they were rinsed in distilled and sterile water. They were then soaked in 8% NaOCL for 5 min and rinsed again, before they were finally soaked in 1.2 g/L $HgCl_2$ for 10 min. After proper rinsing, explants were cultured in media containing 500 mg/L cefotaxime. The last stage of trimming was done to reduce the shoot tips to about 3 × 5 mm which consists of the apical dome, a few leaves primordia, and a thin layer of corm tissue (Strosse et al., 2003). The explants were placed directly on MS-based (Murashige and Skoog, 1962) culture medium contained in reusable Magenta boxes with each containing 50 ml of culture medium. The MS-basal medium was supplemented with commercial-grade sugar (Ganapathi et al., 1995) at a concentration of 30 g/L. Also, a 6-Benzylaminopurine (BAP) concentration of 0.004 g/L was applied as a shooting regulator. The cultures were then kept in varied environments which had different temperatures and light conditions. One of the varied environments was a growth chamber with a regulated temperature of 28 °C, and a light-darkness ratio of 16:8 hours. The second environment was a screen house without a regulated temperature, the temperature ranged from 37 °C to 48 °C, and the light-darkness ratio was in accordance with the one produced by the environment. The research design was a 2*2 factorial experiment in a completely randomized design (CRD). Explants were observed for many dependent variables which include number of shoots, shoot height, shoot health, and number of roots for three and five weeks after initiation. The number of shoots and roots was counted per culture while shoot height was determined using the centimeter calibrated meter rule. Being that the glass is transparent and the culture inside can be fully assessed, the meter rule was placed on the culture glass container and the reading was taken from outside to avoid contamination. Plant health was determined using 5 points scale as specified in Table 1. Each culture was awarded a scale that ranged from 1 to 5 depending on its features.

Table 1: Cultured Plant Health Rating Scale

Scale	State	Features
1	Not healthy	Dead tissue or dying tissue
2	Fairly healthy	Green-coloured shoot with emerging leaves or emerged yellow-coloured leaves.

3	Intermediately healthy	Green-coloured average heightened shoot, few fully emerged narrow leaves with light green colouration.
4	Healthy	Green-coloured heightened shoot with many fully expanded green leaves
5	Very healthy	Green-coloured shoots grew to the top of the culture glass, very many broad green leaves which spread and touched the surfaces of the culture glass

Results

The difference in the shoot proliferation at three and five weeks was very highly significant at $P \leq 0.001$ (Figure 1a). The mean shoot numbers in the growth chamber were 1.01 and 1.96 at both 3 and 5 weeks after initiation, respectively, while for the screen house, mean shoot numbers of 2.56 and 3.46 respectively, were produced at 3 and 5 weeks after initiation. Plant heights across the environments were not significantly different (Figure 1b). Means of 2.98 cm and 4.79 cm for plant heights were produced for the growth chamber at 3 and 5 weeks respectively, while 3.11 cm and 3.82 cm were obtained respectively for the screen house at 3 and 5 weeks after initiation.

Plants' health status increased across weeks for the growth chamber and reversed for the screen house (Figure 1c). Though the difference was not significant, however, in the growth chamber, 2.20 and 2.34 health ratings were produced, at 3 and 5 weeks respectively, after initiation. While screen house produced 2.14 at 3 weeks after initiation and 1.97 at 5 weeks after initiation. A similar trend as in health status was seen in root numbers for their significance and nature of variation across weeks (Figure 1d). Mean numbers of roots in the growth chamber were 1.86 and 2.94 at 3 and 5 weeks after initiation, respectively. For screen house, means of 2.08 and 1.90 were obtained at 3 and 5 weeks after initiation, respectively.

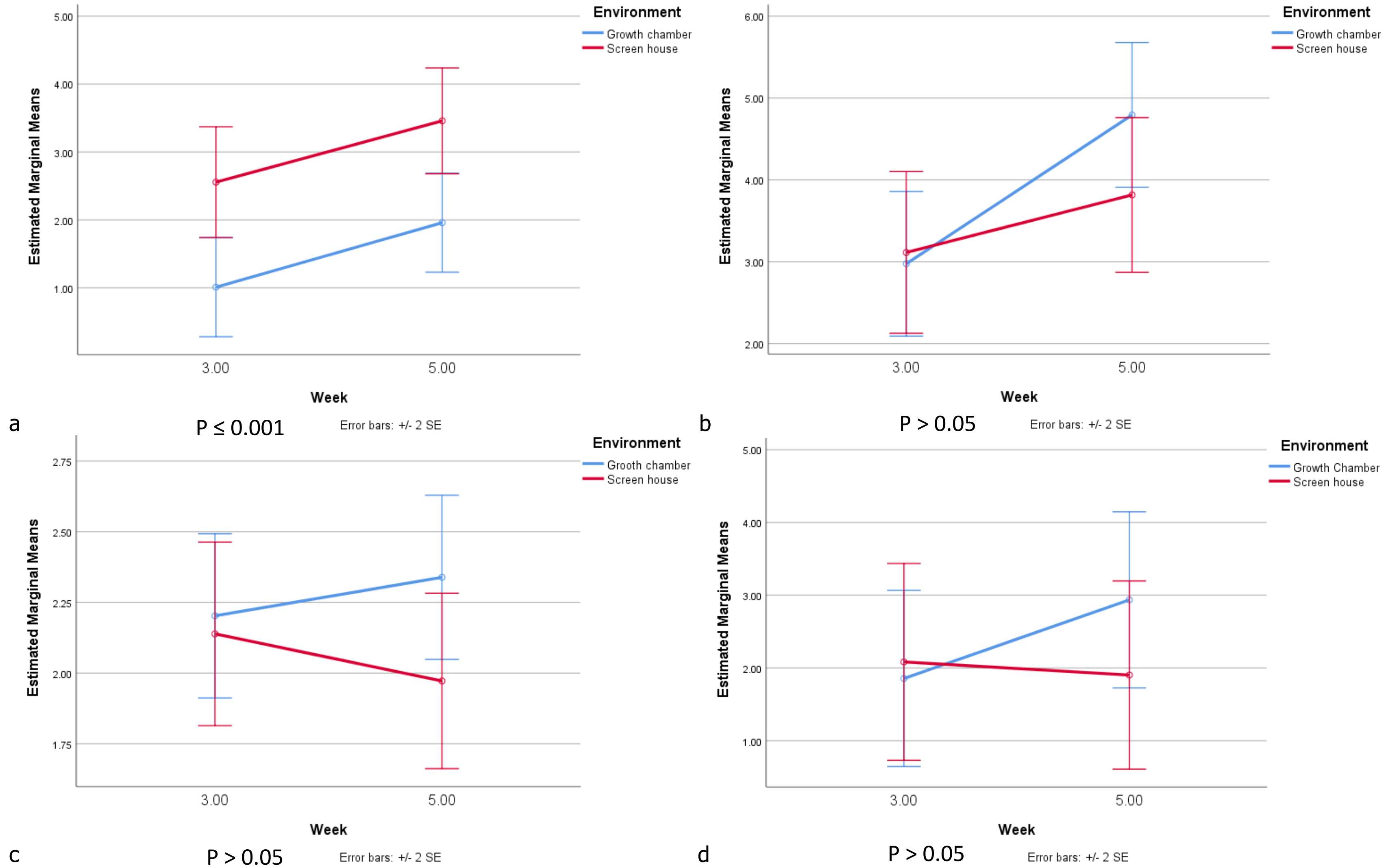

Fig 1. Effect of Culture Environments on *Musa* Micropropagation at 3 and 5 Weeks after Initiation.
Dependent factors: a = shoot number; b = plant height; c = plant health; d = root number. Values were significant at $P \leq 0.05$.

Discussion

The variation obtained from the parameters measured was significant for only the shoot number Figure 1. In the screen house, the temperature varied regularly (37 °C to 48 °C) and this could impose stress on culture which may in turn affect its phenology. According to Hatfield and Prueger (2015), temperature is a basic factor that affects the rate at which plants develop, as warm temperatures of above 30 °C, speed up phenological development. This may have contributed to the high shooting observed for cultures maintained in the screen house environment. Therefore, the screen house favoured shoot proliferation over the growth chamber, though such a high proliferation capacity has not been reported before. The high environmental temperature of the screen house may have increased metabolic activities.

The effect of environment became more evident in different environments used, after 5 weeks of culture initiation. At this level, the number of shoots produced was significantly different across the environments studied (Figure 1a). However, the pronounced plant height recorded for the growth chamber at 5 weeks, although not significant, can be attributed to varying temperatures. Such varying temperature has a dramatic effect on the amount of stem elongation that occurs on a crop (Erwin, 1991). Literature has it that a larger difference between day and nighttime temperatures can induce the production of abscisic acid, which may in turn, enhance stem elongation (Thingnaes et al., 2003)

Conversely, Chiang et al. (2020) reported that in an experiment in which they assessed varied plant performances that were planted in fixed indoor environmental conditions and variable environmental conditions, the varying environmental conditions yielded lower biomass compared to the fixed environmental conditions. The variation in the environment affects plant morphology and photosynthetic capacity (Kaiser et al., 2018).

The high temperature observed in the screen house may have accelerated the photosynthetic rates initially to have resulted in the high response by screen house plants but may have eventually caused damage to the photosynthetic machinery, leading to reduced photosynthetic efficiency and carbon assimilation (Mwambichi, 2023). This resulted in the reduced expression shown after five weeks in the majority of the parameters like health status that were assessed. The smaller number of roots produced in the screen house could be attributed to the higher temperature in the screen house, which may have increased the temperature of the soil in the pot (Chiang et al., 2020), unlike the growth chamber that has a controlled and lower temperature, which may favour anabolic activities.

Additionally, the fewer roots and reduced health status observed for cultures in the screen house at the fifth week could be attributed to the short life cycle of the plants, hence, the death of most of the cultures. According to Taye & Adgo, (2020), warmer temperatures can hasten seed germination and growth rates, which results in shorter crop cycles and increased productivity. The cultures in the screen house had a progressive pattern, whereby the parameters assessed were more expressed in the fifth week, unlike the cultures in the growth chamber. This could be attributed to temperature fluctuations, as it can impact the allocation of resources within plants, thereby affecting biomass partitioning and altering growth patterns (Mwambichi, 2023). Supportively, Pereira et al. (2020), studied the effects of fluctuation in temperature for plant nutrient uptake, assimilation, and utilization. They reported that variations in temperature influenced plant nutrient uptake, nutrient assimilation, and utilization. Hence, affecting their growth and nutritional status.

In summary, as the number of weeks progressed, the features of the cultured plants became more pronounced, and more robust data were collected for analysis. Comparing the features at the weeks' interval, variation in plant height and plant health was not by chance. For both environments, there is a positive relationship between the number of shoots and the number of weeks. The steady temperature variation observed in the screen house hastened phonological activities, hence, reducing the life cycle of the cultures. Therefore, short growth period should be employed while using a screen house as a growth room. Also, cultures from the screen house are less viable, hence, serious care

and attention should be employed during acclimatization and transfer of plantlets to the field.

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