UNIVERSITY OF CAPE COAST

IN VITRO RESPONSE OF FOUR PINEAPPLE (*ANANAS COMOSUS* (L.) MERR.) VARIETIES TO VARYING LEVELS OF PHYTOHORMONES UNDER TWO TEMPERATURE CONDITIONS

JAMES AMOAH NYARKO

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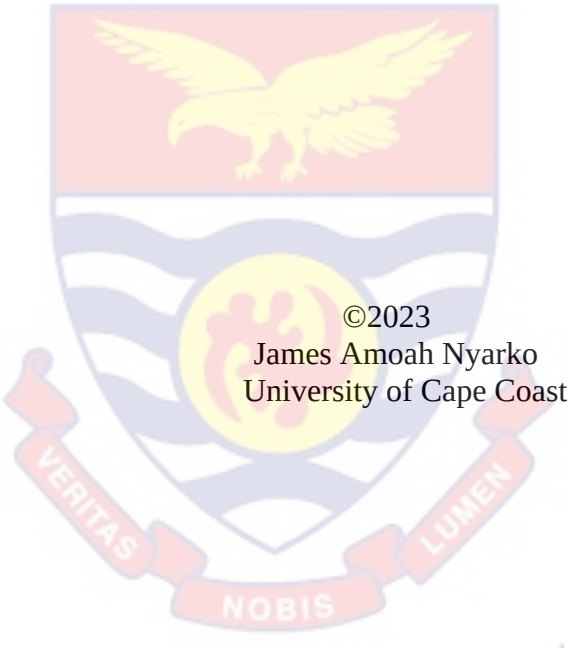

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IN VITRO RESPONSE OF FOUR PINEAPPLE (*ANANAS COMOSUS* (L.) MERR.) VARIETIES TO VARYING LEVELS OF PHYTOHORMONES UNDER TWO TEMPERATURE CONDITIONS

BY

JAMES AMOAH NYARKO

Thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirement for the award of Master of Philosophy degree in Crop Science

JANUARY 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature …… Date ……

Name: James Amoah Nyarko

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature ……..…… Date ….....

Name: Prof. John Nelson Buah

Co-Supervisor's Signature …… Date ……

Name: Dr. Frank Kumi

ABSTRACT

Pineapple is one of the most valuable non-traditional export commodities in Ghana. The production of pineapples in Ghana are extensively engaged by resourced poor smallholder farmers. The advent of *in vitro* propagation of pineapple plantlets is however less exploited. This study was carried to investigate the *in vitro* response of four pineapple varieties on different media and temperature regimes for their initiation, multiplication and rooting. Meristematic buds from the suckers and slips were excised and initiated on six different media formulations, viz., (MS (0), MS + BAP (1.5 mg L^{-1} and 3.0 mg L^{-1}), MS + Kinetin (1.5 mg L^{-1} and 3.0 mg L^{-1}) and IRB505. The Temporary Immersion Bioreactor system (TIBs) together with glass vessels were utilised for culture initiation, multiplication and rooting. A full-strength MS medium supplemented with 3.0 mg L^{-1} BAP and 20 % coconut water was used for culture multiplication. Plantlets were rooted on MS medium supplemented with three different IAA levels, viz., 0.1 mg L^{-1}, 0.5 mg L^{-1} and 1.0 mg L^{-1}. Agar was used as a media solidifier and the pH was adjusted to 5.8. Significant differences were observed in the varietal responses to culture initiation, multiplication and rooting for almost all the parameters measured with Smooth Cayenne being the least responsive to *in vitro* micropropagation. Slips explant significantly ($P < 0.05$) outperformed suckers explant for culture initiation. Cultures at 25 ^{o}C produced the best results over cultures at 19 ^{o}C at all micropropagation stages. Cultures in TIBs recorded the highest multiplication rate of 14 per explant for a cycle and produced better roots. However, the Lateral root score decreased with increasing concentration of the IAA. Rooted plantlets potted in a greenhouse and recorded almost 100 % survival rate on acclimatisation.

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DEDICATION

I dedicate this work to my parents, Mr. Kweku Nyarko and Araba Bosuwa and to my siblings, Jemima, Isaiah, Ruth, Joshua, Obed and Mishael.

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LIST OF EQUATION

LIST OF ABBREVIATONS AND ACRONYMS

2,4-D	2,4-Dichlorophennoxyacetic acid
µmol m^{-2} s^{-1}	Micromoles per metre square per second
2iP	2-isopentenyl adenine
ANOVA	Analysis of variance
B5	Gamborg medium
BAP	6-Benzylaminopurine
Ca	Calcium
$CaCl_2.2H_2O$	Calcium chloride dihydrate
cm	Centimetres
$CoCl_2.6H_2O$	Cobalt (ll) chloride hexahydrate
FAO	Food and Agriculture Organisation
$FeSO_4.7H_2O$	Ferrous sulphate heptahydrate
g	gram
GA_3	Gibberellic acid
g L^{-1}	grams per litre
GLOBALGAP	Global Good Agriculture Practices
GSS	Ghana Statistical Service
H_3BO_3	Boric acid
HCl	Hydrochloric acid
HW	Hellers and White
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IRB505	Micropropagation medium (Iribov West Africa Ltd.)

KH_2PO_4	Potassium dihydrogenphoshphate
KI	Potassium iodide
KIN	Kinetin
KNO_3	Potassium nitrate
L	Litre
LED	Light emission diode
LRS	Lateral root score
LS	Linsmaier and Skoog
LSD	Least significant difference
Mg	Magnesium
mg L^{-1}	milligram per litre
MgSO4	Magnesium sulphate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
ml	millilitre
mmol L^{-1}	Millimole per litre
$MnSO_4.4H_2O$	Manganese (ll) sulphate
MS	Murashige & Skoog
ms^{-1}	metre per second
NA	Not applicable
$Na_2EDTA.2H_2O$	Ethylenediaminetetraacetic acid
NAA	naphthalene acetic acid
$NaMoO_4.2H_2O$	Sodium molybdate
NH_4NO_3	Ammonium nitrate
NN	Nitsch and Nitsch

NOA	Napthoxyacetic acid
^{o}C	Degree Celsius
PB	Paclobutrazol
p-CPA	*para*-chlophenoxyacetic
PCR	Polymerase Chain Reaction
PMWV	Pineapple Mealybug Wilt Virus
RITA®	Bioreactor
Setis	Bioreactor
TIBs	Temporary Immersion Bioreactor system
TZD	Thidiazuron
WAP	Week after planting
$ZnS_4.7H_2O$	Zinc sulphate heptahydrate

CHAPTER ONE

INTRODUCTION

Background of the study

Pineapple (*Ananas comosus*) is one of the most helpful plant species among the Bromeliad. It has a high medicinal rating based on its nutritious fruits. Pineapple is a globally accepted fruit due to its enriched levels of vitamins (A, C & E), minerals and starch-freeness of fruit. The fruit is consumed daily as either fresh or partially processed into jam (Sanewski *et al*., 2018). Pineapple is ranked next to citrus and banana among commercially produced fruit crops (Mohd *et al*., 2020). Global pineapple production has increased from 15 million metric tons to over 18 million (Shahbandeh, 2021). Pineapple production is mainly concentrated in the tropics and countries with similar climatic conditions like Costa Rica (Hossain, 2016).

According to Achaw, (2010), Ghana had been part of the leading pineapple exporters until the introduction of the exotic MD2 variety, which is now the most preferred to the existing varieties; Smooth Cayenne, Sugarloaf and Queen. Currently, all four varieties are under production. Pineapple production in Ghana is categorised into three main groups based on land size for cultivation: small-scaled or out-growers, medium-scaled and large-scaled (Fold *et al*., 2008). The pineapple industry of Ghana, similar to that of Benin, Nigeria and Burkina Faso, is dominated by poor smallholder farmers who cultivate less than two hectares of land (Williams *et al*., 2017). However, smallholder farmers contribute about 50 % of the total export volume; the production is well suited for small-scale farmers for several

reasons including, ease with agronomic practices (Fold *et al*., 2008). Nearly two percent of Ghanaian households cultivate pineapple or are involved in its value chain (GSS, 2008; Wiggins *et al*., 2010).

Tissue culture is one of the most extensively utilised biotechnological tools for agricultural development (Cardoso *et al*., 2018). *In vitro* micropropagation is used extensively to produce disease-free and uniform planting materials (Badou *et al*., 2018). It is one technique that ensures a continuous supply of clean propagules independent of the season effect (Acheampong *et al*., 2015). *In vitro* micropropagation has been used to generate propagules in some fruit crops, such as bananas and other horticultural crops of economic value (Buah, Asare & Arthur 2015; Cardoso *et al*., 2018; Seyoum *et al*., 2021). It is a better approach than conventional plant production, which takes a more extended period of relying on the parent plant to produce offspring. The *In vitro* technique can generate millions of plantlets in a short period when all laid down protocols are followed (Hossain, 2016).

Micropropagation utilises explants of different forms and origins from the parent plant. Explants could either be from a meristematic bud, leaf, shoot tip, node of stem, corm sections, inflorescence, or daughter corm (Kara & Baydar, 2012). To ensure the purity of start-up material, a polymerase chain reaction (PCR) based viral indexing is carried out, translating into the generation of virus-free plantlets (Bhojwani & Dantu, 2013).

The success of micropropagation relies on using ideal phytohormones and other essential elements to help exploit the explant's ability to exhibit inherent

potential that leads to the development of an entire plant. According to Houndédjii & Zandjanakou-Tachin (2016), the choice of explant plays a major role in the success of micropropagation.

Several phytohormones are employed in tissue culture works and notable among them are auxins and cytokinins. Cytokinin promotes cell division (Pessarakli, 2014). Cytokinins are in two broad categories, either endogenous or exogenous. Research has led to the discovery of more active forms of cytokinins that do not occur naturally. According to Badou *et al*. (2018), the inclusion of exogenous cytokinins 6-Benzylaminopurine (BAP) and other synthetic auxin α-Naphthalene acetic acid (NAA) in an *in vitro* culture influences the regeneration and multiplication of pineapple. Similarly, BAP, combined with Indole-3-acetic acid (IAA) in micropropagation, affects the regenerative potential of the Smooth Cayenne pineapple variety used (Kornatskiy, 2020).

Problem statement

Pineapple production in Ghana is confronted with some challenges. There have been several attempts by government and other corporate agencies to address these challenges by introducing several programs (Donkoh & Agboka, 1997; Hotegni *et al*., 2014). Besides marketing and credit-related constraints, diseases and pest infestation and timely access to adequate good quality planting material has also been identified.

Most farmer fields have been found with diseases ranging from severe to fewer devastating ones. Several strains of pineapple mealybug wilt disease (PMWV) and *Phytophthora* have been identified on smallholder farmer fields

(Hotegni *et al*., 2014). These diseases, especially the PMWV, cause tremendous yield loss as it affects the plants' growth and development. The quality of propagules such as slip, sucker and crown from pre-infested farmer fields, possess less vigour due to issues of re-infestation. Access to good quality planting materials is one of the most pressing issues in the Ghanaian pineapple industry (Danso *et al*., 2008) and Nigeria (Adeoye *et al*., 2020). This phenomenon forces most smallholder pineapple farmers in Ghana to buy propagules from other farmers, either within their farming zone or across the border. The propagules that farmers source are of poor quality with high mixtures of off-types (Hotegni *et al.*, 2014). This translates into the production of heterogenous fruit, which does not meet the export and local industrial standard.

There is no structured system that ensure the regular supply of pure and healthy pineapple propagules, as seen in the seed production industry. Many smallholder farmers wait until existing pineapple plantations produce enough propagule before they start successive productions, which limit the potential of many farmers (Sanewski, 2018). Few micropropagation protocols have been developed for pineapple, especially the MD2 and Smooth cayenne. Emphasis has been placed on hormone variance, light and sterilisation techniques (Acheampong *et al*., 2015; Buah *et al*., 2015). Even though temperature variance affects pineapple micropropagation, it has not been fully exploited.

Justification

Pineapple has a higher planting density of about 2400 plantlets per acre. However, it happens to be one of the plants that produces fewer propagules (Kour

et al., 2007). The most common method of propagating pineapple is using vegetative parts, namely suckers, slips and crowns. In many pineapple-producing countries, crowns are the most preferred propagules due to their ability to develop a better rooting system and also support sectioning to increase planting material (Acheampong *et al*., 2015). Nevertheless, sectioning of the crown for more plantlets does not eliminate diseases. There is an increasing demand for pineapple from the export sector (Achaw, 2010; Osei & Aluah, 2021) and locally emerging fruit processing factories.

Micropropagation is the only means that can help generate millions of cleans propagules within the shortest possible time, irrespective of the season. Micropropagation helps to establish commercial farms and expand smallholder farmer fields since the challenge of lack of planting material is eliminated. It also helps in the generation of uniform plantlets. Little scholarly work has been done regarding producing viable pineapple plantlets of all four varieties through *in vitro* culture. The potential of the explant type for these pineapple varieties on *in vitro* initiation culture has not been fully exploited. The conventional pineapple micropropagation relies on solid and liquid cultures in jars which typically makes micropropagation an expensive production technique for commercialisation. Investigating the responses of these varieties to multiplication and rooting in temporary immersion bioreactor system together with the solid cultures will serve as a part of baseline information for potential investors.

General Objective

The study aimed to investigate the *in vitro* performance of different pineapple varieties (MD2, Smooth Cayenne, Sugarloaf and Queen).

Specific Objective(s)

The specific objectives of the study were:

1. To determine the growth of explant types (slip and sucker) under different cultures
2. To evaluate the effect of different temperature regimes (19 ^{o}C and 25 ^{o}C) on the growth and multiplication of pineapple cultures.
3. To determine the regenerative potential of different pineapple varieties under different media formulations (liquid and solid).
4. To determine the rooting characteristics and survival of different pineapple varieties on acclimatisation.

Test of Hypotheses

1. H_0: No significant differences exist in the growth of explant type (slip and sucker) under different cultures.

   H_1: Significant differences exist in the growth of explant type (slip and sucker) under different cultures.

2. H_0: No significant differences exist in the effect of different temperature regimes (19 ^{o}C and 25 ^{o}C) on the growth and multiplication of pineapple cultures.

   H_1: Significant differences exist in the effect of different temperature regimes (19 ^{o}C and 25 ^{o}C) on the growth and multiplication of pineapple cultures.

3. H_0: No significant differences exist in the regenerative potential of different pineapple varieties under different media formulations (liquid and solid).

   H_1: Significant differences exist in the regenerative potential of different pineapple varieties under different media formulations (liquid and solid).

4. H_0: No significant differences exist in the rooting performance and survival of different pineapple varieties on acclimatisation.

   H_1: Significant differences exist in the rooting performance and survival of different pineapple varieties on acclimatisation.

CHAPTER TWO

LITERATURE REVIEW

Importance of pineapple production and production trend

Pineapple is a member of the Bromiliaceae family which offers limitless nutritional and dietary benefits. It serves as a rich source of vitamins and minerals. Pineapple is cultivated commercially and serves as the major export food commodity for many countries (Sanewski *et al*., 2018). The production of pineapple thrives best in the West African sub-region and countries with climatic conditions similar to that of Costa Rica (Achaw, 2010). Pineapple production in Ghana involves many resource-poor smallholder farmers.

The introduction of the MD2 variety from Costa Rica resulted in a decline in Ghana export volumes (Kleemann, 2016). Thus, the MD2 became the export world's favourite. Today, the MD2, Smooth Cayenne, Sugarloaf and Queen are regarded as local pineapple varieties. The pineapple industry continually contributes greatly to Ghana's economic development (Achaw, 2010). The sector serves as the main source of occupation for many farmers and traders involved in its value chain. The government of Ghana made significant contributions to the pineapple industry between 2008 to 2020 which has translated into high production volume (Marie, 1994). However, the decline in the export volumes (Figure 1) is predominantly linked to farmers' inability to meet export (GLOBALGAP) standards. Osei & Aluah (2021) have recommended the empowerment of small-holder pineapple farmers to meet export standards and expansion of the processing industry for pineapple will double up production of the subsector.

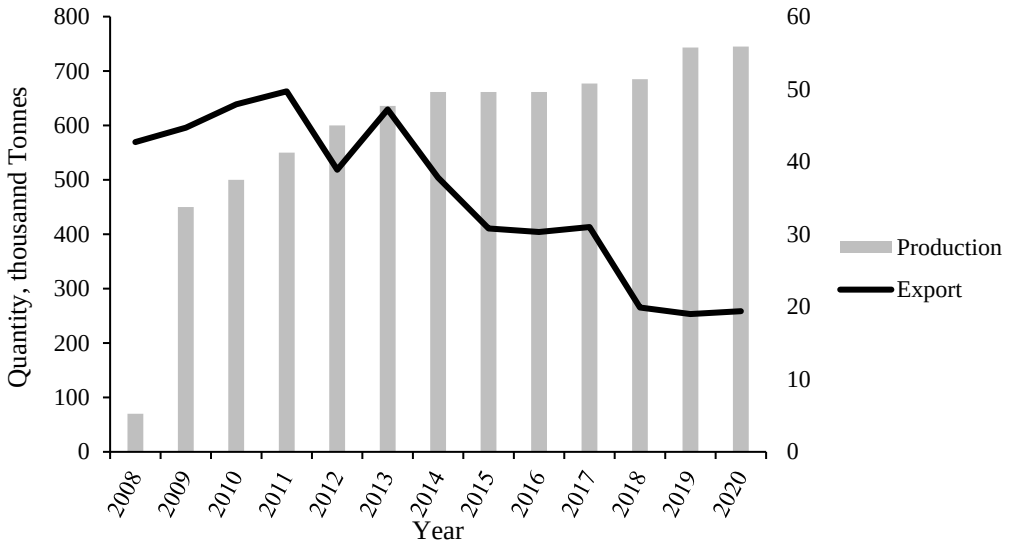

Figure 1: Ghana's pineapple export and production trend
Source: (GEPA, 2021; Osei & Aluah, 2021)

Traditional propagation material for pineapple production

Traditional propagation relies on the few propagules generated by the mother plant. The pineapple plant produces the crown, sucker (side shoots), slips and harpers which serve as new propagules even though their actual and potential regenerative rate of 4 - 5 plants per year is low (Kumar *et al*., 2016; Tegen & Mohammed, 2016). Variations in the size, age, source and form of propagule affect the performance and maturity of the plant on the field.

Suckers arise from the buds in the leaf axil. They are above-ground vegetative material and an average of three are produced per plant. However, sucker production potential is variety-dependent (Kumar *et al*., 2016). Suckers are usually harvested for planting when they are about 35 cm tall (Sanewski et al., 2018).

Ratoon suckers are known to emerge from buds beneath ground level. It is usually characterized by difficulty in planting due to its large size but they produce the highest yield and take a maximum of 18 months to mature (COTVET, 2017).

Crowns are borne on the apex of the fruit. Usually, one crown is produced per fruit however, in some instances fasciation (multiple crowns) does occur. The crown becomes dormant on fruit maturation. Farmers hardly propagate the crown because they are often sold together with the fruit making it unavailable for use as propagule. According to Hotegni *et al*. (2014), the crown has a lower maturation period of almost 24 months. Notwithstanding the maturation defect, the crown produces the most uniform fruits (Kumar *et al*., 2016).

The peduncle just below the base of the fruits hosts the slips. Variations in slip numbers and quality correlates with the plants' health status. The fewer the slips, the higher its quality. However, fruits from slips have an uneven ripening and can take an average of 20 months to mature (Amin *et al*., 2005; Sauls, 1998).

Varieties of pineapple under production in Ghana and their characteristics

There are a few unpopular landraces of pineapple in Ghana. Currently, the MD2, Sugarloaf, Smooth Cayenne and Queen are classified as local varieties of pineapple (Ninsin, 2012). The MD2 has high quality parameters such as brix coupled with low acidity of about 0.45 %. It is the sweetest of all the varieties and has a uniform size on maturity and improved shelf life (COTVET, 2017). The MD2 is currently the most exported variety (Osei & Aluah, 2021). Sugarloaf is very dominant in smallholder farmer fields in the Central Region of Ghana (Acheampong *et al*., 2015). Sugarloaf fruit is conical-shaped and remains green upon ripening. The fruit, however, is relatively difficult to peel (COTVET, 2017). Sugarloaf produces more slips compared to the other varieties.

The Queen variety has moderate productivity. It has a conical-shaped fruit with a sweet golden fresh fruit colour (COTVET, 2017). The leaves of Queen are spiny and create unpleasant working conditions. In many instances, the Queen variety produces fewer slips. Smooth cayenne is one of the most popular varieties that is cultivated outside the boundaries of Ghana. It is a highly productive variety (Bennici & Bussi, 2009) with a large cylindrical fruit shape, and yellow-coloured. The Cayenne fruit is tagged with high grade because it supports canning. Farmers prefer this variety due to its spineless nature (Da Silva *et al*., 2020). Similar to the Queen, the Smooth Cayenne produces fewer slips (COTVET, 2017).

Micropropagation

Importance of micropropagation

The use of infected seedlings is the primary source of disease dissemination. The secure way of controlling this challenge is using disease-free plating material and preferably resistant and not tolerant varieties. Clonal micropropagation, a plant tissue culture technique, has been used to produce several varieties worldwide. Micropropagation has produced seedlings of some crops on large-scale for several farms and biofactories (Datta *et al*., 2017; Guerra *et al*., 1999). Micropropagation can be applied to virtually all plant species; however, those with high economic value are recommended (Datta et al., 2017). Crops such as citrus, banana, cardamom, sweet potato, coffee, black pepper, garlic, turmeric, and ginger are micropropagated (Seyoum *et al*., 2021).

According to Loyola-Vargas & Ochoa-Alejo (2018), ornamentals take pole position among plant species that are now micropropagated for commercial

purposes. The core advantage of micropropagation is the fixation of genetic gain in clonal populations and the rapid production of many healthy, high-quality plantlets in a limited space, regardless of climatic constraints. Plants can be micropropagated in three ways: (1) by encouraging the growth of axillary or apical buds and further rooting, (2) by inducing adventitious buds followed by rooting, (3) by the germination of matured somatic embryos (Loyola-Vargas & Ochoa-Alejo, 2018).

Phytohormones for micropropagation

Growth regulators in micropropagation medium play a crucial role in plant cells, tissues and organs differentiation, growth and development. Auxins and cytokinin are employed for this exercise. Cytokinin promotes bud and shoot development, while auxin favours root induction. A better combination of these hormone categories directly influences the growth pattern of cultured plantlets. The inclusion of cytokinin in tissue culture media releases meristematic buds from apical dormancy enhancing shoot proliferation (Bahadur *et al*., 2015). The commonest micropropagation employed cytokinin is BAP, 2-isopentenyl adenine (2iP), thidiazuron (TDZ) and kinetin. Naturally, auxins are involved in rooting, abscission, tropism, stem internode elongation and apical dominance. Indole-acetic acid (IAA), Naphthalene acetic acid (NAA), and Indole-3-butyric acid (IBA) are the widely used auxin in micropropagation either alone or in combination with some cytokinin (Chokheli *et al*., 2020; Delgado-Paredes *et al*., 2021). Min & Bartholomew (1996) highlights the use of *para*-chlophenoxyacetic acid (*p*-CPA), napthoxyacetic acid (NOA), trichlorphenoxyacetic acid (2,4,5-T) and dichlorophennoxyacetic acid (2,4-D) is used in media for micropropagation of

some species. 2,4,5-T and 2,4-D are reported to be essential in callus induction, growth and somatic embryogenesis (Bahadur *et al*., 2015). Some gibberellins are also included in micropropagation media. GA_3 is the most used gibberellin in micropropagation. However, it is rarely utilised as compared to cytokinins and auxins though it is reported to stimulate tissue growth (Pessarakli, 2014).

Recently, growth retardants such as paclobutrazol are used in the acclimatisation stage of micropropagation for hyperhydricity and control leaf function (Christou & Klee, 2004; Hussain *et al*., 2017). Organic extract of banana, tomato, sugarcane molasses and coconut water have been reported to promote shoot multiplication in micropropagation and reduce media cost (Hussain *et al*., 2017). According to Yong *et al*., (2009), the inclusion of coconut water in tissue culture media is helpful since its physiochemical composition includes vitamins, amino acids, sugar alcohol, natural auxins and cytokinin.

General micropropagation procedure

The micropropagation techniques have been outlined by some researchers (Trigiano & Gray, 2016). All procedures involve obtaining an explant, inoculating the explant, incubation for growth and acclimatisation of the plantlet. An outline of the micropropagation process is the pre-propagation, explant initiation, subculturing rooting and hardening of plantlets (Salvatierra, 2017). Bhatia & Sharma (2015), defined an explant as an excised piece of differentiated tissue or organ which is cultured. The type, size and source of explant are reported to influence the success of micropropagation (Duong *et al*., 2004). Plant meristem is a common explant source. Meristematic buds and the shoot tips are most preferred

to leaf culture which transcends from callus for fear of somaclonal variation (Hussain *et al*., 2017). The source of meristems is of less importance except for micropropagation which aims at producing a virus-free plantlet in which the apical meristem is preferred (Gahakwa *et al*., 2013).

Surface sterilisation of explants in a chemical solution ensures the removal of contaminants while preserving the plant tissues (Amin *et al*., 2005). Chemical solutions often used in disinfecting explants include ethanol, sodium hypochlorite, mercuric chloride, and calcium hypochlorite at different concentrations (Acheampong *et al*., 2015; Buah *et al*., 2015). The dropwise addition of tween 20 to the chlorex solution aggravates the sterility of the explant (Adeoye *et al*., 2020). Following the sterilisation, the explants are cultured under optimal incubation conditions (light, temperature and relative humidity) also referred to as culture initiation.

The explants are multiplied to obtain the desired number through multiple conscious subcultures. Culture multiplication employs media rich in ideal cytokinin either alone or in combination with some auxin (Hussain *et al*., 2017; Sharma & Singh, 1995).

Rooting of plantlets sometimes occurs in the same media as the multiplication culture. For many species, it is advisable to switch the growth regulator to a root-inducing one or possibly increase its concentration to enhance stronger root growth and development (Cardoso *et al*., 2018; Park, 2021). Rooted plantlets are transferred to a substrate of choice (sand, compost, peat or a combination of any) for acclimatisation. The acclimatisation process involves a

gradual adjustment of humidity and light until the plantlets are fully hardened (Hussain *et al*., 2017).

Steps in pineapple micropropagation and some phytohormones used

Initiation

Culture initiation or establishment is the most critical part of plant micropropagation (Acheampong *et al*., 2015). Initiation of pineapple cultures has been possible through the use of meristematic buds from either the crown, slip, sucker or ratoons (Mhatre, 2007; Nikumbhe *et al*., 2013). Explants for the different varieties have been successfully initiated. Notably among them are the popular Smooth Cayenne, MD2, Sugarloaf, Queen and other less-known varieties (Sulaiman *et al*., 2020). Mandal *et al*. (2002) have reported differences in the *in vitro* culture of buds viz matured fruit crown, sucker and slip. Different authors have reported the effectiveness of different sterilant. The commonly used chemicals sterilant includes ethanol, mercuric chloride, sodium hypochlorite, calcium chloride, and silver nitrate. Different concentrations and sterilisation durations have been exploited (Bello *et al*., 2018). Acheampong *et al*. (2015), initiated Sugarloaf *in vitro* with MS media supplemented with BAP alone or in some combination with 0.01 mg L^{-1} and 0.1 mg L^{-1} NAA. The inclusion of BAP and kinetin in MS media enhances the initiation of Smooth Cayenne (Atawia *et al*., 2016). MS media without any hormone also enhances the growth of different bud sizes (Dal Vesco *et al*., 2001).

Multiplication

Shoot multiplication of pineapple aims at increasing the number of shoots through subculturing. In many cases, cytokinin concentration for the initiation

culture is increased together with the auxins. Multiplication cultures are either liquid cultures in shakers, solid cultures in tubes or glass jars or in temporary bioreactors which exponentiate the shoot regenerative potential of explants (Adeoye *et al*., 2020). Among all media types deployed, the Murashige and Skoog media is dominant (Murashige & Skoog, 1962). Escalona *et al*. (1999), obtained the highest shoot rate of 106 MS media was supplemented with 2.1 mg L^{-1} BAP + 0.3 mg L^{-1}. The inclusion of PB (paclobutrazol) and GA_3 produced more uniform shoot. Atawia *et al*., (2016) reported that MS media supplemented with kinetin and BAP at 0.25 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1} and 2.0 mg L^{-1} influenced shoot proliferation however BAP 2.0 mg L^{-1} culture recoded the highest proliferation rate.

The B5 and Woody plant media supplemented with kinetin and BAP of different concentrations had a lower shoot proliferation rate. Similarly, Smooth Cayenne initiated on MS media supplemented with BAP, kinetin and thidiazuron (TDZ) alone or in combination with NAA and observed significant differences where MS media supplemented with BAP 2.0 mg L^{-1} + 0.05 mg L^{-1} NAA gave the highest regenerative rate (Adeoye *et al*. 2020). Adeoye highlighted that transferring the shoot to TDZ 5.0 mg L^{-1} for eight weeks accelerated production and elongated the shoots. In an *in vitro* culture of Smooth Cayenne, MS media supplemented with 1.0 mg L^{-1} BAP outperformed MS media supplemented with the same concentration of 2-Isopentenyl adenine (2iP) (Kornatskiy, 2020).

According to Seyoum *et al*. (2021) the addition of 5 % coconut water to MS media has the potential to spike up shoot generation. The *in vitro* micropropagation of the Queen pineapple variety by Mandal *et al*. (2002) revealed that MS

supplemented with 2.0 mg L^{-1} NAA and IBA, 2.5 mg L^{-1} BAP and 10 % coconut water were the best among other cytokinin, auxin and coconut water combinations as multiplication media. Othman (2016) reported the potential success of the use of 10 and 15 % coconut water in an MS media without any cytokinin for MD2 in-vitro multiplication. Mapes (1973) outlined the procedure for callus induction in modified MS media and further multiplied pineapple shoots with an MS media supplemented with 20 % coconut water and adenine.

Rooting

MS media with 1.0 mg L^{-1} IAA gave the best root performance comparable to IBA 1.0 mg L^{-1}, 2.0 mg L^{-1} and 3.0 mg L^{-1} for Smooth Cayenne rooting culture (Atawia *et al*., 2016). The combination of NAA and IBA at a concentration of 2 mg L^{-1} in an MS medium produced the best root induction which significantly differed from the other plant growth regulator combinations (Adeoye *et al*., 2020). Recently, the inclusion of charcoal in rooting media has become popular (Elias *et al*., 2021; Sani *et al*., 2021). In some instances, charcoal is solely used, or a minimal percentage is combined with IAA or IBA. Jaisy & Ghai (2011), reported the use of charcoal as a cost-effective approach in micropropagation.

Acclimatisation

Improper handling of rooted plantlets may result in plantlets failure. Plantlets' hardening procedures are technical hence researchers have exploited media compositions and microclimatic factors. A combination of peat and sand of different ratios sustained Smooth Cayenne plantlets on acclimatisation (Atawia *et al*., 2016). River sand is reported as a good substrate giving off an acclimatisation

success of about 87 % (Sani *et al*., 2021). Danso *et al*. (2008) reported the survival of rooted MD2 in a jiffy peat moss under greenhouse conditions. Be & Debergh (2006) acclimatised *in vitro* plantlets under a net house and the plantlets survived at a rate of approximately 100 %. Lyam *et al*. (2012) acclimatized pineapple plantlets rooted from a bioreactor system in a medium made from 70 % coconut fibre, 20 % topsoil and 10 % stone dust mix for about 6 days under shade after which they were transferred into plastic bags filled with sand, peat and compost for full-time hardening.

Advances in the use of some micropropagation component

Carbon source

Sucrose is the most familiar carbon source in micropropagation. Several sugar sources have been deployed in culturing potatoes, ginger, chickpea, peanut, lentil, fruit trees and medicinal plants (Prakash *et al*., 2004). The carbon sources contribute 30 % - 40 % of the cost of tissue culture media. (Demo *et al*., 2008). Using table sugar as sucrose is cheaper than other carbon sources and is reported to reduce the medium cost by about 90 % while maintaining the media quality. According to Datta *et al*. (2017) replacing sucrose with table sugar reduced their medium cost to about 100 %.

Similarly, Prakash *et al*. (2012) also reported a cost reduction of about 85 % when they substituted their sucrose with ordinary sugar. (Kodym *et al*., 2001) replacing grade sucrose and gelrite in their banana tissue culture protocol resulted in a reduced total cost. However, resorting to 13 sugars from different countries, light brown and white sugar emerged as the best, having low electrical conductivity.

For potato culture media, Gebre & Sathyanarayana (2001) concluded that the inclusion of table sugar as a carbon source did not just enhance the micropropagation, as was shown in the means of root numbers per plantlet but also lowered the production cost as reported by other researchers. Strawberry plantlets also survived better on acclimation and subsequently on the field when their low-cost rooting medium was supplemented with table sugar, tapioca granule and tap water (Kaur *et al*., 2005).

Water

Sterile distilled water is used for explant washing, hormone preparation and all culture operation that require the use of water are done using (Datta *et al*., 2017; Hannweg & Shezi, 2020; Lal *et al*., 2020). However, tap water devoid of heavy metals and other contaminants is used for the preliminary cleaning of explants such as meristematic buds and leaves (Sharma *et al*., 2013). Due to cost-related issues, smaller facilities use autoclaved tap water rather than distilled water for the stock solution, hormone and media preparation (Savangikar, 2004). Kaur *et al*. (2005), also confirmed the success and cost-effectiveness of using tap water instead of distilled water for their tissue culture protocol for strawberries.

Solidifying agents

Tissue culture media comes in three grades, viz., liquid, semi-solid and solid media. Except for liquid medium, the other two media types have gelling or solidifying constituent (Bhatia & Sharma, 2015; Suthar *et al*., 2011). The use of cassava starch as a gelling agent in potato tissue culture medium is reported to have some limitation that calls for modification (Ogero, 2012). Buah (2014) has reported

the potential of cassava starch alone or in combination with agar as a solidifying agent in banana tissue culture. Several forms of starch have been used, solely as a gelling agent, or in combination with other materials, with their underpinned limitations reported. According to Zimmerman *et al*. (1995), combining corn starch and a lower concentration of gelrite for the *in vitro* propagation of tree crops, ginger, sugarcane and turmeric is possible.

In potato shoot tip culture, tapioca starch as a replacement for 'Bact-agar' yielded good results (Nene & Shiela, 1997). A combination of potato starch, laundry starch and semolina in a 1:2:1 ratio as a component of medium for micropropagation is also valuable (Prakash, 1993). Potato tuber disc has successfully been cultured with barley starch (Sorvari, 1986). Since its introduction over one hundred years ago, agar has been the most widely used solidifying agent in microbial and plant micropropagation media (Neeru & Babbar, 2002). Substrata that have recorded some success in place of agar includes psyllium, starch, gelrite, xanthan gum, guar gum, and agarose (Babbar & Ruchi, 2006; Neeru & Babbar, 2002). The use of expensive agar for commercial tissue culture may not be necessary.

The agar matrix influences nutrient availability to the plant. As a known polysaccharide from seaweed, agar contains Mg, Ca and other trace elements which is better off than the use of Bacto and Noble-agar (Hussain *et al.*, 2017).

Agar, gelrite, micro agar are some common solidifying materials used in pineapple micropropagation protocols. Jain & Häggman (2007) reported that the ideal concentration of agar for pineapple micropropagation is 6 g L^{-1}. A protocol

optimized by Usman *et al*. (2013) indicated that the inclusion of 0.8 % agar in an MS medium helped in the rapid multiplication of Smooth Cayenne. Findings from Awal *et al*. (2010), also revealed that 8 g L^{-1} agar in a medium for the multiplication of Josapine pineapple enhanced shoot growth. Akin-Idowu *et al.* (2014) investigated the effect of growth regulator and medium type using three levels of gelrite (1.0 g L^{-1}, 1.5 g L^{-1} and 2.0 g L^{-1}) and they found that the medium with 1.0 g L^{-1} gelrite supplemented with 0.1 mg L^{-1} and 0.3 mg L^{-1} BAP yielded the best regeneration results.

Light management

In vitro microclimatic factors such as temperature, moisture and light can affect culture responses. Light is a principal environmental cue that influences plantlet growth directly and indirectly. Light serves as the main energy source for photosynthesis. Plants possess various signal-transducing light receptors that control their growth and development owing to the amount, duration and quality of incident light intensity (Kodym & Zapata-Arias, 2001; Pessarakli, 2014). Different authors have reported on the effect of light, its quality, intensity on *in vitro* plantlet growth and development (Domingues *et al*., 2018; Metsoviti *et al*., 2020; Rakshi *et al*., 2017). According to Chen *et al*. (2019), light intensity significantly affects callus and shoot regeneration of ornamental succulent *Haworthia*. Again, Domingues *et al.* (2018) also concluded in their evaluation of different light systems on *Pterospartum tridentatum* that light affects plant *in vitro* response as it greatly affects the mean number of side shoots and weight of plantlet.

Different light sources are used in micropropagation, and plant species respond differently to each type of light. Some plants perform better in darker microclimates; however, according to Domingues *et al.* (2018), dark environment affects chlorophyll content and the multiplication rate of plantlets. White fluorescent light increases the number of side shoots and plantlet weight while light emitting diode (LED) light causes dark green colouration of the explant without a substantive increase in weight (Domingues *et al.*, 2018). Light spectra affect plantlet growth rate and phytochemical characteristics. In C*annabis sativa*, treatment combinations including red-green and red-blue light at various intensities proved better (Boonsnongcheep & Pongkitwitoon, 2020). For photoperiods in micropropagation, lightening duration between 8 and 16 hours have widely exploited (Souza *et al*., 2022). Generally, plantlet rooting is enhanced by far-red light. Fallah & Kahrizi (2016), studied *in vitro* culture of grapes and reported that light intensity of 2500 lux promoted the fastest axillary bud growth over light intensity of 5000 lux. According to Sarikhani & Khorami (2021) light quality affects shoot regeneration and the diameter and length, node number, leave thickness, chlorophyll concentration and stomatal number as well as both fresh and dry weight in micropropagation of Cadaman.

In pineapple micropropagation, different sources of light, photoperiod and intensities, among other illumination characteristics, have been exploited (Acheampong *et al*., 2015; Danso *et al*., 2008; Farahani, 2014; Hamad *et al.*, 2013). These include fluorescent and LED systems, photoperiods ranging from 8 – 20 hrs and light intensity between 1000 lux and 5000 lux. Due to the conservation of

electricity and cost-effectiveness, there is a shift from other lighting systems to LED systems (Pati, 2014).

Culture vessels

Different vessels are used for culture initiations and multiplication depending on the scale of production. Glass test tubes are widely used because their narrow opening reduces the rate of contamination. Conical flasks are used for liquid cultures on a shaker for agitations. Glass petri dishes and conical flasks are reported to be expensive irrespective of their efficiency (Prakash *et al*., 2004). The use of plastic containers in micropropagation in repeated autoclaving have been reported to cause cloudiness, which impedes light penetrance in such plastic containers (FAO, 2004). However, autoclave resistant transparent plastic containers that are autoclave resistant are used for micropropagation. Disposable non-autoclavable sandwich boxes and food containers used in micropropagation are reported to be cost-effective since they are usually made of polystyrene and their use eliminates washing costs (Prakash *et al*., 2004).

Several containers and lids have been manufactured with different technologies for which some require bulk sterilisation with gamma radiation before their usage (Lyam *et al*., 2012). However, the development and use of the sterile plastic bag for commercial micropropagation is one of the biggest achievements for low-cost options in micropropagation (Prakash *et al*., 2004).

Temporary Immersion Bioreactors system (TIBs)

McAlister *et al*. (2005) in their attempt to generate a protocol for eucalyptus, concluded that using the RITA® bioreactor system increased the yield four times

in half the required time comparable to the solid and semisolid system. Moreover, plantlet from the RITA® system were hardier than those raised on an agar-based medium, giving off a higher hardening-off rate during acclimatisation. Temporary immersion bioreactor systems, like RITA®, have several benefits over semisolid techniques (Biruk *et al*., 2013). Temporary immersion systems combine the benefits of both gelled and liquid media, specifically having intermittent 100 % nutrient availability while yet allowing the plants to grow in an air gap (Etienne & Berthouly, 2002). Other benefits of the temporary immersion system are; micropropagule quality, lower labour expenses, lower consumable costs, better leaf growth, lessened hyperhydricity, and decreased tissue asphyxiation (Etienne & Berthouly, 2002; Gichner, 1995). However Akula *et al*. (2000) have reported that the frequency and length of the immersion can affect tea multiplication rate.

In *Hevea brasiliensis*, it has been discovered that the RITA® system promotes root formation (Etienne *et al*., 1997). An effective way of reducing the total cost of micropropagation while increasing the multiplication rate of plantlets is by replacing the agar with a liquid culture through a temporary immersion bioreactor (Biruk *et al*., 2013). Pineapple and other crops have seen a significant multiplication rate through the use of bioreactors (Biruk *et al*., 2013).

Tissue culture media types

Several media have been developed for purposes such as callus induction, organogenesis, cell suspension, micropropagation, and protoplast culture among others (Park, 2021). The variation in tissue culture media types is usually a result of a modification in either the major component, notably, in the organic salt

concentration, vitamins and carbohydrates (Hussain *et al*., 2017). Some of the culture media include; Murashige and Skoog (MS), Linsmaier and Skoog (LS), Nitsch and Nitsch (NN), Gamborg (B5) and Hellers and White media (Park, 2021).

The MS medium has wider utilization in plant tissue culture. The development of the MS media is linked to the researchers' discovery of plant growth regulators. The MS composition is a formulation of nutrients like vitamins, amino acids and inorganic salt. Labels on MS medium indicates the concentration of sucrose per volume. The MS media is used for callus culture, cell suspension, induction of organogenesis and micropropagation (Bhatia, 2015; Bhojwani & Dantu, 2013). It is known to be effective for several species (Cardoso *et al*., 2018; Medina & García, 2005). Modification in the MS medium produced other media types.

The LS media composition is similar to the MS, except for differences in the vitamins. Thiamine concentration is increased to compensate for the absence of inositol. According to Bahadur *et al*. (2015), LS media was first used in optimizing the organic supplement of tobacco culture. LS medium is employed in micropropagation, cell suspension, organogenesis, and callus culture.

The B5 medium is ideal and effective for protoplast culture (Atawia *et al*., 2016). However, in the early ages, it was used for cell suspension culture of Glycine max. Gamborg medium blends components like the MS but has with a higher concentration of potassium and nitrate and a lower concentration of ammonia (Bhojwani & Dantu, 2013; PCT, 2020).

Nitsch and Nitsch developed the NN medium for the *in vitro* anther culture of Nicotiana, a member of the Solanaceae. The NN contains higher concentrations of thiamine, folic acid and biotin a key enhancer of anther callus culture (PCT, 2020).

White medium is recognised as the oldest root culture medium originally developed for tomato root culture. It has a lower salt concentration with a 19 % lower nitrate concentration relative to the MS media (Abobkar & Ahmed, 2012). The $MgSO_4$ concentration of the NN medium is rather higher compared to White medium (PCT, 2020). White media is reported to be successful for *Musa* and *Daucus* species (Bhatia, 2015).

Tissue culture media composition (Murashige and Skoog)

The MS medium is made of macronutrients, micronutrients, organic supplements, and carbon sources. The macroelements are those that are required in concentrations > 0.5 mmol L^{-1} and this includes nitrogen, potassium, phosphorus, calcium, sulphur and chlorine. Contrarily, the microelements include iron, zinc, copper, boron, cobalt, molybdenum and iodine and their requirement is < 0.5 mmol L^{-1} (Bhatia & Sharma, 2015). Detailed compositions of the Murashige and Skoog medium are listed in Table 1.

Table 1: Components of the Murashige and Skoog medium with their respective concentrations in stock solution

Essential Element	Concentration in medium (mg L^{-1})
Microelements	
NH_4NO_3	1650
KNO_3	1900
$MgSO_4.7H_2O$	370
$CaCl_2.2H_2O$	440
KI	0.83
KH_2PO_4	170
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
$MnSO_4.4H_2O$	22.3
Microelements	
$NaMoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Iron source	
$Na_2EDTA.2H_2O$	37.3
$FeSO_4.7H_2O$	27.8
Organic supplements	
Myoinositol	100
Pyridoxine-HCl	0.50
Nicotinic acid	0.5
Glycine	2
Thiamine-HCl	0.1
Carbon source	
Sucrose	30000

Source: (Bhojwani & Razdan, 1996)

Tissue culture media for pineapple

A comparison of Gamborg (B5), woody plant medium and Murashige and Skoog medium on the proliferation of Smooth Cayenne showed that full-strength MS medium was ideal (Atawia *et al*., 2016). MS medium supplemented with different levels of NAA and BAP enhanced shoot proliferation (Danso *et al*., 2008). Aside from varying the concentration of auxin or cytokinin, the MS media is widely

used for pineapple tissue culture initiation, multiplication and rooting (Elias *et al*., 2021; Tegen & Mohammed, 2016). In hydroponic research on the adaptation of Smooth Cayenne, MS medium was a better option (Kornatskiy, 2020). The compatibility of MS medium for pineapple micropropagation has a long history even though other researchers have tried some media variants (Mandal *et al*., 2002).

Challenges in micropropagation

High cost

Despite the benefits of micropropagation, the cost involved restricts its usage and exploitation at an industrial level. This technology is expensive in terms of capital, labour, and energy (Datta *et al*., 2017). To address cost-related issues in micropropagation, some efficient alternative protocols have been optimised (Be & Debergh, 2006; Datta *et al*., 2017; Lyam *et al*., 2012). Many protocols have been refined by replacing most components, specifically those relating to chemicals. Many factors affect micropropagation cultures(Christou & Klee, 2004). Limiting factors could be explant-based, nutrient medium and incubation conditions. Test-tubes, plastic containers, flasks, and other vessels used for micropropagation affect plantlet regenerative potential. Hazra *et al*. (2000), confirmed in their evaluation that culture flasks had a substantial effect on the multiplication and proliferation of shoots as compared to the test-tubes used.

Culture contaminations

Culture contaminations are very common in micropropagation. Microbes feed on the same medium from which the explant strives, making contaminations a menace in all stages of tissue culture (Odutayo *et al*., 2007). Contamination may

principally emerge from the explant, referred to as endophytic, or from wrongful culture procedure (Reddy *et al*., 2021). The presence of microbes in culture vessels increases plantlet mortality, and in situations of latent infestation, shoot and root proliferations reduce (Blake, 1998; Kane, 2003). Sources of contamination may range from poor sterilisation of tools and working cabin, explant used or even from incubation rooms. Odutayo *et al*., (2007) while investigating sources of microbial contamination in plant biotechnology laboratories, realised that not only explants or walls of growing rooms but also the skin of humans (workers) contributed significantly as a source of contaminants. Mites and other insects introduce a greater percentage of contamination in micropropagation (Cassells, 1991). More than half of tissue culture laboratories have reported mites as the prime source of contamination. Mites crawl from one culture vessel to another, transferring bacterial and fungal spores that they carry on their body (Abass, 2013).

Fungi can thrive in virtually every environment provided there is a substrate and a suitable relative humidity of about 60 %. Fungal contamination, specifically for most common fungi such as *Aspergillus niger* and *Aspergillus alternata* in cultures, causes a range of damage, including increasing medium pH and turbidity as well as cell destruction (Abass, 2006; Abass, 2013). According to Reddy *et al*. (2021) yeast, *Penicillium* species and *Aspergillus niger*, are among the major fungal contaminants in tissue culture growth media.

Bacterial infection is latent and is characterised by variable growth of the cultured plants. Bacterial contamination persists in laboratories where tools are not thoroughly flamed or sterilised (Odutayo *et al*., 2007). Culture vessels are generally

loosely fitted for gaseous exchange with the external environment (Kausch, 2018; Reddy *et al*., 2021; Singh *et al*., 2011). Mites and thrips easily enter loosely covered culture vessels and transfer spores of other pathogens they carry. Reddy *et al*. (2021) reports that *Escherichia coli, Pseudomonas fluorescens,* and *Micrococcus sp.*, are bacterial contaminants in cultures.

Management of contaminations

Though microbial contamination is persistent and problematic in micropropagation, adopting sanitary protocols significantly reduces the effect (Kausch, 2018). The severity of mite and thrips infestation can be managed by controlling the microclimate of culture rooms. According to Odutayo *et al*., (2007), microbes proliferate at higher relative humidity; hence regulating the relative humidity of growing rooms will help reduce their population in the growing rooms. Minimising entry frequency in culture incubation rooms also keeps the microbes population in check (Bahadur *et al*., 2015; Chokheli *et al*., 2020; Kausch, 2018). Careful selection of fungicides and antibiotics using moderate concentrations have been reported as functional in eliminating bacteria and fungi. Properties of most effective antibiotics and fungicides include their stability, solubility, combining ability with a medium constituent, inexpensive, nontoxic to humans, and above all, non-resistance inducer (Abass, 2006; Abass *et al*., 2007; Al-Dosary *et al*., 2011). Some approaches have been outlined to control contaminants endogenously embedded in explant or plant tissues. Reddy *et al*. (2021) explain that before the collection of explants, some considerations must be made, and this includes the explant's quality, physiological state, the plant phenotype, the season and location

in which the explant is obtained, and the size of explant and its orientation in the medium. Above all, the explant should be well-trimmed with sterile blades and surface sterilised with different sterilant concentrations and duration in other to control contamination.

CHAPTER THREE

MATERIALS AND METHODS

Study location

The *in vitro* experiment was carried out in the laboratory of Iribov West Africa limited laboratory, Sogakope, Volta Region, Ghana (6°00´29.2´´N 0°34´55.1´´E). The acclimatisation of rooted plantlets was also done in the greenhouse of the same company. The laboratory facility and working conditions conform to International Biosafety standards as described by Neumann (2014).

Experimental design

A completely randomised design with three replicates was used for all experiment. Treatment combinations for culture initiation and bud growth included explant type, six media, and two incubation temperatures, four pineapple varieties. The treatment combination for the multiplication culture was media type and incubation temperature, for the four varieties. Rooting culture treatment was the media type, hormone level, and incubation temperature. Culture durations were 5 weeks for initiation culture, 8 weeks for multiplication culture, and 6 weeks for rooting culture. For cultures using Temporary Immersion Bioreactors systems (TIBs), 20 plantlets were placed in each reactor. The experiment was conducted between December 2021 and August 2022.

Source of experimental material

Slips and suckers of MD2, Smooth Cayenne and Sugarloaf pineapple varieties, were obtained from Milani Ghana Limited. Queen variety were obtained

from Exonas Limited in Swedru in the Central Region of Ghana**.** The experimental materials were well-labelled after collection.

Preparation of explant

The leaves of the experimental plants were gently removed to expose the meristematic buds. With the aid of a blade, the exposed buds were carefully excised with some attached stem tissue, as described by Acheampong *et al*. (2015). The isolated buds were placed into labelled jars. Blades were frequently changed to minimise the rate of cross-contamination. The excised buds were washed thoroughly under running tap water to eliminate dust and other dry matter (Kour *et al*., 2007). The excised buds were then washed with dilute soap solution followed by surface sterilisation with 2% sodium hypochlorite solution for 20 minutes and rinsed thrice with sterile autoclaved water. Two drops of Tween 20 was added to the hypochlorite solution as described by Adeoye *et al*. (2020). This activity was performed under a well surface-sterilised working cabin (clean bench) with an airflow velocity of 0.34 m s^{-1}. The cabin (clean bench) was sterilised with 70 % ethanol.

Figure 2: **A** - MD2 suckers, **B** – excised bud

Water sterilisation

To reduce cost, glass bottles were filled with double-filtered tap water devoid of heavy metals, after which they were autoclaved at 121 ^{o}C for 45 minutes. The autoclaved water was used for the final rinsing of the explants and other activities that required sterile water.

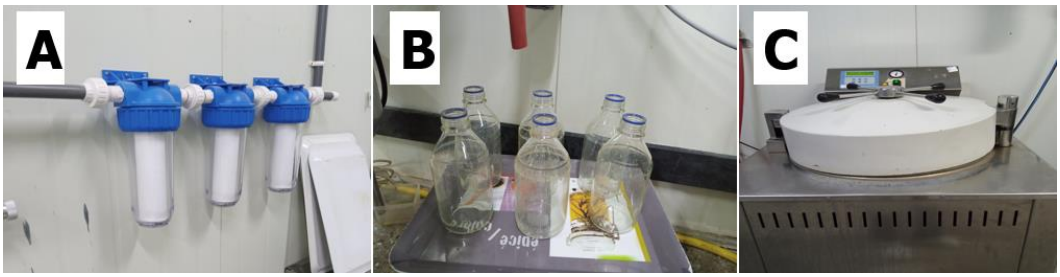

Figure 3: **A** - water filters, **B** - Glass bottles to be filled with filtered tap water, **C** - Autoclave

Media preparation

A commercially formulated MS media with all components, including vitamins from Duchefa Biochemie, was used for this experiment. Standard concentration as recommended on the manufacturer's manual was used. For a litre of media, 600 ml of water was measured and poured into a heating vessel with a magnetic stirrer, to ensure the mixture's homogeneity when added. With the aid of an analytical balance (Ohaus PA64) , 7 g of micro agar, was added to the solution to serve as solidifying agent (Saad & Elshahed, 2012) and 30 g of sugar was added to a boil. The mixture was allowed to warm until the agar was fully dissolved. The boiling lasted approximately 20 minutes on a stove at a temperature of 121 ^{o}C. The magnetic stirrer spined at 100 rpm during the boiling. The MS mixture per litre was also weighed and added to the mixture. Finally, the hormone (BAP or kinetin at 1.5 mg and 3.0 mg) was weighed and added to the mixture. For the accuracy of the pH

meter, the media temperature was lowered to about 50 ºC or less by adding the remaining volume of 400 ml water. The pH probes were then dipped into the media to check the range. NaOH or HCl was added dropwise to adjust the pH of the unmodified media to 5.8. This pH value was maintained for all other media prepared throughout this research. The media was then dispensed at 10 ml per test-tube and labelled accordingly with autoclave tape.

Two media sets were prepared for the multiplication culture: a liquid and a solid media. They had an MS composition with 30 g L^{-1} sucrose, 3 mg L^{-1} BAP and 20 % coconut water per litre. The solid media followed the same procedure described above, with the coconut water being the final media constituent added. The liquid media, on the other hand, had all elements present in the solid media except the agar. Both solid and liquid media were dispensed into test-tubes and glass bottles for autoclaving.

Media for rooting culture were also of two kinds, just like the preceding objective. In this media, the hormone used was Indole acetic acid with three different levels, which were 0.1 mg L^{-1}, 05 mg L^{-1}, and 1.0 mg L^{-1}. The preparation procedure was similar to that already described. Only the hormone type and concentration varied. This media was equally dispensed for sterilisation through autoclaving.

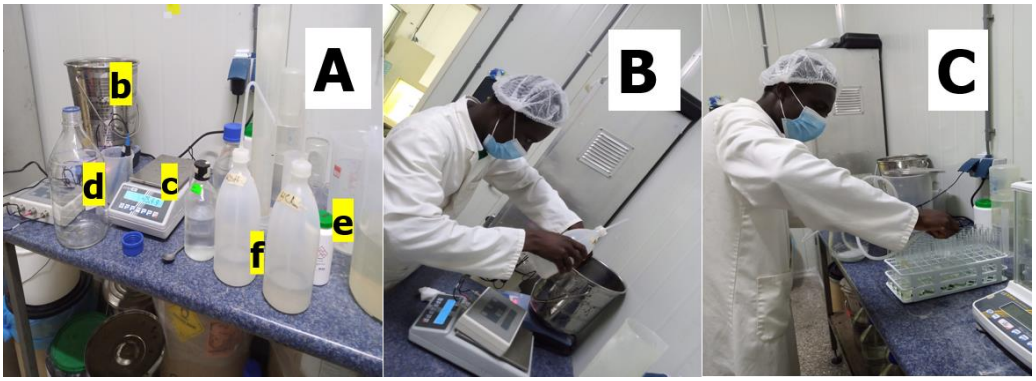

Figure 4: **A** - some component of the preparation room, **b** - cooking vessel, **c** - electric scale, **d** - glassware, **e** - MS medium premix, **f** – HCl / NaOH, **B** - adjusting medium pH with NaOH or HCL, **C** - dispensing medium into test-tubes

Culture procedure and incubation condition

With the aid of a sterile scalpel and forceps, buds of either sucker or slip of the four pineapple varieties were gently trimmed and planted on MS media in test-tubes. It was ensured that the tips of buds faced upwards in the test-tubes; however, in instances where the bud tip was less visible, they were planted side way. The test-tubes were covered with their respective lids and sealed with three layers of transparent sealing tapes. Culture test-tubes were arranged on rags, labelled and sent to incubation rooms. For shoot multiplication, shoots were split longitudinally, spliced at the base, and planted on a medium.

The rooting experiment involved using full plantlets. Uniform plantlets were used. Lower and matured leaves of plantlets were removed with the help of blades and forceps, after which basal incisions were made on plantlets. A batch of the plantlet was planted in the respective solid media while the remaining were placed in the Setis temporary immersion bioreactor. The RITA® temporary immersion bioreactor was used for the multiplication culture over the Setis because the design best fit (McAlister *et al.*, 2005). The media were filled in bioreactor tanks

and connected to programmed pumps that supplied medium to the plantlet at 8 hours intervals in 24 hours and each release lasting for 5 minutes. The maximum stay of culture was eight weeks, as reported by (Abdelhamid, 2021; Hamad & Taha, 2008, 2009)

The setups were kept on sterile shelves in two incubation rooms at 19 ^{o}C or 25 ^{o}C. Each room had a 12-hour light with 3520 lux and relative humidity of 36 - 42%. The culture condition was constant as no power interruption was observed during the culture period.

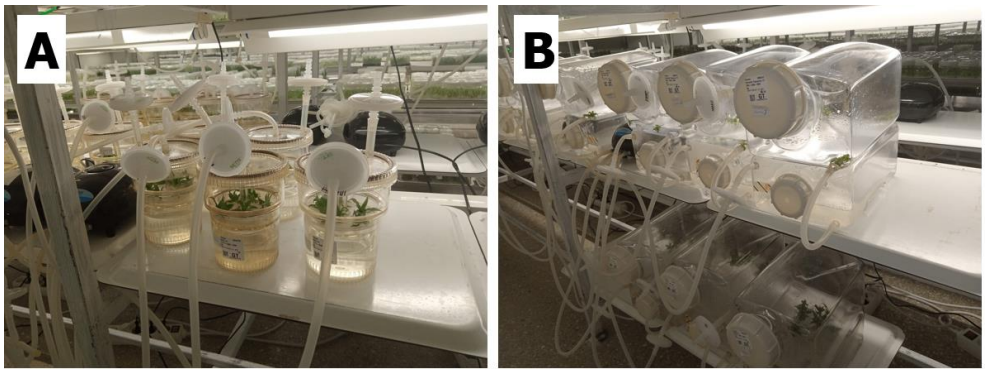

Figure 5: Plantlets in temporary immersion bioreactor: **A** - RITA®, **B** - Setis

Plantlet acclimatisation

After completing the ex-agar exercise and data collection on shoots and roots, the plantlets were sent to the greenhouse for planting. At the greenhouse, a sterile potting mix was moistened and filled in a 48-cell nursery tray with dimensions of approximately 6.35 cm x 3.81 cm. The rooted plantlets were immersed in a fungicide solution and planted in the trays. A low humidity growing chamber was provided for the plantlets for better acclimatisation. The humidity (Figure 6. B) was adjusted over time until the plantlet became fully established

(Hussain *et al*., 2017). The survival rate was recorded after six weeks as described by Farahani (2014).

Plantlet survival on acclimatisation was calculated as:

$$\%\ Survival = \frac{Number\ of\ plantlets\ [xWAP]}{Total\ number\ of\ plantlet\ planted} \times 100$$

Where $\boldsymbol{x}$ is the week number

Table 2: Scale for calli and lateral root scoring

Score	Callus	Root quality
0	Absence of callus	Absence of lateral root (root hairs)
1	Callus to shoot 1:4	Lateral root found on 25% of adventitious roots
2	Callus to shoot 2:4	Lateral root found on 50% of adventitious roots
3	Callus to shoot 3:4	Lateral root found on 75% of adventitious roots
4	NA	Lateral root found on all adventitious roots

NA = Not applicable

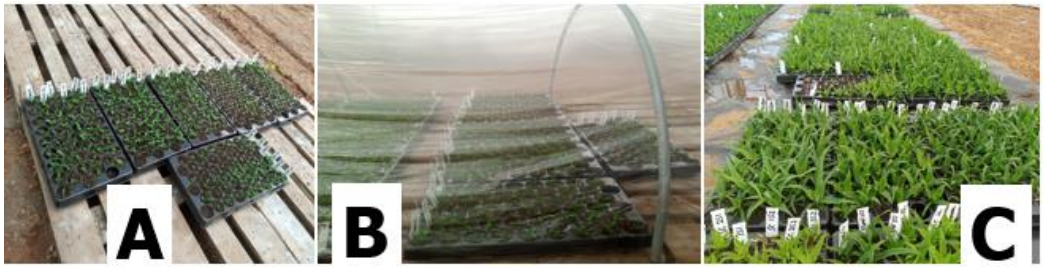

Figure 6: **A** - plantlets just after planting, **B** - plantlets under a humidity chamber, **C** – plantlets six weeks after planting

Data collection

For all cultures, bud growth and development, multiplication and rooting, the percentage contamination and success were recorded. The count and removal of contaminated setup were done at a three-day interval. The rate of multiplication of shoots was determined as described by Adeoye *et al*. (2020). Shoot parameters were recorded in all experimental phases, including the number of leaves, height (cm) and weight (g) of bud and plantlet and the presence of callus (Table 2). The

number of roots per plantlet, root length, lateral root score, plantlet weight, and the number of side shoots after the culture duration and the predetermined shoot parameter were considered. Data were collected during acclimatisation on total number of plants that survived. Observations were also made on the plantlet handling procedure that gave the best result.

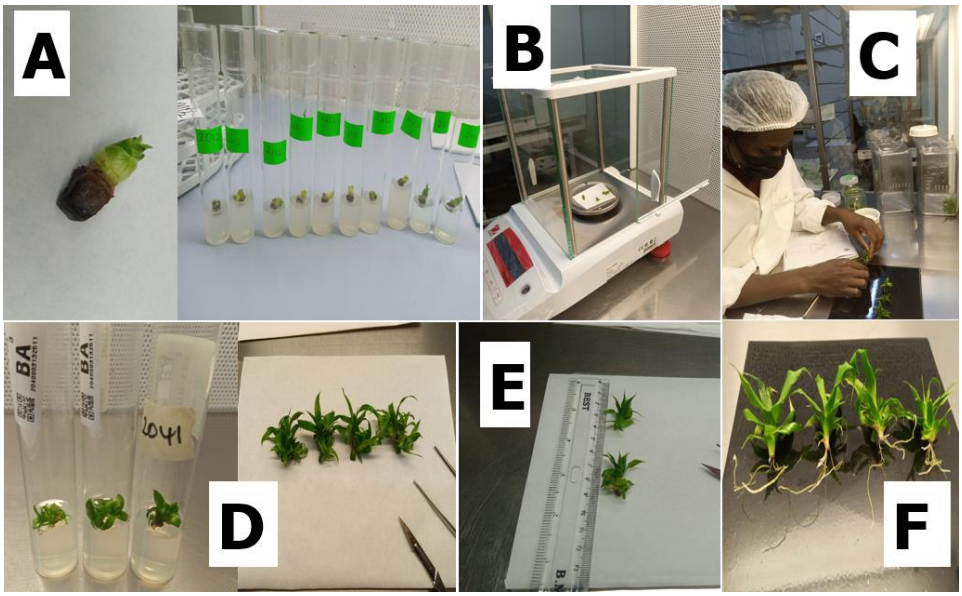

Figure 7: **A** - bud growth, **B** - weighing of plantlet, **C** - measurement of root length, **D** - plantlet with multiple shoots, **E** - measurement of shoot height, **F** - plantlets with root.

Data analysis

The data organisation and plotting of graphs and tables were done using Microsoft Excel version 19. The experimental data were statistically analysed to compare significant differences among the treatments for initiation, multiplication, and rooting cultures using the LSD test at 5 % (Williams & Abdi, 2010). The factors for ANOVA for the number of extracted buds from the experimental material was variety and explant whiles that of the initiation culture were the variety, explant type, incubation temperature, and media. For multiplication culture, the factors

were variety, incubation temperature, and media. In the rooting culture, factors comprised, variety, incubation temperature, media type, and hormone level. The model used was: variety * media type * hormone level / incubation temperature.

Statistical means were separated for the number of buds extracted from the explant type of the varieties using Tukey's test to show the extent of variation from the ANOVA.

Correlation analysis was performed to determine the association among multiplication culture and rooting characteristics. ANOVA were performed using GenStat statistical package version 12 to check statistical significance. Origin 2024 (10.1) was employed for the correlation analysis.

CHAPTER FOUR

RESULTS

ANOVA of buds from experimental materials

ANOVA of buds extracted from the experimental plants is shown in Table 3. The table showed significant differences among the variety, explant type and the interaction of variety and explant type ($P < 0.001$, $P = 0.016$ and $P < 0.001$ respectively).

Table 3: ANOVA of buds from experimental plants

Source of variation	Degree of freedom	Sum of squares	Mean sum of square	Variance ratio	p - value
Variety (V)	3	471.92	157.31	719.12	<0.001
Explant type (E)	1	1.64	1.64	7.5	0.016
V × E	3	38.08	12.69	58.02	<0.001
Residual	14	3.06	0.22		

Buds generated per variety of experimental plants

The extent of variation in the mean number of buds generated from each variety of experimental materials are shown in Figure 8. The mean number of buds generated by each variety significantly differed from one another. Smooth Cayenne generated the highest mean number of buds of 13.4 and this was followed by MD2 which generated a mean of 6.70 buds. Sugarloaf generated the lowest mean number of buds (1.25) and this significantly lower than the other varieties as shown in Figure 8.

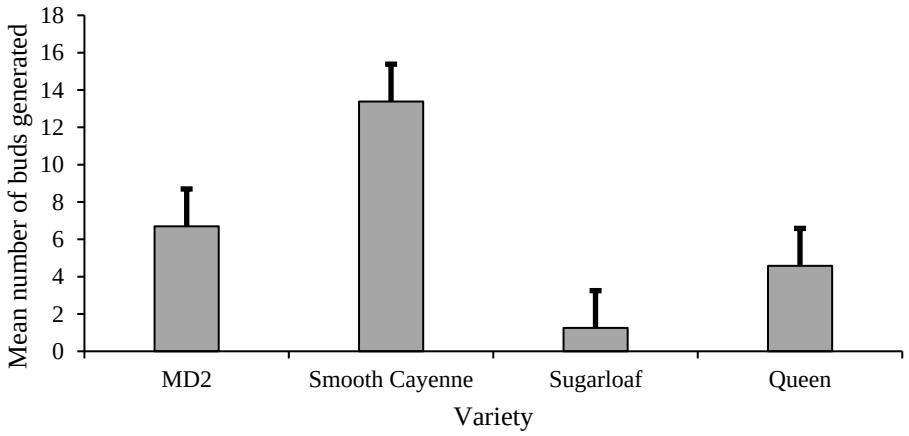

Figure 8: Buds generated per variety of experimental plants

Buds generated per explant type

Slips explant generated higher mean number of buds (6.74), compared to the sucker explant which generated mean number buds of 6.22 (Figure 9).

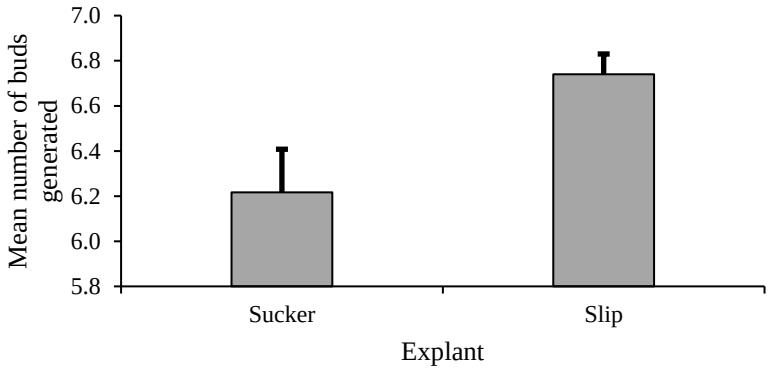

Figure 9: Buds generated from the explant types

Buds generated from the varieties and explant of pineapple

The experimental materials, slips, and suckers of the four pineapple varieties, MD2, Smooth Cayenne, Sugarloaf, and Queen, generated significantly different ($P < 0.001$) mean number of buds. Table 4 shows that the mean number of buds generated from the interaction between variety (MD2, Smooth Cayenne and Queen) and sucker explant were higher than those generated from the interaction between variety (MD2, Smooth Cayenne and Queen) and slip.

However, the opposite was observed in Sugarloaf where it interaction with slip generated buds that were higher than those recorded from its interaction with sucker (Table 4).

Table 4: Buds generate from varieties and explants of pineapple

Variety	Explant type	Mean number of extracted bud
MD2	Sucker	8^{d}
	Slip	6^{c}
Smooth Cayenne	Sucker	2^{b}
	Slip	1^{a}
Sugarloaf	Sucker	2^{b}
	Slip	7^{d}
Queen	Sucker	14^{e}
	Slip	13^{e}

Mean separation by Tukey's test. Means with the same letters are not significantly different. Means were approximated to the nearest whole number

Culture initiation

ANOVA of successfully initiated cultures showed highly significant ($P < 0.001$) differences among the four pineapple varieties. Significant ($P < 0.001$) differences were observed in the explant type and cytokinins concentration (Table 5).

The two-way interaction between variety by explant type as well as variety and cytokinin concentration were significantly different at $P < 0.001$ and $P = 0.008$ respectively. Differences in the three-way interaction of variety by explant type by cytokinin concentration were highly significant ($P < 0.001$) as highlighted in Table 5.

Table 5: ANOVA of successfully initiated cultures

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Variance ratio	P - value
Variety (V)	3	31868.15	10622.72	161.42	<.001
Explant type (E)	1	2496.9	2496.9	37.94	<.001
Cytokinin (C)	5	2094.19	418.84	6.36	<.001
V × E	3	1695.32	565.11	8.59	<.001
V × C	15	2470.64	164.71	2.5	0.008
E × C	5	721.9	144.38	2.19	0.071
V × E × C	15	3756.7	250.45	3.81	<.001
Residual	47	3092.91	126.1		

Varietal response to culture initiation

The extent of variability in the responses among the initiated varieties are detailed in Figure 10. The figure showed that Queen recorded the best of response (66.67 %) followed by the MD2 which recorded 45.79 % and was significantly lower than Queen. Smooth Cayenne and Sugarloaf were almost at par with a response rate of 23.39 % and 22.45 % respectively and were significantly lower than Queen and MD2 (Figure 10).

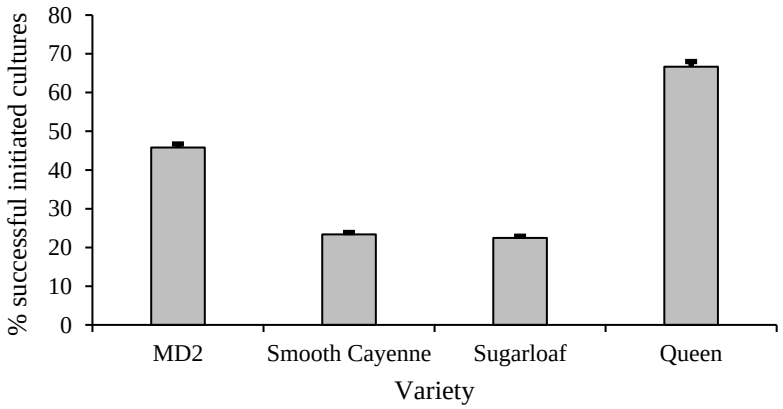

Figure 10: Varietal response to culture initiation
(Means were arcsine transformed)

Response of explant type to initiation

Slip recorded a significantly higher success rate compared sucker in their response to initiation culture (Figure 11).

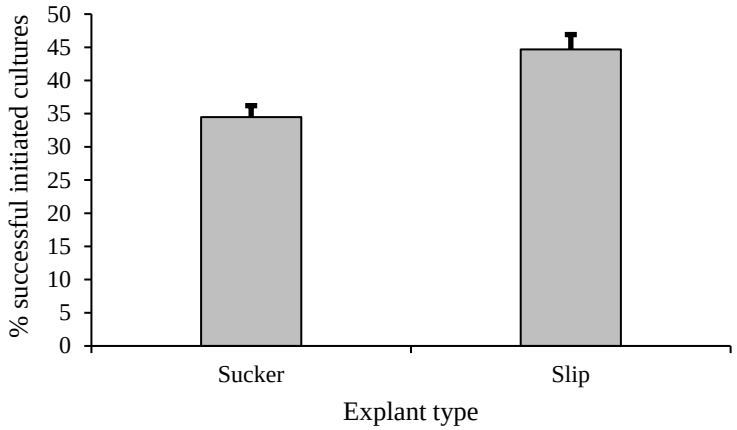

Figure 11: Response of explant type to initiation
(Means were arcsine transformed)

Effect of cytokinins concentration on the success of initiation culture

The influence of cytokinin in culture media on the success rate of initiation is presented in Figure 12. IRB505 recorded the highest success rate of 44.65 % but was not significantly ($P < 0.001$) different from the media supplemented with KIN (1.5 mg L^{-1} and 3.0 mg L^{-1}). The MS medium without any cytokinin recorded the lowest success rate of 31.79 % and was significantly lower than the other media types. Media supplemented with kinetin (1.5 mg L^{-1} and 3.0 mg L^{-1}) recorded a success rate of 42.63 %. The success of BAP 3.0 mg L^{-1} was significantly different than that of BAP 1.5 mg L^{-1} (Figure 12).

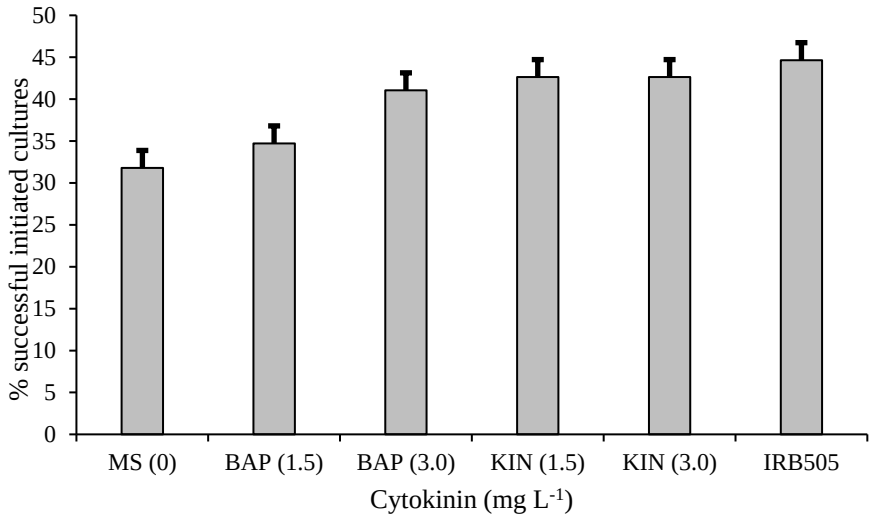

Figure 12: Effect of cytokinins concentration on the success of initiation culture (Means were arcsine transformed)

Variety-explant interaction on initiation culture success

The result of the interaction between variety and explant type showed that the interaction between Queen and slip recorded the highest success rate among cultures initiated and this was significantly different from the interaction between Queen and sucker explant which recorded the next highest success rate (Table 6). MD2's interaction with sucker resulted in a mean success rate of 47. 93 %.

Table 6: Success rate among varieties and explant types in initiated cultures

Variety	Sucker	Slip
MD2	47.93	43.64
Smooth Cayenne	15.24	31.55
Sugarloaf	15.17	29.74
Queen	59.57	73.78
L.S.D	6.66	
S.E	2.34	
% CV	4.1	

Means were arcsine transformed. **MS** = Murashige and Skoog; **BAP** = Benzyl aminopurine; **Kin** = Kinetin; **IRB505** = Private medium (Iribov West Africa Limited).

However, this did not significantly differ from the interaction between MD2 and slip which recorded a mean success rate of 43.64 %. Conversely, Table 6 revealed that the interaction between Sugarloaf and sucker recorded the lowest response rate on initiation and this was significantly different from the other interactions.

Variety-cytokinin interaction on initiated culture success rate

The result of initiation success rate as influenced by the interactive effect of variety and cytokinin concentration is highlighted Table 7. The results showed that the interaction between Queen and medium supplemented with BAP 3.0 mg L^{-1} recorded the highest initiation success rate of 75.91 %. The interaction between Queen and kinetin (1.5 mg L^{-1} and 3.0 mg L^{-1}) recorded a mean initiation success rate of 65.91 %. Conversely, the lowest initiation success rate was recorded from the interaction between Sugarloaf and BAP 1.5 mg L^{-1} (Table 7).

Table 7: Variety-cytokinin interaction on initiation culture success

	Cytokinin (mg L^{-1})					
Variety	MS (0)	BAP (1.5)	BAP (3.0)	KIN (1.5)	KIN (3.0)	IRB505
MD2	26.08	35.36	47.75	53.14	53.14	59.24
Smooth Cayenne	20.75	23.92	23.47	24.38	24.38	23.47
Sugarloaf	18.33	14.21	17.08	27.07	27.07	30.96
Queen	61.99	65.38	75.91	65.91	65.91	64.96
L.S.D	11.54					
S.E	4.06					
% CV	4.1					

Means were arcsine transformed. **MS** = Murashige and Skoog medium; **BAP** = Benzyl aminopurine; **Kin** = Kinetin; **IRB505** = Private medium (Iribov West Africa Limited).

Variety-explant-cytokinin interaction effect on initiation culture success

The extent of variability in the success of initiated cultures as a result of the interaction between variety, explant type and various cytokinin concentration is detailed in Table 8. Table 8 showed that the highest success rate (81.84 %) in initiated cultures were recorded from the interactive effect of Queen, slip and 3.0 mg L^{-1} BAP. This significantly ($P < 0.05$) differed from the interaction between Queen, slip and kinetin (at concentrations of 1.5 mg L^{-1} and 3.0 mg L^{-1}), which recorded the next highest initiation success rate of 77.74 %.

Table 8: Variety-explant-cytokinin interaction effect on initiation culture success

Variety	Explant type	Cytokinin (mg L^{-1})					
		MS (0)	BAP (1.5)	BAP (3.0)	KIN (1.5)	KIN (3.0)	IRB505
MD2	Sucker	4.05	31.41	57.13	63.79	63.79	67.42
	Slip	48.11	39.30	38.37	42.50	42.50	51.05
S. Cayenne	Sucker	16.23	22.91	11.48	13.62	13.62	13.56
	Slip	25.27	24.92	35.46	35.14	35.14	33.37
Sugarloaf	Sucker	4.05	4.05	9.79	24.37	24.37	24.37
	Slip	32.60	24.37	24.37	29.77	29.77	37.54
Queen	Sucker	58.28	60.93	69.98	54.07	54.07	60.09
	Slip	65.70	69.82	81.84	77.74	77.74	69.82
L.S.D		16.32					
S.E		5.74					
% CV		4.1					

Means were arcsine transformed. **MS** = Murashige and Skoog medium; **BAP** = Benzyl aminopurine; **Kin** = Kinetin; **IRB505** = Private medium (Iribov West Africa Limited).

Interestingly, Table 8 revealed that, irrespective of the variety and explant type, their interaction with kinetin either 1.5 mg L^{-1} or 3.0 mg L^{-1} recorded a mean success rate that were not significantly different ($P < 0.05$).

Again, Table 8 revealed that the lowest success rate (4.05) was recorded from the interaction between MD2, sucker and MS medium without any cytokinin

(MS 0). Similarly, Sugarloaf's interaction with sucker and MS (0) recorded the same success rate of 4.05 % but this was significantly lower compared to the interaction between Sugarloaf, sucker explant and medium with BAP 1.5 mg L^{-1}. The interaction between Smooth Cayenne, sucker and the various cytokinin concentrations recorded a fairly-low success rate which was significantly ($P < 0.05$) differed from the other interactions (Table 8).

Contamination in initiation culture

ANOVA of the rate of contamination recorded from the initiation culture are presented in Table 9. The results indicated that differences in the rate of contamination per explant type were highly significant ($P = 0.001$). Additionally, significant differences ($P = 0.025$) were observed in the contamination rate per incubation temperature. The results revealed differences in the rate of contamination due to the interactional effect of explant type, cytokinin concentration and incubation temperature (either two-way or three-way) were not statistically significant ($P < 0.05$) (Table 9).

Table 9: ANOVA of contamination in initiated cultures

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Variance ratio	p - value
Explant type (E)	1	1371.2	1371.2	11.44	0.001
Cytokinin (C)	5	921.5	184.3	1.54	0.189
Temperature (T)	1	630.2	630.2	5.26	0.025
E × C	5	302.2	60.4	0.5	0.772
E × T	1	3	3	0.02	0.875
C × T	5	246.5	49.3	0.41	0.839
E × C × T	5	304.2	60.8	0.51	0.77
Residual	72	8630.8	119.9		

Effect of explant type and incubation temperature on contamination rate in initiated cultures

Initiated cultures involving slips recorded a mean contamination rate of 24.10 % and this was significantly ($P < 0.001$) higher than that of the contaminations recorded in sucker explant (Figure 13). Cultures raised under 25 ℃ also recorded higher mean contamination rate (22.8 %) compared to those raised under 19 ℃.

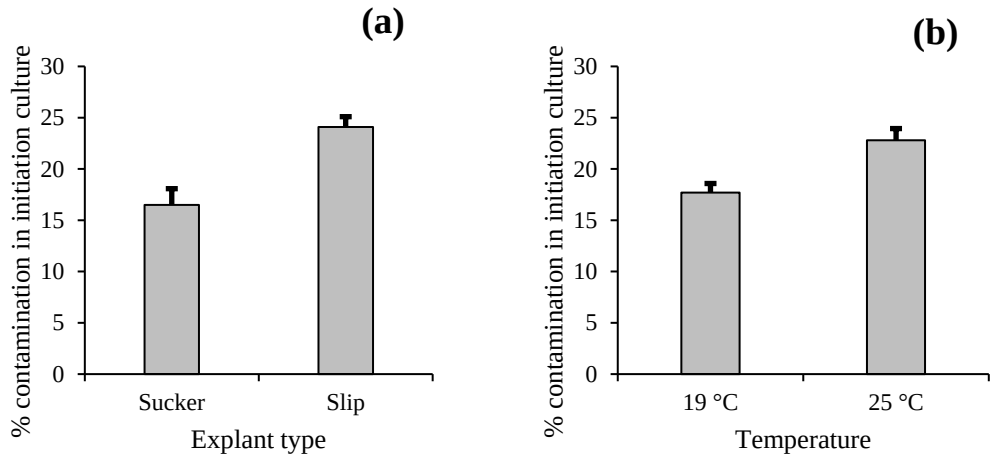

Figure 13: Contamination rate in initiated cultures based on: (**a**) explant type, (**b**) Incubation temperature
(Means were arcsine transformed)

ANOVA for initiation culture parameters

The result from ANOVA showed that the mean number of leaves and shoot height were significantly different ($P < 0.001$) with respect to the incubation temperature (Table 10).

Table 10: Combined ANOVA for initiation culture parameters

Source of variation	Degree of freedom	Mean sum of squares		
		Number of leaves	Shoot height (cm)	Weight of plantlet (g)
Temperature (T)	1	59.009***	12.252***	4.051
Explant type (E)	1	18.792**	1.905*	45.433***
Cytokinin (C)	4	1.062	0.1726	0.297
T × E	1	11.113*	1.7623*	1.41
T × C	4	0.32	0.0888	0.02
E × C	4	1.296	0.0739	0.13
T × E × C	4	1.994	0.0191	0.072
Residual	19	1.854	0.2577	2.863

***, ** and * shows significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively

Additionally, Table 10 indicated that the mean number of leaves, shoot height and weight of plantlet were also significantly different ($P < 0.01$, $P < 0.05$ and $P < 0.001$ respectively) with respect to explant type. Furthermore, the temperature and explant interaction resulted in significant difference in the number of leaves and shoot height (Table 10).

Effect of temperature on the number of leaves and shoot height of plantlets in the initiation culture

The extent of variability in plantlets mean number of leaves and shoot height as influenced by incubation temperature in the initiated culture are depicted in Figure 14. Cultures raised under 25 ℃ produced plantlets with higher mean number of leaves (6.19) and a mean shoot height of 1.87 cm.

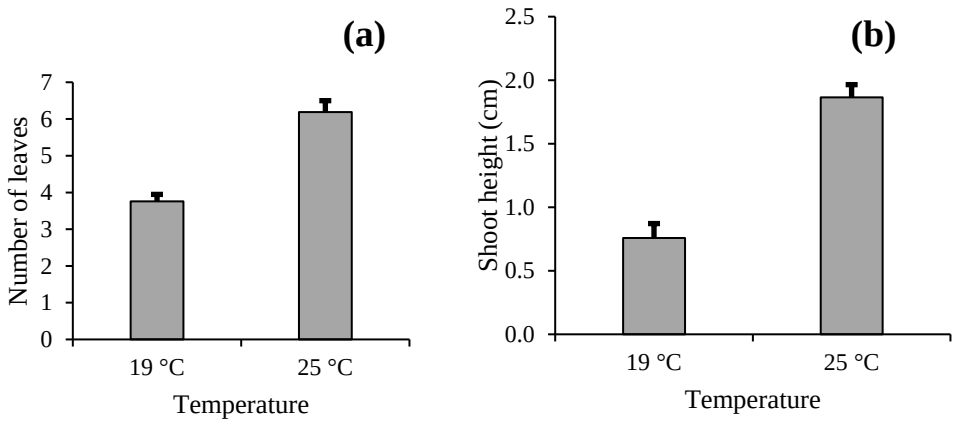

Figure 14: Plantlet's initiation response to temperature: (**a**) number of leaves and (**b**) shoot height.

Effect of explant type on the number of leaves, shoot height and weight of plantlet in the initiation culture

Slip generated plantlet with greater number of leaves (5.66) and plantlet with higher shoot height (1.53 cm) compared to sucker. However, the reserve was observed in the case of weight of initiated plantlet where the sucker was heavier (2.76 g) than the slip (Figure 15).

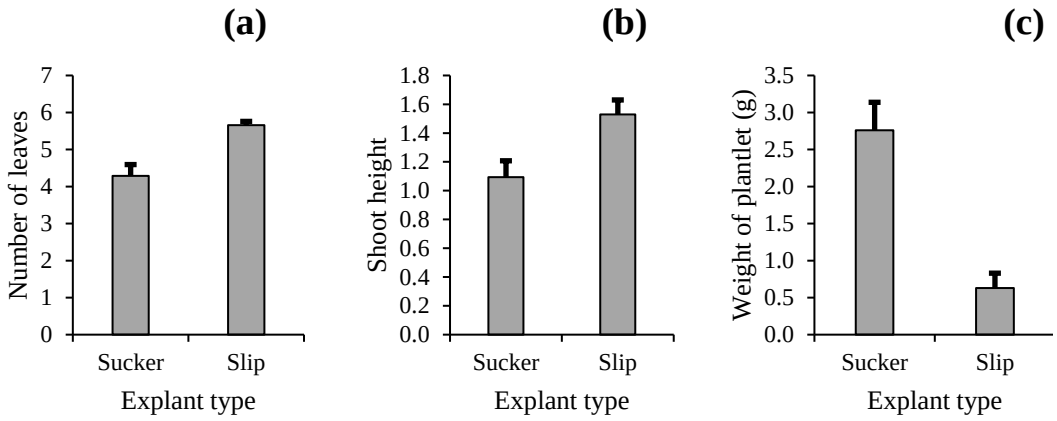

Figure 15: Plantlet's initiation response by explant type: (**a**) Number of leaves, (**b**) shoot height and (**c**) weight

Interaction effect of temperature and explant type on the number of leaves in initiated plantlet

The results of the of interaction between temperature and explant type on the mean number of leaves of plantlet from the initiation culture showed that the combined effect of slip and 25 ℃ generated plantlets with the highest mean number of leaves (7.4) and this was significantly higher compared to other interaction (Figure 16). This was followed by the interaction between sucker and 19 ℃, which also generated initiated plantlet with a mean number of leaves of 4.98. Initiated plantlets with the least number of leaves (3.6) were generated from the interactive effect of sucker and 19 ℃ (Figure 16). However, this was not significantly different from the interaction between slip and 25 ℃ incubation temperature.

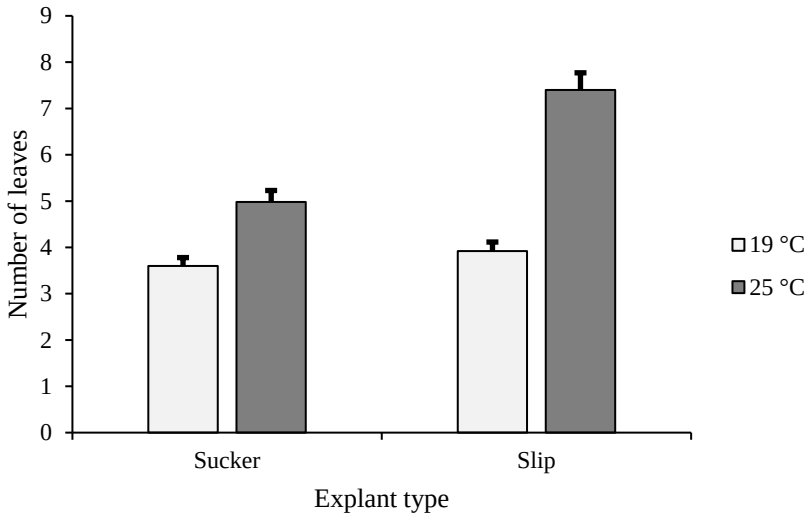

Figure 16: Interaction effect of temperature and explant type on the number of leaves in initiated plantlet
(Means were arcsine transformed)

Analysis of variance for multiplication culture parameters

The experiment focused on the effect of two types of tissue culture media, liquid and solid, on the multiplication and related parameters of four pineapple varieties, namely MD2, Smooth Cayenne Sugarloaf and Queen. ANOVA explicitly showed that differences among the mean number of shoots, the multiplication factor and plantlet weight were statistically significant ($P < 0.001$) among the media (Table 11). Plantlet calli score and plantlet weight showed significant difference at $P < 0.05$ and $P < 0.001$ respectively. Significant difference ($P < 0.05$) was observed for the interaction effect of media and variety on plantlets calli score and weight (Table 11).

Table 11: Combined ANOVA for multiplication culture parameters

Source of variation	Df	Mean sum of squares				
		Number of shoots	Multiplication factor	Callus score	Shoot height (cm)	Weight of plantlet (g)
Media (M)	1	351.562***	637.56***	5.56***	0.02	21.30***
Variety (V)	3	7.854	12.6	1.56*	0.38	4.09***
M × V	3	14.188	14.52	1.48*	0.02	0.91*
Residual	14	5.424	7.52	0.11	0.13	0.28

*** and * shows significant at $P < 0.001$ and $P < 0.05$ respectively

Effect of media type on plantlet number of shoot and multiplication factor

The effect of both liquid and solid medium on plantlets mean number of shoot and multiplication factor from the multiplication culture are shown in Figure 17. The result also showed the outperformance of liquid medium over the solid medium on their influence to plantlets mean number of shoot and multiplication factor (Figure 17).

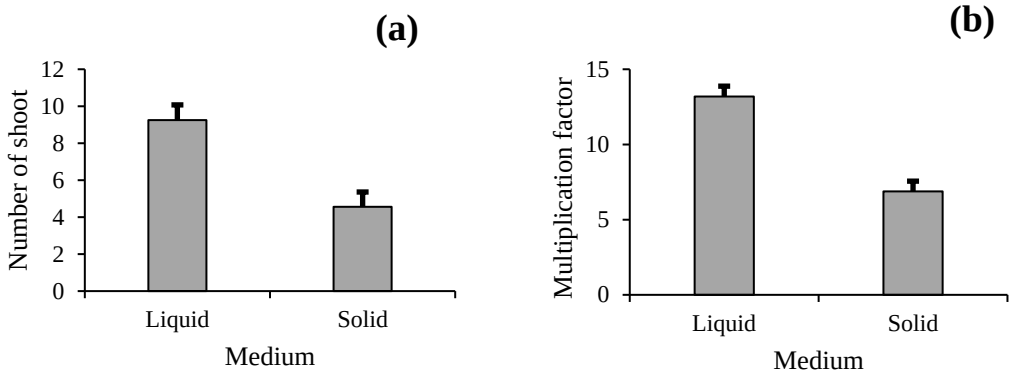

Figure 17: Number of shoot and multiplication factor of plantlet per medium type on multiplication culture

Plantlet calli score and weight per medium type in multiplication culture

Solid medium generated plantlets with the highest mean calli score of 0.56. Contrary, liquid medium generated plantlet with a mean weight greater than those from the solid medium (Figure 18b).

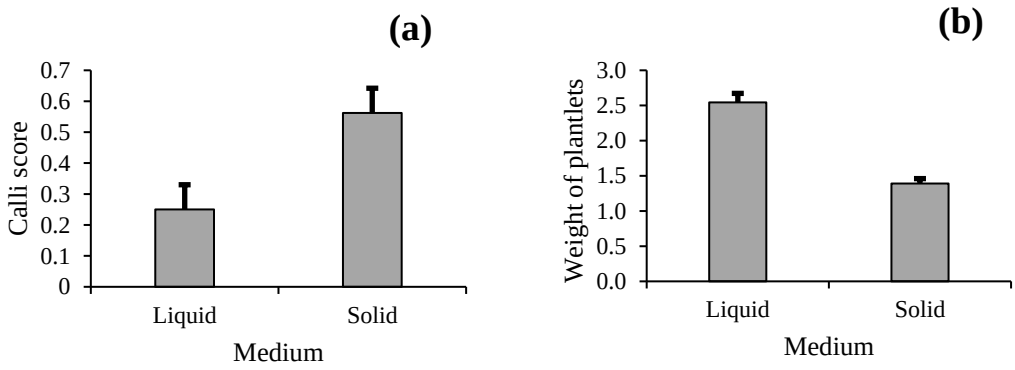

Figure 18: Calli score and weight of plantlet per medium type on multiplication culture

Plantlet calli score and weight per variety in multiplication culture

The extent of variation among the varieties in the multiplication culture regarding their response to mean calli score and mean weight of plantlets are detailed in Figure 19. MD2 and Sugarloaf generated plantlet without calli. Smooth

Cayenne and Queen recorded a mean calli score of 0.38 and 1.25, respectively (Figure 19) and both were significantly different ($P < 0.05$). Queen outperformed by generating plantlets with a mean weight (cluster) of 2.54 g from the multiplication culture and this was significantly different from that of MD2 and Sugarloaf. Smooth Cayenne also generated plantlet with mean weight of 2.21 g and was not significantly different from that of Queen (Figure 19b).

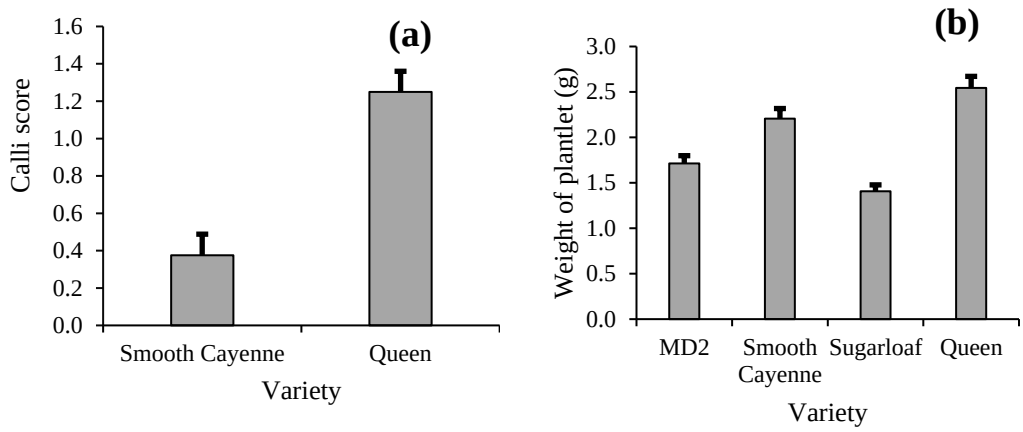

Figure 19: Calli score and weight of plantlet per variety in multiplication culture

Variety-media interaction for plantlet calli score and weight (cluster) in multiplication culture

Plantlets with the highest mean calli score of 1.5 were recorded from the interaction of Queen variety and solid medium and this significantly differed from the other interactions. The interaction between Queen and liquid medium also generated plantlet with a mean calli score of 1. The interaction of MD2 and Sugarloaf with (media irrespective of type) resulted plantlets with 0 mean calli score (Table 12).

Plantlets with the highest mean cluster weight of 3.10 g were generated from the interaction between Smooth Cayenne and liquid medium. Interestingly, this did

not differ significantly from the interaction between Queen and liquid medium which recorded a mean cluster weight of 2.87 g. The lowest mean cluster weight (0.92 g) was observed in plantlet generated from the interaction between Sugarloaf and solid medium (Table 12). However, this also did not differ significantly from the interaction between MD2 and solid medium which recorded a mean plantlet weight of 1.11 g.

Table 12: Medium type-variety interaction on calli score and weight (cluster) of plantlet from multiplication culture

	Calli score		Weight of plantlets (g)	
	Medium type		Medium type	
Variety	Liquid	Solid	Liquid	Solid
MD2	0	0.00	2.32	1.11
Smooth Cayenne	0	0.75	3.10	1.31
Sugarloaf	0	0.00	1.89	0.92
Queen	1	1.5	2.87	2.22
L.S.D	0.537		0.537	
S.E	1.602		0.1895	

Correlation of multiplication culture parameters

The results of the correlation analysis of calli in multiplication culture, plantlets number of shoots, multiplication factor, shoot height and cluster weight of plantlets cultured on both solid and liquid medium showed some significant association (Figure 20). Apart from the cluster weight of plantlets cultured on solid medium which had a weak positive correlation with the shoot height, other parameters on solid medium exhibited significant correlation ($P < 0.05, 0.001$) regardless of the direction (Figure 20b). In the case of liquid medium, the number of shoots showed a significant ($P < 0.05$) positive correlation (0.65) with callus

growth. However, a significant contracting correlation (-0.78, $P < 0.01$) was observed between the number of shoot and calli on solid medium (Figure 20).

Multiplication factor showed a weaker positive correlation (0.31) with the calli. Multiplication factor showed a significant ($P < 0.001$) positive correlation with the number of shoots (0.9) for plantlets cultured on both liquid and solid media. For plantlets cultured on liquid medium, shoot height showed a significant ($P < 0.01$) negative correlation with number of shoot and plantlets multiplication factor. Likewise, shoot height exhibited a significant ($P < 0.001$) negative correlation with both number of shoot and plantlets' multiplication factor when cultured on solid medium (Figure 20b). Interestingly, shoot height recorded a weak negative correlation (0.24) with calli in liquid medium and conversely showed a significant positive correlation ($P < 0.05$, 0.58) with calli growth in plantlet cultured on solid medium (Figure 20b). The plantlets weight also shows a weak (0.21) and fair (0.49) negative correlation with the number of shoots and multiplication factor respectively in the liquid culture. The weight of plantlets cultured on solid medium shows a strong positive correlation (0.94, $P < 0.001$) with the calli and a weak correlation of 0.33 with the shoot height. The plantlets weight also shows a negative correlation of 0.6 with the number of the shoot and the multiplication factor at $P < 0.05$ (Figure 20).

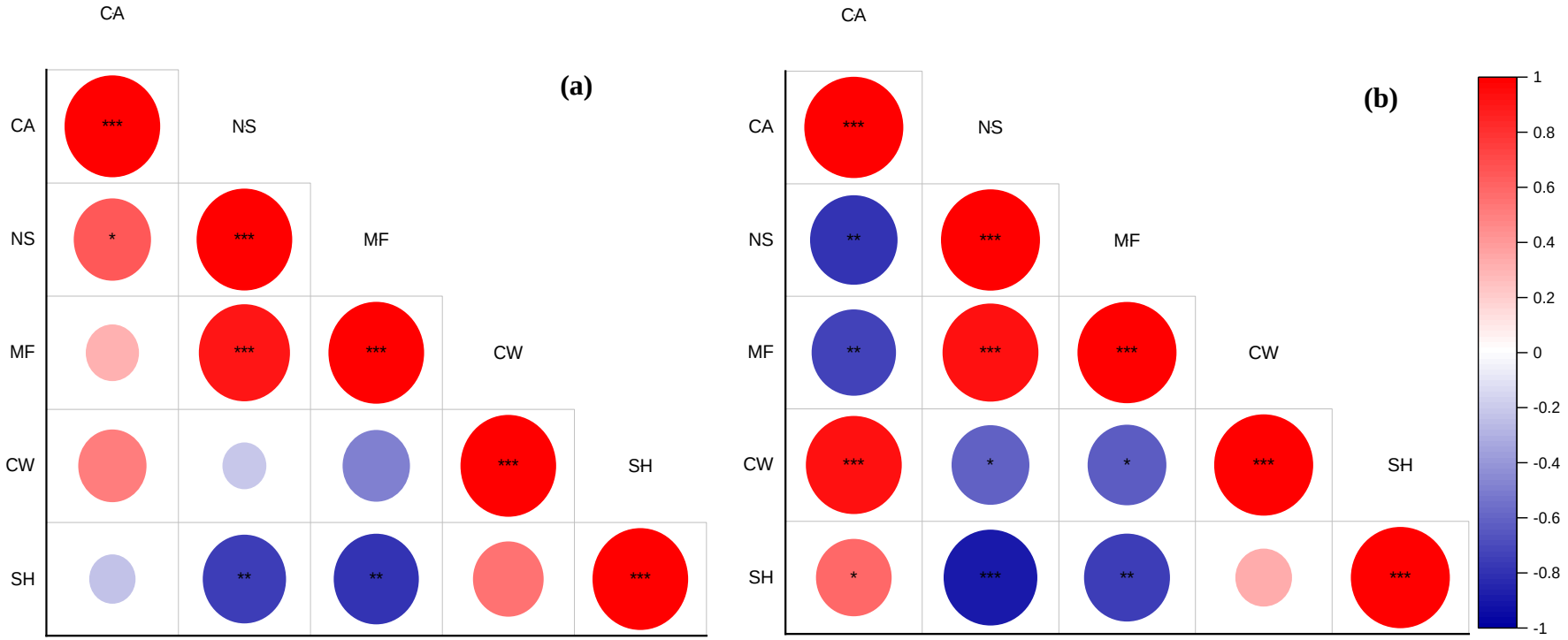

Figure 20: Correlation of multiplication culture parameters

*, ** and *** imply that correlation is significant at P = 0.05, P = 0.01 and P = 0.001 respectively. **a** = Cultured on liquid medium; **b** = Cultured on solid medium; **CA** = calli; **NS** = number of shoots; **MF** = multiplication factor; **SH** = shoots height; **CW** = Cluster weight of plantlet

Rooting in plantlets and their survival on acclimatisation

Percentage of rooted plantlets resulting from various treatments (Auxin levels, media type and incubation temperature) is presented in Table 13. Plantlets cultured under 25 ^{o}C recorded 100 % rooting and survival during acclimatisation six weeks after planting (6 WAP). At 12 weeks after planting, Sugarloaf plantlets cultured on liquid medium supplemented with 1 mg L^{-1} IAA along with those cultured on solid medium supplemented with 0.1 mg L^{-1} IAA recorded 87.5 % of survival.

Smooth Cayenne plantlets cultured on solid medium under the 19 ^{o}C recorded 50 % rooting for 0.1 mg L^{-1} IAA and 0 % for the 0.5 mg L^{-1} and 1 mg L^{-1} IAA on rooting. On percental survival, Smooth Cayenne cultured on solid medium recorded 100 % survival 6 WAP for the 0.1 mg L^{-1} and 1 mg L^{-1} IAA while the 0.5 mg L^{-1} IAA recorded 87.5 % survival. At 12 WAP, Cayenne cultured on solid medium under 19 ^{o}C recorded 100 %, 62.5 % and 75 % survival for 0.1 mg L^{-1}, 0.5 mg L^{-1} and 1 mg L^{-1} IAA respectively.

Sugarloaf on solid culture at 19 ^{o}C had 75 % of rooted plantlets at all IAA levels but recorded 100 % survival at 6 and 12 WAP whilst the 0.5 mg L-1 reduced from 87.5 % to 62.5 % (Table 13). MD2 and Queen cultured under the 19 °C irrespective of IAA level recorded 100 % for rooting and survival at both 6 and 12 WAP.

Table 13: Rooting in plantlets cultured on different treatments (Auxin levels, media type and temperature) and their survival on acclimatisation

Media type	Temp.	Auxin (mg L^{-1})	Variety	% Rooted plantlet	% Survival 6 WAP	% Survival 12 WAP
Liquid	25 °C	IAA (0.1)	MD2	100	100	100
			S. Cayenne	100	100	100
			Sugarloaf	100	100	100
			Queen	100	100	100
		IAA (0.5)	MD2	100	100	100
			S. Cayenne	100	100	100
			Sugarloaf	100	100	100
			Queen	100	100	100
		IAA (1.0)	MD2	100	100	100
			S. Cayenne	100	100	100
			Sugarloaf	100	100	87.5
			Queen	100	100	100
Solid	25 °C	IAA (0.1)	MD2	100	100	100
			S. Cayenne	100	100	87.5
			Sugarloaf	100	100	100
			Queen	100	100	100
		IAA (0.5)	MD2	100	100	100
			S. Cayenne	100	100	100
			Sugarloaf	100	100	100
			Queen	100	100	100
		IAA (1.0)	MD2	100	100	100
			S. Cayenne	100	100	100
			Sugarloaf	100	100	100
			Queen	100	100	100
Solid	19 °C	IAA (0.1)	MD2	100	100	100
			S. Cayenne	50	100	100
			Sugarloaf	75	100	100
			Queen	100	100	100
		IAA (0.5)	MD2	100	100	100
			S. Cayenne	0	87.5	62.5
			Sugarloaf	75	100	100
			Queen	100	100	100
		IAA (1.0)	MD2	100	100	100
			S. Cayenne	0	100	75
			Sugarloaf	75	100	100
			Queen	100	100	100

Analysis of variance of rooting culture parameter

The results of ANOVA of plantlet response to rooting are shown in Table 14.. Plantlets mean number of roots were significant for variety ($P < 0.001$) and incubation temperature ($P < 0.001$) as well as the variety by temperature interaction ($P < 0.001$).

Root length, lateral roots score and shoot height of plantlet showed significant difference ($P < 0.001$) based on variety, hormone level and the incubation temperature. Again, plantlets number of roots, length of roots, lateral root score and number of leaves were significantly different ($P < 0.001$) due to incubation temperature and media type interaction (Table 14).

There were significant differences in the weight of plantlet ($P < 0.001$) with respect to variety, hormone level, incubation temperature, media type and all their interactions (Table 14).

Table 14: Combined ANOVA for root and related parameters from rooting culture

Source of variation	Degree of freedom	Mean sum of squares						
		Number of roots	Root length (cm)	Lateral root score	Number of side shoots	Number of leaves	Shoot height (cm)	Weight of plantlet (g)
Variety (V)	3	14.5498***	11.8431***	5.6667***	8.5278***	27.6794***	10.125***	2.25644***
Hormone level (H)	2	1.6354	2.229***	5.5139***	0.7326	1.8785**	1.5911***	0.25125***
Temperature (T)	1	78.7656***	19.501***	29.3403***	2.5069*	0.69	6.2709***	5.5225***
V × H	6	1.2095	0.1945	0.1806***	0.5938	0.49	0.5275**	0.33755***
V × T	3	6.1638***	0.395	0.3958	1.7431**	3.8935***	1.9861***	0.47954***
H × T	2	0.1615	0.205	0.0903	0.0538	2.7569***	0.035	0.28***
T × Media type (M)	1	14.6302***	17.9256***	6.0208***	0.75	6.75***	0.001	0.65333***
V × H × T	6	1.7332*	0.1001	0.0903	0.0885	1.8171***	0.266	0.08926***
V × T × M	3	1.908*	1.4222	0.0764	0.5972	3.0972***	1.191	0.77556***
H × T × M	2	3.2865*	0.0583	0.1458	0.1094	0.19	0.399	0.16583***
V × H × T × M	6	2.1684*	0.3468	0.2014	0.1441	0.9097*	0.7981***	0.16472***
Residual	35	0.682	0.159	0.1937	0.3571	0.29	0.128	0.01239

*, ** and *** shows significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively

Number of root and length per variety

Mean number of roots and mean root length recorded by each pineapple variety are shown in Figure 21. Queen and MD2 were almost at par with mean root number of 4.3 cm and 4.2 cm respectively. Smooth Cayenne recorded the lowest mean number of roots (2.3 cm) which differed significantly from the other varieties as shown in Figure 21a.

Queen also recorded the highest mean root length (2.4 cm), which did not differ significantly from Sugarloaf, which had a mean root length of 2.07 cm. On the other hand, Smooth Cayenne recorded the lowest mean root length (0.6 cm), differing significantly from the other varieties (Figure 21b).

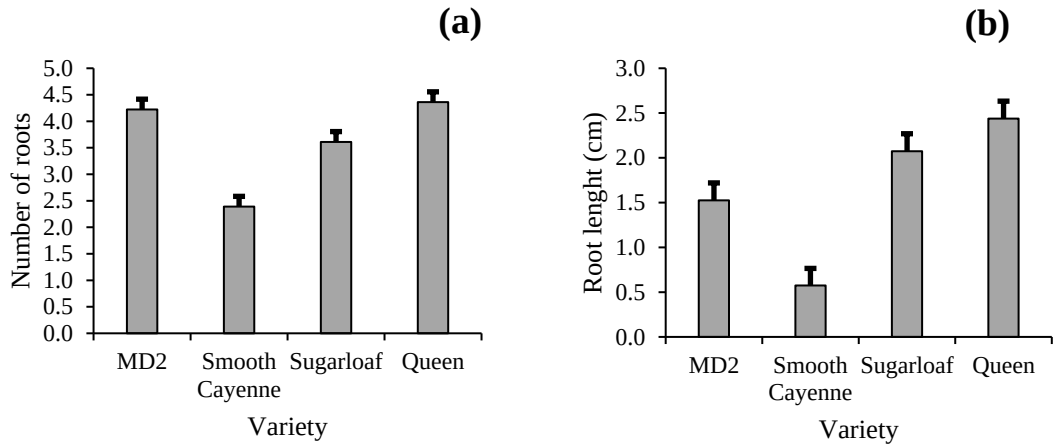

Figure 21: Number of roots (**a**) and root length (**b**) among pineapple varieties

Lateral root score and number of side shoot per variety

MD2 recorded the highest mean lateral root score of 2.5 followed by sugarloaf and Queen which recorded the same mean lateral root sore of 2.3 (Figure 22a). Smooth Cayenne recorded the lowest lateral root score of 1.3 Figure 22a.

However, Smooth cayenne recorded the highest mean number of side shoot Figure 22b.

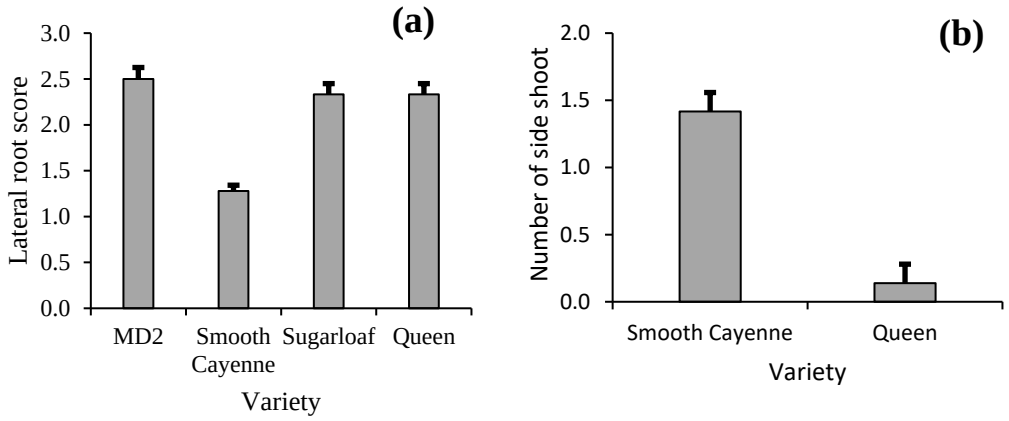

Figure 22: Lateral root score (**a**) and number of side shoots (**b**) among pineapple varieties

Number of roots and shoot height per variety

Plantlets mean number of leaves and mean shoot height are shown in Figure 23. MD2 recorded the highest mean number of leaves (12.3), significantly differing from Sugarloaf (10.5) and Smooth Cayenne (9.2) (Figure 23a).

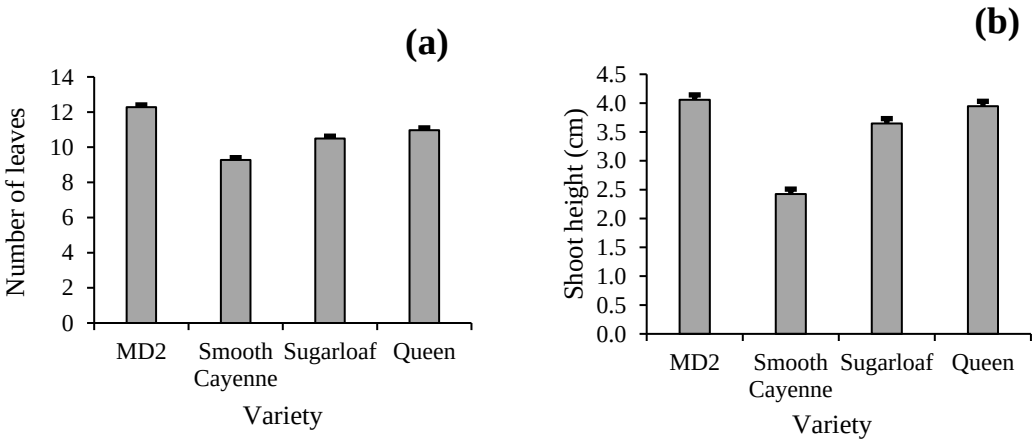

Figure 23: Number of roots and shoot height per variety

Queen recorded a mean number of roots of 10.97, which did not significantly differ from that of Sugarloaf.

A similar pattern is observed in Figure 23b, where MD2 recorded the highest mean shoot (4 cm) significantly differing from Smooth Cayenne, which recorded the lowest shoot height of 2.4 cm. The differences in mean shoot height were not differ between Queen and Sugarloaf, which recorded 3.95 cm and 3.65 cm respectively (Figure 23b).

Weight of plantlet per variety

The mean weight of plantlet per variety are shown in Figure 24. MD2 recorded the highest mean weight (3.4 g) and deferred significantly among other varieties. The weight of smooth cayenne, sugarloaf and queen are 2.7 g, 2.8 g and 2.7 g respectively and were not significantly different (Figure 24).

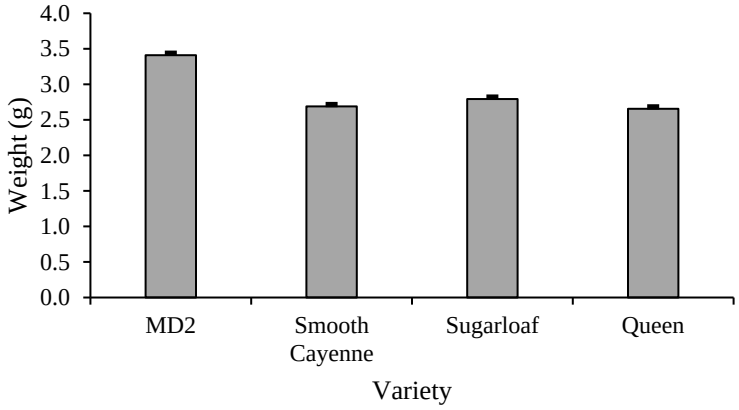

Figure 24: Weight of plantlet per variety

Lateral root score and root length of plantlets as influenced by hormone level

The effect of rooting hormone on plantlets lateral root score and root length are shown in Figure 25. Plantlets' lateral root score significantly decreased from 2.6 to 1.6 as the hormone level increased from 0.1 mg L^{-1} to 1.0 mg L^{-1} (Figure 25a). Media supplemented 0.5 mg L^{-1} IAA generated the highest mean root length (1.92 cm) as shown in Figure 25b.

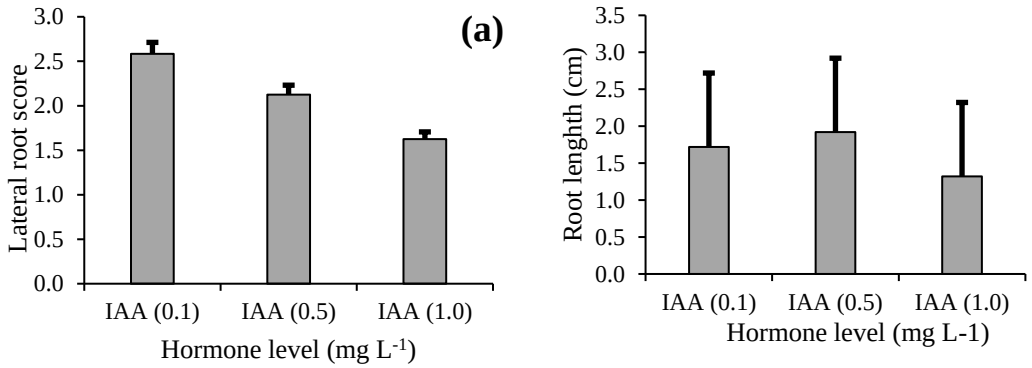

Figure 25: Lateral root score (**a**) and root length (**b**) of plantlet per hormone level

Number of leaves and shoot height of plantlet as influenced by hormone level

The mean number of leaves of plantlets decreased as the level of IAA increased Figure 26a. Plantlets cultured on media supplemented with 0.5 mg L^{-1} IAA produced the highest mean shoot height (3.8 cm), which significantly differed from the mean shoot height of plantlet cultured on media supplemented with 0.1 mg L^{-1} IAA and 0.5 mg L^{-1} IAA (Figure 26b).

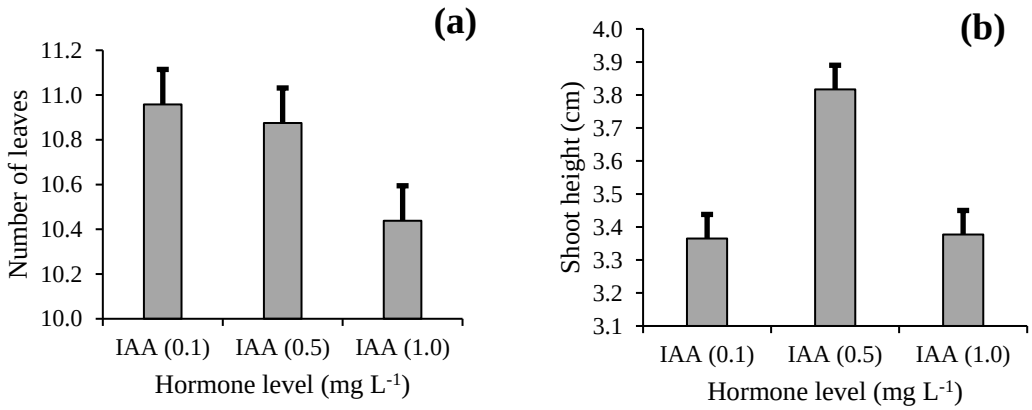

Figure 26: Effect of hormone level on plantlets' number of leaves and shoot height

Weight of plantlet as influenced by hormone level

The effect of hormone level on the weight of plantlets are shown in Figure 27. Plantlets from media supplemented with 0.5 mg L^{-1} IAA recorded the highest mean weight of 3.0 g differing significantly from those cultured on 0.1 mg L^{-1} and 1 mg L^{-1} IAA.

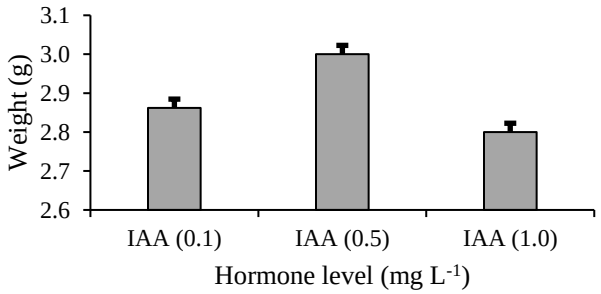

Figure 27: Effect of hormone level on plantlets' weight

Number of roots, root length and lateral root score of plantlets as influenced by temperature

The effect of two incubation temperature (19 °C and 25 °C) on the mean number of roots, root length and lateral root score of plantlets are shown in Figure 28.

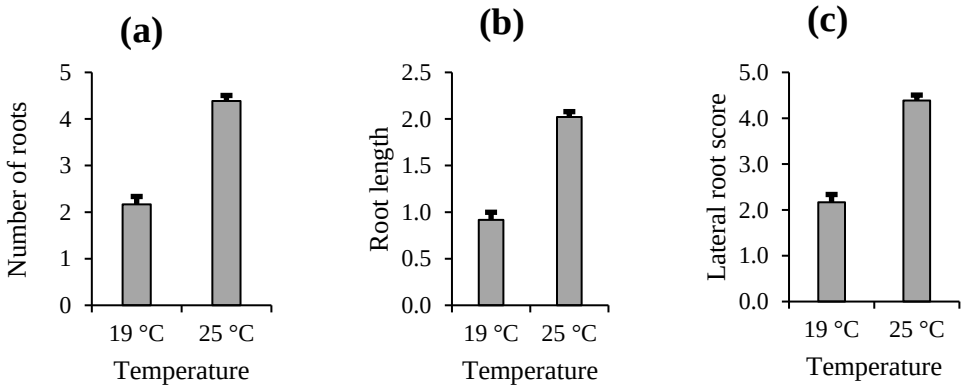

Figure 28: Effect of incubation temperature on plantlets' (**a**) number of roots, (**b**) root length, and (**c**) lateral root score

Plantlet cultured under 25 °C outperformed those that were cultured under 19 °C in terms of their mean number of roots, length of roots and lateral root score (Figure 28).

Number of side shoots, shoot height and weight of plantlets as influenced by incubation temperature

The effect of two incubation temperature (19 °C and 25 °C) on the mean number of side shoot, shoot height and weight of plantlets, is recorded in Figure 29. Plantlet cultured under 25 °C outperformed those that were cultured under 19 °C in terms of their mean number of side shoot, shoot height and weight (Figure 29).

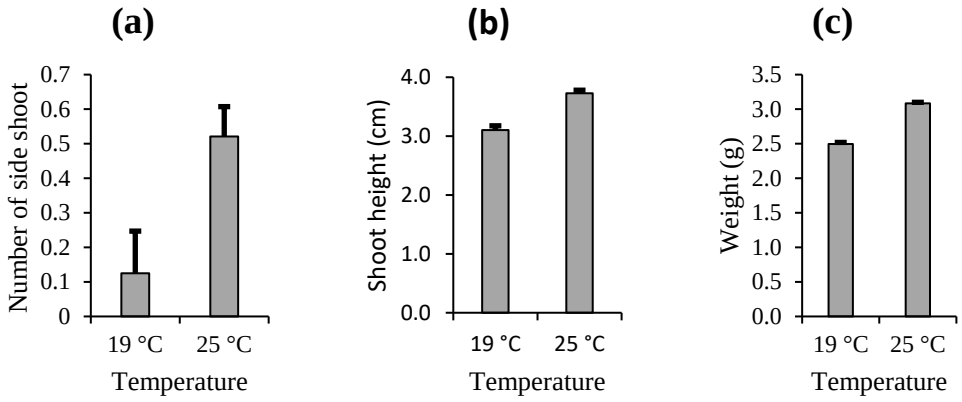

Figure 29: Effect of temperature on plantlets' (**a**) number of side shoot, (**b**) shoot height and (**c**) weight

Variety-hormone level interaction on plantlets mean lateral root score and weight

The results of variety and hormone level interaction is shown in Table 15. The highest lateral root score (3.0) was recorded by Queen with 0.1 mg L^{-1} IAA, significantly differing from other interactions. Generally, lower lateral root scores were recorded for Smooth Cayenne and hormone interaction. The lowest lateral roots score was generated by plantlets from Smooth Cayenne with 1.0 mg L^{-1} IAA

which significantly differed from the interaction between Smooth Cayenne and 0.1 mg L^{-1} IAA (Table 15).

Table 15: Variety and hormone level interaction on plantlet's mean lateral root score and weight

	lateral root score			Weight of plantlet		
	Hormone level (mg L^{-1})			Hormone level (mg L^{-1})		
Variety	IAA (0.1)	IAA (0.5)	IAA (1.0)	IAA (0.1)	IAA (0.5)	IAA (1.0)
MD2	2.83	2.50	2.17	3.40	3.30	3.53
S. Cayenne	1.83	1.17	0.83	2.50	2.80	2.77
Sugarloaf	2.67	2.50	1.83	2.68	3.13	2.57
Queen	3.00	2.33	1.67	2.87	2.77	2.33
L.S.D		0.52			0.13	
S.E		0.18			0.05	
% CV		3.7			2.7	

The heaviest (3.4 g) of plantlets were generated from the interaction between MD2 and 0.1 mg L^{-1} IAA. However, this did not significantly differ from the mean weight observed for the interaction between MD2 and either 0.5 mg L^{-1} IAA and 1.0 mg L^{-1} IAA. Conversely, the lighter plantlets (2.33 g) were generated from the interactive effect of Queen and 1.0 mg L^{-1} IAA, not significantly differing from the result observed between the interaction of Smooth Cayenne and 0.1 mg L^{-1} IAA (Table 15).

Variety-temperature effect on plantlets number of roots, shoot height, leaves and weight.

Results on the interactive effect of pineapple variety and incubation temperature are shown in Table 16. The highest mean number of roots (4.75) was recorded by MD2 and sugarloaf at 25 °C, significantly differing from other interactions except for the mean number of roots generated from the interaction between Queen and 25 °C. Contrarily, plantlets with the significantly lowest mean

number of roots were also generated from the interactive effect of 19 °C and Smooth Cayenne.

That notwithstanding, Smooth Cayenne's interaction with incubation temperature of 25 °C produced plantlets with the highest mean number of side shoot (1.88), significantly different from the other interactions.

Table 16: Interaction effect of variety and incubation temperature on plantlet's mean number of roots, side shoot, leaves and weight

	Number of roots		Number of side shoots		Number of leaves		Weight of plantlet (g)	
Variety	19 °C	25 °C	19 °C	25 °C	19 °C	25 °C	19 °C	25 °C
MD2	3.17	4.75	0.00	0.00	12.00	12.42	2.83	3.70
S. Cayenne	0.33	3.42	0.50	1.88	9.25	9.29	2.20	2.93
Sugarloaf	1.33	4.75	0.00	0.00	10.25	10.63	2.35	3.02
Queen	3.83	4.63	0.00	0.21	12.08	10.42	2.60	2.68
L.S.D	0.68		0.43		0.55		0.11	
S.E	0.24		0.17		0.23		0.03	
% CV	5.1		0		2.1		2.7	

MD2's interaction with 25 °C incubation temperature produced plantlets with the highest number of leaves as well as plantlets with the heaviest mean weight of 3.70 g (Table 16). Plantlets with the least weight were produced from Smooth Cayenne interaction with 19 °C incubation temperature and was significantly different from the other interaction (Table 16).

Temperature-hormone level interaction effect on plantlets' mean number of leaves and weight

The result of plantlets number of leaves and weight as affected by incubation temperature and hormone level interaction are shown in Table 17. The interaction effect between IAA 0.1 mg L^{-1} and 25 °C generated plantlets with the highest mean number of roots (11.5) which did not significantly differ from

interaction effect of 0.1 mg L^{-1} IAA and 19 °C on plantlet mean number of leaves. The lowest mean number of leaves (10.38) were recorded from the interactional effect of the IAA 1.0 mg L^{-1} under 25 °C (Table 17).

The heaviest of plantlets (3.01 g, and 2.98 g) were recorded from the interaction effect of IAA 1.0 mg L^{-1} under 25 °C and IAA 0.1 mg L^{-1} under 25 °C respectively. On the other hand, the lighter weight was recorded for plantlets from the interaction effect of IAA 0.1 mg L^{-1} under 19 °C (Table 17).

Table 17: Interaction effect of incubation temperature and hormone level on plantlet's mean number of leaves and weight

Incubation temperature	Hormone level (mg L^{-1})	Number of leaves	Weight of plantlet (g)
19 °C	IAA (0.1)	10.63	2.64
	IAA (0.5)	11.50	2.48
	IAA (1.0)	10.56	2.38
25 °C	IAA (0.1)	11.13	2.98
	IAA (0.5)	10.56	3.26
	IAA (1.0)	10.38	3.01
L.S.D		0.48	0.10
S.E		0.14	0.03
% CV		2.10	2.70

Temperature-media interaction effect on roots and related parameters

The interaction effect of incubation temperature and media type on plantlet root characteristics are shown in Table 18. The highest mean number of roots (4.94) was recorded from the interaction between 25 °C incubation temperature and liquid medium. A similar observation is shown in plantlets mean lateral root score, number of leaves and weight whose value were 2.92, 11.06, 3.2 g respectively. Contrarily, the interactive effect of solid medium and 25 °C incubation temperature generated the best mean root length of 2.63 cm and a shoot height of 3.73 cm. The

mean shoot height (3.72 cm) observed for the interaction between liquid medium and 25 °C incubation temperature did not significantly differ from that of the shoot height recorded by solid medium and 25 °C incubation temperature (Table 18).

Table 18: Incubation temperature and media type interaction on plantlet's mean number of roots, root length, lateral root score, shoot height, number of leaves and weight

Incubation temperature	Media type	Number of roots	Root length (cm)	Lateral root score	Number of leaves	Shoot height (cm)	Weight of plantlet (g)
19 °C	Liquid	-	-	-	-	-	-
	Solid	2.167	0.92	1.21	10.90	3.10	2.50
25 °C	Liquid	4.94	1.41	2.92	11.06	3.72	3.20
	Solid	3.83	2.63	2.21	10.31	3.73	2.97
L.S.D		0.48	0.23	0.26	0.32	0.21	0.07
S.E		0.17	0.08	0.09	0.16	0.10	0.03
% CV		5.10	11.80	3.70	2.10	2.50	2.7

Interactive effect of variety, hormone level and incubation temperature on the mean number of roots, leaves and weight of plantlets.

Results of the three-way interaction of pineapple variety, hormone level and incubation temperature on the means of plantlet number of roots, number of leaves and weight are detailed in Table 19. For plantlets mean number of roots, MD2 interacting with 0.1 mg L^{-1} IAA at 25 °C incubation temperature generated the highest mean number of roots (5.50). The interactive effect of Smooth Cayenne and 0.5 or 1.0 mg L^{-1} IAA at 19 °C produce plantlet with no root (0). Generally, the interaction effect of pineapple varieties and all hormone levels at 19 °C recorded lower mean number of roots than the interaction effect of pineapple variety and all hormone levels at 25 °C incubation temperature (Table 19).

Table 19: Interaction effect of variety, hormone level and incubation temperature on plantlets mean number of roots, leaves and weight

Variety	Hormone level (m g L^{-1})	Number of roots		Number of leaves		Weight of plantlet (g)	
		19 °C	25 °C	19 °C	25 °C	19 °C	25 °C
MD2	IAA (0.1)	3.75	5.50	12.00	12.88	3.20	3.50
	IAA (0.5)	3.75	4.38	12.00	12.38	2.50	3.70
	IAA (1.0)	2.00	4.38	12.00	12.00	2.80	3.90
Smooth Cayenne	IAA (0.1)	1.00	2.38	8.75	9.50	2.20	2.65
	IAA (0.5)	0.00	4.38	9.75	9.50	2.10	3.15
	IAA (1.0)	0.00	3.50	9.25	8.88	2.30	3.00
Sugarloaf	IAA (0.1)	1.00	5.13	8.75	11.38	2.35	2.85
	IAA (0.5)	1.25	5.00	12.00	10.38	2.50	3.45
	IAA (1.0)	1.75	4.13	10.00	10.13	2.20	2.75
Queen	IAA (0.1)	3.50	4.38	13.00	10.75	2.80	2.90
	IAA (0.5)	4.25	5.00	12.25	10.00	2.80	2.75
	IAA (1.0)	3.75	4.50	11.00	10.50	2.20	2.40
L.S.D		1.45		0.95		0.20	
S.E		0.41		0.27		0.06	
% CV		5.10		2.10		2.70	

Plantlets with the highest mean number of leaves (13) was observed from the interaction of Queen by 0.1 mg L-1 IAA at 25 °C. The next highest plantlets mean number of leaves resulted from the interaction of MD2 variety and 0.1 mg L-1 IAA at 25 °C. Mean number of leaves of 8.75 was recorded for the interaction of Smooth Cayenne and 0.1 mg L-1 IAA at 19 °C and this was the observed lowest number of leaves recorded for the interaction among varieties, hormone level and incubation temperature (Table 19).

The heaviest of plantlet in terms of the mean weight (3.9 g) was recorded from the interaction of MD2, 1.0 mg L-1 IAA and 25 °C incubation temperature while the lightest plantlets mean weight (2.1 g) was as a result of the interaction of Smooth Cayenne, 0.5 mg L-1 IAA and 19 °C incubation temperature (Table 19).

Interactive effect of variety, media type and incubation temperature on the mean number of roots, leaves and weight of plantlets

The effect of a three-way interaction of variety, media type and incubation temperature on plantlets mean number of roots, leaves and weight is shown in Table 20. A combined effect of MD2, liquid medium from bioreactor and an incubation temperature of 25 °C produced plantlets with the highest mean number of roots of 3.93. The effect of MD2, solid medium and an incubation temperature of 25 °C produced plantlets with the next highest mean number of roots of 3.47 (Table 20). For interactions involving 25 °C incubation temperature, its interaction with Smooth Cayenne and solid medium produced plantlets with the lowest mean number of roots. Similarly, the interaction effect of Smooth Cayenne, solid medium and 19 °C incubation temperature generated plantlets with a mean number of roots of 2.2.

Plantlets that possessed the highest mean number of leaves of 5.67 were observed from the interaction effect of Sugarloaf variety with liquid medium and 25 °C incubation temperature and this slightly outperformed the mean number of leaves from the interaction effect of Queen variety, liquid medium and 25 °C incubation temperature which recorded a mean of 5.50. On the other hand, plantlets with the lowest number of leaves of 0.33 was observed from the of Smooth Cayenne variety, solid medium and 19 °C (Table 20).

Again, the result showed that plantlets that were lighter (2.2 g) emerged from the interaction of Smooth Cayenne, solid medium and 19 °C incubation temperature (Table 20). Contrary, the heaviest of plantlets (3.93 g) were seen from

the interaction of MD2, liquid medium and 25 °C incubation temperature, significantly ($P < 0.05$) different from the other interactions.

Table 20: Interaction effect of variety, media type and incubation temperature on plantlet's mean number of roots, leaves and weight

Variety	Media type	Number of roots		Number of leaves		Plantlet weight (g)	
		19 °C	25 °C	19 °C	25 °C	19 °C	25 °C
MD2	Liquid	-	3.93	-	4.92	-	3.93
	Solid	2.83	3.47	3.17	4.58	2.83	3.47
Smooth Cayenne	Liquid	-	3.33	-	3.67	-	3.33
	Solid	2.2	2.53	0.333	3.17	2.20	2.53
Sugarloaf	Liquid	-	2.83	-	5.67	-	2.83
	Solid	2.35	3.20	1.333	3.83	2.35	3.20
Queen	Liquid	-	2.70	-	5.50	-	2.70
	Solid	2.6	2.67	3.833	3.75	2.60	2.67
L.S.D		0.97		0.64		0.13	
S.E		0.34		0.22		0.05	
% CV		5.10		2.10		2.70	

Interaction effect of hormone level, media type and incubation temperature on the mean number of roots, leaves and weight of plantlets

Mean number of roots and weight of plantlets as influenced by the interaction of hormone level, media type and incubation temperature are shown in Table 21. The interaction effect of 0.5 mg L^{-1} IAA, solid medium and 25 °C incubation temperature generated plantlets with a mean number of roots of 5.67. That of liquid medium, 1.0 mg L^{-1} IAA and 25 °C also generated plantlet with a mean of 4.69 number of roots. The interaction effect of solid medium, 0.1 mg L^{-1} IAA and 19 °C incubation temperature generated plantlet with mean number of roots of 2.31 and this was the same as the mean number of roots from the interaction effect of 0.5 mg L^{-1} IAA, solid medium and 19 °C incubation temperature.

Table 21: Interaction effect of hormone level, media type and incubation temperature on plantlet's mean number of roots, and weight

Hormone level (mg L^{-1})	Media type	Number of roots		Plantlet weight (g)	
		19 °C	25 °C	19 °C	25 °C
IAA (0.1)	Liquid	-	4.44	-	2.98
	Solid	2.31	4.25	2.64	2.98
IAA (0.5)	Liquid	-	5.69	-	3.43
	Solid	2.31	3.69	2.48	3.10
IAA (1.0)	Liquid	-	4.69	-	3.20
	Solid	1.88	3.56	2.38	2.83
L.S.D		0.84		0.11	
S.E		0.29		0.04	
% CV		5.10		2.70	

Again, plantlets with the highest mean weight (3.43 g) were from the interaction effect 0.5 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature. However, plantlets that recorded the least weight of 2.38 g were generated from the interactive effect of 1.0 mg L^{-1} IAA, solid medium and 19 °C incubation temperature and this same interactive effect generated plantlet with the lowest mean number of roots of 1.88 (Table 21).

Mean number of roots, number of leaves, shoot height, and weight of plantlets as influenced by the interaction between variety, hormone level, media type, and incubation temperature

The result showed that plantlet with the highest mean number of root (7) were generated by the interaction of Queen variety, 0.5 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature (Table 22). The interaction between Sugarloaf, 0.1 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature also generated plantlet with mean number of roots of 6.5. Contrary, the interaction between Smooth Cayenne, 0.1 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature

generated plantlet with the lower mean number of roots of 1.5. Again, the interaction between Smooth Cayenne, 0.5 mg L^{-1} IAA, solid medium and 19 °C incubation temperature also generated plantlet without roots (0) (Table 22).

The interactions produced plantlets with varying number of roots. Plantlets with the highest mean number of leaves (14) were a product of MD2, 0.1 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature. Table 22 shows that the lowest mean number of leaves were recorded from plantlet from the interaction between Smooth Cayenne, 0.1 mg L^{-1} IAA, solid medium and 25 °C incubation temperature.

A combined effect of Queen, 0.5 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature gave off plantlet with the highest mean shoot height of 5.45 cm and this was followed by a mean shoot height of 4.88 cm which was a product of the interaction between MD2, 1.0 mg L^{-1} IAA, solid medium and 25 °C incubation temperature (Table 22).

Plantlets with the highest mean weight (4.20 g) were generated by the interaction between MD2, 1.0 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature. The interaction between MD2, 0.5 mg L^{-1} IAA, liquid medium and 25 °C incubation temperature also generated plantlet with a mean weight of 4.1 g (Table 22).

Table 22: Interaction effect of variety, hormone level, media type and incubation temperature on plantlet's mean number of roots, leaves, shoot height and weight

Variety	Hormone level (mg L^{-1})	Media type	Number of roots		Number of leaves		Shoot height (cm)		Weight of plantlet (g)	
			19 °C	25 °C	19 °C	25 °C	19 °C	25 °C	19 °C	25 °C
MD2	IAA (0.1)	Liquid	-	5.50	-	14.00	-	4.70	-	3.50
		Solid	3.75	5.50	12	11.75	2.95	4.50	3.2	3.50
	IAA (0.5)	Liquid	-	5.00	-	12.75	-	4.70	-	4.10
		Solid	3.75	3.75	12	12.00	3	4.35	2.5	3.30
	IAA (1.0)	Liquid	-	4.25	-	12.50	-	4.33	-	4.20
		Solid	2	4.50	12	11.50	3.13	4.88	2.8	3.60
S. Cayenne	IAA (0.1)	Liquid	-	1.50	-	10.75	-	2.70	-	3.20
		Solid	1	3.25	8.75	8.25	2.1	2.13	2.2	2.10
	IAA (0.5)	Liquid	-	5.00	-	10.25	-	3.10	-	3.60
		Solid	0	3.75	9.75	8.75	2.3	2.45	2.1	2.70
	IAA (1.0)	Liquid	-	4.50	-	9.00	-	2.78	-	3.20
		Solid	0	2.50	9.25	8.75	1.95	2.33	2.3	2.80
Sugarloaf	IAA (0.1)	Liquid	-	6.50	-	10.50	-	3.43	-	2.50
		Solid	1	3.75	8.75	12.25	2.73	4.43	2.35	3.20
	IAA (0.5)	Liquid	-	5.75	-	10.00	-	3.43	-	3.30
		Solid	1.25	4.25	12	10.75	3.36	4.98	2.5	3.60
	IAA (1.0)	Liquid	-	4.75	-	10.25	-	3.40	-	2.70
		Solid	1.75	3.50	10	10.00	3.58	3.53	2.2	2.80
Queen	IAA (0.1)	Liquid	-	4.25	-	11.25	-	2.93	-	2.70
		Solid	3.5	4.50	13	10.25	3.78	4.03	2.8	3.10
	IAA (0.5)	Liquid	-	7.00	-	10.50	-	5.45	-	2.70
		Solid	4.25	3.00	12.25	9.50	5	3.70	2.8	2.80
	IAA (1.0)	Liquid	-	5.25	-	11.00	-	3.75	-	2.70
		Solid	3.75	3.75	11	10.00	3.53	3.53	2.2	2.10
L.S.D			1.6766		1.1007		0.7264		0.226	
S.E			0.5840		0.3834		0.2530		0.0787	
% CV			5.1		2.1		2.5		2.7	

Correlation among rooting culture parameters

The results of the correlation analysis of the parameters measured in the rooting experiment showed some significant association (Figure 30). Plantlets' number of roots showed a significant ($P < 0.05$) positive correlation (0.48) with the root length. Lateral root score showed a significant ($P < 0.001$) positive correlation (0.68) with number of roots. LRS also shared a significant ($P < 0.05$) positive correlation with root length (0.48).

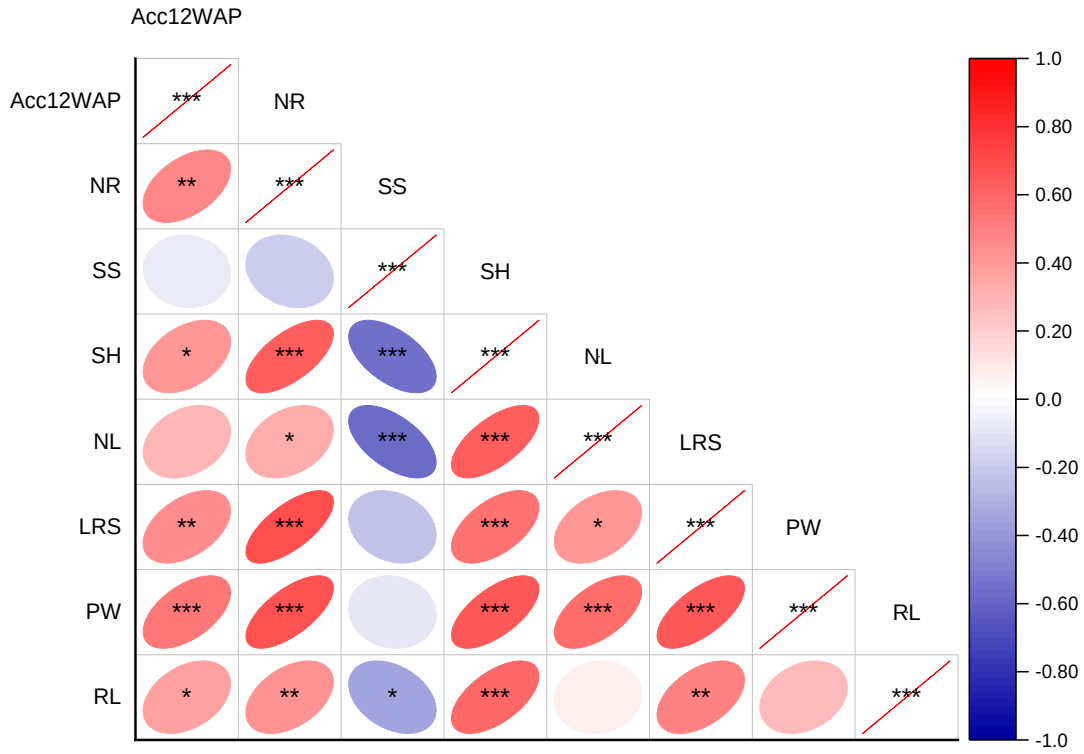

Figure 30: Correlation of roots and related parameters

*, ** and *** imply that correlation is significant at P = 0.05, P = 0.01 and P = 0.001 respectively. **NR** = number of roots; **RL** = root length; **LRS** = lateral root score; **SS** = number of side shoots; **SH** = shoot height; **NL** = number of leaves; **PW** = plantlet weight; **Acc12WAP** = plantlets acclimatisation 12 weeks after planting

Number of side shoots showed a significant ($P < 0.05$) negative correlation (0.48) with the root length. Shoot height shared a significant ($P < 0.001$) positive

correlation with number of roots, root length, and lateral root score (Figure 30). Contrarily, shoot height showed a significant ($P < 0.001$) negative correlation with number of side shoots (0.55). Number of leaves together with the root number and lateral root score exhibited a significant ($P < 0.05$) positive correlation of 0.3 and 0.4 respectively. Additionally, number of leaves showed a significant ($P < 0.001$) negative correlation (0.57) with number of side shoots.

Weight of the plantlet showed a significant ($P < 0.001$) positive correlation (0.6) with the number of roots, the lateral root score, and the shoot height. Plantlets on acclimatisation 12 WAP showed a significant ($P < 0.05$) positive correlation of 0.47, 0.36 and 0.45 with number of roots, root length and lateral root score respectively (Figure 30). Above all, the plantlets' acclimatisation at 12 WAP showed a significant ($P < 0.01$) positive correlation with the plantlet weight (0.53).

CHAPTER FIVE

DISCUSSION

Culture success

Significant variations were observed in the success rate of the four varieties during culture initiation. The differences in the varietal response could be attributed to their genetic differences. Different varieties of a known crop respond differently to micropropagation (Houndédjii & Zandjanakou-Tachin, 2016). According to COTVET (2017), MD2, Queen, Smooth Cayenne, Sugarloaf and other less-known pineapple varieties respond differently in terms of regeneration for vegetative material on farmer fields. Differential *in vitro* regeneration of pineapple varieties and their response on farmer fields can be linked to the varietal response.

The significant differences observed among pineapple varieties and their response to culture initiations corroborates with the results of Badou *et al*. (2018), which revealed that Smooth Cayenne (Hilo) variety was better than Sugarloaf variety. Findings from this initiation regarding varietal variance is consistent with the conclusions drawn by Guerra *et al*. (1999) who studied the micropropagation performance of two pineapple varieties (Perolera and Primavaria) from Brazil and reported that although these varieties have good adaptability in the area, they responded differently to micropropagation just as these popular Ghanaian varieties have shown in this study.

Bhatia (2015), and Bhatia & Sharma (2015), reported that different species and varieties may respond differently to micropropagation. Again, in cannabis micropropagation, Boonsnongcheep & Pongkitwitoon (2020), confirmed that the

variety and the source of explant affected the success of cultures. These findings are similar to Kara & Baydar (2012), who reported that for lavender varietal response to micropropagation, the Silver variety performed better than Super A variety. Hazra *et al*. (2000), while investigating the sprouting and proliferation of cotton concluded that genotype played a significant role in micropropagation success. The variations in genetic potential of the varieties used in the current study could be responsible for the observed differences in culture initiation. The genetic potentials of these varieties might as well influence the observed differences in shoot multiplication cultures and its associated parameters, especially, calli and weight. Furthermore, similar observations were recorded for the varying response of plantlets to rooting.

Response of explant type to micropropagation

The observed statistical differences in explants success in micropropagation were not a deviation. Bhatia (2015), reported that the source of explant is a major contributor to the success of their culture establishment. According to COTVET (2017), sucker and slip respond differently in terms of growth and yield and this differential response may also be link their success rate during culture initiation. Mandal *et al*. (2002), also observed varying responses among sucker, crown and slip explant types for Queen variety on initiation and subsequent growth *in vitro*. Additionally, the response rate of initiated slip and sucker were significant and also higher than the results reported from this study even though it was limited to Queen variety (Mandal *et al*., 2002). Nikumbhe *et al*. (2013), confirmed that vegetative slips produced the best *in vitro* result compared to the crown and shoot tip on a test

medium. Explorations on the effects of the type of explant on micropropagation in lavender revealed that the shoot tip of the silver variety was more responsive than buds from the stem internode (Kara & Baydar, 2012). Escalona *et al*. (1999), studied the *in vitro* response of Smooth Cayenne and reported that crown and slip bud explants, responded differently in all aspect of micropropagation.

Influence of hormone-free medium on initiation cultures

The results shows that the full-strength MS medium without a cytokinin was able to influence bud growth in the initiation cultures just as the medium supplemented with either BAP or Kinetin. This is in conformity with the results by Sani *et al*. (2021), who used a hormone-free MS media to enhance the growth of Smooth Cayenne buds even though the MS supplemented with BAP and activated charcoal outperformed the regenerative potential of the former. The success of the hormone-free MS medium was also confirmed by Zerihun *et al*. (2009), when they investigated the *in vitro* response of Queen variety of pineapple and cardamon. Their results showed that a full-strength MS medium without Kinetin or BAP was capable of enhancing bud growth. A similar result was published by Zuraida *et al*. (2011), on the *in vitro* growth of pineapple where shoot proliferation was enhanced by a hormone-free MS medium. Some other authors have reported the success of a hormone-free MS on the *in vitro* growth of pineapple and these give credence to the results of this study (Adeoye *et al*., 2020; Akin-Idowu *et al*., 2014; Atawia *et al*., 2016; Kiss *et al*., 1995).

Cytokinin-supplemented medium on culture initiations

The type and concentration of cytokinin used in micropropagation affects response of cultures. The results of this study showed better performance of both kinetin levels (1.5 mg L^{-1} and 3.0 mg L^{-1}) over the BAP in the MS media for the percentage culture success (bud growth). This corroborates with Kiss *et al*. (1995), who reported a better shoot regeneration potential of *in vitro* pineapple on an MS media supplemented with Kinetin to media with BAP supplements. It however contradicts the findings from Zerihun *et al*. (2009), which suggested that kinetin-supplemented MS media recorded an inferior performance to BAP or a combination of BAP and kinetin for their *in vitro* experiment on Smooth Cayenne. Adeoye *et al*. (2020), reported better performance of MS media supplemented with BAP 3.0 mg L^{-1} over all other combinations of thidiazuron and Kinetin for bud and shoot growth. Differences between their findings and that of this study can be attributed to culture conditions.

The performance of the IRB505 can be attributed to protocol optimization through testing and modification over a longer period to suit the commercial micropropagation of pineapple.

Findings from this study also showed significant interactions among the varieties, explant type and cytokinin concentrations. It was observed that Queen variety recorded a better performance on all BAP levels over the kinetin-supplemented media. The highest percentage of culture success was recorded in Queen cultured on the MS media supplemented with 3.0 mg L^{-1} BAP. The three-way interaction also showed that slip of Queen variety cultured on MS medium

supplemented with 3.0 mg L^{-1} BAP recorded the overall highest initiation culture succuss. It could be that slips of Queen variety have better nutrient uptake and nutrient use efficiency in vitro and interact better with BAP than the varieties do with sucker explant.

Contamination

The percentage of contaminated cultures from the study was relatively low because none of the setups recorded not more than 25 % contamination. The results showed that the levels of contamination were statistically not significant for the media (cytokinin). However, the levels of contaminants associated with the explant (sucker and slip) and incubation temperature and the interaction between the explant type and temperature varied significantly ($P < 0.0.5$). Air and soil-borne pathogens pose several degrees of risk of contamination to field-grown plants and hence aseptic techniques are pre-requisite for the establishment of cultures in vitro (Parveen *et al*., 2019). Contaminations account heavily for the difficulty in culture initiations. According to Reddy *et al*. (2021), the commonest source of contamination in micropropagation is the source of explant and this could explain the significant differences in the level of contaminations for the slip and sucker explant. The generally low level of contamination from the study could be attributed to the stringent and effective sterilisation procedure used. According to Odutayo *et al*., (2007), the use of an ideal explant, a standard media, and a good microclimate under full asepsis may result in a clean culture establishment.

The low level of contamination in the growing rooms may be attributed to the hygienic condition and the unfavourable microclimate for mites, thrips and

other microbes' proliferation. Mites crawl from one culture vessel to another, transmitting pathogens (Abass, 2013). Bahadur *et al*. (2015), Chokheli *et al*. (2020), and Kausch (2018), reported that stringent adherance to sanitation conditions significantly reduces contamination.

Although the levels of contamination recorded in initiated cultures were generally low, the results showed that differences in contamination levels associated with cultures raised under 19 °C were significantly lower compared to those raised under 25 °C. The observed significant difference in the contamination rate affirms findings of Odutayo *et al*., (2007) and Schiraldi & De Rosa (2014) which indicates that most microbes that causes contaminations in tissue culture thrive best at temperatures above 22 °C. Therefore, their proliferation rate might have been lowered by the lower temperature specifically 19 °C.

The observed signification interaction that resulted in slips cultured under 25 °C recording the highest level of contamination may be due to the fact the slips harbored some contaminants that the sterilisation techniques could not compltetely eliminate. These microbes may have latently thrived better under the 25 °C growing room.

Effect of temperature on shoot growth

Plant growth is generally an outcome of cell division, enlargement, and differentiation into different tissues. A normal growth process is accompanied by irreversible changes in size, weight, and length. The outcome of the shoots growth rate of this study viz.; the length, the number of leaves and weight showed significant differences for the two incubation temperatures (19 ^{o}C and 25 ^{o}C) and

the type of explant (slip and sucker) as well as their interaction. Aside, from the quality of the explant and the nutrient composition of culture media, the growth and development of tissue culture heavily rely on the availability of a suitable microclimatic condition for which temperature is the topmost (Neumann, 2014). Temperatures above the optimum requirement of a micropropagated species results in tissue death (Bhojwani & Dantu, 2013; Trigiano & Gray, 2016). Contrary, culture temperatures lower than the optimum requirement for a species tends to slow the rate of tissue differentiation and ultimately reduced growth rate (Bhatia & Sharma, 2015; Neumann, 2014).

According to De Capite (1955), in micropropagation, growth is a function of temperature and light when the tissue temperature is above 26 ± 1 ^{o}C on the other hand, growth becomes a function of only temperature when tissue temperature is below 25 ± 1 ^{o}C. Reports show that pineapple can adapt to field temperatures of between 16 ^{o}C and 32 ^{o}C. The result of the study suggest that the *in vitro* growth of pineapple cultures is better at 25 ^{o}C which is within the range of 16 ^{o}C and 32 ^{o}C (Desjardins & Borroto, 1999). The poor performance of the cultures under the 19 ^{o}C for all micropropagation stages in this study may be due to the lower rate of cell of division and the expression of genes that coordinate cellular differentiation.

Again, the superior performance of slip cultured at 25 ^{o}C in terms of the number of leaves, shoot height and weight compared to cultures raised under 19 ^{o}C, as well as cultures that involved sucker explant, might be attributed to the fact that slips exhibit a more favourable response to nutrient uptake at 25 ^{o}C compared to sucker explants.

Influence of media type on multiplication culture parameters

The multiplication medium significantly affected various parameters, including the multiplication rate, the number of side shoots, cluster weight and the number of leaves per plantlet. These results were expected, as several authors have confirmed in their studies (Akin-Idowu *et al*., 2014; Almeida *et al*., 2002; Danso *et al*., 2008; Lyam *et al*., 2012; McAlister *et al*., 2005; Yabor *et al*., 2006). The results also revealed significant interaction between the varieties and medium type concerning the measured shoot parameter. Notably, differences have been reported in shoot multiplications between agar-based medium and liquid culture on shakers (Rezali *et al*., 2017). Similarly, variations in shoot multiplication have been observed for cultures in temporary immersion bioreactors, semi-solid and solid media (Akin-Idowu *et al*., 2014).

The lower multiplication rate of plantlets on solid medium compared to the liquid medium in the temporary immersion bioreactor system is in agreement with the results of Escalona *et al*., (1999), who found that the *in vitro* multiplication of pineapple in bioreactor systems produced higher numbers and superior plantlets compared to those on the solid medium. The significantly higher multiplication rate and other shoot characteristic recorded from the current study are consistent with finding from Firoozabady & Gutterson, (2003) which showed that Smooth Cayenne cultured in periodic immersion system recorded exponentialy higher multiplication rate compared to their cultures raised on solid medium. Similar resullts have also been confirmed by Biruk *et al*. (2013), where *in vitro* Smooth Cayenne cultured in a temporary immersion system showed higher multiplication rate compared to

those cultured on solid medium. Taken togerther, these findings, along with the current study, affirm the efficiency of using temporary immersion bioreactor system (TIBs) for pineapple micropropagation as previously reported by Sani *et al*. (2021) in their investigation of Smooth Cayenne cultured in TIBs. The current study again tallies with findings of Lyam *et al*. (2012), who reported outstanding multiplication and rooting of pineapple in the temporary immersion bioreactor system. Similar results have also been published for the micropropagation of eucalyptus in TIBs. According to McAlister *et al*. (2015), cultures in TIBs exhibit a higher multiplication rate (four-to-six folds) of plantlets in half the time required by cultures in solid and semi-solid medium.

The agar matrix might have affected the nutrient uptake process by the cultured plantlet, which could be linked to the lower multiplication rate of the plantlet cultured on the solid medium. Danso *et al*. (2008) reported that plantlets raised on solid medium have less surface area contact with the medium compared to those raised on liquid medium, resulting in their lower multiplication rate. The high multiplication rate and shoot characteristics of cultures in the TIBs might have been due to the intermittent flow of nutrient media to the plantlets and their growth in an air space (Etienne & Berthouly, 2002). It is possible that in the absence of nutrient medium, stress adaptive hormones are released by plantlet to increase the nutrient uptake during the intermittent feeding and this translates into the higher growth and development.

Effect of coconut water in multiplication cultures

The inclusion of coconut water in the multiplication media influenced plantlet regeneration. Almeida *et al*. (2002), have reported the mass proliferation of shoot on MS media supplemented with 3 mgL^{-1}BAP. The inclusion of the coconut water in the MS media supplemented with BAP could also be responsible for this success. The potential of coconut water has been reported by some researchers (Mandal *et al*., 2002; PCT, 2020). For pineapple micropropagation, the reported successful volumes of coconut water used are 5 % L^{-1}, 10 % L^{-1}, 15 % L^{-1} and 20 % L^{-1} in MS media either alone or in combination with some cytokinin (Mandal *et al*., 2002; PCT, 2020; Zepeda, 1979). Although the variety and age of the coconut fruit may influence its biochemical properties, it serves as a rich source of amino acids, auxin, and cytokinin among others (Yong *et al*., 2009).

The observed significant differences in the varietal callus formation could be partly attributed to the inclusion of coconut water in the media. Despite the reported benefits of coconut water on shoot proliferation, it is reported to aid in callus induction and organogenesis when combined with casein hydrolysate and NAA on Murashige and Tucker media (Fitchet, 1990).

Rooting

Findings from the current study revealed significant differences in the number of root numbers and root lengths per variety, hormone contration, incubation temperature and their interactions. These resultlts were expectged as they been reported by some authors (Adeoye *et al*. 2020; Atawia *et al*. 2016; Bhojwani & Dantu 2013; Monthony *et al*. 2021). Atawia *et al*. (2016) reported that

the highest numbers of roots for their *in vitro* culture of pineapple were obtained from MS medium supplemented with 1 mg L^{-1} IAA over the 3 mg L^{-1}. In the case of this study, the MS medium with 0.1 mg L^{-1} IAA performed better than the medium with 1 mg L^{-1} IAA in terms of rooting. The result of the current study on root proliferation conforms to the findings of Lakho *et al*. (2023), who investigated the effect and concentrations of different auxins on *in vitro* pineapple and found out that even though the performance of IBA surpassed that of IAA, however, the MS medium supplemented with 0.1mg L^{-1} IAA produced plantlets with higher number of root compared to those supplemented with 1 mg L^{-1} and 3 mg L^{-1} IAA. It can be concluded that there is an inverse relationship of rooting with increasing concentrations of IAA. Pierik (1997), considered IAA as a weak auxin however its inclusion in an MS medium at low concentrations either alone or in combination with other auxins can induce root growth and development.

The results of the current study showed that as the concentration of IAA increased, the lateral root score of plantlets reduced. This was observed in plantlets cultured on solid and liquid medium. Generally, higher concentration of auxins in micropropagation medium is known to affects root numbers, length, density, lateral root characteristic and branching among others. The influence of IAA on lateral roots in pineapple micropropagation have not yet been established however, that of other auxins in the case of other crops have been studied. For example, Buah *et al*. (1998), investigated the root characteristics of auxin-supplemented and auxin-free media on *in vitro* Musa plantlets and found out that NAA does not favour lateral root development on *in vitro* Musa plantlets. Buah *et al*. (1998) also added that

NAA had an inhibitory effect on the development of lateral root. A similar deduction can be made for in IAA in pineapple micropropagation: increasing the concentration of IAA has an inhibitory effect on the development of lateral roots.

Correlations

The main goal of culture multiplication is to increase the number of plantlets on successive subcultures. The number of shoots from one culture is sub-cultured by splitting the shoot into several propagatable pieces and re-culturing on fresh media. This fundamental principle of pineapple micropropagation could have accounted for the observed significant strong Pearson correlation (0.9) between the number of the shoot and the multiplication factor. Cells differentiate into tissues, organs and plantlets in totality. The plantlets' height and number of leaves usually correspond with their total weight. In vegetative propagated species, the weight of minisetts is reported to affect their rooting and sprouting potential. Employing such a principle in micropropagation may mean that, the heavier the weight of an explant, the likelihood of developing more roots on an ideal media under an optimum microclimate. This could have also accounted for the positive correlation between the plantlet weight and the quality of roots developed.

The observed positive Pearson correlation of plantlets' weight with the acclimatisation 12 WAP suggests that heavier plantlets have better adaptability on weaning than lighter ones. It is possible to record higher mortality in less heavy plantlets on acclimation should there be a fluctuation in microclimatic conditions. The primary function of the root is for the absorption of nutrients for the efficient survival of the plant. In principle, plants with more active roots tend to have higher

nutrient uptake and hence better growth and development. This relation could have accounted for the observed positive correlation between root numbers and plantlets' survival on acclimation. Similarly, the longer a root, the better its ability to exploit the substrate on which it is planted, and this could have possibly accounted for the observed positive correlation of root length and plantlets survival 12 WAP. The inclusion of the auxin IAA concentrations in the rooting media was to primarily induce and promote root growth. The auxin function could be linked to the observed negative correlation in the root length and the number of side shoots. The hormone function could have also led to the observed negative correlation between shoot height and the number of side shoots.

Acclimatisation

The observed 90 % and over survival of rooted plantlets on acclimatisation 6 and 12 WAP signifies proper handling and hardening off. Micropropagated plantlets are fragile therefore a little mishandling such as non-fungicide treatment before planting, improper and the use of nonsterile planting medium and poor microclimate may result in plantlets mortality (Suthar *et al.*, 2011). Apart from the cost implications, the use of peat for micropropagule nurseries is highly recommended (Datta *et al.*, 2017). The success of this survival rate could be linked to the adherence to standard plantlet handling protocols and the microclimates provided (Bhojwani & Dantu, 2013). Zuraida *et al*. (2011), recorded almost 100 % survival for Maspine pineapple plantlets on acclimatisation. Similarly, Atawia *et al*. (2016), have reported successful acclimatisation of their rooted micropropagated plantlets and obtained a higher leaf count for their various

combination of potting mix. However, a 1:2 combination of peat sand and peat produced a survival rate of 88 %. Sani *et al*. (2021), also used river sand as a substrate for acclimation and obtained 87 % success. The survival in the current study is in agreement with the results of Zerihun *et al*. (2009), whose work focused on the shoot regenerative rate and survival of pineapple and cadmium. Again, these finding are similar to that of several other reports (Danso *et al*., 2008; Soto *et al*., 2020). Danso *et al*. (2008), observed an optimal growth of rooted MD2 plantlets in peat moss just as Lakho *et al*. (2023) also reported on the performance of Smooth Cayenne plantlet planted in peat moss over other substrates used on the acclimatisation of their micropropagated plantlets. The survival of plantlets in peatmoss can be attributed to the uniqueness of the peatmoss including high structural stability, uniform composition, high air space and water-holding capacity, low and readily changeable pH, nutritional status, and absence of weed seeds, insects, pests, and diseases (Durło *et al*., 2018). The peat used in the current study possed similar attribute resulting in successful plantlet acclimatisation.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The findings of this study are summarised as follows:

1. The mean number of buds generated from the experimental materials significantly differed across varieties and explant types.
2. Significant differences were observed among the varieties, the explant type, and the MS medium with varying cytokinin on culture initiation.
3. Queen was the most successful variety on culture initiation, followed by MD2 while Sugarloaf and Smooth Cayenne performed similarly with poor results.
4. The MS media without any cytokinin had the least influence on bud growth on initiation.
5. Explants cultured on IRB505 performed a little above those on media supplemented with kinetin and that of BAP (3.0 mg L^{-1}) on the initiation.
6. Incubation temperature significantly influenced bud growth, multiplication of shoots and the rooting of plantlets.
7. A strong positive correlation was found between the number of shoots and the multiplication factor in the multiplication culture.
8. Contrasting correlations were observed in the amount of calli and the number of shoots from the multiplication cultures due to the media type.
9. A strong positive correlation existed between the number of roots and the root length.

10. Differences in the varietal response to rooting were significant.
11. Irrespective of the media type, the lateral root score decreased with increasing concentration of IAA.
12. Plantlets weight correlated positively with their survival and more than 90 % of rooted microplants survived on acclimatisation 12 WAP.

Conclusions

1. The slips were more successful on initiation cultures compared to suckers.
2. The four pineapple varieties responded differently to initiation and subsequent growth.
3. Shoots multiplication and related indices from the temporary immersion bioreactor were significantly higher than those cultured on solid media.
4. Rooting and related parameters of plantlets cultured in the TIBs were better than plantlets cultured on solid media. Similarly, rooting and other measured parameters on plantlets cultured at 25 ^{o}C were better than those cultured at 19 ^{o}C.

Recommendations

1. Further research should be conducted on the flow rate of the nutrient medium onto plantlets in bioreactor cultures. Thus, the adjustment of pulse intervals, to ascertain the possibility of higher readings.
2. The acclimatized plantlets should be assessed on the field for their agronomic performance and possible occurence of somaclonal variation.

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