Original Research Article

Acclimatization of Tissue Culture Pineapple Plantlet Using Semi-Autotrophic Hydroponics Technique in Comparison With Other Conventional Substrates

ABSTRACT

Aims: This research is aimed at optimizing the protocol for acclimatization of tissue culture ~~cultured~~ pineapple plantlets. Previously conventional substrates consisting of topsoil mixtures have produced low yield and low survival rate of the tissue culture plantlets. Semi-Autotrophic Hydroponics (SAH) technique is being compared with Sawdust (SD) and Topsoil (TS) as a suitable method of acclimatization and further rooting of the plantlets.

Study design: Experimental Research Design

Place and Duration of Study: The experiment was conducted at the Department of Biotechnology and Tissue Culture, National Horticultural Research Institute, Jericho-Idishin, Ibadan. Feb 2021 – April 2021.

Methodology: The crown of *Ananas ~~comosus~~ u* (pineapple) was extracted and was cultivated in test tubes containing full MS media. The plantlets were sub-cultured twice, after which they were taken into the hardening chamber. The plantlets were acclimatized in the sterile substrate - Semi-Autotrophic Hydroponics substrate and other unsterile substrates – Topsoil(TS), Sawdust (SD), Sawdust and Topsoil (3:1) and Sawdust and Topsoil (1:3).

Results: The result shows that the mean difference in Plant Height and Root Length for SAH substrate was significant (*P*=.05). A 100% survival rate was observed for the plantlets grown SAH media as compared to the ones acclimatized on Topsoil and Sawdust combinations. The SAH media also enhanced further rooting of the plantlets, and there was a significant increase in plant height.

Conclusion: In conclusion, SAH media is a very effective media for the hardening and acclimatization of micro-propagated plantlets.

Keywords: (Semi-Autotrophic Hydroponics, Acclimatization, Tissue Culture, Micro-propagation)

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1. INTRODUCTION

Acclimatization is an important step in the micropropagation of plants. During in vitro culture, plantlets grow under special conditions in air-tight vessels, thereby increasing humidity and controlling the temperature, unlike conventional culture. (Yaya et al., (2015)). The aseptic environment in vitro reduces the stress of pathogenic organisms. Several micro-propagated plants do not survive the transition from in vitro environment to the field due to changes in temperature, humidity, and lightning. *Hazarika B.N. (2003).* The ultimate success of tissue cultured plants on a commercial scale depends is the ability to transfer the clean plantlets from a controlled, aseptic environment to land successfully while maintaining a low cost and high survival rate. During the process of Tissue Culture, plantlets are handled with utmost care in a stable and well-controlled atmosphere. The culture media serves as the nutrient source for the growing plantlet. The temperature, light, and humidity of the laboratory are also controlled to suit the need of the plantlet.

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The transfer of tissue culture plantlets from the lab to soil usually leads to them being exposed to abiotic stresses, like altered temperature, light intensity, and humidity conditions, and biotic stresses, like soil microflora. The transfer of tissue culture plantlets from laboratory to soil needs to be slow and stepwise. This process is known as ACCLIMATISATION. Acclimatization is the adaptation of organisms to a new environment.

1.1. Problem Statement

Various unsterilized substrates have been tried in the past for the acclimatization of micro propagated plantlets, leading to poor survival rates and poor yield. Ubalua and Okorafor (2013) reported a 58% survival rate for sweet potato plantlets grown on unsterilized substrates. There is, therefore, a need to develop a low cost easily accessible technology that would produce clean, virus-free, sterile plantlets in large quantity within a short period, hence the introduction of the Semi-Autotrophic Hydroponics Technology

Semi-Autotrophic Hydroponics (SAH) is a low-cost novel technology, licensed and rapid alternative method for the acclimatization of tissue culture cultivated plantlet and successful transfer to the field. (Pelemo O. 2019).

2. MATERIAL AND METHODS

The experiment was carried out at the tissue culture laboratory of the National Horticultural Research Institute, Ibadan, Nigeria. Pineapple crowns were obtained from the research field of the National Horticultural Research Institute. The crowns were sterilized with 50% Clorox (Sodium Hypochlorite 5.2%, (15mins)), 20% Clorox (10mins), and rinsed with sterile distilled water. Sterile crowns were cultured in the prepared medium which comprised of Murashige and Skoog (MS) (1962), supplemented with ~~macro and microelements~~, vitamins (Nitsch and Nitsch, 1965), 3% sucrose, and 0.1 g/L Myo-inositol. Cultures were incubated at 25 ± 2^{o}C for four weeks for shooting and rooting induction.

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Comment [M3]: MS medium includes of macro and micro elements

2.1. Tissue Culture Media Composition

Full MS (Murashige and Skoog) Media
Vitamins
30% Sucrose
0.1g/L Myo-inositol
0.02g/L Cysteine
Hormones

Comment [M4]: What?

2.2. Hardening and Acclimatization in SAH Media

Clean, mature virus-free plantlets were transferred into SAH (Semi-Autotrophic Hydroponics) Media for acclimatization to take place. SAH nutrient solution is added fortnightly to enhance growth. 15 micro-propagated plantlets were used per SAH container.

2.2.1. SAH Media Composition

SAH substrate consists of Nitrogen (N), Phosphorus (P), and Potassium (K)

- **Nutrient Solution A:** 35.4g of Solution Calcium Nitrate in 15L of distilled water

- **Nutrient Solution B:**

14.7g of Magnesium Sulphate
4.08g of Potassium Monophosphate
15.5g of Potassium Nitrate
0.02g of Ferrous Sulphate
Makeup to 15L with distilled water

- **Nutrient Solution C:**

Mix 500ml of Solution A and 500ml of Solution B + 2litres of water to form Solution C
Note that Solution B must be kept in a black keg or container to prevent the oxidation of iron compounds.

2.2.2. Hardening and Acclimatization in Saw Dust and Soil Mixtures

The micro-propagated plantain plantlets were subjected to primary hardening treatment using different formulated substrates i.e. Sawdust only; sawdust + top-soil (1:3) ratio; sawdust + top-soil (3:1) ratio; and topsoil only. Ten micro-propagated plantain plantlets were used per treatment in a round tray placed in a humidity chamber with a relative humidity of 75% and a temperature of 35^{o}C.

Comment [M5]: Plantain?

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3. RESULTS AND DISCUSSION

There was a significant effect of substrate on root length, plantlet height, and leaf number (*p<0.05).* for the acclimatization period (Table 1). Mean values for all substrate combinations were illustrated in figure 1.
There was a significant increase in Plant Height for the substrate SAH, as against other substrates *(p<0.05).* The plantlets in SAH, SD, and SD: TS 1:3 showed a significant increase in root length, with SAH having the highest value, followed by SD. The plantlets in SAH also had a 100% survival rate as against SD: TD 3:1 which had a 50% survival. It was demonstrated that pineapple acclimatization was the most efficient in Semi Autotropic Hydroponics media because the growth yield increased significantly compared to the conventional method in sawdust and topsoil under the same environmental conditions. These findings are in agreement with the findings of Ariadne et al (2015) who agreed that the use of SAH with in vitro rooted plants increased efficiency in the 'transfer from culture' process because it improved the survival percentage and facilitated management of them.

Table 1: Showing Least Squares Means for the plant growth parameters

SUBSTRATE	PLANT HEIGHT		NO OF LEAVES		ROOT LENGTH	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
SAH	5.33 ± 0.3*	7.5 ± 0.3*	6.67 ± 0.6	8.83 ± 0.6	1.15 ± 0.1*	1.38 ± 0.1*
SD	4.33 ± 0.3	5.15 ± 0.3	6.33 ± 0.6	7.83 ± 0.6	0.82 ± 0.1*	0.88 ± 0.1*
TS	4.45 ± 0.3	5.30 ± 0.3	7.00 ± 0.6	7.5 ± 0.6	0.33 ± 0.1	0.61 ± 0.1
SD:TS 1:3	3.50 ± 0.3	3.72 ± 0.4	5.00 ± 0.6	6.33 ± 0.7	0.38 ± 0.1*	0.42 ± 0.1*
SD:TS 3:1	4.42 ± 0.3	5.05 ± 0.3	7.50 ± 0.6	7.83 ± 0.6	0.37 ± 0.1	0.42 ± 0.1

Note: Mean ± S.D. with the asterisks shows population means which are significantly different. Mean separation done at p<0.05
Mean ± S.D = Mean values ± Standard deviation of means of five experiments

LSD for plant height: 1.412
LSD for no leaves: 2.652
LSD for Root length: 0.9864

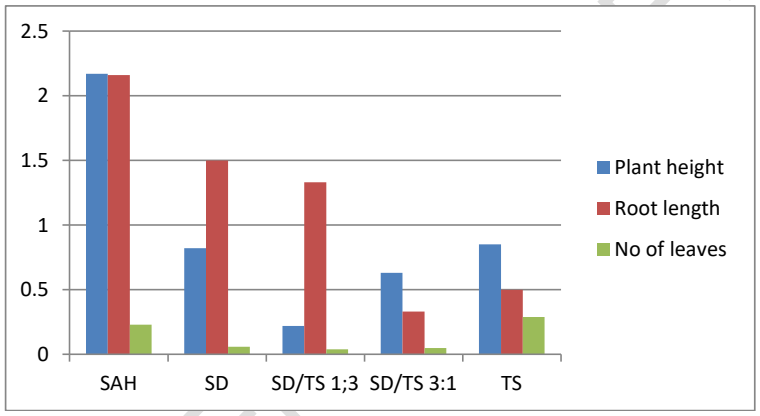

Figure 1: showing the mean difference between the growth parameters

*Mean difference at * P < 0.05.*

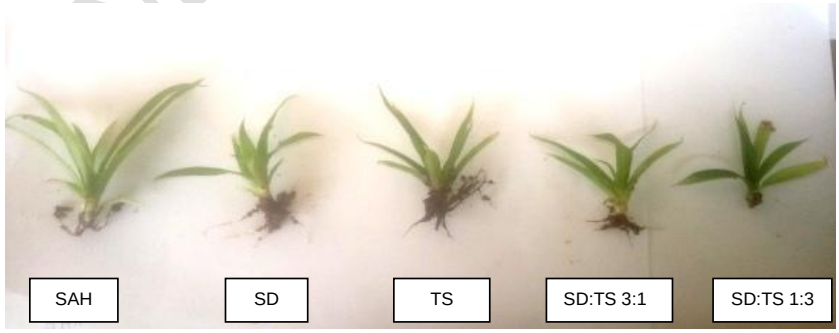

Fig 2: Showing the final plantlets after 15 days of hardening in various media.
SAH: Semi autotrophic hydroponics, SD: Sawdust alone, TS: Topsoil alone

Fig 3: (a) Tissue Culture pineapple plantlets (b) SAH culture pineapple plantlets

4. CONCLUSION

The substrate Semi Autotrophic Hydroponics (SAH) presents the best condition for the acclimatization and growth of the tissue culture pineapple seedlings.

Comment [M7]: Seedling?

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

5. REFERENCES

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