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# Hardening Of In -Vitro Propagated Pineapple Plantlets (Ananas Comosus L. Var. Smooth Cayene), To Ex-Vitro Condition, Using Different Substrates In Ibadan, Nigeria.

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***Abstract***
***Background***
*Hardening of fully grown in-vitro propagated plantlets is highly crucial under ex-vitro conditions,to avoid sudden shock,that might lead to high mortality rate and also encourage gradual exposure of plantlets to their natural habitat (ex-vitro). Acclimatization of in-vitro pineapple plantlets is the intermediate stage between the laboratory and the field establishment. This study was undertaken to determine the best substrates (Sterile soil compositions) for the hardening of fully grown in-vitro propagated pineapple plantlets under ex-vitro conditions. Fully grown pineapple plantlets between 3.0 to 3.5cm heights, were subjected to different standard concentrations of top soil, carbonated rice husk, melon husk and river sand respectively. Each treatment with varying standard concentrations were subjected to dry sterilization condition for 1hour under temperature condition of $250^0_{C.}$ Treatment with carbonated rice husk and top soil, with or without river sand gave highestpercentage for survival rate, while treatments with melon husk gavehighest percentage of mortality rate. The best result for plant height, was attained from substrate with carbonated rice husk, top soil and river sand at ratio 1:2:1, while the best result for the highest new leaf formed was recorded in treatment with rice husk and top soil only at ratio 1: 1. The results gotten showed that carbonated rice husk and top soil are good constituents of substrate, that can enhance optimum growth and development of shoot systems via hardening of Ananas comosus ex-vitro.*
*This was achieved using stepwise process and gradual exposure of in-vitro propagated pineapple plantlets to abiotic factors under hardening chamber prior transplanting to the screen house for further growth and development.*

***Keywords****: In-vitro; Ex-vitro; Hardening; Ananas comosus; Acclimatization; Substrates.*

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## I. Introduction

The ultimate success of *in-vitro* produced plantlets depends upon the successful transfer and establishment of plants in *ex vitro* conditions. High loss or damage of *in vitro* raised plants can occurred when transferred to *ex vitro* conditions because of the transfer shock (Pospisilova*et al*., 1999). This is due to the exposure of plants to many new *ex vitro* situations such as low humidity, high level of irradiation, water deficit because of the poor hy- draulic conductivity of the roots and low root-stem connection (Fila *et al*.,1998). Therefore, plants under *in vitro* condition need to be acclimatized by different options. The threats for survival in *ex vitro* can be overcome by acclimatizing the plantlets with gradual reduction of relative humidity temperature, air flow and irradiation level (Pospisilova *et al*., 1999).

The successful establishment of *in vitro* raised plants on the soil and later on field is the major success of *in vitro* propagation (Teixeira *et al*., 2001). Thus, *in vitro* pineapple plantlets need to be acclimatized carefully (Ayelign *et al*.,2013).

The hardening period varies from 3-4 weeks under the acclimatization chamber, until the plants reach an appropriate size (5 –10cm height) for screen house transferand(20 – 30cm height) before been transplanted to the field. The losses during acclimatization and the lengthy period are the major limitations of a widespread use of micropropagated pineapple plantlets. To acclimatize *in vitro* plants, various approaches are employed by different researchers towards successful establishment of *in vitro* plants under *ex vitro* condition(Davis *et al*., 2008) For instance, substrates and the type of pots used during acclimatization are crucial factors determining the survival rate and performance of plants under *ex vitro* conditions (Vasane and Kothari, 2006).

Pineapple (*Ananas comosus* L.) is one of the most popular and delicious triploid fruit. It is esteemed for its pronounced flavor and nutritive elements. Pineapple is propagated vegetatively through suckers, slips or crowns(Eeckenbrugge and Leal, 2003). However, these planting materials have their limitations including transmission of diseases, less uniformity and inadequacy for commercial production.

Existing of severe shortage of planting materials is the key bottle- neck for expanding pineapple cultivation in the country. Efforts have been exerted on conventional pineapple production through suckers, slips and crowns(Firoozabady and Gutterson, 2003). However, the demand and availability of planting materials during planting period are not well synchronized since the planting time in the country relies on the raining season mostly. Hence, *in vitro* culture techniques have been applied worldwide to address these problems(Abebe *et al*.,2003). *In vitro* propagation is a crucial technique for disease free, rapid, uniform and mass production of pineapple plantlets(Teixeira *et al*., 2001).

## II. Materials And Methods

**Grown cultures collection and source**

Fully grown Pineapple plantlets that were rooted, elongated, with uniform height (not less than3-3.5cm) , were selected from the growth room of Plant tissue culture Laboratory , National Centre For Genetic Resources And Biotechnology(NACGRAB) Moor Plantation, Apata, Ibadan, Nigeria (PLATE 1). Hardening and transplanting of the plantlets(*Ananas comosus*) were achieved inside the acclimatization chamber and Screen house of Plant tissue culture Laboratory,(NACGRAB), Moor Plantation (PLATE 2 &3).

Different concentrations of substrates(sterile soil compositions) were prepared, using dried sterilization technique(oven), at $250^0{}_C$ temperature for 3 to 4 hours. Three replicates per treatment(three treatments with controls), were designed for the preparation of the sterile soil media; using coconut fiber, melon husk and rice husk respectively.The treatments are as follow:

**Coconut fiber;** (coconut fibre$^1$+ top soil$^1$, coconut fibre$^1$ + top soil$^2$+ river sand$^1$and coconut fiber only).

**Melon husk;**(melon husk$^1$ + top soil$^1$,melon husk$^1$+ top soil$^2$ + river sand$^1$ and melon husk only).

**Rice husk(carbonated);**( rice husk$^1$+ top soil$^1$, rice husk$^1$+ top soil$^2$ + river sand$^1$and rice husk only).

**Controls**

(i) River sand only and

(ii) Top soil only.

Threemain treatments with controls were used in the experiment, each treatment has three replicates, including the controls.

**Hardening condition and substrates for *ex-vitro* propagation**

All the sterile soil media compositions used were measured, based on different standard concentration respectively. Different proportions of substrates prepared were subjected to dry sterilization technique(oven), at $250^o$cfor4 hours.The sterile soil compositions (substrates) were loaded in metal jarrespectively.Thereafter dried sterilization was achieved, via oven and the sterile media(substrates) were left for 24 hours till the temperature was at the minimal level($0^0$c), prior utilization.

The sterile soil treatments (all the replicates) were mixed with tap water and distributed inside different small white nylons, using big trays. Fully grown plantlets were selected and removed gradually from the vials. The *in-vitro*media were gently washed off from the root of the plantlets and were transplanted to the different standard concentrations of prepared sterile soil media, which were enclosed with big transparent nylons with the addition of some droplets of water and later hanged under the hardening chamber to create high relative humidity condition. White labelling tags were used to document vital information about each treatment and all the replicates. Furthermore, after three to five days of hardening, the nylons were punched with 3 to 6 holes to allow gradual release of the relative humidity condition and exposure of plantlets to abiotic factors(*ex-vitro)* condition.

Subsequently, the plantlets were properly monitored for two to fiveweeks during hardening process and later transplanted to the screen house(weaning), using black polythene bags with only top soil(loamy soil), for further growth and development. These plantlets stayed in the screen house for 4 to 6weeks before it was successfully established on the field.

**Statistical analysis**

The experimental design was a Completely RandomizedDesign (CRD) with five main treatments and three replicates each.The data generated were subjected to analysisof variance(ANOVA) using the statistical software(SPSS).

## III. Results

Hardening of fully grown *in-vitro* propagated pineapple plantlets was done using different compositions Table 1. Results showed thatsubstrate with mixture of carbonated rice husk, top soil and river sand gave the highest Plant height. While the best number of leaf was developed from substrate/ sterile soil composition of rice husk with top soil. This showed that there was significant differences among the treatments ($P \leq 0.05$).

Furthermore, combination of coconut fiber with top soil and river sand gave growth response which was minimal to that of carbonated rice husk. Rice husk(carbonated) and top soil with or without river sand had optimum performance to growth and development *ex-vitro.*

**Table 1**: Response of *in-vitro* propagated *Ananas comosus* ondifferent sterile soil compositions (substrates) under *ex-vitro* condition.

| TREATMENT | PLANT HIEGHT (CM) | NUMBER OF LEAVES |
|---|---|---|
| CF + TS | 5.97b | 10.70b |
| CF + TS +RS | 5.71bc | 8.07cd |
| RH + TS + RS | **6.89a** | 9.33bc |
| RH + TS | 3.59de | **12.28a** |
| MH + TS | 1.41f | 1.23ef |
| MH + TS + RS | 2.71e | 0.79f |
| CF CONTROL | 5.27c | 0.76bc |
| RH CONTROL | 4.23d | 8.29c |
| MH CONTROL | 1.67ef | 3.63e |
| TS CONTROL | 3.93cd | 8.22c |
| RS CONTROL | 3.89 d | 6.50d |

Values are means and standard errors of three replicates. Values with the same letter are not significantly difference at $P \leq 0.05$, using Duncan Multiple Range Test.

CF(coconut fibre), TS( topsoil), RS(river sand), RH(carbonated rice husk), MH(melon husk),

**Plate 1**: Fully grown *in-vitro*Pineapple plantlets(*Ananas comosus*).

**Plate2**: Hardening of*Ananas comosus*, using(Rice husk, Top soil, and River sand), at ratio: 1:2 :1under acclimatization chamber (*ex- vitro* condition).

**Plate3**:Transplanted Pineapple seedlings, inside screen house conditions.

## IV. Discussion

This study showed that different constituents have effects on the hardening of *in-vitro*propagated *Ananascomosus*plantlets ( PLATE 1), via *ex-vitro*condition. Melon husk, coconut fiber and carbonated rice husk were used to enhance soil texture for proper growth and development of *A. comosus*. For treatments augmented with Melon husk, it was observed that at first week of hardening, 80 percent of the acclimatized *Ananas comosus* plantlets had decayed/ rotten via the roots, which led tohighestmortality rate, while other treatments has little or no mortality rate. The loss of plantlets subjected to melon husk treatments can be as a result of some nutritional components present in the melon husk which enhanced easy fermentation with microbes. Melon husk contained appreciable amounts of carbohydrate, protein and minerals, which help the microorganisms to break down some tannins and aid in the secretion of enzyme, that resulted to variations in fermentation of samples, under certain conditions (Ogbe *et al*., 2012).Treatments with coconut fiber had about 40 percent mortality rate, which was better, compared to that of melon husk constituents. The treatments with carbonated rice husk gave the best and highest growth response under hardening condition (PLATE 2). *Ananas comosus* plantlets hardened with rice husk treatmentswere further transplanted to the screen house for further establishment on the field (PLATE 3).

## V. Conclusion

Acclimatization of *in-vitro* propagated pineapple plantlets in *ex-vitro* conditions is a critical step for survival and growth performance of plant (Ayelign*et al*., 2013). Despite the advance protocol development derived for *in-vitro* propagation of plants, there are still limitations in acclimatization protocols, especially concerning plant development via *ex-vitro* (Gonzalez-olmedo *et al*., 2005). The risk of plantlets to become wilt when transferred to *ex-vitro* conditions is high, due to the transfer shock ( Posposilova *et al*., 1999 ).

In this study, experimental protocols were conducted to determine the best substrates or sterile soil compositions for the hardening of pineapple plantlets in *ex-vitro* conditions, especially in south west zone of Nigeria to prevent high mortality rate of *in-vitro* plantlets,due to seasonal variation in climatic factors, prior transplanting and establishment to the field for further utilization. The research findings showed that (substrate components): carbonated rice husk with topsoil and river sand gave best result for optimum growth performance and vigor compared to other substrates components. According to research findings, top soil is a major component of substrate for hardening of propagated *in-vitro*plantlets, but for hardening of *A. comosus* , it was discoveredthat carbonated rice husk, river sand and top soil are good constituents that enhanced high level of nutrients, porosity and texture that aidedin the growth and development of*A. comosus*plantlets under *ex-vitro* condition for high productivity level and reduction in mortality rate.

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