Original Research Article

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

ABSTRACT

Aims: 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. This research is aimed at optimizing the protocol for acclimatization of tissue culture pineapple plantlets.

Study design: Experimental Research Design

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

Methodology: The technique employed was a Randomized Complete Block Design (RCBD) with 10 samples per treatment in 6 replicates. 5 treatments were investigated in this research. Data analysis was done with the use of SAS statistical analysis software ($p<0.05$). Fresh crowns of *Ananas comosus* (pineapple) were extracted and were 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 Semi-Autotrophic Hydroponics substrate and other substrates – Topsoil(TS), Sawdust(SD), Sawdust + Topsoil (3:1), and Sawdust + Topsoil (1:3).

Results: The result shows that the mean difference in Plant Height and Root Length for SAH substrate was significant as against other substrates used in this study *($p<0.05$)* with SAH having the highest value of 3.50cm for plant height and 2.53cm for root length. A 100% survival rate was observed for the plantlets grown SAH media as compared to the ones acclimatized on Topsoil and Sawdust combinations which showed a 50% survival rate. There was no significant difference in the number of leaves among all the groups of substrates.

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

Keywords: (Acclimatization, Micropropagation, Semi-Autotrophic Hydroponics, Tissue Culture.)

1. INTRODUCTION

Acclimatization is an important step in the micropropagation of plants. It is the process of adapting a plant or organism to its new environment. During in vitro culture, plantlets grow under special conditions in air-tight vessels, thereby increasing humidity and controlling the temperature, unlike conventional culture (1). The aseptic environment in vitro reduces the stress of pathogenic organisms. Several micropropagated plants do not survive the transition from in vitro environment to the field due to changes in temperature, humidity, and lightning (2). The ultimate success of tissue cultured plants on a commercial scale depends on the ability to transfer the clean plantlets from a controlled, aseptic environment to land successfully while maintaining a low cost and high survival rate (3).

The process of tissue culture can be summarized in four stages; initiation, multiplication, rooting, and acclimatization (4, 5). The initiation stage involves explants selection, followed by surface sterilization. The sterilized plants are then transferred to a nutrient medium under a sterile environment to establish the plant in culture (6). The next stage is multiplication; this involves the proliferation and multiplication of shoots. This stage is normally repeated through a process known as sub-culturing until the desired numbers of plantlets are achieved. The rooting stage prepares the propagated plants for planting out into the soil. The plantlets are often transferred to a rooting medium which serves the purpose of inducing root formation (6). The final stage in the tissue culture process is acclimatization. This is where the plants are transferred from the culture medium to a substrate and acclimatized to external conditions. The plants are kept in a shade or greenhouse where they become adapted to the external environment by modifications to leaf morphology and anatomy (7, 8).

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, humidity conditions, and biotic stresses, like soil microflora. The transfer of tissue culture plantlets from laboratory to soil needs to be slow and stepwise. Acclimatization is the adaptation of organisms to a new environment (9). The substrates used in the acclimatization process are quite important as it determines the survival and overall outcome of the plants. The aim of this research, therefore, is to optimize the protocol for the acclimatization of tissue cultured pineapple plantlets for a higher rate of survival and better growth parameters (23, 28)

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 (28). 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, a licensed and rapid alternative method for the acclimatization of tissue culture cultivated plantlet and successful transfer to the field. (10).

2. MATERIAL AND METHODS

The experimental design adopted was that of Randomized Complete Block Design (RCBD) in a 3x5 factorial scheme, with five substrates (Semi autotrophic hydroponics, sawdust, topsoil, sawdust+topsoil mix (1:3) and sawdust + topsoil mix (3:1), three growth parameters (plant height, number of leaves and root length) with six replicates. The experimental unit consisted of six trays containing ten plantlets each.

The technique employed was a Randomized Complete Block Design (RCBD) with 10 samples per treatment in 6 replicates. 5 treatment parameters were investigated in this research. Data analysis was done with the use of SAS statistical analysis software. ($p<0.05$) (21).

2.1. Plant Material

The experiment was carried out at the Tissue Culture Laboratory of the National Horticultural Research Institute, Ibadan, Nigeria. Freshly harvested pineapple crowns were obtained from the research field of the National Horticultural Research Institute. The outer leaves of the crowns were gently removed, until the inner bud was exposed.

2.2. Sterilization of pineapple explants

The exposed pineapple crowns were washed thouroughly under running water, amd then sterilized with 50% Clorox (Sodium Hypochlorite 3,5 v/v) for 15min and 20% Clorox (Sodium Hypochlorite 3,5 v/v)for 10min. Explants were rinsed thrice with sterile distilled water (15).

2.3. Initiation of sterilized explants

Explants were initiated using Nelson and Somogi medium and reported by Nagata et al (11). This medium contained mineral salts of Murashige and Skoog (1962) medium, (12). Indole-3-butyric acid [IBA] (2.0 mg/l), Napthaleneacetic [NAA] (2.0 mg/l), Benzylaminopurine [BA] (2.5 mg/l), Sucrose (30 g/l), Plant Preservation Mixture [PPM] (1 ml/l) and Phytagel (2.5 g/l). Sterilized pineapple buds were transferred to the MS initiation media under sterile conditions using a Labconco laminar flow hood (15).

2.4. Multiplication

Upon establishment, approximately 6 weeks after initiation, the plantlets were transferred to a multiplication medium in 200 mL glass food jars. The multiplication medium (11) was made up of Murashige and Skoog (1962) medium, IBA (2.0 mg/l), NAA (1.8 mg/l), Kinetin (2.0 mg/l), Sucrose (30 g/l), PPM (1 ml/l) and Phytagel (2.5 g/l). Further sub-culturing was done every 6 weeks to increase plantlet numbers. Mature rooting systems were established after the plantlets were placed on a rooting medium containing mg NAA (16).

2.5. Acclimatization in SAH Substrate

The plantlets were transferred to Semi Autotrophic Hydroponics substrate mix (SAH). The SAH substrate mix (containing sterilized compost peat material) was obtained from Dr. Matsumoto Ryo, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. (10, 14).

Procedure
SAH uses clean virus-free Tissue culture plantlets as planting materials (Figure 1). It consists of sterilized compost peat materials or cocopeat, and formulated nutrient solutions A & B; (see composition below) as a growth medium. Growth medium per box is contained of 500ml of SAH substrate moistened with 250ml of combined solutions A & B. Lid of SAH boxes though provided with 2-4 holes for aeration are kept closed for the first 10 days to reduce transpiration. The temperature requirement for SAH plants is 25±2^{O}C (14).

2.5.1. Composition of SAH Nutrient Solution

Nutrient Solution A: 35.4g of Solution Calcium Nitrate in 15L of distilled water (14).

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 (14).

Nutrient Solution C:
500ml of Solution A and 500ml of Solution B was made up to 2 L of water to form Solution C. Solution B was kept in a black container to prevent the oxidation of iron compounds (14).

2.6. Acclimatization in Saw Dust and Top Soil Mixtures

The micropropagated pineapple 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 micropropagated pineapple plantlets were used per treatment in round trays placed in a humidity chamber with a relative humidity of 75%. (23, 26).

3. RESULTS AND DISCUSSION

There was a significant effect of substrate used on mean root length, mean plantlet height, and mean leaf number ($p<0.05$) during the 15 days acclimatization period (Table 2). Mean values for all treatment combinations were illustrated in Table 2. There was a significant increase in the mean plant height for the SAH substrate, as against other substrates used in this study *($p<0.05$)* with SAH having the highest value of 3.50cm, followed by SD/TS 1:3 with a plant height of 1.98cm.

The plantlets in SAH and TS showed a significant increase in the mean root length, with SAH having the highest value of 2.53cm followed by TS with a value of 1.13cm. There was no statistically significant difference in the root length of SD, SD/TS (1:3), and SD/TS 3:1. There was no significant difference in the number of leaves among all the groups of substrates. The plantlets in SAH also had a 100% survival rate as against SD: TD 3:1 which had a 50% survival (Table 3).

It was demonstrated that pineapple acclimatization was the most efficient in Semi Autotrophic 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 Pelemo et al (10, 14), 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 the plant management. Ogwuche et al also agreed that SAH supplemented with nutrients aids the rapid multiplication of cassava; which is very important in production of cassava planting material.

(29). He also concluded that the success of plantlet acclimatization and field survival depends on the medium used for propagation (29)

An overall increase in the mean plant height, mean root length, and percentage survival indicates better plant growth in harsh conditions. The plant will ultimately be able to better adapt to conditions on the field when transplanted (16, 17, 18)

Tissue culture pineapple plants are in more demand by local and commercial farmers. They are clean and uniform. Acclimatization of tissue culture pineapple plantlets in SAH substrate is the best option to increase the high production of pineapple tissue culture plantlets (19, 20, 21).

Table 1: Analysis of variance summary for plant height, number of leaves, and root length for acclimatized pineapple explants

	PlantHeight (cm)	NoofLeaves (cm)	RootLength (cm)
Substrate (s)	8.79*	2.30ns	3.43**
Reps (R)	1.08ns	2.45ns	0.46ns
Error	0.49	0.92	0.03

Table 2: Interaction effect of substrate on plant height, number of leaves, and root length of acclimatized pineapple explants

Substrate	Plant Height (cm)	No of Leaves (cm)	Root Length (cm)
SAH	3.50a±034	2.17a±0.83	2.53a±0.12
SD	0.82c±0.38	1.50ab±0.22	0.78c±0.06
SD/TS 1:3	1.98b±0.42	1.00ab±0.01	0.800c±0.05
SD/TS 3:1	0.63c±0.18	1.17ab±0.48	0.78c±0.06
TS	0.85c±0.20	0.50b±0.22	1.13b±0.08

Note: Mean with the same letter are not significantly different p<0.05
Mean ± SEM = Mean values ± Standard error of means of five experiments

Table 3: Showing the percentage survival of the pineapple plantlets after acclimatization

Substrate	Survival %
SAH	100
SD	95
SD/TS 1:3	70
SD/TS 3:1	50
TS	50

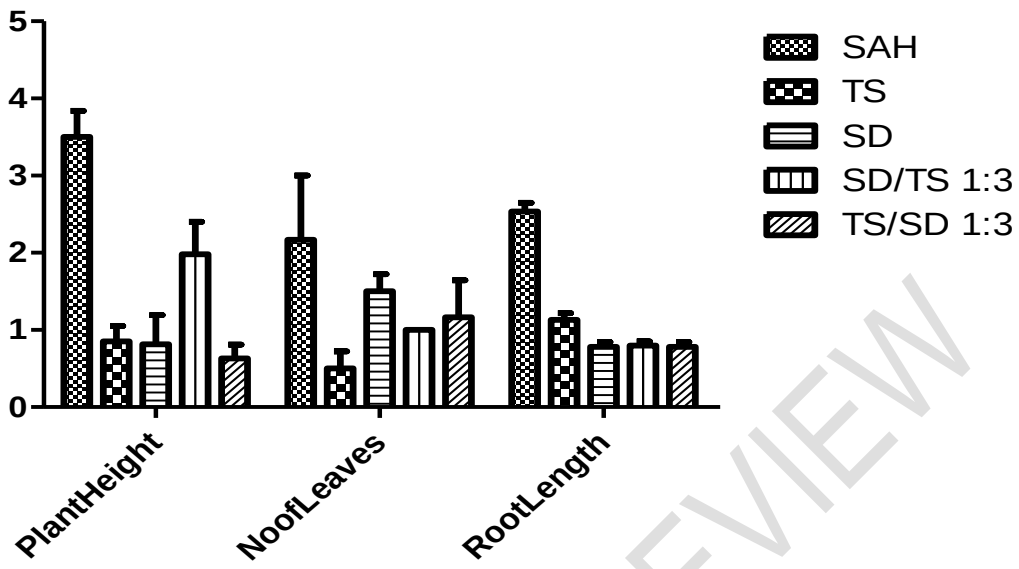

Figure 1: showing the mean difference between the growth parameters (p<0.05)

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 in baby food jar and (B) Tissue culture pineapple plantlets acclimatized in SAH substrate.

4. CONCLUSION

The Semi Autotrophics Hydroponics (SAH) substrate presents the best condition for the acclimatization and growth of the tissue culture pineapple plantlets. This offers a great opportunity to assist developing countries to produce more pineapple plantlets for food security regularly. There is also a need to study local SAH substrates that are easily available in the communities for easy adoption by subsistence and commercial farmers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

5. REFERENCES

1. Yaya, FV., Suh, C. ; Lendzemo, V., Akume, ND. Plantain acclimatization in relation to substrate type. International Journal of Agriculture Innovations and Research. 2015: Vol.3 No.6 pp. 1757-1760 ref.17.
2. Hazarika BN. Acclimatization of tissue cultured plants. Current Science Journal. 2003: 85, 1704-1712.
3. Chandra S, Bandopadhyay R, Kumar V, Chandra R. Acclimatization of tissue cultured plantlets: From laboratory to land. Biotechnology Letters. 2010: 32(9):1199-205

4. Al-SaifAM, Hossain AS, Taha RM. Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro*. African Journal of Biotechnology. 2013: 10: 5291-5295.
5. Davey MR, Butler-Bowdon T, Anthony P. Plant Cell Culture: Essential Methods. John Wiley and Sons, Sussex, UK. 2010.
6. Jackson D, Williams S, Newby D, Hall S, Higgins S, Francis R, and Smith A. Tissue Cultured Versus Traditionally Grown Pineapples: Growth and Nutrient Profile. Journal of Biotechnology Biomater. 2016: Vol 6(3): 237.
7. Zuraida AR, Shahnadz AN, Harteeni A, Roowi S, Radziah CC, et al. A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus* L.) shoots using liquid shake culture system. African Journal of Biotechnology. 2013: 10: 3859-3866.
8. Tassew AA. Evaluation of leaf bud cuttings from different sized crowns for rapid propagation of pineapple (*Ananas comosus* L. [Merr.]). Biology Agriculture and Healthcare. 2014: 4: 1-7.
9. Hazarika BN, Teixeira da Silva JA, Talukdar A. Effective Acclimatization of in Vitro Cultured Plants: Methods, Physiology, and Genetics. Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues. 2006: (Vol. II). Edition: 1. Chapter: 55.
10. Pelemo O, Benjamin G, Adejumobi I, Olusola T, Odom-Kolombia O, Adeosun T, et al. Semi-Autotrophics Hydroponics: A potential seed system technology for reduced breeding cycle and rapid quality seed delivery. 2019; DOI:10.13140/RG.2.2.33676.77449.
11. Nagata I, Lorz H, Widholm JM. Biotechnology in agriculture and forestry. Transgenic crops V. Springer, New York. 2007.
12. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962: 15: 473-479.
13. Usman IS, Abdulmalik MM, Sani LA, Muhammad AN. Development of an efficient protocol for micropropagation of pineapple (*Ananas comosus* L. var. smooth cayenne). African Journal of Biotechnology. 2013: 8: 2053-2056.
14. Pelemo O., de Koeyer D., Matsumoto R., Agre P., Asiedu R., Asfaw A., Semi-Autotrophic Hydroponics: A robust technique for accelerated basic seed yam production. Enhancing yam breeding for increased productivity and improved quality in West Africa (AfricaYam). Out-scaling improved yam varieties and seed technologies for enhanced food security (in view). 2019.
15. Dutta I, Bhadra J, Ghosh P, Saha B, and Datta S. An efficient and cost-effective protocol for in vitro propagation of pineapple. Journal of Ornamental Plants. 2013: 3: 229-234.
16. Mendes PDS, Araújo WF, Antunes F, Chagas EA, Couceiro MA. In vitro cultivation of pineapple seedlings using filters, artificial ventilation, and sucrose. 2015; 9: 202-207.
17. Ab Rahman, NA, Abdul Latif, N, Udin EZ, Awal A, and Shamshia A. In Vitro Regeneration and Acclimatization of Pineapple. (*Ananas comosus L. Merr. Var. MD2*). Journal of Food Research. 2020: 4 (suppl. 5)164-172.
18. Colombo RC, Favetta V, Aparecida da Cruz M, Tadeu de Faria R, Lilian de Carvalho D, and Roberto S. Acclimatization and growth of ornamental pineapple seedlings under organic substrates. Journal of Ornamental Horticulture. 2017: 23(3):257.
19. Ab Rahman Z, Nurl Shahnadz AH, Harteeni A, Sreeramanan S. A novel approach for rapid micropropagation of maspine pineapple (*Ananas comosus L*.) shoots using liquid shake culture system. African journal of biotechnology. 2011: 10(19):3859-3866.

20. Buah JN, Paul AA, and Ransford AJ. In vitro growth and multiplication of pineapple under different duration of sterilization and different concentrations of Benzylaminopurine and sucrose. Journal of Biotechnology. 2015: 14(1): 35-40.
21. Ariadne VG, Yanet S, Ohitza G, Andy D, José GA, Alexandra S. et al. Micropropagation of papaya plants in temporary immersion recipients from axillary shoots. Colombian Journal of Biotechnology. 2015: Vol. XVII No. 1, 70-78.
22. Nitsch JP, Nitsch C. Haploid plants from pollen grains. Science (1969); 163: 85- 87.
23. Eden A. Perez and Cerruti RH. Preparing tissue-cultured banana plantlets for field planting. Biotechnology Unit, Cooperative Extension Service, College of Tropical Agriculture and HR, University of Hawai. 2008.
24. Kone T, Brahima André S, Kpatolo Zana C, Mongomake K, Siaka T, Kone D. Effects of substrates, weight and physiological stage of suckers on massive propagation of plantain (Musa paradisiacal). International journal of research – granthaalayah. 2016: 4(1):1-13
25. Burgel L, Hartung J, Graeff S. Impact of Different Growing Substrates on Growth, Yield and Cannabinoid Content of Two Cannabis sativa L. Genotypes in a Pot Culture. Journal of Horticulturae. 2020: 6(4).
26. Pacual JA, Ceglie FG, Tuzel Y, Tittarelli F, Koller M, Koren A, and Hitchings R. Organic substrate for transplant production in organic nurseries; A review. Journal of Agronomy for Sustainable Development. 2018: 38(3).
27. Carr MKV; The water relations and irrigation requirements of pineapple (*Ananas comosus* var. *comosus*). A review. Exp Agric. 2012: 48: 488-501.
28. Ubalua AO, Okoroafor UE. Micropropagation and postflask management of sweet potato using locally available materials as substrates for hardening. Plant Knowl J.2013:2(2):56-61.
29. Ogwuche, TO, Adesanya TA, Diebiru-Ojo EM, Adetoro NA, Olasupo TK, Kumar PL, at al. Influence of Growth Nutrient and Rooting Hormone on Survival and Growth of Semi-Autotrophic Hydroponics (SAH™) Cassava Plantlets. Review paper; International Institute of Tropical Agriculture. 2020.