ASSESSMENT OF SOME HIGH-RATIO PROPAGATION TECHNOLOGIES FOR QUALITY SEED YAM TUBER PRODUCTION IN *Dioscorea alata* L. AND *Dioscorea rotundata* Poir

BY

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A Thesis in the Department of Agronomy
submitted to the Faculty of Agriculture and Forestry
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

OCTOBER 2021

CERTIFICATION

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ACKNOWLEDGEMENTS

All glory and honour to God, the immortal, the invisible, the only wise; by whose infinite grace and mercy I have my being and progress.

I would like to express my profound gratitude to my supervisor and mentor, Prof. M.O. Akoroda of the Department of Crop and Horticultural Sciences, formally Department of Agronomy, University of Ibadan. Sir, I am forever grateful to him for accepting to supervise me despite the heavy demand of his office as the Executive Director of Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The Anthem "see all, read all, edit all," which he taught me since the M.Sc. days, kept resonating as I prepared this thesis, line by line, word for word. I appreciate my co-Supervisor, Dr. N. G. Maroya, Project Leader, Yam Improvement for Income and Food Security in West Africa (YIIFSWA), International Institute of Tropical Agriculture for the Scholarship offered me his immense support and patience throughout the period of this study.

I am also highly grateful to Prof. Bola. A. Olaniyan of the Department of Crop and Horticultural Sciences, University of Ibadan and Dr. Morufat. O. Balogun of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, for their guidance, comments, and suggestions in preparing this thesis. I thank the aforementioned for being the driving force behind this work. Professor Dr. ir. S. Werbrouck of the Department of Applied Bioscience Laboratory of Applied *in vitro* Plant Biotechnology, University of Ghent, Belgium, is highly appreciated for the warm reception and mentorship he offered during my visits to his Laboratory to conduct part of this study. The entire staff at this Laboratory are highly appreciated for their assistance. To all these mentors mentioned here, your painstaking efforts and contributions to the successful completion of this study will forever be in my sweet memory. Again, I thank them for believing in me and for the training I had the privilege to receive from them.

I am so grateful to Dr. R. Asiedu, the IITA Yam Community of Practice and IITA-BMGF/YIIFSWA project, for the scholarship offered to pursue this study. I also thank Dr. H. Kikuno, who was my first Instructor at IITA Ibadan, Nigeria, for the training and exposure I had under his watch when I joined IITA as an Industrial Trainee and later as a fresh graduate and as an M.Sc. fellow.

Teachers are gods. Gratitude and appreciation to eminent scholars from whose fountains of knowledge I drank to gain knowledge and rectitude are a demand of my present academic status. I appreciate Prof. J. A. Fagbayide, the Head of the Department of Crop Science and Horticulture University of Ibadan, Ibadan, Nigeria, for his commitment to completing this Ph.D. study. I thank Professor V. O. Adetimirin for recommending my admission to the graduate college while serving as the Head of the Department of Agronomy, which is now split into the Departments of Crop and Horticultural Sciences and the Department of Soil Resources Management. I will forever be grateful to him for the privilege to pursue my postgraduate study at this prestigious University. I wish I could find enough words to appreciate Dr. S. O. Osunsanya for his immense sacrifices in registering my abstract and this thesis from the Department up till Postgraduate College. I thank him for being the driving force behind the registration of this thesis at the postgraduate college University of Ibadan, Nigeria.

My sincere appreciation goes to the Departmental lecturers: Professors M. E. Aken'Ova of blessed memory, G. O. Adeoye (retired), A. O. Ogunkunle (retired), H. Tijani-Eniola, E. A. Aiyelari, G. E. Akinbola, O. Fagbola, K. O. Oluwasemire, as well as Drs. O. O. Adeoluwa, A. Abe, B. Olasanmi, J. R., Orimoloye, E. Y. Thomas, O. W. Olaniyi, Akinrinola and Engr O. A. Sadiku for their tremendous support. The Non-Academic Staff and others who have contributed to the success of this academic journey are well appreciated.

The supports given by Messrs M. Oyetayo and J. Taiwo in the field, Aeroponics and Temporary Immersion Bioreactor Systems experiments is highly appreciated. I express my gratitude to Drs A. Paterne, M. G. Akinwale, N. A. Adetoro, and O. Alabi for the motivation and encouragement received. I thank Messrs T. Ayankanmi and O. Azeez for their tremendous assistance. Also appreciated for their supports are Messrs Adeosun Tunde, A. Kabiru, A. Edumodu, T. Olusola, I. Adejumobi, Y. Kolombia, and the entire membership of the Yam Barn at IITA.

Also worthy of appreciation are my colleagues in the Agronomy Club, University of Ibadan, the International Association of Research Scholars and Fellows (IARSAF-IITA). A. A. Bello and E. Oketade are well appreciated for their immense

contribution towards the success of this work while drinking together from the well of knowledge of our supervisor (Prof. M. O. Akoroda).

I express my profound appreciation to Reverend S. I Ajetomobi, Pastor (Dr) S. Ajayi, other Pastors and brethren of the Redemption Faith Church Ibadan for their prayers and encouragement. I cannot but recognize the immense contributions of my good friends, A. Oluwamuye, S. Ogidan, O. Amusa, B. Odewumi O. Ajayi, O. Akanbi and O. O. Fayemi, towards the successful completion of this program. To all who have supported me in one way or the other in the course of this program, I pray God in his infinite mercies to bring great helpers your ways. I thanked Dr. S. Adeleke, Elders J. Uponi, P. Igboba, W. Odianarewo, Dr. E. Parkes, I. Odey, O. Akinboade, B. A. Ojelade, C. Okoruwa and all other members of the IITA Bible Study Group for their prayers and words of encouragement.

May I now register my heartfelt thanks to my wife, **Abosede Esther Pelemo,** for her companionship, love, and support throughout the course of my study. Masters Ayoola Cornelius, Erastus Oladipupo, and my girl Eniola Emmanuella are all appreciated for enduring my absence during my research trips to the University of Gent, Belgium. I am greatly indebted to them all. I am eternally grateful to my parents, Late Most Senior Apostle Prophet Andrew Tolorunloju Pelemo and Beatrice Pelemo. They denied themselves the comfort and pleasure of this World to raise my siblings and me. Their prayers, counsels, and sacrifices were inestimable. The unflinching supports received from my siblings: Mr. M. A. Pelemo, Ms. Olayinka A. Andrew, Architect S. O. Andrew, Mr. O. J. Andrew, Mrs. Olabimpe. A. Adeyeri, Mr. A. O. Andrew, Ms. Oluranti Andrew, and Mrs. Olufisayo. O. Babanola were instrumental to the psychological strength and vigour that fuelled and sustained this academic pursuit. I thank my cousin: Ogunsakin Oluwasesan, for being there for me always especially at crucial times.I am most grateful to them all. I am also thankful to uncle Jimola Pelemo who was a source of encouragement to me in my academic pursuit.

DEDICATION

This thesis is dedicated to God,

and to the needy who acquired knowledge

with tears

ABSTRACT

Edible yams are widely cultivated staple food crops in the tropics, but their production is constrained by low multiplication ratio, which results in short supply of Seed Yam Tubers (SYT). The use of High-ratio Propagation Technologies (HrPT) could enhance quality and quantity of SYT. However, limited information is available on the use of HrPT and the amenability of yam varieties to HrPT for SYT production. In this study, the uses of some HrPT for quality SYT production in *Dioscorea alata* and *Dioscorea rotundata* were investigated.

Three HrPTs: Conventional Tissue Culture (CTC), Aeroponics System (AS) and field-based Yam Minisetts Technique (YMT) were evaluated for yam propagation using standard procedures. Five yam varieties (TDr9519177, TDr9518544, TDr8902665, TDa291 and TDa9801176) cultured in six growth media [4.43 g/L Murashige and Skoog (MS) basal medium and 7.0 g of agar-agar supplemented with each of 30 g/L sucrose-M_1, 60 g/L sucrose-M_2, 0.1mg/L Jasmonic Acid (JA)+30 g/L sucrose-M_3, 0.1 mg/L JA+60 g/L sucrose-M_4, 1mg/L-Naphthalene Acetic Acid (NAA)+30 g/L sucrose-M_5 and 0.1 mg/L NAA+60 g/L sucrose-M_6] using three light types [blue-Light Emitting Diode (LED), red-LED and white-LED] in CTC experiment were evaluated for Number of Nodes-NN, Number of Vines-NV and Vine Length-VL (cm). Explants from Acclimatised Tissue Cultured Plants-ATCP, Direct Vine Cuttings-DVC and Rooted Vine Cuttings-RVC of the five yam varieties were grown in AS and evaluated for Plant Survival-PS, Number of Tubers-NT and Fresh Tuber Weight-FTW (g). Using the YMT, four *D. alata* and 12 *D. rotundata* varieties were evaluated on the field using five Sett Weights-SW (10, 20, 30, 40 and 50 g). Data on Plant Emergence-PE (%), NT, SYT (%) and Fresh Tuber Yield-FTY (t/ha) were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Across varieties, NN, NV and VL differed significantly among media and light types. The NN, NV and VL ranged from 3.3±0.9 (M_6, red-LED) to 15.9±1.1 (M_3, white-LED), 1.2±0.3 (M_2, Blue-LED) to 3.2±0.8 (M_4, white-LED) and 4.3±0.9 (M_6, red-LED) to 10.5±1.0 (M_6, red-LED), respectively. The PS, NT and FTW varied significantly among yam varieties and explant sources. The PS across varieties was in the order: ATCP (52.0±14.5)>DVC (35.4±11.6)>RVC (28.3±16.0). The NT ranged from 12.3±0.6 (TDa9801176, DVC) to no tuber (TDa291, RVC). The FTW obtained from ATCP, DVC and RVC ranged from 6.2±15.1 (TDa291) to 257.8±3.2 (TDr9518544), 0.0 (TDr8902665) to 157.0±3.5 (TDr9518544) and 0.0 (TDa291) to 147.8±3.3 (TDa9801176), respectively. Effects of variety, SW and variety×SW interaction in YMT were significant on PE, NT and FTY. Across SW, PE declined from 97.7±9.5 (TDa9801176) to 40.5±7.6 (Danacha). Across varieties, NT ranged from 13.1±1.3 (10 g SW) to 20.3±3.8 (50 g SW), while FTY ranged from 8.6±2.6 (10 g SW) to 20.7±4.3 (50 g SW). The proportion of SYT was highest (66.9±6.0) in 20 g SW and lowest (55.1±13.1) in 50 g SW.

Jasmonic acid supplemented medium, white light emitting diode, tissue culture plants, sett weights of 20 g and varietal effect enhanced propagation and seed yam tuber production in *Dioscorea alata* and *Dioscorea rotundata.*

Keywords: Tissue culture media, Vine cuttings, Sett weights, minisett, Aeroponics
Word count: 489

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LIST OF ABBREVIATIONS AND ACRONYMS

ADP	Agricultural Development Program
AS	Aeroponics System
B LED	Blue Light Emitting Diode
CRD	Completely Randomised Design
CRH	Carbonised Rice Husk
CRI	Crop Research Institute, Ghana
CTC	Conventional Tissue Culture
DAS	Days after Sprouting
DAP	Days after Planting
DAC	Days After Cutting
DAT	Days after Transplanting
DF	Degree of Freedom
DVC	Direct Vine Cutting
EC	Emulsifiable Concentrate
FAO	Food and Agricultural Organisation
GH	Glass House
Ha	Hectare
HrPT	High-ratio Propagation Technologies
IITA	International Institute of Tropical Agriculture, HQ Nigeria
JA	Jasmonic Acid
LED	Light Emitting Diode
MAC	Months After Cutting
MAP	Months After Planting
MoU	Memorandum of Understanding
MS	Murashige and Skoog
MT	Metric Tonnes
NAA	Naphthalene Acetic Acid
NACGRAB	National Centre for Genetic Resources and Biotechnology

NRCRI	National Root Crop Research Institute, Nigeria
RVC	Rooted Vine Cutting
RLED	Red Light Emitting Diode
RCBD	Randomised Complete Block Design
SE	Standard Error
SNC	Single Nodal Cutting
SSA	Sub-Sahara Africa
ST	Sterilised Topsoil
SW	Sett Weights
SYT	Seed Yam Tuber
TDa	Tropical *Dioscorea alata*
TDr	Tropical *Dioscorea rotundata*
TIBs	Temporary Immersion Bioreactor system
USD	United State Dollar
VCT	Vine Cutting Technique
WAC	Weeks After Cutting
WAP	Weeks After Planting
YIIFSWA	Yam Improvement for Income and Food Security in West Africa
YMT	Yam Minisett Technique

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Yam, a tropical tuber crop of the family *Dioscoreaceae* and genus *Dioscorea,* has over 600 species worldwide. About 60 of these species are being grown for food, alcohol, beverages and medicine. But six of these species are of economic significance as a food crop (Lebot, 2009; Nweke, 2016). Yam species that are important as food crops are *D. rotundata* Poir. (White yam), *D. alata* L. (water yam) and *D. cayennensis* Lam. (yellow yam). The minor yam species include *D. dumetorum* (Kunth), Pax (bitter yam), *D. bulbifera* L. (aerial yam) and *D. esculenta* (lesser yam). The distribution of yam in the tropics is wide, whereas a few members of *Discoreacea* are grown in the temperate regions of the World. In terms of production, it is the third most important tropical tuber crop after Cassava (*Manihot esculenta* Crantz) and Sweet Potato (*Ipomoea batatas* Lam.) (Scott *et al.,* 2000). Over 300 million people depend primarily on yam for livelihood and food security in sub-Saharan Africa (IITA, 2010).

Though a staple food crop in West Africa, Southeast Asia and the Caribbean, Yam is classified as starchy food, it is consumed in the fried or boiled tuber, pounded yam, amala, boiled soup, or boiled soup porridge. The crop is also attaining a degree of industrial use. The flour produced from some *D. alata* cultivars has been found suitable for making flour needed in the confectionery industries. Aside from the starch for which yam is known, bioactive such as mucin, dioscin, dioscorin, choline, polyphenols, diosgenin and vitamins such as carotenoids have been extracted from some yam species. Other bioactive such as hypoglycemics, antimicrobial and antioxidant extracts have been reported (Chandrasekara and Josheph, 2016). Food yams can be boiled, roasted, grilled, or fried, parboiled and flaked, but mostly eaten as a pounded yam in its growing belt of West Africa. Yam's contribution to the gross income of farmers from arable crops in West Africa is about 32% (Orkwor and Asadu, 1998). It is mainly grown from whole tuber, otherwise known as seed yam tuber (SYT)

or yam setts produced by sectioning whole tubers into parts. Yam tubers, therefore, plays a dual role of food storage and seed organ. This dual function of the yam tuber as a food and seed organ has endangered its ability to adequately meet yam growers' and consumers' seed and food needs (Aighewi *et al*., 2014).

The conversion of food into seeds has resulted in high prices for yams in the marketplace, making yams inaccessible to low-income earners.

Also, the sett multiplication ratio for yam is low (1−4) compared to other clonal crops (Ondo *et al.*, 2016; Asiedu, 2003). In contrast to sweet potatoes, mainly grown from the vine, the planting material in yam production is primarily obtained from the tubers among yam growers. Yam sett produced by cutting ware yam into setts, seed yam tubers (SYT) selected from bulk harvest and the seed from the second harvest in previously milked yam plants had been relied upon by yam farmers as sources of planting materials. Nevertheless, these have been insufficient to meet the seed demand of yam growers. Also, as mentioned above, these modes of generating planting materials cannot eliminate diseases since they are carried out in an uncontrolled environment, where the plant and its tuber(s) are open to pest infestation. Diseases such as viruses, fungi, bacteria and soil-borne pathogens such as nematodes also have adverse effects on seed production through these techniques. Therefore, a single SYT production technique is not adequate to address the SYT deficit.

In addition to seed challenge, labour requirement of 300–400 man-days per hectare for various production operations is also a constraint to yam production (Orkwor *and* Asadu, 1998). The concept of dry season planting even in uplands is an age-long practice used by farmers to avoid seed loss and seed storage cost and prevent the conversion of seed to food (Morse, 2018). This has also become counter-productive as prolonged drought, especially in recent times, has caused seed loss through seed desiccation and rots.

The resultant scarcity of SYT has caused a decline in yam production. Aighewi *et al.* (2015) also assert that yam, a clonally propagated crop, is prone to seed quality decline as it is affected by viruses, bacteria and fungi. In the further attempt to address the challenges of the SYT deficit, yam has also been propagated by cutting ware or food yam tuber (≥ 1 kg) into pieces (≤ 200 g), otherwise known as yam setts. This last resort must have contributed significantly to the high cost of food yam, which has raised it beyond the reach of the poor. The lack of propagation materials is one of the considerable challenges to yam production. There is a need to improve the methods to

increase seed and ware yam produced yearly and reduce the time for developing lines in breeding programs. In order to alleviate the SYT scarcity among yam growers, the minisett technique was reevaluated for SYT production, using both released and Farmers' varieties. A combination of more High-ratio Propagation Technologies (HrPT) required to address the challenge of obtaining quality seed in significant quantity was attempted.

1.2 Statement of the Problem

Addressing the high seed deficit in yam could remain a mirage if the potentials and complementarity of HrPT remain untapped or un-optimized. The main inputs in yam production are SYT (100−300 g whole tuber), labour and staking material. These inputs account for 45%, 21% and 16% of yam production costs, respectively (Ezeh, 2004). Seeds account for almost 50% of the total outlay for yam production. This information was corroborated by the previous works (Okoli and Akoroda, 1995; Nweke, 1994), which reported that the cost of planting materials for yam ranged from 33− 50%. Also, Sanginga *et al.* (2015) assert that SYTs account for 63% of total variable production costs. Seed tuber quality in terms of health status and purity are neglected due to the wide gap between required and actual seed need.

The multiplication ratio (1:4) achievable in yam production using traditional methods is not sustainable. The fresh tuber yield (t/ha) of yam is low and has further reduced from 11.5 t/ha in 2010 to 8.3 t/ha in 2019 (FAO 2021) due to the scarcity of quality and adequate amount of seeds. The scarcity of SYT resulting in the high seed cost of food yam is also of concern, constituting a critical challenge. The lack of SYT has contributed to a significant decline in yam production among farmers in the yam growing belt of West Africa (Aighewi *et al.,* 2014).

The non-availability of a formal seed system influences high seed costs in yam production. The unavailability of high-ratio propagation technologies (HrPT) facilities and poor understanding of relatively known and attempted SYT production technologies have contributed to the scarcity and the continuous use of infested seed for yam production (Aighewi *et al.,* 2015; Maroya *et al*., 2014a). The non-availability of quality SYT has continued to be a critical challenge, particularly to smallholder farmers in yam-producing communities.

1.3 Aim and Objectives of the Study

Understanding the need, interest and limitations of these techniques are crucial to ensure that the required SYT need of yam growers are met. The production, distribution and sustenance of healthy SYT have the potential to enhance yam production. Therefore, these technologies are complementary, inter-related and were assessed in this study to contribute to knowledge on SYT production in quality and quantity, thereby reducing the critical gap between required and actual SYT produced annually (FAO, 2017). Therefore, the main objective of this study was to assess quantitative and qualitative SYT production using the selected techniques. The sub-objectives were to:

1. determine the status of available SYT production techniques among key yam researchers and farmers with a structured questionnaire;
2. determine best media composition light type and photoperiod suitable for plant growth and microtuber production in TIBS and CTC;
3. determine best explant sources for AS and vine cutting seedling production;
4. identify varietal and sett weight effect on sprouting, survival and tuber yield of different genotypes of two yam species under YMT;
5. determine the efficiency of different rapid seed production techniques in generating SYT for various end-users.

1.4 Significance of the Study

To date, there does not appear to be a specific protocol for the production of microtubers from clean plants needed in the production of SYT. Using virus-free mother plants, obtained through *in vitro* and semi in-vitro techniques is critical to quality SYT production. Even though the yam minisetts technique has existed for decades, with several research findings reported, information on variety by sett weight interaction is limited. Through evaluation of some basic principles, this study provided information lacking in some of the previous works. Also, reports from planting to harvest were provided by previous researchers but with little or no information on the relative performance of breeder lines known mostly to yam researchers and landraces often referred to as popular market varieties. This study provides adequate information on the performance of the selected yam varieties across yam growing regions in Nigeria, in addition to the elite breeder line. Thus, establishing appropriate Minisetts weight for SYT tuber production with the likelihood of high economic returns. In this

study, in the quest to find a sustainable solution to a healthy SYT deficit, alternative techniques such as vine cutting technique (VCT), aeroponics system (AS), temporary immersion bioreactor system (TIBS) and conventional tissue culture CTC were investigated.

It is hoped that the yam breeding scheme will be enhanced through reductions in the breeding cycle if these technologies are optimally employed. Also of significance is the helpful information that this study provides for the rapid multiplication of breeder seeds (BS) and other seed classes, i.e., foundation seeds-FS and commercial seeds CS). Ultimately, yam production could increase with adequate seed supply in terms of quality and quantity produced. This establishment of a formal seed system (FSS) for yam and other clonal crops is hinged on understanding the reviewed HrPT developed in this study. Significant production of healthy seeds using HrPT could enhance seed availability at an affordable rate. The yield of yam could increase from its current value of 8.0 t/ha to an attainable yield of 22.0 t/ha if quality seeds in an adequate amount are made available. The production and dissemination of quality seeds of recently improved varieties could also be enhanced with the application of HrPT under consideration.

1.5 Scope of the Study

The HrPT, namely: YMT, VCT, AS, TIBS and CTC, were deployed to assess STY production using varieties of two yam species (*D alata and D. rotundata*) as test crops. The performances of two yam species in these HrPT were evaluated in the field and laboratory at the International Institutes of Tropical Agriculture Ibadan, Nigeria and the *in-vitro* Plant Biotechnology Laboratory of the Department of the Applied Biosciences University of Ghent, Belgium. An on-farm, farmer participatory trial was also conducted at Agunrege, Oyo state. The production of *in-vitro* microtubers, minitubers which are primary SYT and the production of SYT (100−500) using the HrPT as mentioned above were the focus. The microtubers obtained from CTC could be planted directly, harvested as primary or standard SYT. The harvested SYT is further subjected to YMT among seed growers to produce FS and then QDS. The QDS are tubers, otherwise classified as commercial SYT (Aighewi *et al*., 2015). The location used for the on-farm trial and demonstration of YMT to local farmers was Agunrege via Saki, Oyo State, Nigeria.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and distribution of yam

Yams are mostly dioecious plants of the Dioscoreaceae family, consisting of over 600 climbing vines and woody shrubs. Most members of this family produce subterranean tubers, aerial tubers, bulbils, or tuberous stems. They have heart-shaped leaves, small green or white flowers and fruits bearing winged capsules or berries. Yams are distributed widely throughout the world's tropical and warm temperate regions, though human translocations have certainly influenced current geographic distribution (Barton, 2014).

Today yams are part of the essential staple food in Africa, Asia, the Caribbean, Pacific Islands and the Americas. Yam belongs to the family *Dioscoreaceae* and genus *Dioscorea.* This genus includes about 600 species, of which 50−60 are cultivated or gathered for food or pharmaceutical purposes (Craufurd *et al.,* 2001). There are about ten species that are of economic importance as foods. The most important food species are *D. rotundata, D. alata and D. cayennensis* (commonly known as white or guinea yam, water yam and yellow yam, respectively). Others include *D. esculenta, D. dumetorum, D. opposita and D. bulbifera*. Among these species, *D. rotundata, D. alata and* D. *cayennensis* are the most important, constituting up to 90% of the world food yam produced (Craufurd *et al.,* 2006a).

These yam species originated from the tropics of Africa, Southeast Asia and South America (Orkwor *and* Asadu, 1998). The *D. alata* is referred to as Asiatic yam and must have originated from the tropics of Burma and Thailand (Ayensu and Coursey 1972). *Dioscorea rotundata, D. cayennensis and D. dumetorum* were first domesticated in West and Central Africa (Orkwor *et al.*, 1998). *Dioscorea esculenta* originated from China, while *D. trifida* is believed to have originated from South America as it dates to pre-Columbian times (Ayensu and Coursey, 1972;). The *D. alata, D. rotundata, D. cayennensis* are known to Asia and West Africa has spread westward as far as South America.

The area, now referred to as the African yam belt, covers the West of Cameroon and the Bandama River in central Cote d'Ivoire. This spread was hinged on the potential and importance of the crop, particularly as food (Orkwor *and* Asadu, 1998).

2.2 Botany of yam

Yam is almost perennial but is grown as an annual crop (Orkwor *and* Asadu, 1998). Yam possesses two underground structures: the fibrous root system and the thick storage organ (the tuber) in which starch is deposited (O'Sullivan and Jenner 2006). Primitive species produce rhizomes with the above-ground part: the stem twining vines or stem, which can grow up to 30 m in length and have moderate to profuse branches in many species (Bai and Ekanayeke, 1998). The above-ground part of the yam plant consists of vine-like stems on which leaves and inflorescence are formed.

Yam vine length varies within and among species. The vines can grow several meters long if provided with rigid support (stake) or may climb vertically on other herbaceous species, which is why most yam plants require staking for optimum development (Crauford *et al*., 2001). The direction of twining while climbing could either be clockwise or anticlockwise, depending on the species. The *D. rotundata, D. alata, D. japonica and D. opposita* are characterised by the vine twining in the right or clockwise direction. Whereas species such as *D. dumetorum, D. esculenta, D. trifida* twine anticlockwise (Bai and Ekanayeke, 1998, Malaurie *et al.,* 1995).

Yam leaves are borne on long petioles and are usually simple, cordate, or acuminate but are lobed or palmate in some species. The leaves are glabrous, which is broad, non-hairy and primarily glossy. *Dioscorea rotundata* bears simple, cordate leaves which have an opposite arrangement. There is considerable variation in the size and colour of yam leaves. In most cases, the lamina is superficial and without serration on the margins. The colour of the leaf tip is usually green except for young leaves of *D. alata* cultivars in which anthocyanin pigment may mask the green colour of the young leaves. Yams are light-loving plants; the petioles grow or twist in such a way as to expose the lamina to the maximal amount of sunlight. (O'Sullivan, 2008).

2.3 Morphology of yam

Yams have a vegetative system composed of root apparatus (some extend throughout the upper layers of the soil, others consist of root hairs), a stem apparatus and a foliar apparatus. The adventitious roots arising from the stem base absorb

mineral nutrients and water (Orkwor *et al*., 1998). The aerial yam stem is usually a thin twining vine allowing the plants to climb. The stem is winged in some species (*D. alata*) and commonly spiny in others, especially *D. rotundata, D. dumetorum D. cayennensis,* among others. Several species have deep striations on their vine, some contain anthocyanins and others have large thorns. The direction of the twining is used as a taxonomic feature. The leaves are petiolate except for *D. dumetorum*, *D. hispida and D. pentaphylla*, which have trifoliate leaves and hairs on their vines. Opposite to alternate leaf arrangement is exhibited with axillary buds (Degras, 1993).

The genus *Dioscorea* is dioecious but irregular in male and female flowers, which insects pollinate. The seed is flat, has a wing-like structure and usually goes through a dormancy period of three to four months before germination can occur. As flowering is rare, yam is clonally propagated using the tuber and the bulbils (Degras, 1993). But of recent, vine cutting technique, which involves the use of 1− 2 nodal cutting from growing mother plants for seed production, is being explored at IITA and popularised among seed producers and farmers (Kikuno *et al*., 2010; Maroya *et al.,* 2014b)

Tuber, as earlier mentioned, is the economically vital part of the yam plant in most *Dioscorea* species. It is made of starch and moisture, proteins, minerals (calcium, iron), vitamins (B and C) and crude fibre (South Pacific Commission, 1990). They are sometimes referred to as stem tubers because they are modified stem structures. The non-edible bulbils which are suitable for planting are a characteristic of some *D. alata* cultivars. These bulbils are produced on the leaf axis and sometimes weigh about 20 to 100 g per bulbil. *Dioscorea bulbifera,* on the contrary, produces only aerial tubers weighing up to1kg (Degras, 1993). This bulbil also serves the dual purpose of food storage organ and planting material as obtained in tubers.

2.4 Socio-economic importance of yam

Yam is a staple food crop for over 100 million people in the humid and sub-humid tropics. It constitutes an average of 32% of farmers' gross income derived from arable crops (Orkwor *et al*., 1998). The world annual tuber production is approximately 50 million tonnes (MT) from about 50 countries. The *D.rotundata* FTY is about 11 t/ha in the major producing countries of West Africa. According to FAO (2021) statistics, 74.3 MT of yam were produced Worldwide in 2019. About 97.4% of this was from sub-Saharan Africa. Nigeria is the leading producer in the World,

Table 2.1: World yam production (Mt (000)) statistics over ten years

	Country	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
World		56.7	53.1	53.1	56.8	66.9	67.5	74.2	77.7	73.4	74.3
Africa		54.5	50.9	51.0	54.5	64.9	65.6	72.3	75.8	71.7	72.4
	Nigeria	37.3	33.1	32.3	35.6	45.2	45.7	51.4	54.1	50.0	50.1
	Ghana	6.0	6.3	6.6	7.1	7.1	7.3	7.4	7.9	7.9	8.3
	Cote d' Ivoire	5.4	5.5	5.7	5.7	6.2	6.7	6.9	7.1	7.2	7.2
	Benin	2.6	2.7	2.8	3.0	3.2	2.7	3.0	3.2	2.9	3.1
	Togo	0.7	0.7	0.9	0.7	0.8	0.8	0.8	0.8	0.9	0.9
Asia		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Americas		1.7	1.7	1.6	1.7	1.4	1.3	1.3	1.3	1.0	1.3
Oceania		0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

Source: FAOSTAT| ©

FAO Statistics Division May 13, 2021

followed by Ghana, Côte' Ivoire, Benin and Togo in West Africa (Table 2.1). While Ethiopia (0.47mt) is the major producer in East Africa, Colombia (0.28 mt) leads the production in South America, followed by Brazil (0.23 mt) and Japan (0.14 mt), leads in yam production in Asia (FAO, 2021).

In Nigeria, yam cultivation spread from the humid rainforest to the Northern guinea savannah. However, the production is at its peak in Niger state through Benue to the Taraba States. States of the humid Tropics which had this peak value are Calabar, Ebonyi, and Enugu. The derived guinea savannah areas of Kwara and Ondo States have high production values compared to the states situated in the Mangrove rain forest (Figure 2.1). This near-ubiquitous presence of yam across Nigeria suggests it as a suitable crop to alleviate food insecurity and reduce poverty among resource-poor farmers. Yam tubers serve a dual agricultural function: first, as a food source for millions of people and, secondly, as planting material (Hahn *et al*., 1995; Akoroda *et al*., 2007).

Yam is produced commercially and for domestic consumption (Aighewi *et al.,* 2003, Mignouna *et al.,* 2014b). Also, Nweke *et al.* (1991) reported yam as an essential staple food in the West African countries, representing the primary yam belt of the World. This belt extends from Cote d'Ivoire to Cameroon. Yam is said to provide more than 200 dietary calories for some 60 million people in this belt. However, yam has significant genetic diversity, while cultural practice and usage vary within the yam growing belt. About 48 mt of yam (95% of global supply) is produced on 4 million hectares annually, mainly in five countries: Benin, Côte d'Ivoire, Ghana, Nigeria and Togo. Nigeria alone accounts for 70% of global yam supply (FAO, 2014). Yam constitutes an average of 32% of farmers' gross income derived from arable crops (FAO, 2009).

The demand for yam has been on the increase. Percent increase (99.2%) over ten years (2010−2019) is the highest with maize (86.6) and cassava (66.6) in a distance of second and third, respectively among significant food crops of sub-Sahara Africa (Table 2.2). Though grown for its carbohydrate content, yam is also an essential source of protein, fats and vitamins. Yam is the most nutritious of the tropical root and tuber crops; it contains approximately four times as much protein in cassava and is the only major tuber crop that exceeds rice in protein content in a proportion of digestible energy (O'Sullivan, 2010). Yam is therefore regarded as more nutritious than cassava

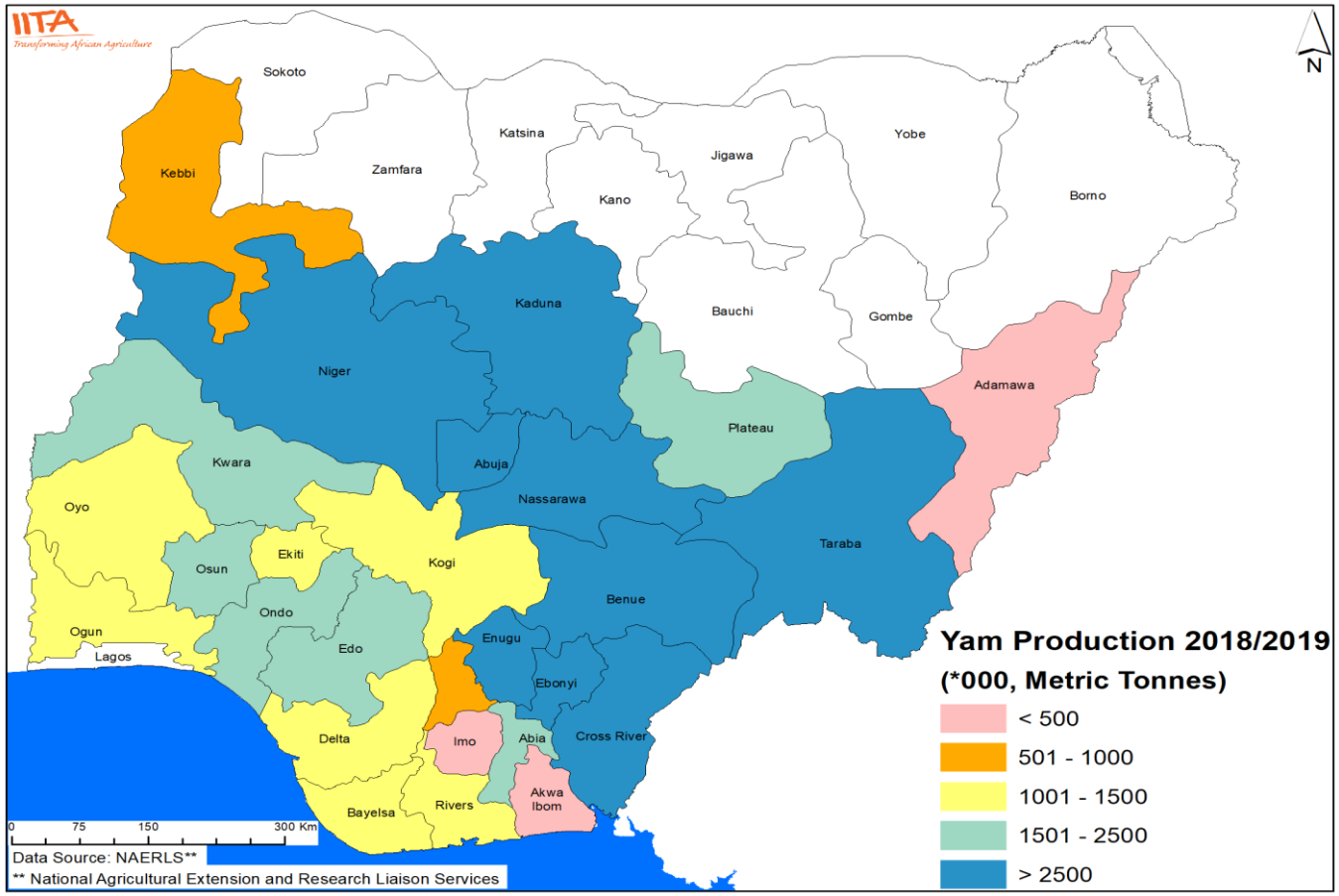


Figure 2.1: Map of yam Production (Metric tonnes) in Nigeria.

Source: Geographic Information System (GIS) Unit, IITA-Ibadan, Nigeria

Table 2.2: Comparative production trends among five of the food crops in the World.

	Production-Mt by Year							Increase-Mt (2010–2019)	
Crop	2010	2014	2015	2016	2017	2018	2019	Net	Percent
Maize	616	844	1052.6	1127	1139	1125	1149	533	86.6
Rice	4.4	701	488.2	493.2	501.4	508.8	503.9	63.9	14.5
Cassava	182	230	291.1	290.6	286.7	295.1	303.6	121.4	66.6
Yam	37.3	45	67.5	74.2	77.7	73.4	74.3	37	99.2
Beans	18.2	24	28	28.9	31.4	30	28.9	10.7	58.8

Source: FAOSTAT| © FAO Statistics Division February 11, 2021

and potato because yam products generally have a lower value for glycemic index. This low glycemic value indicates that yam provides a more sustained form of energy and better protects against obesity and diabetes (Thottappilly *et al.*, 1999).

2.5 Yam as a source of food and income

Food Yam is conventionally being described as a cash crop. This is due to its increasing role in enhancing food security and alleviating hunger among yam farmers and other stakeholders across the yam growing belts (Asiedu and Sartie, 2010). Tubers of the essential yam species are edible. Apart from the six edible species earlier mentioned, some wild species are also of economic importance and edible after much processing, such as soaking in water to detoxicate the tubers (Oke, 1985). Yam is prepared in different ways and these include boiling either before or after peeling, roasting and frying, which is the most preferred in terms of conservation of nutritive value (Bell and Favier, 1980). Apart from the daily uses of yam tubers as food, yam has social and cultural importance, particularly in Nigeria, where it is used in religious, marriages, birth and death ceremonies. The ceremonial yam is planted in high mounds for use as gifts or ritualized exchanges.

Although toxic yam species are being used as fish baits by local fishers, other species are used in medicine as alkaloid drugs (Ene and Okoli, 1985). *Dioscorea rotundata*, compared with *D. alata* is considered a more valuable species in market value, cultural demand, rituals, religion and rites in West Africa (Osunde and Orhevba, 2009). Although *D. alata* has been reported to be superior in emergence, yield and ease of cultivation in terms of less input, demand can be harnessed for industrial use as it grows well and suppresses weed.

2.6 Cultivation and land preparation practices in yam

Yam is usually grown on mounds or ridges with stakes or live support (Ranor *et al*., 1992). Yam is primarily produced by smallholder farmers who rely on traditional methods for cultivation and seed generation. Depending on the species, yam tubers mature after 6−12 months (Ikotun, 1989). Tubers are usually stored for up to six months for the purposes of consumption and as plant materials needed for the next season's cropping (Okoro and Nwakiti, 2004). Yam tubers are commonly stored in barns which provide good ventilation. Protection from termites, mealybugs and aphids

attacks can be minimised by dipping the tubers in a mixture of bactericide or fungicide, which are cypermethrin-based, before long-term storage up to 4 months.

Yam tubers can also store well in dry, dark, cool and ventilated places such as storage huts (South Pacific Commission, 1990), mainly for tubers meant for consumption within a short period. Yam is frequently grown with other types of plants. Yam intercropping with grain legumes such as African yam bean (*Sphenostylis stenocarpa* Hochst ex A. Rich), okra and melon is common for weed management (Odurukwe, 1980; and Onwueme, 1978). Yams perform less in both shoot and tuber yield under the intercrop sys, especially when intercropped with heavy N-feeder crops such as maize and deep feeder crops like cassava. They are primarily planted in sorghum fields before sorghum harvesting. A common practice in guinea savannah zones (Ekanayake and Asiedu, 2003). The critical need for the stake, especially in *D. rotundata,* necessitates the practice of succeeding sorghum with yam. The sorghum straws serve as stakes for the succeeding yam.

Land preparation involves ridge, heap and mound making at approximately 1 m apart. Though heaps and mounds are preferred in yam cultivation, the ridge method is more economical since it yields more yam tubers and enhances space optimization (Asiedu and Sartie., 2010). The ridging technique maximizes land use and plants can be planted more closely together (0.50 m) in yam purposed for the export market. However, the ridge has not been able to replace mound or heap in ware yam production, but in SYT production, ridges area most appropriate. The Plant density per area can be enhanced in the ridge method. Seed yam tubers (SYT) are produced by planting minitubers or cut sett from ware tubers. The yam setts are usually treated with wood ash (local method) or the combination of fungicide and bactericide chemicals: thiabendazole, lambda-cyhalothrin, cypermethrin and mancozeb 80WP, etc. However, these come with different trade names.

Depending on the availability of these chemicals, they could be combined or used as a straight application. Mulching is essential for October to December planting. It is done with dry grass or plant debris and then weighed down with balls of earth. Atu *et al.* (1983) reported that cultural and chemical methods could control pests and diseases, but the most appropriate integrated pest management (IPM) approach. Plant-parasitic nematodes such as root-knot nematode (*Meloidogyne* spp.) and yam nematode *(Scutellonema bradys)* cause enormous damage to yam tubers. Insects such as beetles and crickets cause severe damage to yam by burrowing into the tubers.

Weeding in the yam field is usually done three to four times, depending on adopted agronomic practice and the rate of weed growth. Korada *et al.* (2010) recommended disease-resistant cultivars for use, perhaps as a means to attain optimum sprout and yield. But this could pose a breeding challenge as consumer-preferred varieties may not bear this trait. Harvesting is done after complete senescence but before the soil becomes dry and hard. Generally, the yield of 10–15 tonnes per hectare (ha) for *D. rotundata* and 16–25 tonnes per ha for *D. alata* are obtainable under good agronomic or management practices. The harvested yam is sorted, graded and treated based on need or storage purposes. Yam tubers are traditionally stored in the barns by tying them with ropes. Yam tubers can also be stored *in situ* or harvested and covered with much in a cool-dry place for a short duration. Ware yam tubers are best stored in rodent-proof wood or metal base shelves with wire netting (Terry *et al.,* 1984). Sprout decreases starch in stored tubers. Therefore, the removal of shoots in yam tubers is required to maintain their quality.

2.7 Growth and Development in yam

Yam growth and development are significantly restricted at temperatures below 20°C and cannot tolerate frost conditions. This temperature has limited the spread of yam to most of the temperate parts of the World. Yam generally requires an optimum temperature range of 25−30°C, resulting in good crop growth and yield between April and September (Orkwor, 1998). The lower temperature of 15°C and higher temperature above 35°C retard sprouting in yam setts. This higher temperature accounts for the desiccation of yam setts planted, especially in shallow depths or on unmulched heaps and mounds (Asiedu and Sartie., 2010). The light plays a significant role in the morphological development of yam. It is an essential environmental factor in yam production.

Yam, like many other Tropical plants, is light-loving. Even though water and nutrient requirements are met, light becomes the most frequent limiting factor (Crauford *et al*., 2006). Day length of about 12 hours has influenced tuber initiation in yam (Shiwachi *et al*., 2002). Short-day length enhances tuber formation and growth, while long days favour vine growth (Ayankanmi *et al*., 2006). Tuber yield is mainly determined by the total vine production and its partitioning between the various plant organs. Soil moisture conservation is necessary for yam planted in the dry season, hence the need for mulching. Besides conserving soil moisture, mulch also prevents

SYT from rotting before sprouting and the young vines from being scorched by soil heat (Orkwor *et al.*, 1998).

2.8 Staking and trailing in yam

Staking is an agronomic practice targeted at providing support structures for the elevation of creeping plants. The stake raises plants above ground level, thereby enhancing photosynthesis, increasing plant growth, development and ultimately the tuber yield. Staking reduces the infection and spread of the soil-borne pathogen from one plant to another, especially fungi diseases such as Anthracnose and bacterial blight. The use of stake also has the potential to reduce disease severity. It makes field management to be efficient as it enhances the use of herbicides for post-emergence weed control. Materials used as stakes include Bamboo, dead or live stick, rubber, or metal poles. Wire, twine, or ropes are used for trellising. Also, Tsado (2012) asserts that yam produced under the staked system out-grows and out-yields those in the non-staked system. Staking contributes to increased growth and development of yam.

The *D. alata* varieties and some *D. rotundata* cultivars do perform equally with minimal to no-staking. Staking is an essential but costly input in yam production. It is necessary for yam cultivation in the humid forest to enhance the synthesis and partitioning of dry matter in the twining yam vine. The leaves are displayed to attract adequate photosynthetic active radiation (PAR). Most yam genotypes yield well when their vines are staked because staking increases light interception by leaves. It also facilitates the ease of weeding (Ogunniyan and Akoroda, 2004). The use of stake in yam cultivation is expensive, laborious, encourages deforestation and creates bottle-neck to yam mechanization (Onwueme and Haverkort, 1991). The use of trellis methods which is cost-effective, less labour-intensive and environmentally friendly, has been devised to address the challenges mentioned above in the stake method. The practice of converting Sorghum straw to stakes within a field in a rotational cropping system has also simplified staking among farmers. Also, selecting varieties that perform optimally under little or no staking is critical, reducing labour and input costs.

2.9 Harvesting and post-harvest practices

Single and double harvesting are usual practices among yam growers in the Tropics. The double harvest is achieved by first milking the tuber at the active growth stage and then final harvesting at senescence. The essence of milking mainly in early maturing varieties is to obtain SYT. The SYT after milking is characterized by

irregular shapes, multiple buds and thick periderm, which reduces water loss and enhances storage. Seeds from either harvest have been considered inadequate in the farmers' quest to meet their seed needs. In single harvesting, plants are usually harvested at senescence at the end of the season (Onwueme and Charles, 1994). Single harvesting is often than not a characteristic of the late bulking varieties. This single harvest type can also be when the rainfall duration of the agro-ecoregion is less than six months.

The planting or emergence to maturity and harvesting ranges from 6–7 months in *D. rotundata* and up to 10 months in other species (Onwueme and Charles, 1994). The first harvest or "milking" at five−six months after planting and the second harvest at three to four months later have been reported (Bencini, 1991). Traditionally, yam is cured by drying the tubers in the sun for a few days. The optimum conditions for curing are 29−32°C at 90−96% relative humidity for four−eight days. Storing at 15°C with prompt removal of sprouts improved the eating quality of tubers (Coursey, 1976), presumably due to the water loss associated with curing and the inhibition of the biochemical synthesis that accompanies sprouting.

2.10 Breeding challenges and prospects in yam

Yam improvement through germplasm collection, crossing parent parents with desirable traits to obtain a progeny with higher breeding value than the parent, is key to meeting farmers' preference. Breeding and the development of better stress-tolerant yam is also a challenge. Reynold *et al.* (2015) cited abiotic and biotic factors as potential yield reductions in yam like any other crop. Yam productivity is affected by declining soil fertility, diseases and pests associated with intensive cropping systems (Frossard *et al.,* 2017). Yam cultivation has a high environmental impact due to its high nutrient demand. Hence, developing low N-tolerant varieties will be the antidote to continual forest depletion for yam cultivation while making a frantic effort to abate this menace. Consumer preferences are in different forms. Breeding programs can contribute significantly to addressing these challenges (De Koeyer *et al*., 2016).

Developments of new breeder lines and the improvement of the existing landraces provide opportunities for expanding yam markets across the World's growing regions. Essential traits for breeding include nutrient use efficiency, tolerance to low N, tuber yield, tuber quality, resistance or tolerance to diseases, high sugar, non-oxidation, *et cetera* (Craita and Tom, 2013). Yam breeding is challenging because of

the relatively long crop cycle, low seed multiplication ratio, poor understanding of genetic diversity and limited breeding enabling techniques, contributing to the accelerated breeding of other clonal crops such as potato and cassava (De Koeyer *et al.*, 2016). Using high-ratio propagation techniques, the multiplication of yam can be optimized to reduce the long breeding cycle of 10 years and above in yam by up to 50%. This high ratio propagation is achievable, using single nodal cuttings to produce quality seedlings in quantity to conduct screening and evaluation trials beginning from the seedling stage.

2.11 Determinants of maturity and dormancy in yam

Tuber maturity and dormancy are critical to SYT quality in storage, viability, and the crop cycle. A matured yam crop is distinguishable by cessation of plant growth and leaf senescence. The yam crop cycle, which spans from planting and field emergence to tuber formation and maturation, depends on the species and varieties within a species. The proportion of whitish to dark brown colour of the tuber periderm is often used to measure maturity. Other crude indices have been reported based on the percentage of tuber portion non-friable after cooking or bitter after cooking. The most frequently reported measure is the period from planting to harvest (growing period). Still, it has been reported that the period from emergence to maturity provides a better estimate (Onwueme and Charles, 1994).

Dormancy, a physiological rest period without an apparent external sign of physiological or biochemical activity, is a process that constitutes a programmed inability of the tuber to sprout and grow. Despite suitable environmental conditions, dormancy prevents active cell division in plant parts, i.e., meristematic apices, buds, rhizomes, and tubers especially in yam. Regardless of environmental conditions, Yam tubers usually undergo dormancy (Park *et al.*, 2003b, Lang *et al*., 1987). The period of dormancy in yam can be adjudged as its shelf life. Yam tubers with a long duration of dormancy of 4 months and above can be stored for off-season planting or can be used for consumption. This assertion is justified because the onset of sprouting in tubers marks the beginning of the loss in quality and quantity of stored starch accompanied by shriveling and reduction of tuber and its cooking quality. Dormancy is controlled by the regulatory processes which involve: endo-dormancy (which is governed by endogenous conditions), para-dormancy or correlative inhibition (which is controlled outside the affected organ but within the parent plant) and exo-dormancy or quiescence

(which is governed by conditions in the external environment, i.e., the abiotic factors) (Lang *et al.*,1987).

These definitions apply equally to the tuber, meristems, botanical seeds and aerial buds. The total duration of dormancy is of great ecological significance and appears to adapt to the prevailing environmental conditions (Okagami and Tanno, 1991). Nevertheless, the length of inactivity constitutes the major physiological limitation to the successful year-round propagation of yam. This limitation results in a reduced number of cropping cycles and a general short supply of yam tubers to the end-users. This period also could have accounted for the long crop cycle (Craufurd *et al.,* 2006b).

Dormancy in *D. rotundata* commences as soon as tuber maturation is completed, signaled by the field's total senescence of foliage and vines. Dormancy promoters such as endogenous gibberellins (GA_1−GA_4), implicated in this senescence, are also responsible for the dormancy mechanism in *D. alata* and *D. rotundata.* These hormones, which cause senescence in the vine part of the plant, are soon deposited in the tuber, thereby inhibiting vine formation (Park *et al.,* 2003a). Cultivars of *D. rotundata* and *D. alata* vary in their dormancy period. This variability vis-à-vis sprouting led Onwueme (1978) to suggest that it is more appropriate to describe the growing period of yam in the field as being from sprouting or budding instead of the time from planting. This assertion may not hold where a non-dormant, i.e., already sprouted tubers, was used as planting material.

This attribute of yam, i.e., exhibiting up to 4 months of dormancy and viability, is advantageous in agro-ecologies where the rainfall period is succeeded by 4− 5 months of the dry spell. Unlike in other root and tuber crops, yam has the advantage of a long shelf life like that of cereals and legumes. Curing and storing SYT for a more extended period is premised on the long dormancy period in yam (Ravi *et al.,* 2009**).** Yam researchers, farmers and processors often face the hurdle imposed by the perishability of the tuber. This challenge is evident in the field establishment, processing and storage. The tuber serves as the organ for consumption and field propagation. The cost of planting material, susceptibility to storage and field pests, loss and pilfering have posed challenges to yam researchers and farmers. In the breeding activities, varietal propagation and selection scheme involves the repeated evaluation of clones in preliminary uniform and advance trials often measured in yield. The natural break in the evaluation cycle of yam often results in tuber losses. These losses

are advantageous to the yam selection process since materials most susceptible to storage pests and pathogens are identified and weeded out.

2.12 Environmental factors influencing yam growth and development

Yam growth is severely restricted at a temperature below 20^{o}C and cannot tolerate frost conditions. Yam is therefore not a popular crop in the temperate regions of the World. They generally require an optimum temperature of 25^{o}C−30^{o}C resulting in good crop growth and yield between April and September in the Tropics. The lower temperature of 15^{o}C and higher temperature above 35^{o}C have been found to retard sprouting in yam. These temperatures account for desiccation and rots, respectively, in yam setts planted in shallow depth since they are exposed to very high soil temperatures (Waziri *et al*., 2016).

Light is an essential environmental factor in yam production. When water and nutrient requirements are met, the light then becomes the most frequently limiting resource. Light plays a significant role in the morphological development of yam. Day length of about 12 hours has been found to influence tuber initiation in yam (Shiwachi *et al*., 2002). Short-day length favours tuber formation and growth, while long days favour vine growth (Ayankanmi *et al*., 2006). The wet tropics such as the Pacific with a yield record of up to 46 t/ha are believed to be the most suitable agro-ecoregion for yam (Sotomayor-Ramirez *et al*., 2003).

In the Caribbean, where the average annual rainfall ranges from 800−3500 mm, the yield recorded ranges from 18−25 t/ha. On marginal land with less than 400 mm average annual rainfall and low water holding capacity, the yield declines to less than 5 t/ha. However, the average yield of fresh yam tuber in Africa is about 10 t/ha. The yield is generally higher in the West Indies, with a yield range of 11−14 t/ha depending on cultivar and management practices (FAO, 2013).

Yam requires a well-distributed rainfall of 6−7 months, i.e., during its active growth (Orkwor and Asadu, 1998). The shoot and tuber yield is affected by drought, contributing to yield loss with an impact factor of 6.15% (Table 2.3). When minisetts planting is succeeded by drought, the setts often dry up. Caking and rot of these minisetts occur especially when they sprout before the onset of seasonal rains as practiced by yam farmers. Prolonged drought and excessive soil heat are reasons for poor plant performance, especially in YMT. Shoot and particularly FTY (t/ha) in yam is negatively impacted affected by other factors such as Agronomic practices, diseases (fungi and virus), nematode infestations, poor soils, among others (Table 2.3).

Table 2.3: Estimated yield gap and intervention effects in yam

Constraints	Mitigating Factors	FTY (t/ha)	Impact (%)	Expected gain in FTY (t/ha)
Actual		10.7		
Attainable		22.0		
Agronomic	Poor soil		12.98	1.45
	Drought		6.15	0.69
	Weed		11.61	1.30
	Scarcity & Poor-quality planting materials		16.39	1.84
Sub-total Gain from interventions				5.28
Biotic	Anthracnose		17.76	1.01
	Virus		9.56	1.07
	Nematode		8.88	0.99
	Rot fungi		6.42	0.72
Sub-total Gain from interventions				3.79
Technique transfer	Improved varieties		6.15	1.38
	Improved agronomic practices		4.10	0.73
Sub-total Gain from interventions				2.11

Source: YIIFSWA project proposal document, IITA, 2011; Aighewi *et al.*,(2021)

FTY: fresh tuber yield.

2.13 Soil and soil nutrient requirement in yam

Yam performance is influenced by soil quality. Unlike cassava and sweet potatoes that can thrive well in marginal soils, yam generally requires a well-pulverized and drained soil, consistent with high organic matter content (Carsky *et al*., 2007). Organic matters enhance tuber formation, adventitious root penetration and tubers' development in yam (Lebot, 2009). Usually, yam is planted as the first crop and on mounds or ridges in the quest to meet its demand for fertile and well-pulverised soil to ensure optimum yield. Most of the yam farmers in West Africa only meet the high fertility need of yam by growing it as the first crop after land clearing (O'Sullivan and Jenner, 2006). Nitrogen and potassium are often insufficient and need to be supplemented by inorganic fertilizer (O'Sullivan and Ernest, 2008). Fertilizer recommended rates for yam in Nigeria is 70 kg N/ha (as urea); 50 kg P_2O_5/ha (super phosphate); 20 kg K_2O/ha (muriate of potash); and 2 kg MgO/ha (Magnesium sulphate) (Adeniji *et al.,* 2001). Oshunsanya and Akinrinola (2013) reported that the application of organo-mineral fertilizer at the rate of 2.0–3.0 t/ha ameliorates physically degraded soil.

Bulk density (a soil physical property) associated with zero tillage has a more deleterious effect on yam tuber yield and shape than the soil chemical properties (Agbede and Adekiya, 2012). Bulk density affects yam performance. Therefore, sandy soils with gravel, clayey hardpan, and compacted soils must be avoided. The optimum pH that yam can tolerate is 5.5−6.5 (Degras, 1993). However, nitrogen and potassium are often insufficient. They need to be supplemented by inorganic fertilizer. It has been reported that a yield of 29 t/ha in yam would remove 133, 10, 85 kg/ha of nitrogen, phosphorus and potassium, respectively, from the soil (O'Sullivan and Ernest, 2008).

2.14 Constraints to yam production

Yam production in Nigeria is experiencing a decline due to the high cost of planting materials and labour, which account for about 50% and 40%, respectively (Onwueme and Charles., 1994). The high cost of planting material has necessitated the search for alternative means of massive SYT tuber production at minimum cost and high efficiency. Seed yam tuber (SYT) scarcity has compelled farmers to neglect seed health, contributing to a loss in yield and tuber quality (Lutaladio *et al*., 2009). Yam growers often convert table yam to seed by cutting them into setts to make up for the very high SYT deficit. An indication that Seed yam production is an economically

viable Agribusiness. Abiotic factors such as drought, biotic factors (disease and pest) and other factors have widened the yam's actual and potential yield gap.

The gap between the required and the actual seed produced is wide (Table 2.4). This yield gap is due to the lack or inadequacy of quality planting material (seed). It is also hinged on poor understanding and utilisation of SYT production techniques to reduce and eliminate diseases. The development and transfer of seed production techniques are critical toward bridging this gap. Also, this gap is further widened as both the area under yam cultivation and total yam output is declining (IITA 2009). The decline in average yield per hectare has been more drastic, as it dropped from 14.9% in 1986−1990 to 2.5% in 1996−1999 (CBN, 2002, Agbaje *et al*., 2005 and FAO, 2007). This declining trend may not be unconnected with resource use and allocation (Nwosu and Okoli, 2010). The relative increase in yam production as presented in Table 2.3 observed over a decade is because of the increase in the harvested area per year and not due to any other factor. The yield in relation to the harvested area showed a continual yield decline to corroborate this report. Although, Manyong *et al.*, 2001 reported a relative increase in production due to fresh tuber yield up to 5% and a 7 % increase in output due to the harvested area (ha).

This can be attributed to the use of poor soil and ultimately lack of good quality seed. An increase in seed production in the last five years coincides with the increase in yam production. This positive relationship indicates that more seed will increase output and seed quality positively correlates with tuber yield (Table 2.4). This shift can be attributed to the fact that yam has more demanding requirements regarding soil nutrient, labour and volume of planting and, ultimately, production costs. Increased production is believed to be constrained mainly by the high cost of SYT, which is significantly higher than cassava (IITA, 2013). In Nigeria,

the three significant inputs in yam production are SYT, labour and staking material. These inputs account for 45, 21 and 16% of yam production costs, respectively (Ezeh, 2004). High labour demand of 300−400-man days per hectare is required for various production operations such as land preparation, planting, weeding, trailing of vines, harvesting, pest and disease control both in the field and in storage were also reported as a constraint (Orkwor *et al.,* 1998). A significant limitation in yam production has been the large quantity of planting material required per hectare basis and low multiplication ratio, which hinder the dissemination of new cultivars.

Table 2.4: Food yam × seed tuber production statistics

Year	Yam production (MT)	Area harvested per ha(10^6)	Fresh tuber yield (t/ha)	Actual seeds prod. (MT)	Required seeds (MT)
2006	52.3	4.7	11.2	1.9	14.1
2007	47.2	4.8	9.7	2.0	14.7
2008	52.9	4.9	10.7	2.0	14.7
2009	47.7	4.8	10.0	2.1	14.4
2010	56.7	4.9	11.5	2.0	14.7
2011	53.1	6.5	8.1	2.1	19.5
2012	53.1	6.7	8.0	2.2	20.1
2013	56.8	7.3	7.8	2.3	21.6
2014	66.9	7.6	8.8	2.4	23.1
2015	67.5	7.7	8.7	2.8	23.28
2016	74.2	8.5	8.7	3.2	26.3
2017	77.7	9.0	8.6	3.1	25.6
2018	73.4	8.7	8.4	3.1	24.7
2019	74.3	8.9	8.3	3.0	25.3

Source: FAOSTAT| © FAO Statistics Division May 13, 2021

Ha: Hectare; T: tonnes; Mt: Metric tonnes

Actual and required seeds were calculated base on Area harvested

Ten thousand regular SYT tubers are needed to plant one hectare of yam at an optimum spacing of 100 ×100 cm to produce an economic quantity of ware yam.
The scarcity and high cost of purchasing SYT aggravate this problem. The increased demand for soil nutrient content by yam is another constraint. Due to the increase in urbanization and industrialization, primary and secondary fallows have become scarce. This has drastically reduced yam production in urban areas (Orkwor and Asadu, 1998).

Conserving germplasm in tuber form is difficult and undesirable because of bulkiness, poor storability and clonal propagation, encouraging disease proliferation (Asiedu, 1994). Tuber damage due to plant-parasitic nematode is significant in tuber quality reduction and yield loss in the field and storage (Adegbite *et al.,* 2006). In yam, underground tubers are seriously affected by pathogen accumulation, reducing the quality of planting material (Malauri *et al.,* 1995). Transportation of high volumes of cumbersome planting materials for field planting is equally a challenge. About 2.5 to 3.0 tonnes of planting material are required to plant one hectare. Thus, the high cost of transportation of planting material also increases the cost of production. In the light of this, a new paradigm must be pursued using yam seedlings, minitubers and single nodal vines cuttings as initial materials for producing SYT tubers. Adopting new yam cultivars that are tolerant to diseases and low-cost methods to combat seed challenges in yam can help smallholder farmers cope with the increased pest pressure and nematodes (Coyne *et al.,* 2006).

2.15 Diseases of yam

As earlier stated, an overview of yam diseases is critical to this study mainly because yam is a clonally propagated crop. Transmission and spread of diseases have grossly affected shoot and tuber yield, causing near extinction of some varieties and total loss of germplasm in some cases (Personal interview of farmers, 2015). Rodents and birds usually cause damages ranging from yield loss to tuber quality and quantity deterioration under storage. These pests singly or in combination cause deterioration and total loss both in the field and in storage (Adegbite *et al*., 2006). Besides, severe losses also occur during storage due to tuber physical injuries and tuber metabolic activities. Physical injuries are caused during the growing period by pests, yam beetles, nematodes, *et cetera*. A significant loss can be incurred during harvesting and digging (especially in large tubers), loading and off-loading, transportation over bad roads and rodent attacks (in storage).

Further losses also occur due to tuber respiration and transpiration (Coursey, 1976). However, yam beetles and nematodes are the major pests of yam in West Africa. Yield losses due to damage by yam beetles have been reported to be as high as 77% (Tobih *et al.,* 2007). Even though several nematodes have been reported to attack yam, only three; namely the yam nematodes (*Scutellonema bradys*), the root-knot nematodes (*Meloidogyne* spp.) and the lesion nematodes (*Pratylenchus* spp.) are recognized as constituting significant constraints to yam production (Coyne *et al.,* 2006; Adegbite and Agbaje, 2007) (Plates 2.1). Storage losses appear after the dormancy period with the growth of tuber sprouts, which leads to a heavy loss of water and weight of the tuber (Okoro and Nwankiti, 2004). The microorganisms responsible for yam diseases include fungi, bacteria and viruses.

Yam is affected by numerous pests and diseases and pathogens both in the field and in storage. This includes insects, nematodes, vertebrate pests, fungal and bacterial infection and viruses which are either singly or in combination are responsible for sub-optimal yield and deterioration in the quality of stored tubers (Adegbite *et al*., 2006). However, yam beetles and nematodes are the essential yam pests in West Africa (Tobih *et al.*, 2007). Yield losses due to damage by yam beetles have been reported to be as high as 77%. Yam production is hindered, as in many other crops, by pests and diseases. These can be classified as insects, nematodes, fungi, viruses and bacteria (Onwueme, 1978 and Hahn *et al*., 1987).

Insects, such as a yam beetle (*Heterogonous* species), are a significant pest of yam in West Africa, which causes a severe problem in yam production. The insects feed on the tuber though they rarely kill the yam plant, but cause damage to the tubers, rendering them unmarketable (Plates 2.1) and predisposing them to rot during storage. These insects can be controlled by dusting the yam with insecticidal dust just before planting. Examples of such insecticides are Aldrin, cypermethrin, Actellic, Karate©, Mancozeb DF, which are most effective for rainy-season planting. Other insects that affect yam include chrysomelid beetles which damage the young yam plant, Scales and mealybugs. These are the primary insect pests of yam tuber in storage. Scales and mealybugs form a thick whitish colony on the surface of the tuber and obtain nourishment by sucking sap from the tuber. As such, tubers infested shrivel up more rapidly than healthy ones and often increase the sugar content. Scales and mealybugs on tubers can be controlled by scrubbing them off with brush before planting, soaking SYT tubers and cutting yam sett in a mixture of Karate and mancozeb for 20 minutes.

Mag. x 5

Sources: A, B and D: Pelemo O. S.; Tafa yam market, Niger State, Nigeria. December 2014; C: Pelemo O. S; Salaga, northern region, Ghana. September 2012

Plate 2.1 Nematode infested tubers (a, b, d) and yam beetle-infested tuber (c)

A: yam tuber infested with *Scutelonema. Bradys* resulting in dry rot

B: yam tuber attacked by gall and root-knot nematode

C: Yam tuber damaged by yam beetle

D: Nematode infested ware yam cluster at Tafa market, Niger State, Nigeria

2.15.1 Fungi diseases of yam

Anthracnose (dieback or scorch) caused by *Colletotrichum gloeosporioides (Cg)* is yam's most crucial fungal disease, causing significant yield losses. The Cg pathogen causes brown spots on the leaves, spreading rapidly in rainy seasons (Brunt *et al*., 1989). While *D. alata*, *D. nummularia* and *D. trifida* are highly susceptible to *Cg, D. esculenta*, *D. rotundata and D. pentaphylla* are more resistant (Winch *et al*., 1984). *Sclerotium* wilt disease, caused by *Sclerotium rolfsii*, is also a critical leaf disease of yam. All yam species, except possibly *D. rotundata*, are affected by this fungus (Ikotun, 1989).

Another yam foliar disease that is caused by fungus is *fusarium* wilt. Though localized, it can be virulent on yam planted on sand-loam soil. Incidence is most observed in *D. rotundata* and has been reported in some parts of Nigeria (Ikotun, 1990). Thirty-six different fungi are known to attack yam in storage across the continents of Africa, Asia and America (including the Caribbean). Still, only 16 species have been reported to be associated with severe storage deterioration (Ikotun, 1989). Rots of yam generated by fungi often start in the field and progress in storage (Ikotun, 1989).

Fungal diseases are divided into three groups: dry rots, responsible for causing soft spots and decay; wet rots, disintegrating tissues into a watery mass; and hard rots. Tuber rots of various kinds are the most significant diseases caused by fungi in yam, whether in storage, the planted settlers, or growing tubers in the field. Indeed, fungus rots account for a more significant loss of stored tubers and planted minisetts in the field. The success of minisett for SYT production depends largely on overcoming fungal infections.

Coursey (1976) also reported several fungi diseases that attack yam, which causes both dry and soft rots in the tuber. The soft rots are due to *penicillium, fusarium* and *Botrydiplodia* species. Rhizopus or Basiodiplodia may cause soft rots. *Rosellinia* and *Sphaerostilbe* are organisms that have been identified to cause dry rot. Control measures include treating the sett with fungicide or alkaline material (wood ash, mancozeb©, Rodomil©, neem extract, etc.) before planting. Harvesting carefully to avoid wounding the tubers and removing rotted tubers before minisett generation and planting are preventive measures. (Sung *et al.,* 2010).

2.15.2 Bacteria diseases of yam

A few bacterial pathogens have been reported, but only *Corynebacterium* species are pathogenic in the field (Emehute *et al*., 1998). These bacteria are associated with the yam nematode, *Scutellonema bradys*, causing dry rot in storage (Ikotun, 1989). However, the primary problematic bacterium in yam storage is *Erwinia carotovora*, which causes tuber rot under high relative humidity and cooler temperatures and is generally associated with *S. bradys* (Ekundayo and Naqvi, 1972).

2.15.3. Viral diseases of yam

In sub-Saharan Africa, where more than 90% of the world yam is produced, Virus is the most difficult to control or eliminate all the diseases affecting yam. Challenges of lack of quality seed are often associated with the virus (Adeniji *et al*., (2012), Salazar, 1996). They are difficult to identify and most farmers can neither remember nor eliminate them. This is because they bring about a gradual and not a sudden reduction in the affected plant's growth, development and tuber yield over a period that could span a decade before the total loss is attained. Viruses that have been associated with yam include yam *mosaic virus* (YMV, *D. alata virus* (DAV), *D. alata* bacilliform virus (DaBV), genus Badna virus and *D. dumetorum* virus (DdV) and *Dioscorea* mottle virus (DMoV). These viruses have been identified, indexed and differentiated using symptomatology (Goudou-Urbino *et al.,* 1996). Transmission and proliferation studies show that YMV, DaV, DaBV and CMV can be transmitted by aphids and mealybugs (Odu, *et al.,* 2001). The primary method for virus control is to use clean planting material but some resistance to YMV has been observed in some breeding lines of *D. rotundata* (Odu *et al*., 2004). These viral infections and other diseases of yam have contributed to the reduction in tuber/sett sprouting, survival and overall yield per area in yam.

In recent times, efforts have been made through viral indexing, on-the-field positive selection, chemicals and bio-control measures to reduce infestation. Varietal selection for disease resistance has also been one of the breeding focus at IITA. Yam farmers are limited to positive selection and rarely acquire clean seed to combat the menace posed by yam disease. CTC and TIBs are suitable for multiplying viral index plants. The technicality and cost required to do this are unaffordable to resource-poor farmers. Approaching formal seed companies who can afford CTC, TIBS and AS to

obtain healthy pre-basic and basic seed tubers, which can be multiplied by the farmer through YMT, is a sure way of attaining the desired healthy yam plant and tuber.

2.16 Seed yam tuber production and challenges

Yam can alleviate the food deficit in Africa in this 21st century if efforts are made to address critical challenges associated with its production (Tetteh and Saakwa, 1994). Seed yam could be a whole tuber with weight ranging from 1− 99 g (minitubers); a tuber weighing 100−300 g (regular SYT) or a tuber weighing 500−1000 g (large SYT for production of ceremonial yam of up to 5−10 kg and above). Seeds are strategic input in ware yam production. Yam production cannot expand without propagation techniques to address rapid SYT multiplication with enhanced quality (Aighewi *et al.,* 2014). Quality and high ratio seed production in yam is attainable by refining existing seed production techniques, developing or adapting novel approaches and promoting these techniques to different end-users. The short, medium and long-term approach to the sustainable establishment of a formal seed system for yam is critical for sustainable SYT production. The scarcity of SYT is highly pronounced in some yam-growing areas of the West Africa yam belt. Farmers sometimes travel through difficult terrain to procure and transport SYT across rivers and sometimes lover several kilometers (Plate 2.2). Farmers from the north bank of Niger in Idah, Kogi State, Nigeria, assume their land is unsuitable for seed production. So they come to the south bank of Niger Ilushi, Edo-State) to acquire planting material per season.

Sustainable yam production is contingent upon an adequate supply of healthy SYT (Beckford, 2009). Seed yam on which the future of the farmer and the entire population of the yam growing regions depend has been so challenging to produce, maintain, increase and improve. The major constraint of yam production over the years has been producing healthy SYT (a whole tuber propagule of between 200−300 g). The conversion of SYT tuber to “ikokoro” (a peeled, parboiled and dried tuber for dry milling into flour used in food preparation) among Nigeria's Yoruba-speaking people has also led to a not favourable competition. This is because SYT, when processed into “ikokoro” increases in value by over 100%. The SYT, when converted to ‘’ikokoro’’ for yam flour, could cost as high as 240 dollars per 100 kg bag while it is less than 50 dollars when sold afresh as SYT (source: personal investigation).

Also, the preference of SYT for roadside roasting and marketing has contributed to a colossal seed deficit, which made the situation more challenging.

Plate 2.2: Seed yam tubers being ferried over River Niger (Nigeria) by Marketers and Farmers.

Source: Pelemo O. S., Ilushi yam market, Edo State, Nigeria. November 2012.

Roasters and consumers often opt for SYT tubers since they are easy to roast and very handy compared to large tubers. The specification of yam for export (3.5−4.5 cm diameter and 15−40 cm tuber length) in terms of size, which falls within the SYT tubers range, also negatively impacts the availability of SYT planting material. The poor adoption of the yam minisett technique design to combat this seed deficit has also been poorly adopted.

2.17.1 Seed production through milking

In most yam growing areas, next season SYT is often produced by pre-harvest of actively bulking tubers, often called fresh tubers (around July-August), to obtain the second harvest, which is considered most suitable as seed. Production of second harvest seed is primarily possible in regions with bi-modal rainfall or areas with up to 8 months of rain. This seed produces tough skin and is less prone to damage by high soil temperature and nematode attack (Degras 1993; Aighewi *et al.,* 2015).

However, late bulking, occasional drought, tediousness and high production costs discourage its wide adoption. The need to make SYT available in quality and quantity has led to various techniques ranging from botanical seeds through the minisetts technique to the vine cutting technique (Aighewi *et al.,* 2015). Before the advent of these techniques, farmers generate SYT through milking, selecting SYT sizes during harvesting, especially among varieties that produce multiple tubers. Seed yam production requires a combination of Minisetts, peel-sett, minitubers and vine cuttings techniques. Seed from previously milked yam plant is unpredictable and unreliable for regular SYT supply.

Reliance on traditional ways of producing SYT has failed because milking has not met SYT demand and is only possible with the early maturing varieties. There is also the disadvantage of partial to total plant loss where milking is succeeded by occasional drought or prolong (August) break. The use of ware yam as starting material in an SYT production is paradoxical in that it takes away from the food supply (Ezeh, 2004). The prospect of SYT depends on refining the current development of novel SYT production techniques and the effective transfer of these techniques to end-users.

2.17.2 Yam minisett technique

The Yam Minisetts Technique (YMT) involves about 25 g sett to produce the whole tuber, which serves as SYT (Okoli and Akoroda, 1995). This technique is helpful for rapid, high-volume SYT production (IITA, 1985; Igwilo *et al.,* 2009). The technique has increased the multiplication ratio in SYT production from 1:5 to 1:30 (Orkwor *et al*., 1998). Production of SYT through YMT has been found to be economically viable (Mignouna *et al*., 2014a).

Despite this and notwithstanding its existence for about four decades, YMT is still unpopular because most of the manuals earlier developed to out-scale YMT were not readily available to farmers (Aighewi *et al.,* 2014). Plant populations of 40,000, 50,000 and 60,000 can be obtained per hectare in YMT using a spacing of 25×100, 20×100 and 15×100 cm, respectively (Ezeh, 2004). The low adoption of YMT is mostly occasioned by failures in poor sprouting and sensitivity to moisture stress in the soil have been its bane or shortcomings (Ayankanmi *et al.,* 2006). The average yields for seed multiplication are usually low, 3−5 t/ha depending on management practices. Under good management, about one ton of planting material gave yields of 8–24 t/ha depending on the cultivar.

The YMT has excellent potential to alleviate the SYT deficit, but only 46.6% of farmers are aware of it in Nigeria, while only about 22.4% are practicing YMT (Okoro, 2008). Low sprout rate in minisetts, partly due to inadequate rainfall, insufficient planting time and the challenges militating against adopting and using this technique. The poor understanding of the technique, as mentioned, is one of the reasons why yam producers must take the risk of ferrying SYT across river Niger in Nigeria (plate 2.2). Lack of technical details (39.7%), high labour and skill requirement (38.3%), environmental challenges (34.4%), dearth of inputs (17.8%) and inadequate storage facilities (1.7%) (Okoro, 2008) are among the obstacles militating against SYT production. This can reduce loss by over 50%. The rate and uniformity of sprouting of the Minisetts technique with white guinea yam (*D. rotundata*) were lacking. The pre-sprouting technique of Minisetts SYT production developed by IITA was found to give more uniform plant establishment on the field and tuber size (Otoo *et al.,* 1985).

There are knowledge gaps in the appropriate sett weights that provide the required SYT (100−500 g). IITA, in conjunction with European Initiative on Agricultural Research for development in WREN media (2013), reported the use of 80

g sett weight for SYT production under YMT. Oguntade *et al.* (2010), on the economics of SYT production under YMT, used 20−50 g sett weight for economic analysis (Mignouna *et al*., 2014a). Aighewi *et al.* (2014) considered a more comprehensive range of 25−100 g minisetts weight for SYT production. The disparity in the usage of minisett weights irrespective of variety or species was confirmed in Emokaro and Law-Ogbomo (2008) report. They further reported that an increase in the sett weight of 25−50 g results in the increase in seed cost alone by 62%. Therefore, optimizing the actual sett weight needed per variety for optimum SYT production is critical to breaking even for a profitable SYT business.

Another challenge of YMT is its inability to eliminate diseases and pests than other technologies (Jules and Zareen, 2015). But positive selection, which entails choosing a healthy mother plant for seed production, is an alternative. Positive selection practice is not widespread among yam farmers (Aighewi et al., 2020). They often ignorantly market their healthy and bulky tubers that command market value while unknowingly keeping the virally infected tuber below one kilogram as planting material. The seed class tubers emerged because of low FTY from virally infected mother plants (Personal interview). Accumulation of viruses and other diseases due to the reuse of tubers also results in a continuous decline in yield, productivity and eventual loss of germplasm. This is often preceded by foliar symptoms and reduced vine mass which farmers often ignore.

2.17.3 Vine cutting technique

The use of vine cutting to produce vine seedlings was based on the pioneering work of Njoku (1963), which drew attention to the possibility of raising seedlings of food yam through vine cuttings as an alternative to propagation by tuber. Given the large quantities of tubers/bulbils committed to producing new yam plants, which otherwise would have been available for human consumption, other methods of yam propagation using vine cuttings (VC) have been sought after. The vine cuttings can be used to produce minitubers between 100 and 120days which could be re-planted to produce SYT (Acha *et al*., 2004; Shiwachi *et al*., 2005; Lebot, 2009).

A cutting usually involves a node, made so that about 2.5 cm of vine tissue is left attached below and above the node with the leaf intact (Okonkwo, 1985). Different methods of growing 1−3 nodal cuttings have been practiced by scientists at the International Institute of Tropical Agriculture, Ibadan, Nigeria. These include pre-

nursery of vine cuttings in cups with a mixture of Sterilized Top Soil (STS) and Carbonized rice husk (CRH) in ratio 1:1 and 1:2 respectively or *vice versa* depending on the rationale for rooting and or source of the mother plant. The nursing of vine cutting with a nursery bag and its subsequent transplant to the field has been used extensively in vine seedling production.

Depending on the mother plants, day length and age, the VC may just form roots and minitubers. Plants above three months in age (those in the reproductive phase where rapid tuber formation and flowering have occurred) will produce only roots and minitubers while single nodal cutting excised from young but matured plant produces seedlings with the new shoot (Shiwachi *et al*., 2005). The use of vine cuttings as propagative material has received a lot of interest, particularly with the use of vine cuttings from the Aeroponics system to generate seedlings for SYT production (Maroya *et al*., 2015). Yam propagation using vine cuttings was first reported in non-food yam; it has since been extensively reported in food yam (Vander Zaag and Fox, 1981; Akoroda and Okonma 1982; Shiwachi *et al*., 2005; Kikuno *et al.,* 2008). Different explant sources for VC, including screen houses, field plants, acclimatized tissue culture plantlets and Aeroponics plants, have all been attempted.

Vine cuttings have been reported to take to the age of the mother plants. Vines excised from actively growing plants: plants in the vegetative growth stage produce new shoots. Whereas vine cuttings excised from plants above 90 days after emergence or tuber formation stage tends to produce minitubers only without new shoots. This age effect of the mother plant in relation to VC survival and tuber production has also been reported (Kikuno *et al.,* 2007). But information on varietal response to this technique is still sketchy. Identifying the suitable explant source for establishing mother plants needed for VC and screening of yam varieties for response to this technique will contribute to the optimisation of this technique for SYT.

In recent times, the growth of vine cuttings in suspended vertical sacks with an automated irrigation facility that can take 10−20 two nodal cuttings per sack was introduced at IITA Ibadan (Lopez-Montes, 2014). Also, non-rooted and rooted vines have been used as the source of planting material for the AS (Maroya *et al*., 2014). A significant improvement in the rooting, survival, vine growth, tuber formation and yield from vine cuttings have recorded considerable success. Rooted vine cuttings of 1−3 nodes (Acha *et al.,* 2004; Kikuno *et al.,* 2007; and Agele *et al.,* 2010) produced minitubers of 50−600 g after eight months (Aighewi *et al.,* 2015). This implies that

single nodal cuttings with good variety (Breeder lines) and source (virus indexed clean plantlet of tissue culture origin), if well cultured, have the potential to produce above seed to ware yam.

Several yam species have been rooted in different media without hormones, although some hormone treatments accelerate root development in yam vines (Acha *et al.,* 2004). The ease with which cutting can root and establish varies with species and cultivar and is influenced by physiological factors related to plant growth (Hartman *et al.,* 1997). Onwueme and Haverkort (1991) reported that *D. alata* roots more readily than *D. rotundata* while *D. esculenta* and *D. trifida* are difficult to root from vine cuttings (Hartman *et al.,* 1997). Rooted vine cuttings could give a higher multiplication rate than propagation through Minisetts (Okoli *et al.,* 1982). Studies have been conducted on yam propagation using vine cuttings. Hormones such as auxin, indole-butyric acid (IBA) and naphthaleneacetic acid (NAA) had been used in the recent past to either initiate or enhance rooting (Okoli and Akoroda, 1995; Acha *et al.,* 2004).

The explants' sources and age of vine cuttings had been reported to affect rooting, shoot and tuber formation in yam (Kikuno *et al.,* 2007). Acha *et al.* (2004) reported that hormones could enhance root and shoot formation in vine cuttings, especially in non-responsive clones of *D. rotundata.* The treatment of nodal cuttings with different hormones and hormone concentrations before planting in a suitable rooting medium could be valuable in determining the optimum hormone concentration required for effective rooting. The cost implication of using synthetic hormones (IBA, NAA) is high and the ease of obtaining them in a developing country like Nigeria is limited. Agele *et al.* (2010) suggest suitable and cheaper natural compounds (growth-promoting substances) at optimum concentration for yam vine rooting. In IITA-Ibadan, modification of vine cutting techniques over the years has been ongoing.

Vine cutting of *D. rotundata* can be used to produce minitubers within 100−120 days that could be used for germplasm exchange and production of yam (Shiwachi *et al.,* 2005). Rooting of vine cuttings decreased with the age of the mother plant; the young plant (2−3 months) had better new vine and root formation than old plants (4-5 months) (Kikuno *et al.,* 2007). Natural compounds have been found to enhance the percentage of vines that form tubers (Kikuno *et al.,* 2007). Hybrid yam varieties were also reported to respond better to the vine cutting method of propagation than local varieties (Ikeorgu *et al.,* 2008). The established vine cutting rate was lower in field materials than plant materials from tissue culture (Kikuno *et al.,* 2007; Okunade,

2011). Therefore, better results are obtained using tissue culture-derived plants, previously potted and grown in the screen house for vine seedling generation. Seed tuber production through vine cuttings increases the multiplication of clones beyond the minisetts level (Nwankwo *et al*., 2017).

The need to develop this technique is informed by the inability of an average farmer to acquire equipment, tool, technicalities, or expertise presently in use under research. The screen house, which has played a significant role in screening for the amount of insolation received by plants and preventing pests, diseases and human attacks, is unaffordable to small-scale farmers. The procurement of healthy SYT and *in vitro* plantlets to produce mother plants for vine seedling production has also been perceived to contribute a stumbling block towards rural farmers' successful adoption of this technique when released. Seed tuber production through vine cuttings increases the multiplication of clones beyond the minisetts level. It also results in the production of SYT that is free of nematodes and soil-borne pathogens if a sterilized medium of topsoil and carbonized rice husk (CRH) is used. Thus, it offers potential for 'cleaning up' declining clones (Lebot, 2009). The yam nodal vine cuttings have offered valuable hope to overcome the challenges of inadequate planting material. Among other methods of rapid multiplication of yam seeds, the vine cutting technique stands out as the most promising in terms of adaptability and multiplication ratio. Multiplication of yam by *in vitro* growth of the nodal segment is practical for rapid clonal multiplication. Seed production through vine cuttings increases the rapid proliferation of clones, resulting in seeds free of nematodes and soil-borne pathogens if a sterilized medium is used (Akoroda and Okonmah, 1982).

The vine cuttings propagation method is helpful for plant breeders, seed companies and elite seed growers, and yam farmers because of the technicalities and resources required for its setup. Propagation through vine cuttings offers higher multiplication rates and healthy seed tuber. It reduces the use of tubers that can be used for foods or seed tubers for the next cropping season and the rapid propagation of improved varieties (Aighewi *et al*., 2015). Also, the vine cutting technique ensures better quality SYT since soil-born pathogen such as nematode is avoided.

2.17.4 Production of Yam minitubers

Minitubers are whole but small yam tubers that range in size from l−20 g. They can be sown directly in the field with a better sprouting rate for SYT production. It has

been reported that minitubers eliminate non-uniform sprout and fungal attacks associated with minisetts (Ikeorgu *et al.*, 2008). The use of minitubers for SYT production was developed to augment the YMT earlier promoted by IITA and the yam research program of the National Root Crop Research Institute (NRCRI) to complement the yam minisetts technique developed over two decades ago (Ikeorgu and Nwokocha, 2001).

This technique aims at producing minitubers (whole but small tubers 30–150 g) from 6−10 g minisetts that could be distributed to farmers to be sown directly into prepared seedbeds for seed and ware yam production. Micro-setts are planted at a close spacing of 20×10 cm to give a population of 500,000 plants per hectare. Experience shows that you do not need a particular medium to raise the seedlings. Still, the use of shade nets capable of 40–50% shading improves the sprouting percentage and, therefore, the minitubers yield relative to the un-covered plots (Ikeorgu and Ogbonna, 2009). It requires a seed agency to produce the minitubers of desired varieties and supply them to the farmers. Thus, it eliminates the inherent drudgery for the farmer in cutting setts, treating with chemicals and curing, which the farmer often complains as a setback to the adoption of the yam Minisetts technique. Minituber yields so far achieved from trials range from 1.5 t/ha to 5.0 t/ha depending on the yam species and variety (Ikeorgu and Ogbonna, 2009).

Single nodal vine cuttings are a good source of minitubers production as 85.5% of vine cuttings from the screen house have a weight range of 10−50 g and field-derived vine cutting materials have tubers 2–10 g weight range at 100% (Okunade, 2011).

2.17.5 Aeroponics System (AS)

Aeroponics System is the process of growing plants in an air or mist environment without soil or an aggregate medium (known as geoponics). Soil-less yam propagation system will increase seed and ware yam productivity and effectively reduce diseases and pest incidence and severity (no soil-borne or vector transmitted pests and diseases during the vegetative phase). Greenhouse for AS food production was first used in 1986. The AS was now involved in the application of large-scale closed-loop in greenhouses for commercial crop production (Stoner and Clawson, 2000). The AS has been used to grow vegetables and seeds in potatoes (Otazu, 2010), yam (Maroya *et al.*, 2015)

The AS has rowing plants in small spaces, especially indoors. The roots of plants are suspended or hung in a dark chamber and periodically sprayed with a nutrient-rich solution. The AS plants are suspended (usually inserted in the top) over a reservoir within some sealed container. Feeding for AS is accomplished using a pump and sprinkler system, which periodically sprays nutrient-rich solutions to the plant roots. Due to the disease-free environment unique to AS, plants could be grown at a higher density (per square meter). The AS was also described as a valuable, rapid and straightforward method for preliminary screening of genotypes as roots are easily accessible for screening (du Toit and Perderson, 1997). The roots are most suitable for DNA extraction and the growth trend can easily be measured as applicable in the shooting part.

Plants grown using AS spend intermittent time in the air and in direct contact with hydro-atomized nutrient solution. The time spent without water allows the roots to capture oxygen more efficiently (Otazu, 2010). Furthermore, the hydro-atomized mist also significantly contributes to root oxygenation and plant growth. The relatively low solution volumes used in AS, coupled with the minimal amount of time that the roots are exposed to the hydro-atomized mist, minimize root-to-root contact and spread pathogens between plants. The AS for seed tuber and seedling production by design can eliminate soilborne pathogens, thereby producing healthy SYT.

The AS adopted for yam in this study has been widely used in potato tuber production. Propagating yam by directly planting vine cuttings in AS boxes to produce mini-tubers in the air is a novel technique requiring modification. Still, a test trial conducted at IITA-Ibadan shortly after establishment shows that AS technique can work perfectly in yam. The use of pre-rooted and direct vines cuttings of 2−3 nodes has been reported to be successful and minitubers of 0.2−2.7 were harvested from the preliminary trial conducted at IITA-Ibadan (Maroya *et al.*, 2014a).

Direct planting of unacclimatized *in vitro* and Bioreactor plantlets into the field readily exposes the plants to harsh environmental conditions and field pests and diseases. The AS provides a sustainable mechanism to grow such clean plants. The As grown plants produce vines, seedlings and tubers planted in the field, thereby increasing the success rate of clean plants introduced to the field using these systems. The production and distribution of healthy SYT tuber will be a breakthrough in yam production.

Healthy seed tuber can increase to 70.0% (Maroya *et al.,* 2014; Balogun *et al*., 2014a). The development of AS procedures to produce VCs and standard seed tuber is

needed to strengthen healthy SYT in yam. This is the basis for evaluating the performance of explants of varied sources and the nutrient type and rate required for optimum vine development and tuber formation in yam under AS system.

2.18 Microtuber production in conventional tissue culture

Plant Tissue Culture (PTC) may be defined as a process whereby small pieces of living tissue and or explants are isolated from an organism and grown aseptically on a nutrient medium under controlled conditions (Gamborg, 1991; Amelia and Lii-Jang, 1982). Economical, the use of PTC is for the rapid propagation of plants for clean seed tuber production in yam. Plant Tissue culture facilitates the exchange of plant breeding material. *In vitro* culture combined with other techniques allows the elimination of viruses and other diseases from valuable clones. Germplasm conservation *in vitro* is also essential for the preservation of genetic resources (Kameswara, 2004). The optimisation of CTC and TIBS could enhance the multiplication of plantlets and microtuber production in yam if the appropriate media, light colour, photoperiod and hormone concentration are known (Alizadeh *et al*., 1998; Cabrera *et al*., 2005). Hogue (2010) also reported that 3% sucrose without hormone could not produce any micro-tuber under *in vitro* conditions, while 6% sucrose without hormone showed a non-significant effect. Murashige and Skoog (MS) medium supplemented with 4mg/L KIN + 6% sucrose showed the best result. Alizadeh (1998) reported that 80−100% sucrose added to MS media compared to MS only significantly induced microtuber production in yam. Growth regulators, media types and compositions influence micro-tuber formation in yam (Alizadeh *et al*., 1998; Cabrera *et al*., 2005).

2.18.1 Media formulation and preparation for *in vitro* yam culture

Murashige and Skoog (1962) gave a comprehensive outline of the generalized nutrient medium, which is the most used today. This is usually modified to suit various species and explants responses. The results achieved have been remarkable Gamborg *et al.* (1982) classified the media for plant tissue culture into five parts: inorganic salts, carbon sources, vitamins, growth regulators and organic supplements. Different media concentrations are being used, either liquid (without agar) or semisolid (with agar) form. Tissue culture enhances rapid multiplication and also reduces labour and space requirements. Despite the improvement in yam propagation from setts (Minisetts) and seeds, the methods are still slow and unsuitable for clean and healthy seed production.

2.19 Temporary Immersion Bioreactor system

Temporary immersion bioreactor systems (TIBS) for plant micropropagation support plants' cell, tissue, organ growth and development. It comprises plant and medium chambers in separate or identical containers, air compressors, shelves, timer, LED or fluorescent lights, *etc.* TIBS for plant growth are in different categories based on operation:

1. tilting and rocker machines.
2. complete immersion of plant material and renewal of the nutrient medium.
3. partial immersion and a liquid nutrient renewal mechanism.
4. complete immersion by pneumatic driven transfer of liquid medium and without nutrient medium renewal (Etienne and Berthouly 2002).

TIBS is a great advantage compared to the conventional tissue culture (CTC) in that it allows for aeration of the plants leading to rapid growth and healthy plantlet. It is used in achieving the following:

a. vine proliferation and micro-cuttings.
b. minituber production.
c. somatic embryogenesis (Etienne and Berthouly 2002, Ossai *et al.*, 2018).

Teisson *et al.* (1999) listed the following conditions as necessary for efficient and productive TIBS irrespective of the type:

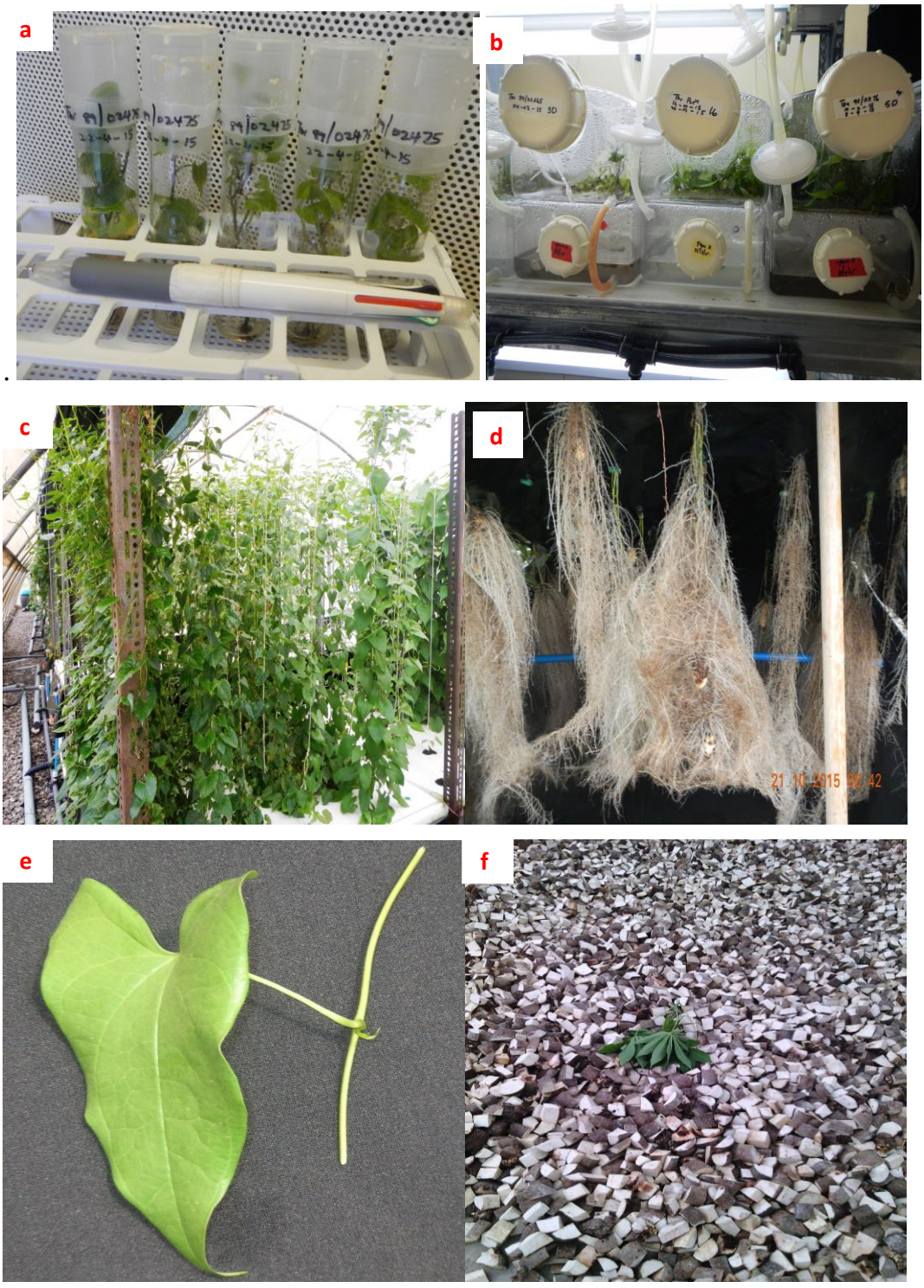
i. avoidance of continuous immersion, which adversely affects growth and morphogenesis
ii. provision of adequate oxygen transfer
iii. provision of sufficient mixing
iv. limits shear levels
v. enable sequential medium changes and automation
vi. reduced contamination
vii. cheap and easy to acquire

In addition, sustainable TIBS must be easy to repair and well adapted with accessible parts that can easily be improvised. Although minisett and vine cutting techniques are being refined, these techniques cannot yield virus-free seeds, more so as yam is a clonally propagated crop. Of all these techniques, conventional tissue culture, TIBS and the AS system ensure producing healthy, clean SYT tuber. This is possible since the environments are either aseptic/*in vitro* (TIBS and Tissue culture) or partially controlled (AS system). TIBS has the assurance of producing healthy, clean tuber at a very high multiplication rate of up to 32,768 in 365 days per TIBS (Balogun *et al.*,

2014b). Also, yam microtubers formation in TIBS has been reported to have higher yield and better plant vigour when compared to plants from the conventional propagation (Cabrera *et al.,* 2011).

The optimisation of TIBS for the multiplication of plantlets and microtuber production in yam could be enhanced if the appropriate medium, light and immersion frequency are known. Hogue (2010) reported that 3% sucrose without hormone could not produce any microtuber under *in vitro* conditions, while 6% sucrose without hormone showed a non-significant effect. But Murashige and Skoog (MS) medium supplemented with 4 mg/l KIN + 6% sucrose showed the best result. Alizadeh et al., (1998) reported that 80−100% sucrose added to MS medium compared to MS only significantly induced microtuber in yam under TIBS. Growth regulators, media types and composition influence microtuber formation in yam (Alizadeh *et al.,* 1998; Cabrera *et al.,* 2005). Hogue (2010) reported that dark conditions took a minimum of 22 days for tuber production against the light condition (35 days). Also, yam microtubers formed in TIBS have been reported to have higher yield and better plant vigour when compared to plants from the conventional propagation (Cabrera *et al.,* 2011). Many factors (variety, immersion frequency, medium type, medium composition, light regime) were reported to affect micro-tuber inducement. So this work seeks to narrow the conditions down to the more critical ones. Combining these factors using a stepwise approach while relying on literature to prioritize the actual contributory factor(s) to micro-tuber formation in yam under *in vitro* conditions.

Although Minisetts and vine cutting techniques are being refined, these techniques cannot generate virus-free seeds, more so as yam is a clonally propagated crop. Plant material propagated by temporary immersion can perform better during the acclimatization phase than material obtained on semi-solid or liquid media (Etienne and Berthouly 2002). Some of the SYT production techniques assessed in this study have been used in potato and vegetable production or refined for yam propagation. Among the techniques of interest considered CTC and TIBs are techniques for clean plantlet or pre-basic seed production (Plate 2.3 a & b). The AS showed promising performance for yam culture. Its adaptation for yam culture is premised on its performance for the growth and multiplication of related clonal crops. It can yield nematode-free SYT tuber, bulbils and vine cutting suitable for seedling production (Plate 2.3 b & c).



Plates 2.3 Some of the propagation techniques for SYT production

a. Conventional Tissue culture; source of virus-free plantlet or minitubers
b. Yam plantlets culture in TIBS; TIBs enhance rapid multiplication
c. Yam plants growing in AS.
d. Yam tubers produced in AS chamber.
e. Single nodal cutting for vine seedling production.
f. Minisett generated from whole clean tubers for SYT production.

Among the propagation techniques under assessment, vine cutting and yam minisett are relatively known among yam researchers and a few farmers (Plate 2.3 e & f). Minisett and vine cutting techniques have multiplication ratios of 1:10 (Mbanaso, 2011; Balogun *et al.*, 2014a) and 1: 80, respectively (Acha *et al*., 2004). TIBS offers a faster (and better) multiplication rate while culture aeration, growth, development, system automation and productivity are enhanced compared to CTC (Balogun *et al.,* 2014b). It is also a helpful tool that provides yam plants of high quality in vigour and ease of microtuber production (Akita and Ohta, 2002). Despite the advantages in TIBS, there is a limited number of reports on TIBS application to produce seedling and or storage organ microtuber in yam (Akita and Ohta, 2002).

The current trend is that microtubers can be produced, mainly in *D. alata* while *D. rotundata,* which is the most important yam species in the SSA had recorded a sporadic micro tuber formation under TIBs and CTC. The optimisation of CTC and TIBS for tuber formation through the review of photoperiods and nutrient composition. It is also necessary to know how these microtubers will perform in term of sprouting potential, plant establishment and the overall tuber yield.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experiments and experimental sites

3.1.1 Locations of the experimental site

The International Institute of Tropical Agriculture, Ibadan, where most of this work was carried out, is located at the 7º26'N latitude and 3°54'E longitude in the transition rain forest zone. This zone is at an altitude of 210 m above sea level and has a mean annual rainfall of 1400 mm spread between April and October, followed by 5-month dry weather (Table 3.1). The soil is a derived basement complex rock with sandy-loam surface texture overlying a layer of angular to sub-angular quartz gravel merging into an argillic horizon (Lal, 1974). *In vitro* tuber initiation was conducted at the Applied *in vitro* Plant Biotechnology Laboratory, Department of Applied Bioscience, University of Gent, Belgium.

The minisett refining was carried out on-station (IITA-Ibadan) for two years. For two years, one year, an on-farm experiment was conducted at Agunrege, Atisbo Local Government Area, Oyo State. Agunrege is in the derived savannah with 08° 39' N latitude and 03° 38' E longitude at an altitude of 253 m above sea level. The physical and chemical properties of the fields used before land preparation in each cropping season and location are as shown in Table 3.2.

3.1.2 Varieties used in this study

A total of 18 yam varieties, including 13 varieties of *D. rotundata* (five breeder lines and eight landraces) and five varieties of *D. alata* (three breeder lines and two landraces), were used in this study (Table 3.3). The maximum number of varieties used was 16 in the minisett experiment conducted on-station. Based on the performances and status of the selected varieties used in the on-station experiment, ten varieties were further selected and used in the on-farm minisett demonstration in conjunction with yam farmers. Five varieties were used in the *in vitro* experiment, conducted at the University of Ghent and Aeroponics experiment conducted at IITA, Ibadan, Nigeria.

Table 3.1: Monthly weather report for IITA-Ibadan, Oyo state, Nigeria as obtained before and during the field trial

Month	Rainfall (mm)	Min Temp (° C)	Max Temp (° C)	Min RH (%)	Max RH (%)
2013					
January	32.9	20.7	34.0	29.0	88.0
February	30.1	22.8	34.4	32.0	91.0
March	101.1	23.7	33.9	47.0	94.0
April	242.6	30.0	32.5	52.0	96.0
May	102.5	22.4	31.3	57.0	95.0
June	155.0	22.2	30.1	60.0	96.0
July	178.6	21.5	28.4	69.0	97.0
August	46.0	21.0	27.9	65.0	96.0
September	169.0	21.9	29.3	62.0	94.0
October	107.1	22.3	30.5	57.0	95.0
November	20.5	23.1	32.2	50.0	95.0
December	114.5	21.7	32.2	39.0	92.0
2014					
January	0.5	22.6	33.2	37.0	96.0
February	83.8	22.7	34.6	30.0	92.0
March	153.6	23.7	33.5	45.0	95.0
April	164.6	23.1	32.3	54.0	94.0
May	178.1	22.8	40.8	57.0	94.0
June	367.2	22.6	30.5	63.0	95.0
July	324.2	22.5	28.5	68.0	91.0
August	98.6	21.5	26.9	72.0	93.0
September	134.2	22.1	28.6	66.0	92.0
October	177.1	22.0	30.1	61.0	92.0
November	49.1	22.8	31.4	50.0	90.0
December	0.0	21.5	32.5	30.0	86.0

Source: Agro-climatology unit IITA, Ibadan (2015)

RH = Relative humidity

Table 3.2 Soil physical and chemical properties of the experimental sites

Soil properties	Unit	IITA_BN19 Value	IITA_D17 Value	On-farm (Agunrege) Value
pH (H_2O (1:1))	-	5.3	6.3	6.2
Organic Carbon	g/kg	0.9	1.1	1.1
Available P	mg/kg	4.9	4.2	5.3
Total N	g/kg	0.9	1.0	1.0
Exchangeable bases (Cmol /kg)				
Ca		2.04	1.44	1.65
K		0.18	0.13	0.04
Mg		0.31	0.32	0.28
Na		0.05	0.08	0.09
Exch. Acidity		0.31	0.34	0.30
ECEC		2.89	1.97	2.06
Particle size distribution (%)				
Sand		67	62	76
Silt		11	11	9
Clay		22	27	15
Textural classification (USDA)		Sandy Loam	Sandy Loam	Sandy Loam

P = Phosphorus; N = Nitrogen; Ca = Calcium; K = Potassium; Mg = Magnesium; Na = Sodium; Exch. Acidity = Exchangeable Acidity; ECEC = Exchangeable Cation Exchange Capacity; USDA = United States Department of Agriculture

Table 3.3: Varieties of *D. rotundata* and *D. alata* yam species evaluated in this study

Serial no	Varieties	Source	Status	Experiment	Country of release/Origin
	Dioscorea rotundata				
1	TDr9518544	YIP-IITA	Released	1, 3, 4, 5	Benin
2	TDr8902677	YIP-IITA	Released	4	Nigeria
3	TDr9519177	YIP-IITA	Released	1, 3, 4, 5	Nigeria
4	TDr8902665	YIP-IITA	Released	1, 3, 4, 5	Nigeria
5	TDr8902475	YIP-IITA	Released	3, 4, 5	Nigeria
6	TDr13-1(Hembakwase)	YIP-IITA	Landrace	4	Nigeria
7	TDr04-219 (Amula)	YIP-IITA	Landrace	4, 5	Nigeria
8	Pona	YIP-IITA	Landrace	4, 5	Ghana
9	Obiaturugo	Ilushi	Landrace	4	Nigeria
10	Alumaco	YIP IITA	Landrace	4	Nigeria
11	Danacha	Ilushi	Landrace	4	Nigeria
12	Meccakusa	Ilushi	Landrace	4, 5	Nigeria
	Dioscorea alata				
13	TDa0000194	YIP-IITA	Released	4, 5	Nigeria
14	TDa93-36	YIP-IITA	Landrace	4	Nigeria
15	TDa9801176	YIP-IITA	Released	1,3, 4, 5	Nigeria
16	TDa291(Agbon)	YIP-IITA	Landrace	1, 2, 3, 4, 5	Nigeria

Source: National Centre for Genetic Resources and Biotechnology (NACGRAB), (2012); Nweke (2016);
Experiments: 1. Tissue culture; 2. Temporary immersion bioreactor system; 3. Aeroponics; 4. On-station minisett; 5. On-farm minisett.
YIP: Yam improvement program; GRC: Genetic resources centre; IITA: International Institute of Tropical Agriculture

One variety (TDa291) was used in the minitubers experiment involving a Temporary immersion bioreactor system (TIBS).

3.2 Determination of the statuses of available SYT production techniques among key respondents

Structured questionnaires were used to retrieve information from 35 yam researchers. These spread across Savanah Agricultural Research Institute (SARI), Nyankpala, Ghana and Crop Research Institute (CRI), Kumasi, Ghana; International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and National Root Crop Research Institute (NRCRI), Umudike, Abia State Nigeria. Eighty-five percent of these were retrieved and used to process the information on awareness, usage and cost of SYT production technologies known to these researchers. Similarly, structured questionnaires were used to interview 50 key yam farmers at high and low yam production locations in Oyo State, Nigeria.

The questions stated in the structured questionnaire were interpreted into local languages (Yoruba or Pidgin English) for the farmers by the trained enumerators sourced from the Justice Development and Peace Commission of the Catholic mission. These enumerators were those that have been working directly with these farmers in both locations. The farmers interviewed spread across fourteen villages in Oyo state. Data were collected on the background information, the status of the respondent, farm size, years of farming, awareness and use status of SYT production technologies known to farmers. Also, setup cost (researchers only), the prospect of the techniques, estimated tuber weight (g), among other information from the researchers, were retrieved and processed using descriptive analysis.

3.3 Tuber production in selected yam varieties under conventional tissue culture

This experiment assessed the effects of basal medium composition, light quality and light duration for micro-tuber production in Conventional Tissue Culture (CTC). Three basal media compositions, two hormones concentration, with a no-hormone as control (1), blue and red-Light emitting Diodes (LED) with white fluorescent as control (2) and two photoperiods (16 and 8 hours) were assessed. Parameters measured include shoot growth and microtuber initiation in selected yam varieties. The procedures below were used in this experiment conducted at the

Bioscience Centre, Department of the Applied Bioscience, Laboratory of *In-vitro* Plant Biotechnology, University of Ghent, Belgium.

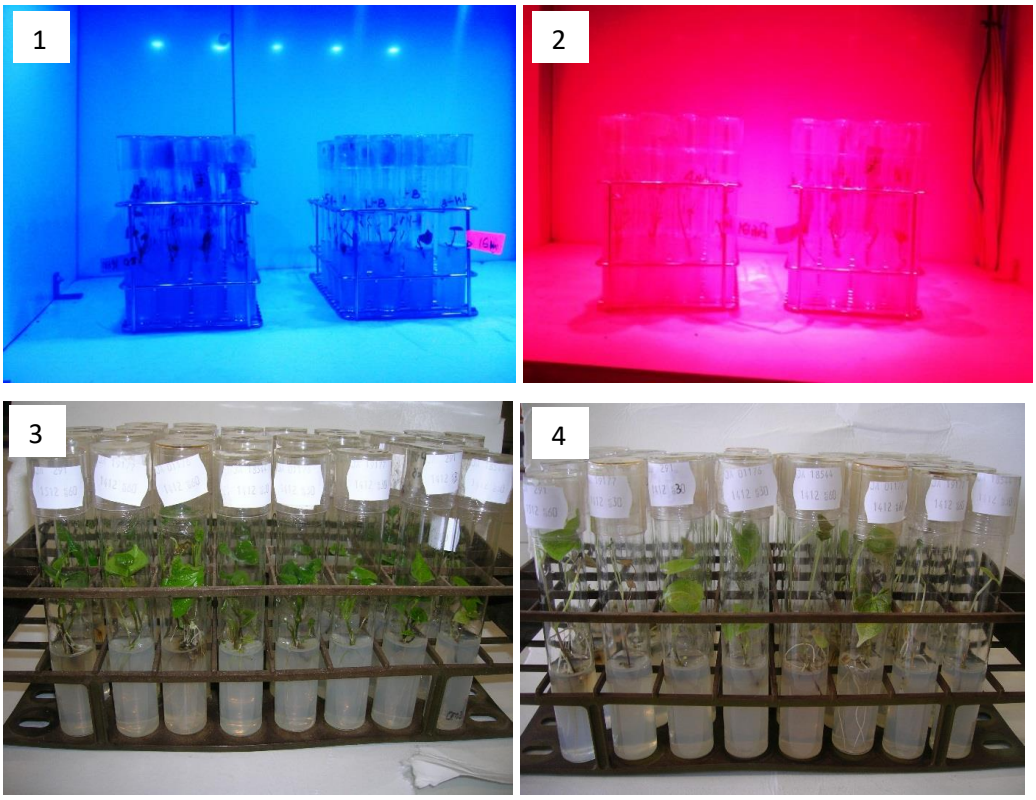
3.3.1 Plantlets preparation at IITA-Ibadan

The equipment and materials used were: flow hood, plastics, test-tubes, tool-kits, media reagents, light (fluorescent, Red and Blue LED), plantlets of selected five yam varieties (TDa 291, TDa 98/01176, TDr 95/18544, TDr 95/19177 and TDr 89/02665), mouth and nose guards, disposable gloves and overall. Clean (virus indexed) plants initially cultured in 250 mL test tubes filled with 30 mL semi-solid media at IITA-Ibadan were transported to the laboratory in Belgium within 24 hours. The initial multiplication media used at IITA-Ibadan comprised of full Murashige and Skoog (MS) basal medium (4.43 g/L) (1962), with vitamins, sucrose 30 g/L and purified agar at seven g/L. These test-tube plants were kept in the growth room at a temperature of 25±2 ºC for four weeks. At four weeks after culture, fully grown test-tube plants that showed no contamination upon visual observation were selected and transferred to the *In vitro* Plant Biotechnology Laboratory of the Department of the Applied Biosciences University of Ghent, Belgium for microtuber initiation experiment using three different light colours.

3.3.2 Microtuber induction in plants cultured *in vitro*

The factors imposed were: light type (red and blue LED lights) with white fluorescent as control−1 ((Plate 3.1: (1−4)), hormones (jasmonic, naphthalene Acetic Acid and No-hormone as Control−2), photoperiod regimes (8 and 16 hours) and varieties (5) were combined using a stepwise design. Before the subculturing of CTC plants in this experiment, plantlets cultured into these factors were further observed for bacteria and fungi contamination. Only clean test-tube plants were selected and used.

Materials: Flow hood, plastics, test-tubes, tool-kits, media reagents, light (White Fluorescent (45 µmol $m^{-2}s^{-1}$PAR)., Red LED (660 nm photons) and Blue LED (454 nm photons)), plantlets of five varieties of yam (TDa 291, TDa 98/01176, TDr 95/18544, TDr 95/19177 and TDr 89/02665) sourced from IITA. Other items used were: mouth and nose guards, disposable gloves and a laboratory coat.



Plates 3.1 Coloured Light emitting diode as its influence yam growth

1) *CTC plantlet under blue LED.*
2) *CTC plantlet under red LED.*
3) *Normal CTC plants*
4) *Etiolated CTC plants.*

Media Preparation: There were six media compositions, namely: (M1) 4.43 g/L MS with vitamins, No-hormone, 30 g/L sucrose; M2). 4.43 g/L MS with vitamins, No-hormone, 60 g/L sucrose; M3). 4.43 g/L MS with vitamins, 0.1 mg/L Jasmonic acids, 30 g/L sucrose; M4). 4.43 g/L MS with vitamins, 0.1 mg/L Jasmonic acids, 60 g/L Sucrose, M5). 4.43 g/L MS with vitamins, 0.1 mg/L NAA, 30 g/L sucrose and M_6). 4.43 g/L MS with vitamins, 0.1 mg/L NAA, 60 g/L sucrose. Naphthalene Acetic Acid (NAA), 30 g/L sucrose and f). 4.43 g/L MS with vitamins, 1 mg/L NAA, 60 g/L sucrose was used to culture plants in this experiment. Kinetin at 1 mg/L, L-cysteine at 20 mg/L and purified agar at 7 g/L were used for media composition. Hormones were added after autoclaving before dispensing into test tubes. Media were dispensed into test tubes at 30 mL per 250 mL glass test tubes and were left for 72 hours to observe them for contamination.

Clean (virus and bacterial indexed) plants prepared from IITA-Ibadan were sub-cultured into single nodal cuttings and then transferred into the test tubes. This exercise was conducted under an aseptic environment using a lamina flow hood. These cultured plantlets were placed in the growth room at a temperature of 25±2ºC. Cultured plantlets were placed under Red and Blue LED lights with white fluorescent (WF) as control-1 in boxed wooding shelves. As stated in the media composition above, two photoperiod regimes (16 and 8 hours) and two sucrose levels (30 and 60 g/L) were applied.

Experimental Design: A step-wise completely randomized design (CRD) was used. Three varieties of *D. rotundata* and two varieties of *D. alata.* Four tissue culture plantlets were assigned per factor combination.

Data Collection: Data on the establishment, number of shoots, leaves and calli formation were collected at 1 MAC, while at 4 MAC, data were collected on the number of initiated tubers, number of calli, number of leaves, number of nodes, number of branches and length of vine. These data were collected from 188 test tube plants. The values obtained were used to step down the treatment combinations to 48 experimental units, which were the best. These data were collated and analysed using analysis of variance and descriptive statistics.

3.4 Assessing the performance of in vitro plant growth under TIBS

Nodal cuttings were obtained from plants previously cultured from CTC-derived plants grown in test tubes and TIBS sources. The CTC plants were sourced from plantlets cultured in 250 mL test tubes containing 30 mL of semi-solid media, while TIBS plants were obtained from setis, which comprises a 2.5 L tank containing 1 L nutrient solution and a mounted plant growth chamber.

3.4.1 Experimental design and procedure

A completely randomized design with four replications was used in this experiment. Media containing Murashige and Skoog (MS) basal medium with vitamins (4.43 g/L), Myo-inositol (100 mg/L), sucrose (30 g/L), Kinetin (1 mg/L) and L-cysteine (20 mg/L) and purified Agar (7 g/L) was used. Media were dispensed at 100 mL per 500 mL transparent plastic vessel. The lids of these vessels were ventilated with paper film capable of preventing fungi and bacteria spores. Plants obtained from the two sources were excised into two nodal cuttings. Transfer of cuttings into plastic vessels was done at five cuttings per vessel containing the growth media. The nodal cuttings in culture were placed under fluorescent light in the growth room at a temperature of 25±2 ºC. These *in vitro* cultured plants were observed for the number of roots, vine, leaves and tuber formed at 4 and 8 weeks after culture (WAC).

Data collection: Data were collected on the number of leaves, the numbers of nodes, the number and the length of vine, plant vigour (score of 1−5), leaf colour, calli formation and oxidation (score) at four and eight weeks after planting. These data were analysed using descriptive analysis.

3.5 Microtuber production under temporary immersion bioreactor system

Nodal segments of TDa 291 (*D. alata*) clones were obtained from previously cultivated plants were grown in a 250 mL test tube containing30 mL semi-solid multiplication media. The media comprised of kinetin supplemented with MS medium at 4.43 mg/L (1962), 30 g/L of sucrose and 7.0 g/L of purified agar (Gelrite). the protocol used by Cabrera *et al.* (2005), which associated microtuber production in TIBS with increased sucrose level up to 100 g/L was followed. A preliminary test-tube experiment indicated that sucrose plays a predominant role in tuber formation. Therefore, 50, 60 and 80 g/L of sucrose were used in this microtuber initiation experiment. Hence, three separate media formulations were used. There were (i) 1

mg/L NAA + 2.215 g/L MS + 60 g/L sucrose; (ii) 0 g/L NAA + 4.43 g/L MS + 80 g/L sucrose; and (iii) 0 g/L NAA + 2.215 g/L MS + 50 g/L sucrose. These formulations were randomly selected., Two light types were further tested. These light types were (i) combined red LED of 660 nm photons and (ii) blue LED of 454 nm photons. The white fluorescent light of 45 µmol $m^{-2}s^{-1}$PAR was used as the control (Plate 3.1; 1–4) and the testing was done in a dark chamber at the TIBS facility, Bioscience Centre, IITA, Ibadan, Nigeria. This testing was done using the following procedure: three media formulations were prepared at one litre per TIBS setis using a pH of 5.7. The formulated media were autoclaved to prevent the likelihood of fungi and bacteria contamination. Fifty nodal cuttings in four yam varieties were sub-cultured and introduced into the TIBS setup. These plants under TIBS culture were observed at 4 WAC and terminated at 12 WAC. Plant growth parameters and microtubers produced were evaluated. Harvested tubers were planted into potting soil and observed for plant growth.

Data on the number of microtubers produced, leaves, nodes and roots were collected at 4, 8 and 12 WAC. The evaluation of tuber formation was done at 12 WAC only when the experiment was terminated. The number of leaves, nodes, tubers and tuber weight were taken per factor combination. Data collected were processed and the mean value of performance was reported using descriptive statistics.

3.6 Evaluating explants sources and the responses of yam varieties under Aeroponics System

The evaluation of two yam varieties for survival, growth and tuber production was carried out in 2014 and 2015 at IITA-Ibadan. Three different planting materials, i.e., direct vine cutting (DVC) (two nodal cuttings), indirect (Rooted), single nodal cuttings with new vine and Bioreactor (*in vitro*) generated plantlets, were used. A Fertiliser composition comprising ammonium nitrate (272.7 g), calcium nitrate (195.5 g), potassium sulphate (60g), triple superphosphate (65.2 g), Magnesium Sulphate (98.3 g) and fetrilon C (5 g) were dissolved into 800 L of water. The pH (H_2O: acid; 1:1) of the nutrient solution was 5.7. This composed nutrient solution was used in fertigating the root zone of suspended plants in the lid box Aeroponics chamber.

Sterilization of soil for mother plant establishment: the soil sterilized for 10 hours at a temperature of 80°C using an electronic sterilizer (Pro-Grow Electric Soil Steriliser, Model SST 60R) was used in planting.

3.6.1 Establishing mother plants for direct and pre-rooted vine cuttings

In this experiment, there are three varieties of D. rotundata (TDr 95/18544, TDr 95/19177, TDr 89/02665) and two of D. alata (TDa 98/01176) TDa 291) were used. Seed yam tubers (100−200 g) were selected and cut into 20 g minisett weight. These minisetts were immersed n a mixture of insecticide (lambda-cyhalothrin at 2 ml/L) and fungicide (dithiocarbamate at 10 g/L) for 15 minutes. Air drying was done in a cool, dry place for 24 hours, after which the setts were buried in a moist carbonized rice husk (CRH) for pre-sprouting. Setts with sprouts and roots were transplanted into potted soils two weeks after pre-sprouting.

Experimental Design and procedure: Pots were laid in a completely randomized design (CRD) at the Glasshouse, IITA-Ibadan. Plastic pipes were used to stake the yam plants while watering was done as at when due. At two months after planting (MAP), symptomless mother plants were selected for a vine-cutting generation. Vines of these two months old plants were excised into two nodal cuttings for direct planting in the AS. The leaves of the lower node were excised with scissors and then inserted into the holes on the chamber lids. Each of the varieties was planted at 12 cuttings per replication. Only two replications were possible due to the inadequate number of Aeroponics tables as of the time of this experiment. These two nodal cuttings were supported with Styrofoam at the point of insertion into the holes.

3.6.2 Production and transfer of explants in Aeroponics System (AS)

Experimental Design and procedure: The experimental design was a split-plot where plant sources were the main plot, whereas varieties were sub-plot. The aeroponics system introduced explanations sourced from direct vine cuttings (DVC), rooted vine cuttings (RVC) and acclimatized TIBS plants. The 18 explants with two nodal cuttings were excised using scissors from one or two healthy mother plants cultured using experiment 1 procedure. These two nodal cuttings per variety were planted directly into the AS was the case in Experiment 1. Similarly, 30 single nodal cuttings excised from the same plants were planted into nursery bags containing STS and CRH at ratio 2:1. The leaves of the 1st nodes immersed into the chamber in the DVC method were removed such that the lateral meristem and the leaf stalk are intact. Roots and tuber(s) emerged from this node while the uppermost leaves/nodes above the chamber lid were left for photosynthesis, transpiration and the subsequent

emergence of a new vine. Also, vine cuttings with root and new shoot herein referred to as rooted vine cuttings (RVC) were produced using STS and CRH.

Transplanting the pre-rooted cuttings into the chamber was such that root and mother-leaf petiole were within the chamber while the leaves blade and the new vine were above the chamber. Plantlets obtained from the TIBS, DVC and RVC were compared with one another in the AS facility. Eight weeks old bioreactor plantlets were acclimatized for two weeks in the insect-proof screen house. The TIBS plantlets and RVC were transplanted into AS simultaneously, while the DVC preceded the TIBS and RVC materials by four weeks.

Data collection and analysis: Data on plant survival and plant growth parameters were recorded at 14, 28 and 56 days after planting (DAP), while the yield parameters were taken at 4, 8 and 12 MAP. Data on the number of established vine seedlings after transplanting and the number of tubers produced in AS at harvest was recorded. The data collected were subjected to analysis of variance while means were separated using the least significant difference (LSD) at a 5 % probability level.

3.7 Assessing the influence of sett weights and varieties on fresh tuber yield

This experiment was carried out in two seasons at IITA, Ibadan. Minisetts were generated from selected healthy (nematode and rot-free) seed tubers of 100−400 g weight that have broken dormancy. Thus, five sett weights (SW) (10, 20, 30, 40 and 50 g) were obtained from 12 varieties of *D. rotundata* (Alumaco, TDr 04-219, Danacha, TDr13-1, Meccakusa, Obiaturugo, Pona, TDr 89/02475, TDr 89/02665, TDr 89/02677, TDr 95/18544 and TDr 95/19177) and four varieties of *D. alata* (TDa 291, TDa 93-36, TDa 00/00194 and TDa 98/01176) using a digital weighing balance. This experiment was carried out in the 2013/2014 cropping season and repeated in the 2014/2015 season.

3.7.1 Pre-planting Operations

Cutting of sett was done such that tubers were first to cut into 50 and 40 g SW and then the smaller setts (10 – 30 g) were obtained from these initial SW, i. e. 40−50 g. In all the SW produced, the cut surface to area bearing the periderm was ensured at a minimum ratio of 1:2. This implies that a sett has a minimum of 33% periderm cover. Each sett was placed on a scale to determine its weight using an error margin of ±1. Twenty setts per factor (Variety × SW × Rep) were produced. Setts were loosely

bagged using a net bag to avoid bruises and then dipped in a solution of ethylene bisdi-thiocarbamate (EBDC)) applied at 20 g/L of water and lambda-cyhalothrin at 20 mL/L of water for 10 minutes. Air drying of these minisetts was done in a cool, dry place. Minisetts were then buried in moist carbonised rice husk (CRH) and kept for two weeks to ensure the healing of cut surfaces.

3.7.2 Field Preparation, design and layout

Field preparation was carried out a week before planting and the layout of the field was in three replications. A split-plot design was used with sett weight as main plot and variety as sub-plot. The plots were separated with a 1 m alley between blocks and 1 m space within plots. The length of each plot was 5 m. 20 setts per plot per variety at 0.25 m intra-row and 1 m inter-row spacing was used. *D. rotundata* and *D. alata* were planted in separate plots to avoid bias due to shading. Planting was done by carefully selecting setts that met any criteria for planting, i.e., healed cut surface and initiated root and vine. Planting was done by burying setts at a depth of 4−6 cm using a hand hoe. Care was taken to ensure that the periderm surface was placed on the soil while the cut faced the planter before covering it with soil. Herbicide application was carried out immediately after planting using a combination of Pre-emergence and contact herbicides.

3.7.3 Agronomic operations

Emergent yam vines were trellised on 2 mm diameter nylon ropes vertically attached to horizontal 10 mm diameter nylon ropes attached to the top of 2 m high bamboo poles. Each pair of bamboo poles was placed at both ends of each plot. 10 mm nylon rope was at the top ends to form a line. The 2 mm nylon ropes were loosely tied to each emergent vine just above the first node and then attached to the horizontal 10 mm rope, which is 2 m above ground level. Weedings on the field were carried out at intervals of 25− 30 days and these were supplemented with roguing during data collection to maintain a weed-free field.

Data collection and analysis: Data were collected on sprout emergence at 1, 2, 4 MAP plant establishment, Yield and yield-related data collected include the number of stands at harvest, number of tubers and tuber weight (Table 3.4). Data collated were analysed using SAS 2003 version 9.1.4, while means were separated using Tukey's honest significant difference (HSD).

3.8 Re-assessing yam minisett technique among farmers

An on-farm trial was conducted among farmers in *Agunrege*, Oyo State, Nigeria. Agunrege is surrounded by about 18 other communities and has the derived guinea savannah agro-climatic condition (08°39'369″N, 03°38'723″E, 253 masl). Three sett weight (SW) categories (10, 30 and 50 g) were used in this experiment. A total of 10 varieties, including seven varieties of *D. rotundata* (TDr 04-219, Meccakusa, Pona, TDr 89/02475, TDr 89/02665, TDr 95/18544, TDr 95/19177) and three varieties of *D. alata* (TDa 291, TDa 00/00194 and TDa 98/01176) were used. Seed yam tubers (100−300 g) with broken dormancy were selected and then cut into 10, 30 and 50 g SW with a sharp knife and a sensitive weighing balance. An error margin of ±1 was allowed in all the three SWs generated.

The minisetts were loosely bagged using net bags to avoid bruises and then dipped in a solution of ethylene bisdi-thiocarbamate (EBDC) applied at 2 g/L of water and lambda-cyhalothrin at 20 mL/L of water for 10 minutes. Planting was done on a plot of four ridges at 0.25 m intra-row and 1m inter-row spacing. The minisetts were planted on each plot using 40 setts per factor (Var × SW × Rep) at ten setts per line. Unlike the 2−year trials at IITA, the on-farm trial was planted directly without the stake to reduce cost and make it attractive. At the same time, only three SWs were used to ease communication of experimental procedure and output to farmers to enhance the adoption of a practical outcome. The experimental design used was Randomized Complete Block Design (RCBD) fitted into a split-plot arrangement in three replications. Field operations and practices were carried out with the farmers in the on-station minisett trial. Two field visits at the plant growth stage (4 MAP) and at harvesting (6 MAP) were organised for farmers. Tubers were harvested at complete senescence (6 MAP), with harvest data collected and analysed using SAS 2003 version 9.1.4, while means were separated using Tukey's honest significant difference (HSD).

Table 3.4: Morphological and agronomic data collected

Data collected	Method of assessment	When collected	Unit of measure
Sprout or vine emergence	Vine emergence from soil level was counted and collated.	7- 49 DAP	Number
Plant establishment till harvest	Counting of the existing plants at monthly interval till harvest	1-6MAP	percentage
Tuber weight	All tubers were harvested and weighed at harvest per plot using a calibrated scale.	6 MAP	Gram
Number of tubers and category (Ware, Seed and less than seed tuber)	Tubers harvested were counted per plot and sorted by categories using a calibrated scale	6 MAP	Nil
Dry matter weight of tuber at harvest	100 g standard weight sample weight was chop per tuber and oven-dried to constant weight at a temperature of 75 ºC for 72 hours	6 MAP	Percentage

DAP Days After Planting; MAP = Months after Planting

CHAPTER FOUR
RESULTS

4.1 Responses on awareness and use of SYT production techniques

4.1.1 Background data of key farmers consulted and interviewed

Finding from the interview of yam key stakeholders (Researchers and yam farmers) showed that the average age of yam farmers was 50 years. Although, 55.0% of those farmers interviewed were below the age of 50 years. An indication that yam is still not a crop of interest among the youths. Furthermore, farmers surveyed in the study area cultivated yam on less than 2 ha. The gender imbalance was also high among yam growers in the surveyed area, given that less than 10.0% of yam farmers interviewed were women (male to female yam farmers were in the ratio (44:3). All the farmers claimed not to have received any improved yam variety and training on SYT production techniques.

4.1.2 Awareness and use of potential SYT production techniques among Researchers and farmers

The yam researchers interviewed showed that Yam Minisett Technique (YMT) was the most known and utilized technique. All the researchers (100.0%) were aware of YMT, while 71.0% used this technique for Seed Yam Tuber (SYT) production (Figure 4.1). Researchers' awareness and use of Vine Cutting Technique (VCT) and minitubers for SYT production were 77.0% and 32.0%, respectively. About 67.7% of researchers were aware of the use of Conventional Tissue Culture (CTC). The percentage (9.7%) of researchers who were using Tissue Culture for this purpose was low. Temporary Immersion Bioreactor System (TIBS) and Aeroponics System (AS) had awareness values of 3.2% and 41.9%, respectively, among researchers. None of the researchers interviewed with the aid of a stuructured questionnaire used AS and TIBS as both had 0.0% usage (Figure 4.1). Similarly, 51.0% of the farmers were aware of YMT (Figure 4.2). Vine cuttings and minitubers had low awareness ratings of 12.0% and 7.0%, respectively. The farmers interviewed had no awareness (0.0%) of AS, TIBS and CTC.

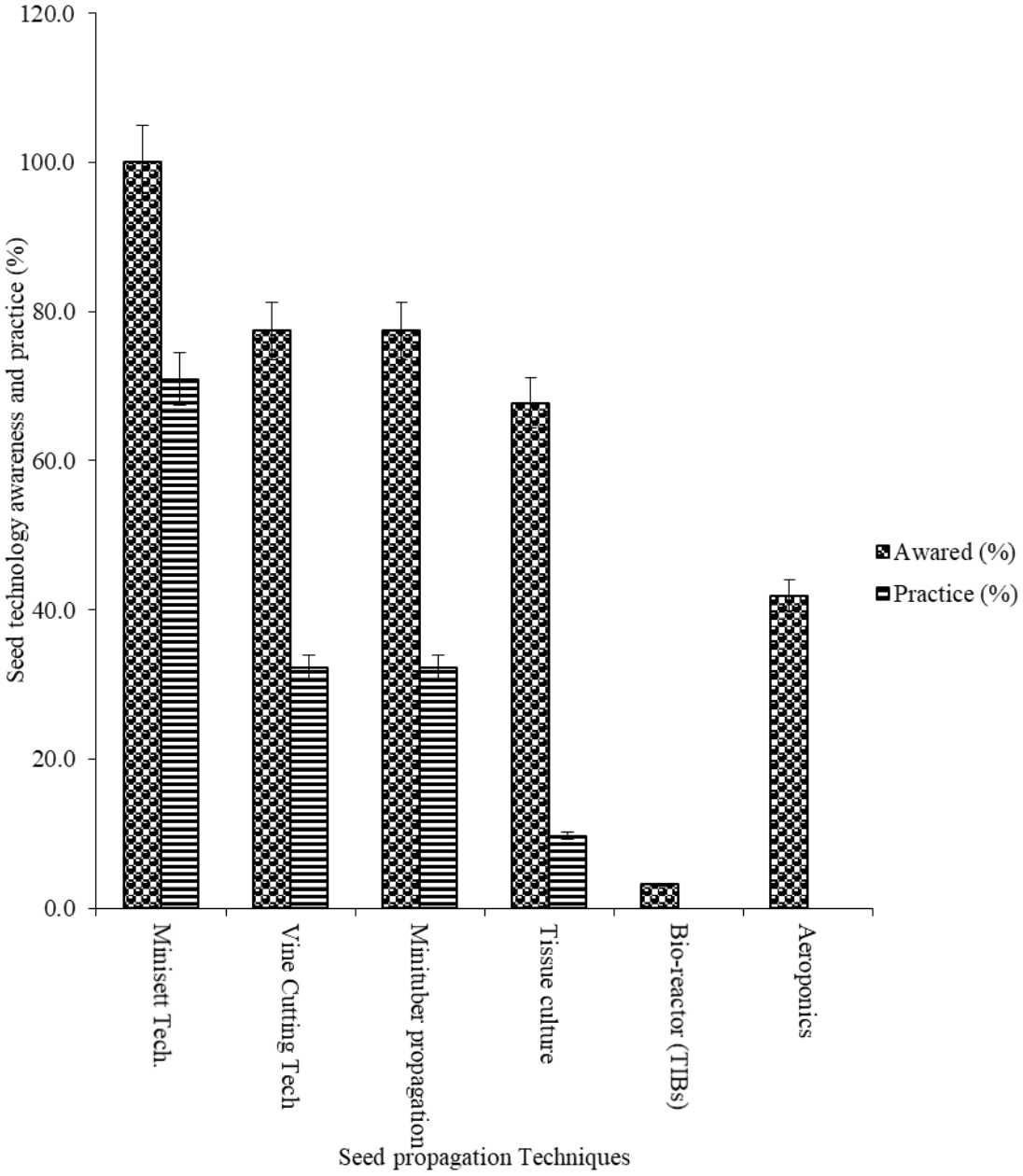


Figure 4.1: Awareness and use status of different SYT production techniques among yam researchers

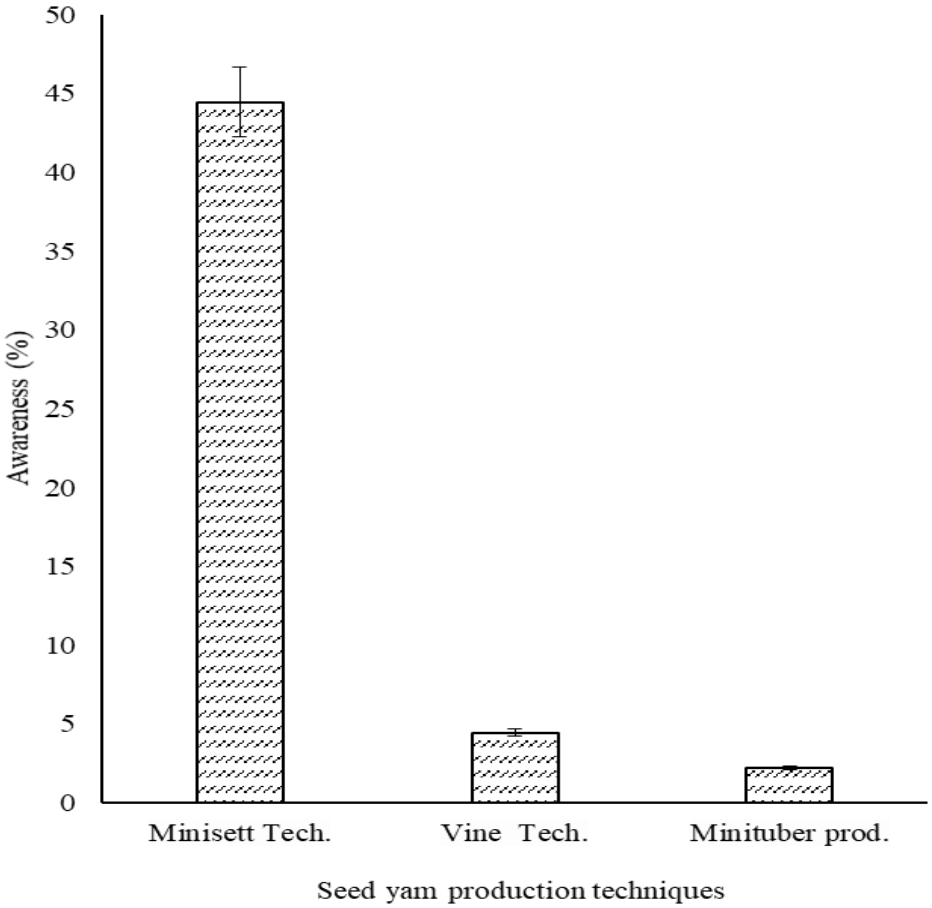


Figure 4.2: Levels of awareness of different SYT production techniques among farmers

4.1.3 Estimated output on SYT production techniques among researchers

The estimated success rate obtained from the respondents, who had used at least one of these techniques, indicated that the yam minisett technique (65.9%) had the highest survival rate, closely followed by conventional tissue culture (CTC) (57.1%). The vine-cutting technique (43.1%) had the lowest survival rate (Figure 4.3). Similarly, the minisett technique gave the highest value of the average tuber weight (269.2 g), followed by vine cuttings (58.3 g), while cultures from CTC rarely produce tubers (Figure 4.3).

4.1.4 Cost estimates of high-ratio propagation techniques of SYT

Information retrieved from the researchers on the projected cost of setting up SYT production technologies indicated that, on an estimated basis, it would take approximately US $ 20,000 to establish TIBS and AS (Figure 4.4). The surveyed researchers cited the initial capital requirements for CTC, TIBS and AS and their high technical requirements as the most important challenges limiting their use.

Vine cutting technique required an estimated value of about 10,000 USD for setup, while YMT had the least capital of about 1,000 USD as setup cost. All the respondents ranked YMT as the technique that required the lowest setup cost (Figure 4.4). The setup cost ranging from 1,000 USD in minisett technique to 20,000 USD for CTC, TIBS and AS described the variation in adopting these techniques.

4.1.5 Seed sources, forms and tuber portion knowledge among farmers

Information retrieved from farmers interviewed on their seed types and sources showed that 65.0% of the farmers purchased their seed from markets, followed equally by farmers who claim to have gotten their seeds from their father (17.5%) and friends (17.5%). (Figure 4.5). Information on farmer's seed types showed that seed from milked yam plant (35.0%), yam sett (dissected whole tuber) (35.0%) and Seed yam tubers (30.0%) (tubers of 100−500 g) are the sources of seed form according to the farmers. Head (a portion of the tuber, bearing the crown) was the most used tuber portion (67.5%). Farmers who attest to using all tuber portions without recourse for particular tuber portions were 32.5% (Figure 4.5).

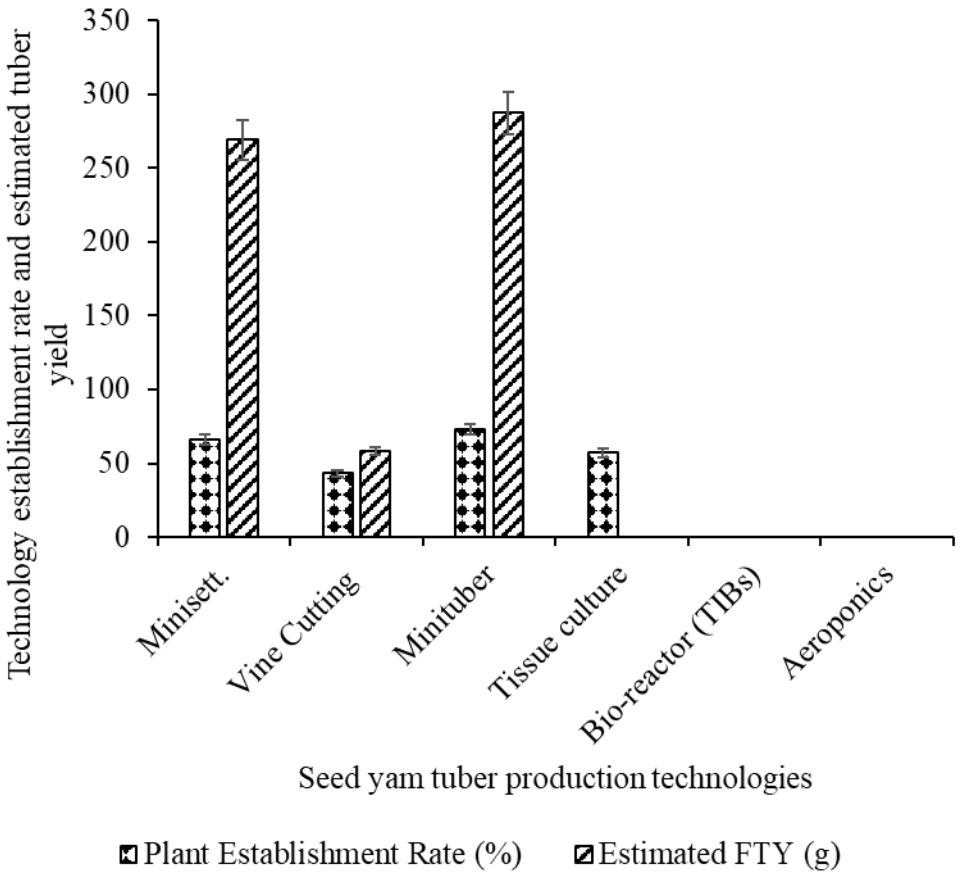


Figure 4.3: Estimated plant establishment rate and fresh tuber yield (g) among SYT production techniques

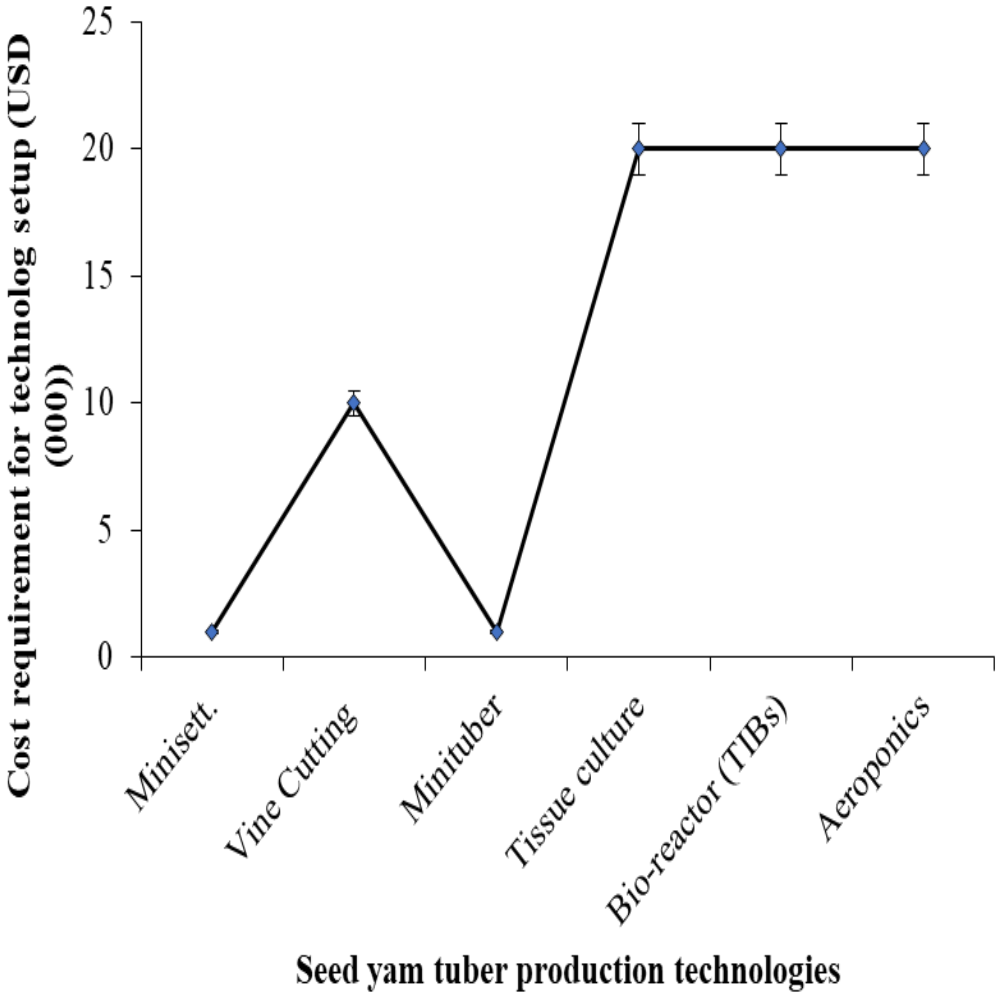


Figure 4.4: Estimated cost of establishing SYT production techniques

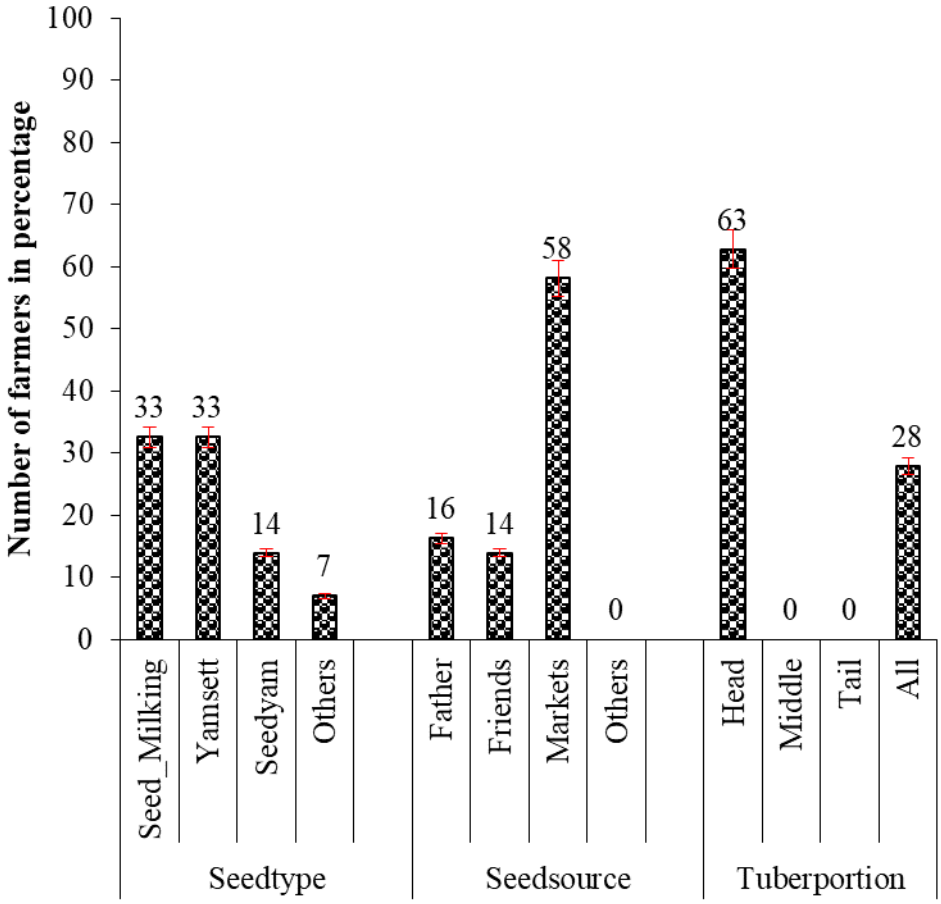


Figure 4.5: Farmers preferred seed types, seed sources and understanding of tuber portion effect in yam production

4.2 *In vitro* yam multiplication

4.2.1 Yam plant growth as influenced by explant sources under *in vitro* culture

The mean values of plant growth parameters of yam sourced from TIBS and CTC were presented in Figure 4.6. All the variables except oxidation score in TIBS-sourced single nodal cuttings had a higher value than those obtained from CTC-sourced explants. The number of nodes, the most critical parameter, had a mean value of 5.3 from TIBS-sourced explants whereas, CTC-sourced explants had a mean value of 3.5. Mean values for the number of roots (5.1), the number of leaves (5.6) and the number of vines (1.3) in plants of TIBS origin were higher than the corresponding values (1.3, 2.3 and 1.0 respectively) in plants of CTC origin.

Furthermore, the TIBS-sourced plants were three times more vigorous than CTC-sourced plants. Oxidation or darkening, which is occasioned by the release of toxic exudates, was higher in Tissue culture sourced plants (3.2) than in TIBS-sourced plants (1.7) (Figure 4.6).

Results of the selected three varieties of *D. rotundata* (TDr 89/02665, TDr 95/18544, TDr 95/19177) and two varieties of *D. alata* (TDa 98/01176, TDa 291) sourced from TIBS only and cultured in semi-solid media showed a marked varietal influence (Table 4.1). Among the *D. alata* varieties used, TDa 98/01176 was superior to TDa 291 based on parameters measured at 8WAC except for the number of roots. Among the *D. rotundata* varieties, TDr 95/18544 had the highest mean value for the number of nodes (12.5), followed by TDr 95/19177 (8.5), while TDr 89/02665 had the lowest mean value of 4.4 for the number of nodes. Thus, the number of nodes that define the multiplication rate in any yam under *in vitro* culture was least in TDa 291 and TDr 89/02665 (Table 4.1).

4.2.2 Plants responses to light types

Responses of TIB-cultured *D. alata* genotype TDa291 to 2 light types, as shown in Figure 4.7, showed that lights had a varied influence on plant growth. The mean value of 23.6 cm for vine length in plants cultured under combined blue and red LED light (BR-LED) was higher than the mean value for vine length (16.3 cm) in plants cultivated under the white fluorescent light. Although, while BR_LED plants were relatively etiolated when compared with WF culture plants, a mean value for the number of branches were higher in plants cultured under white fluorescent light (WF) (1.3) than that for the plants cultivated under BR-LED light (0.5) (Figure 4.7).

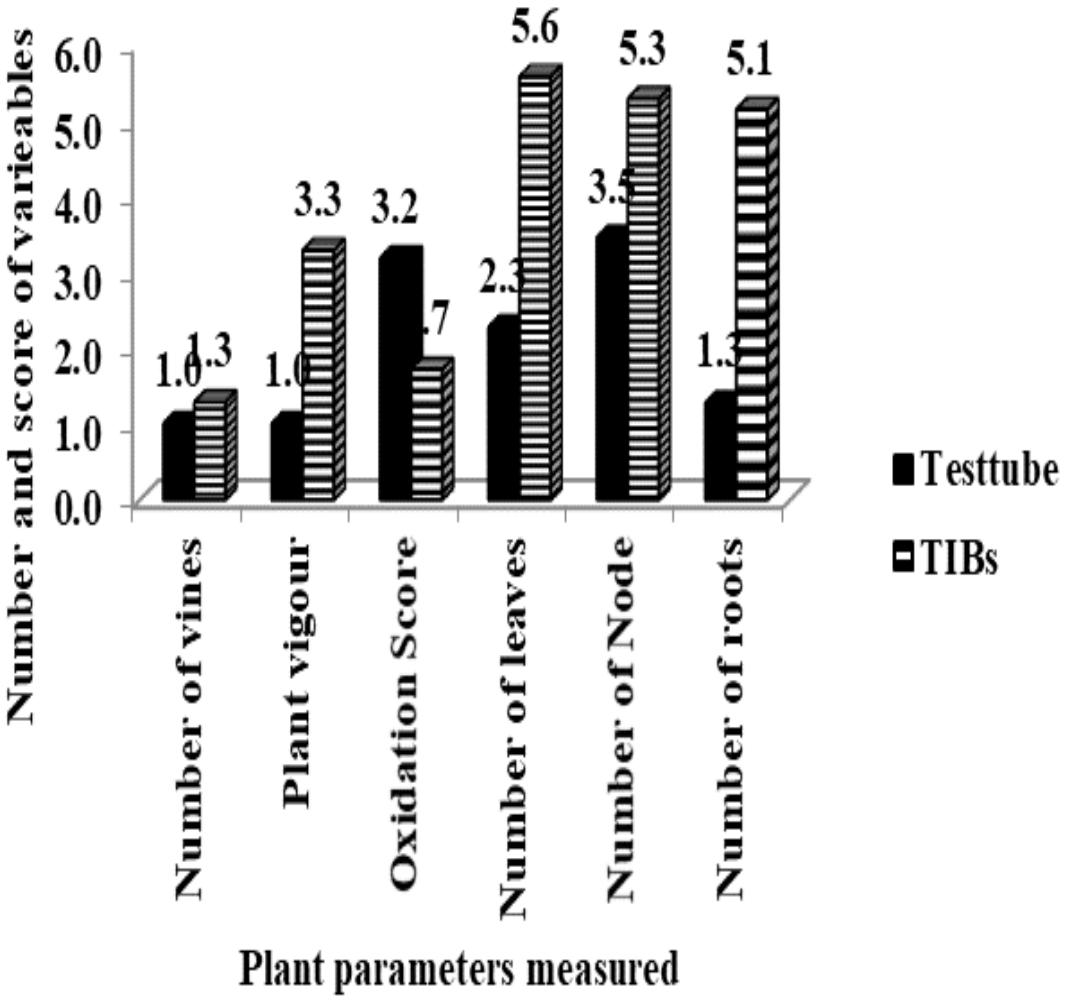


Figure 4.6: Mean values of *D. alata* cultivar as affected by explant source at 4WAC

TIBS: Temporary Immersion Bioreactor System

Table 4.1: Mean values of plant growth traits recorded at 8WAC in five yam varieties culture under conventional tissue culture

Varieties	Number of vines	Number of leaves	Number of roots	Number of nodes	Number of branches
TDr 95/19177	2.±0.02	10.2±0.40	6.7±0.13	8.5±0.11	2.4±0.13
TDr 95/18544	2.7±0.51	12.8±1.0	8.5±0.54	12.5±1.0	3.2±0.31
TDr 89/02665	2.3±0.01	4.1±1.0	3.9±0.49	4.4±0.81	0±0.40
TDa 291	1.5±0.13	7.2±0.27	6.2±0.02	7±0.22	1.5±0.07
TDa 98/01176	2±0.02	7.5±0.20	5.2±0.20	7.5±0.11	1.7±0.02
Mean	2.10	8.40	6.10	8.00	1.80
SE (±)	0.40	3.00	1.60	2.60	1.00
CV (%)	20.80	39.40	28.10	36.90	67.50

CV = Coefficient of variation; SE = standard error

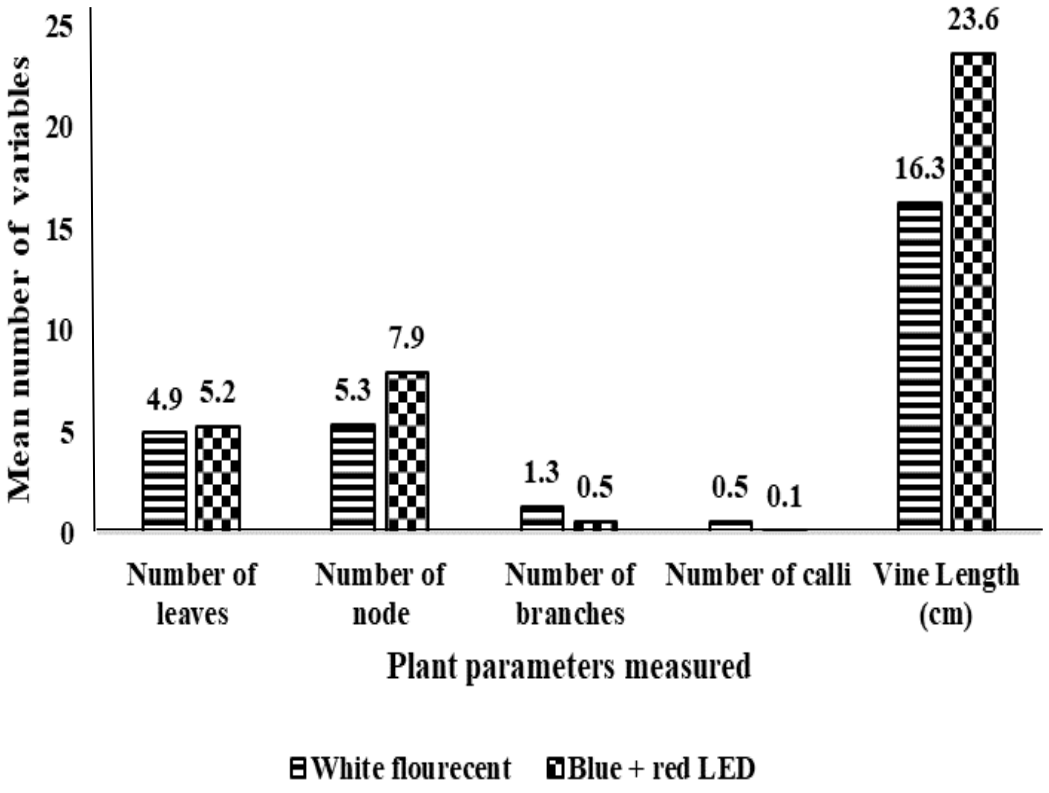


Figure 4.7: Mean values of measured parameters of yam cultivar cultured in Temporary Immersion Bioreactor System using two light types at 12 WAC

The BR-LED plants also had a higher mean value for the number of leaves (5.2) and nodes (7.9) than WF cultivated plants, which had mean values of 4.9 and 5.3 for leaves and nodes.

4.2.3 Responses of *in vitro* yam plants to media compositions, photoperiods and light types

Results obtained on the selected yam varieties' performances, using *in-vitro* protocols, showed that plant development and minituber production were influenced by sugar concentration. The difference in sugar concentration was highly significant ($p < 0.001$) for the number of tubers produced and also significant ($p < 0.05$) for the number of vines (Table 4.2). The Hormones used were highly significant ($p < 0.001$) for the number of nodes, number of tubers, number of calli, number of leaves and vine length but was not significant for the number of vines. Light types were highly significant ($p < 0.001$) for the number of nodes and leaves formed.

Also, light types significantly influenced tuber initiation but were not significant for calli formation and the number and length of the vine (Table 4.2). Varietal influence on tuber initiated, number and length of the vine were also significant ($p < 0.05$). Even though there was no significant interaction between variety and photoperiod, hormones by variety interaction were highly significant ($p < 0.001$) for vine length and was also significant ($p < 0.05$) for the number of nodes and tuber initiates (Table 4.2).

The result showed that plants under red LED light (RL), as observed visually, were characterized by etiolation. Pale green to total loss of green pigmentations was also observed in plants cultured under RL. Petioles ending in needle-like leaf rudiments (without leaf blade) were also observed in plants under RL. Light type significantly ($p < 0.05$) influences the number of nodes, number of leaves and number of tubers initiated *in vitro* (Table 4.3). White fluorescent (WF) and BL had a mean value of 8.88 and 6.41 for the number of leaves, respectively, while RL had a mean value of 3.17 (Table 4.3). The WL also had the highest mean value for nodes (12.58), followed by BL (8.23) and RL (6.71) (Table 4.3).

Mean values of plants growth parameters obtained in plants cultured under 16-hours per day photoperiod were higher than those obtained in plants cultivated under 8 hours per day photoperiod for all the parameters observed. These values were significant ($p < 0.05$) for the number of nodes and leaves.

Table 4.2: Combined analysis of variance of some yield and yield-related parameters in yam as evaluated under *in vitro* culture

Source of variation	DF	Number of nodes	Number of tubers	Number of calli	Number of leaves	Vine length (cm)	Number of vines
REP	1	1.25ns	0.39ns	0.10ns	7.05ns	2.54ns	1.10ns
Light type	2	198.80***	3.80*	0.17ns	273.06***	20.69ns	0.98ns
Photoperiod (PP)	1	37.90*	0.22ns	0.00ns	243.57*	3.91ns	1.16ns
Hormone	2	466.40***	8.32***	138.90***	367.14***	268.00***	2.58ns
Sugar (g/L)	1	46.55ns	17.90***	0.01ns	29.87ns	11.56ns	11.81*
Variety (Var)	4	35.00ns	2.20*	0.03ns	25.90ns	34.00*	5.57*
PP*Var	4	8.10ns	0.06ns	0.16ns	10.45ns	6.70ns	2.33ns
Hormone*Variety	7	45.23*	1.50*	0.09ns	18.10ns	39.22***	2.22ns

*** = highly significant ($p \leq 0.001$); * = significant ($p \leq 0.05$), ns = not Significant DF = Degree of freedom

Table 4.3 Mean values of some plant growth traits of yam cultured *in vitro* as influenced by light, photoperiod, sucrose and hormones

Variables	Number of Nodes	Number of Tubers	Number of Vines	Vine Length (cm)	Number of Calli	Number of Leaves
			Light type			
White Light	12.58a	0.79a	2.13ns	7.72ns	0.79ns	8.88a
Blue LED	8.23b	0.20b	2.13ns	7.18ns	0.66ns	6.41b
Red LED	6.71b	0.49c	1.90ns	7.94ns	0.62ns	3.17c
			Photoperiod			
16hours	8.90a	0.43ns	2.11ns	7.49ns	0.70ns	6.56a
8hours	7.10b	0.38ns	1.91ns	7.73ns	0.60ns	3.54b
			Sugar concentration			
60 g/L	8.80ns	0.79a	2.40a	7.88ns	0.81a	4.90ns
30 g/L	7.64ns	0.12b	1.81b	7.35ns	0.55b	5.55ns
			Hormones			
JA	11.28a	0.80a	2.00a	9.83a	0.05b	8.00a
NAA	5.96b	0.16b	2.24a	5.89b	1.44a	3.25b
No-hormone	5.97b	0.07b	1.48b	6.58b	0.00b	3.93b

Mean values having the same letter along the same column under each treatment category were not significantly different. LED = Light Emitting Diode; JA = Jasmonic acid; NAA = Naphthalene acetic acid

The plants subjected to the photoperiod of 16-hours had the highest mean values of 8.90 and 6.56 for the number of nodes and number of leaves, respectively. Whereas, 8 hours photoperiod had low mean values of 7.10 and 3.54 for the same parameters (Table 4.3). Although, 8-hours photoperiod plants had a higher mean value for vine length (7.7 cm) when compared to plants cultured under 16-hours photoperiod, which had a mean value of 7.5 cm for the vine length (Table 4.3).

The differences in mean values for the number of nodes, number of vines, number of tubers, number of calli and number of leaves produced were significant ($p < 0.05$) for hormones (Naphthalene acetic acid (NAA), Jasmonic acid (JA) and No-hormone) (Table 4.3). The media supplemented with JA were significantly higher ($p < 0.05$) in the mean value for number nodes, number of tubers, number of leaves and vine length (cm) (Table 4.3). Plants cultured in media supplemented with NAA and the media with no-hormone were not significantly different ($p < 0.05$) for the number of nodes, tubers and leaves but were significant for the number of vines. Calli formation (a negative attribute in plant tissue culture) was significantly higher for NAA plants, while the mean value for calli in JA and no-hormone were not significant ($p < 0.05$).

The higher sugar concentration, 60 g/L of sucrose, gave higher values (8.80), 0.80, 2.40, 7.90 cm, 0.81 and 4.90 respectively for all the parameters observed when compared with the values (7.60, 0.10, 1.80, 7.40 cm, 0.55 and 5.55 respectively) obtained for the lower concentration (30 g/L of sucrose). However, the values were only significant for the number of tubers (0.81 and 0.10 at $p < 0.001$), number of vines (2.40 and 1.80 at $p < 0.05$) and number of calli (0.81 and 0.55). Combination of WF light, 16-hour photoperiod, 60 g/L sucrose and Jasmonic acid significantly enhances tuber production. The media supplemented with 1 mg L^{-1} NAA induced callus in 98.0% of all factor combinations containing the hormone (Table 4.3).

Across varieties, mean values presented in Table 4.4 showed that the number of nodes ranked across varieties ranged from media 15.9 (M_3) under WLED to 3.3 (M6) under Red-LED. The number of microtuber initiated was observed across media supplemented with 60 g sucrose: M_4 (1.7, 1.5) for White and Red LED, respectively and M_2 under Red (1.6). The *D. rotundata* (TDr 95/18544, TDr 95/19177 and TDr 89/02665) varieties used in this experiment, in this order, had significantly higher ($p < 0.05$) mean values for the number of vines and vine length. (Table 4.5). Variety TDr

Table 4.4: Mean values of plant growth traits as influenced by media and light types under *in vitro* condition in selected yam varieties

Media*Light (LED)	No. of vines	Shoot L. (cm)	No. of nodes	No. of leaves	No. of calli	No. of tubers	No. of branches	Callus Score (1–5)
M1_Blue	1.7	5.1	6.9	5.3	0	0.1	0.6	4.8
M1_Red	1.3	7.7	5.7	1.6	0	0	0.3	5
M1_White	1.5	6.4	5.3	4.8	0	0	0.2	1
M2_White	1.9	9.1	6.4	5.4	0	0	0.3	1
M2_Blue	1.2	8.7	7.3	6.2	0	0	0.3	5
M2_Red	1.5	6.6	5.9	2.1	0	1.6	0	1
M3_Blue	1.5	7.9	10	7.2	0	0	1.2	5
M3_Red	1.6	9.3	8	4	0	0.2	1	5
M3_White	2.4	7.1	15.9	14	0	0.3	2.5	4.8
M4_Blue	2	9.1	11.3	8.3	0.2	0.4	2	4.7
M4_Red	2.2	10.5	11.3	4.3	0	1.5	2.1	5
M4_White	3.2	7.4	15.7	10.7	0.1	1.7	2.8	4.9
M5_Blue	2.5	6	8.6	6.7	1.5	0.1	1.7	2.8
M5_Red	1.9	5.9	5.6	2.5	1.2	0.1	0.7	3
M5_White	1.5	7.7	7.3	3.6	2	0	0.7	2
M6_Blue	2.1	5.6	5.3	3	1.3	0	1	2.4
M6_Red	2.5	4.3	3.3	1.2	1.5	0.2	0.3	2.4
M6_White	2.1	6.9	9.6	3.6	1	0.7	1.8	2.3
Mean	1.9	7.3	8.3	5.3	0.5	0.4	1.1	3.5
SE (±)	0.1	0.4	0.8	0.8	0.2	0.1	0.2	0.4
CV (%)	25.9	21.7	40.7	60.7	139.5	149.4	77.5	45.1

LED= light emitting diode; WF= White fluorescentM1: MS+30g/Lsucrose; M2: MS+60g/L-sucrose; M3: MS+0.1mg/L JA+30 g/L sucrose; M4: MS+0.1 mg/L-JA+60g/Lsucrose; M5: MS+1mg/L-NAA+30g/Lsucrose; M6: MS+0.1g/L-NAA+60g/Lsucrose

Table 4.5: Mean values of plant traits as influenced by varietal differences in two yam species under *in vitro* condition

Variety	Number of tubers	Number of leaves	Number of nodes	Number of calli	Vine length (cm)	Number of vines
TDa291	0.6ab	6.2a	9.7a	0.7	9.1a	2.2ab
TDa98/01176	0.8a	6.0a	8.8a	0.7	8.2a	1.9b
TDr95/19177	0.3bc	4.1a	7.2a	0.7	6.4b	1.7b
TDr95/18544	0.1c	6.3a	8.8a	0.5	7.9a	2.6a
TDr89/02665	0.0c	2.3b	3.9b	0.9	4.8c	1.6b

Means followed by the same letter along the same column were not significantly different ($p < 0.05$).

89/02665 was significantly low for the mean number of nodes (3.9), the number of tubers (0) and the number of leaves (2.3). Whereas TDr 95/19177 and TDr 95/18544recorded a mean value of 0.3, 0.1, respectively, for the number of tubers. Variety TDr 89/02665 had the lowest mean value for the number of tubers and a significantly low ($p < 0.05$) mean value for the number of leaves, number of nodes, number of vines and vine length (Table 4.5). Selected *D. alata* varieties used were significantly higher ($p < 0.05$) than *D. rotundata* varieties for the number of tubers. Variety TDa 98/01176 recorded the highest mean values of 0.8 for the number of tubers, followed by TDa 291 (0.6). Overall, *D. alata* varieties were superior to the *D. rotundata* varieties as 79.0% of the tubers produced were accounted for by TDa 98/01176 and TDa 291 (Table 4.5).

4.3 Effects of media composition and light type on plant growth and tuber production in TIBS cultured plants

The experiment reported in Table 4.6 compared three media types; they were: 1. 1.0 mg/L Naphthalene Acetic Acid (NAA) + 2.215g/L MS + 60g sucrose, 2. 0.0g/L NAA + 4.43g/L MS + 80g/L sucrose and 3. 30.0 g/L NAA + 2.215 g/L MS + 50 g/L sucrose. Plants cultured in media 2 and 3, which no NAA had a higher number of leaves, the number of nodes and the number of tubers initiates compared to media 1 with NAA as observed at four weeks after culture (WAC). There were relationships between media and light types. All the parameters measured except the number of calli were higher in plants cultured under white fluorescent (WF) lights using media 1 and 3.

The combined blue and red LED light under media 2 and 3 had higher mean values for the number of vines, number of leaves, number of nodes and tuber initiates (Table 4.6). Media 3 with half MS and 50 g/L of sucrose but without NAA had a higher number of vines (108), number of leaves (120), number of nodes (108) and number of tubers initiates (11) for plants cultured under WF light when compared to plants cultured under same light but in media 1. Media 2 and 3 had an aggregate of 8 and 21 tuber initiates at 4 WAC, while media 1 (with NAA) had a total of 2 for all light types (Table 4.6).

Similarly, at 12 WAC, plants cultured in media 2 and 3 had a mean value of 19.0 and 20.0 respectively for the number of tubers under white fluorescent while plants cultivated using the same media but under a combination of blue and red (BR)

Table 4.6: Mean values of plant growth parameters and microtuber formation as affected by different lights and media types in D. alata cultivar (TDa291) at 4 and 12 weeks after culture in the temporary immersion bioreactor system

	Media T_1		Media T_2		Media T_3	
	Combined Blue and Red LED	White Fluorescent	Combined Blue and Red LED	White Fluorescent	Combined Blue and Red LED	White Fluorescent
			4 WAC			
Number of Vines	22	63	92	15	88	108
Number of Leaves	13	56	99	9	34	120
Number of Tuber	1	1	7	1	10	11
Number of Nodes	22	76	114	15	92	108
Number of Calli	49	1	3	33	3	1
Root formed (Scored on a scale of 1−5)	1	4	4	2	4	3
			12 WAC			
Number of Vines	23	30	384	82	198	150
Number of Leaves	24	22	1320	738	143	200
Number of Tubers	0	0	19	19	12	20
Number of Nodes	120	148	900	486	460	320
Number of Calli	48	50	11	10	0	0
Root formed (Scored on a scale of 1−5)	3	1	4	4	3	4
Mean Tuber weight (g)	0	0	2	2	1	3

Media 1= 1.0 mg/L NAA + 2.215 g/L MS + 60 g Sucrose; Media 2 = 0.0 g/L NAA + 4.43g/L MS + 80 g/L Sucrose and Media 3 = 0.0 g/L NAA + 2.215 g/L MS + 50 g/L Sucrose; WAC: Weeks After Culture; LED = Light Emitting Diode

LED had a mean value of 19.0 and 12.0 respectively for the number of tubers. Thus, media 2 and 3 had an aggregate of 38 and 32 tubers, respectively.

Whereas plants cultured in media 1 produced no tuber. Also, mean values obtained for plant growth parameters measured: number of leaves, number of nodes, number of vines and number of roots formed were higher in media 2 and 3 than media 1.

Of the three media tested in this experiment, media 2 was best for measured plant growth parameters. However, media 1, which had NAA, was found to induce calli almost equally in WF (50) and BR LED (48) (Table 4.6). Also, high sucrose concentrations of 50 and 80g/L without NAA in media-2 and media-3, respectively, had tuber initiates at 4 WAC and these matured into viable tubers at 12 WAC. Plant etiolation was observed in plants cultured under LED with a mixture of blue and red lights, unlike plants cultivated under WF. Microtubers were harvested from plants cultured in TIBS at 12 WAC. These microtubers harvested from TIBS from observation broke dormancy and sprouted within two weeks of harvest. (Plate 4.1).

4.4 Performances of selected yam varieties cultured in an aeroponics system

Growth parameter

Plant growth parameters of six yam varieties cultured in the Aeroponics system showed that TDr 95/18544 had the highest mean value for new vine formation (2.7 per stand), followed by TDr 89/02665 (2.1 per stand). The mean value for the number of roots and leaves produced were also higher in varieties TDr 95/18544 (8.5 and 12.8) and TDr 95/19177 (6.7 and 10.2), respectively compared to the values obtained for the same parameters in TDr 89/02665 (3.9 and 4.1) and TDr 89/02475 (4.0 and 6.2). Varieties TDr 95/18544 and TDr 95/19177 with 12.5 and 8.5 respectively as the mean values of the number of nodes also performed better than TDr 89/02665 and TDr 89/02475, whose mean values for the same parameters were 4.4 and 5.6, respectively (Table 4.7).

This experiment also showed that vine rooting of two nodal cuttings in the Aeroponics System is significantly different ($p < 0.05$) among varieties used. Based on the percentages of vine cuttings rooted at 0.5 Months After planting (MAP), variety TDa 291 had a significantly higher value than that of variety TDa 98/01176. All the *D. rotundata* species were used except TDr 95/19177. However, among the *D. rotundata* varieties used, varieties TDr 95/19177 (38.2%) and TDr 89/02665 (38.1%) had significantly higher ($p < 0.05$) values for root formation (Table 4.8). Variety TDr

Plate 4.1: Plant responses to light and tuber formation in different media under the temporary immersion bioreactor system

A: Tubers formed within root mat of a vine cluster in TIBS; B: Tubers/bulbils in vine nodal portions; C: normal vine length as influenced by fluorescent light; D: Etiolated plants; E: effect of NAA hormone on plants; F: tubers harvested from TIBS.

Table 4.7: Mean values of plant growth traits at two months after planting among selected yam varieties cultured in Aeroponics system

Varieties	Number of emergent new vines per stand	Number of leaves	Number of roots	Number of nodes	Number of branches
TDa 291	1.5	7.2	6.2	7.0	2.5
TDa98/01176	2.0	7.5	5.2	7.5	2.7
TDr 89/02475	1.6	6.2	4.0	5.6	1.0
TDr 89/02665	2.1	4.1	3.9	4.4	1.0
TDr 95/18544	2.7	12.8	8.5	12.5	4.2
TDr 95/19177	2.0	10.2	6.7	8.5	3.4
Mean	2.0	8.0	5.8	7.6	2.5
CV (%)	21.5	38.4	30.6	37.0	52.0
LSD (0.05)	0.4	3.1	1.8	2.8	1.3

CV = Coefficient of variation; LSD = Tukey's honest Significant difference

Table 4.8: Mean values of plant parameters in direct vine cuttings of selected yam varieties cultured in aeroponics system

Variety	Rooted vine cuttings (%) at_0.5MAP	Emerged vines (%) at 1 MAP	Harvested stand (%) at 4MAP	Mean tubers per stand	Mean Tuber weight (g)
TDa 291	42.7a	65.1bc	47.5b	4.7a	4.9a
TDa 98/01176	32.1c	89.5a	53.3ab	4.4a	5.2a
TDr 89/02475	27.5d	82.5a	34.2c	3.9b	2.5b
TDr 89/02665	38.1b	69.8b	55.0a	3.6b	2.2b
TDr 95/18544	32.7c	62.5bc	45.8b	4.3a	4.1a
TDr 95/19177	38.2ab	55.6c	46.7b	4.7a	2.7b

Means followed by the same letter along the same column were not significantly different ($p < 0.05$). MAP = Months after planting

95/18544 also had a significantly higher value than that of variety TDr 89/02475, with the least value of 27.5%. This variety had the lowest plant survival at harvest (4MAP), which was significantly different ($p < 0.05$) from other values. In contrast, varieties TDr 89/02665 and TDa 98/01176 had the highest plant survival of 55.0% and 53.3%, respectively, among the two species tested (Table 4.8). Variety TDa 98/01176 had the highest overall average tuber weight of 5.2 g while TDa 291 and TDr 95/19177 had the highest mean values of 4.7 g for tuber weight at 4 MAP (Table 4.8). All the varieties used had multiple tubers in the Aeroponics system.

4.4.2 Plant survival and tuber formation in Aeroponics System

The combined ANOVA values presented in Table 4.9 showed significant differences ($p < 0.001$) among varieties, sources and variety by source interaction for all the explants introduced into the Aeroponics system. Mean values for the number of tubers harvested at 12 MAP were also significantly different ($p < 0.001$) for variety, sources and variety by source interaction. In contrast, tuber weight was only significantly different ($p < 0.001$) for variety (Table 4.9). Variety × Source interaction was also significantly different ($p < 0.05$) for survival, plant growth parameters and tuber weight obtained at 6 MAP.

The mean values of measured traits obtained for plant survival in the Aeroponics System at 1 MAP, 4 MAP and 12 MAP were presented in Table 4.10. Varieties TDr 95/18544, TDr 95/19177, TDa 98/01176 and TDa 291 had a percent survival of 87.5, 83.3, 75.0 and 25 respectively TIB- sourced plants at 4 MAP. These varieties in this exact order had mean values of 79.2, 58.3, 62.5 and 25%, respectively, for DVC plants. In contrast, the RVC had a lower survival value of 42.1, 14.3 in TDr 95/18544 and TDr 95/19177, respectively, but there was no difference in the survival of RVC and DVC sourced TDa 98/01176 under AS system (Table 4.10).

Mean values for plant establishment in AS among yam varieties of different sources, as presented in Table 4.10, showed that the source and variety influenced the mean values for the number of tubers and the length of tubers (cm). The mean values for the number of tubers, tuber weight and tuber length for DVC-sourced plants were also highest in TDr 95/18544 among the *D. rotundata* used. Variety TDa 98/01176 had mean values of 13.0 for the number of tubers for TIBS-sourced plants, while DVC and RVC-sourced plants both had a mean value of 18.0 for the number of tubers harvested. The yam tubers in SYT class (100−300 g) were found in TDr 95/18544

Table 4.9: Combined analysis of variance for survival and tuber weight of selected yam varieties from three explant sources as cultured in aeroponics system

Source of Variations	Number of survived plants at 1 MAP	Number of survived plants at 4 MAP	Number of survived plants at harvest (12 MAP)	Number of tubers harvested	Tuber length (cm)	Tuber weight (g)
Variety	11.17***	30.90***	56.27***	366.24***	90.29*	2270.65*
Source	28.16***	32.00***	53.49***	45.07*	94.08ns	1842.18ns
Variety ×Source	17.51***	7.44*	7.50*	364.23***	12.98*	1902.05ns

*, **, *** = Significant at 5%, 1% and 0.1% probability levels respectively; ns = not significant

Table 4.10: Mean values for periodic plant establishment (%) in Aeroponics (%) as influenced by variety and explant sources

Variety	Mean Survival @1MAP			Mean Survival @ 4MAP			Mean Survival @ 12MAP		
	TIBS	DVC	RVC	TIBS	DVC	RVC	TIBS	DVC	RVC
TDa 291	100.0	95.8	0.0	25.0	25.0	0.0	20.8	20.8	0.0
TDa 98/01176	100.0	100.0	100.0	75.0	62.5	70.8	66.7	62.5	54.2
TDr 89/02665	78.6	100.0	0.0	57.1	41.7	0.0	14.3	0.0	17.1
TDr 95/18544	100.0	100.0	85.7	87.5	79.2	42.9	87.5	75.0	0.0
TDr 95/19177	100.0	100.0	100.0	83.3	58.3	14.3	70.8	54.2	0.0
Mean	95.7	99.3	95.2	65.6	50.7	42.7	52.0	35.4	23.8
CV (%)	10.0	1.9	55.1	38.9	40.9	72.0	62.5	87.9	98.8
LSD (0.05)	8.6	1.7	47.1	22.9	18.6	27.6	29.1	27.9	21.1

DVC = Direct Vine Cutting; RVC = Rooted Vine Cutting; TIBS = Temporary Immersion Bioreactor System; MAP = Months After Planting; CV = Coefficient of Variation, LSD = least significant difference

(257.8 g), TDr 95/19177 (132.8 g) and TDa 98/01176 (201.40 g) for TIBS-source plants. This tuber category was also obtained, but in TDr 95/18544 (157.0) and TDa 98/01176 (183.2g) only for DVC-sourced plants and TDa 98/01176 (147.8 g) only for RVC-sourced plants (Table 4.11).

4.5 Performances of yam varieties and sett weights under minisett technique

4.5.1 Plant emergence and survival

The mean square values of the performances of the different sett weights, varieties and their interactions tested in years and across years were significantly different for most of the traits measured. Early emergence was highly significant ($p < 0.01$) for variety × sett weight (SW) interaction and highly significant ($p < 0.001$) for variety and variety × year interaction. The total number of emerged plants at 60 days after planting (DAP) was highly significant ($p < 0.001$) for variety, SW as well as variety × SW, variety × year and variety × year × SW interactions. However, this parameter was not significant for the year. The number of stands at harvest was not significant for the year and variety × SW interaction. Still, it was significant ($p < 0.01$) for the Year × Variety × SW interaction and highly significant ($p < 0.001$) for variety, SW, as well as the year × variety interaction (Table 4.12).

Mean square values for the number of tubers harvested were highly significant ($p < 0.001$) for variety, SW and the year × variety interactions. The mean square value for fresh tuber weights was also highly significant ($p < 0.001$) for variety, SW, year and year × variety and year × SW interactions. The year and variety × SW interaction were significant at $p < 0.05$ and $p < 0.01$ probability levels, respectively, variety × SW × year interaction was not significant. The number of tubers per weight categories: tubers greater than 500 g, SYT (100−500 g) and tubers less than 100 g were highly significant for varieties, SW, year as well as variety × SW, year × variety and year × SW interactions but was not significant for year × variety × SW interactions. Table (4.13). Variety × sett weight interactions were highly significant ($p < 0.001$), indicating that earliness and overall plant emergence across varieties were influenced by SW (Table 4.13).

The mean values for early emergence among the improved varieties of *D. rotundata* (TDr 95/18544, TDr 95/19177, TDr 89/02665, TDr 89/02475, TDr 89/02677) *and D. alata* (TDa 00/00194, TDa 98/01176), at 0.5 MAP respectively

Table 4.11: Yield and yield parameters of aeroponics system tubers as influenced by source and variety at 12 months after planting

	TIBS Plantlets			Direct Vine Cutting			Rooted Vine cutting		
Variety	Number of tubers	Tuber weight (g)	Tuber length (cm)	Number of tubers	Tuber weight (g)	Tuber length (cm)	Number of tubers	Tuber weight (g)	Tuber length (cm)
TDa 291	5.0	6.2	4.3	3.0	53.7	8.0	0.0	0.0	0.0
TDa 98/01176	13.0	201.4	23.4	18.0	183.2	11.0	18.0	147.8	14.3
TDr 89/02665	3.0	85.7	9.1	1.0	1.3	0.5	0.0	0.0	0.0
TDr 95/18544	13.0	257.8	38.4	7.0	157.0	21.0	7.0	42.1	14.4
TDr 95/19177	9.0	132.8	11.1	3.0	21.0	13.0	0.0	0.0	0.0

TIBS = Temporary Immersion Bioreaactor System

Table 4.12: Combined analysis of variance for some of the agronomic traits measured in yam minisett experiment conducted in two planting seasons

Source	Df	Number of emerged plants at 0.5MAP	Number of emerged plants at 1 MAP	Number of emerged plants at 2 MAP	Number of stands at harvest (6MAP)
Rep	2	1732.9	39.7	70.4	71.3
Year	1	18625.2*	38.8	187.5	4471.3
Error (a)	2	852.6	68.6	377	507.9
Sett Weight	4	1097.3	4054.5***	2802.0***	7291.1***
Year*Sett Weight	4	295.4	625.7	495.4	752.2
Error (b)	16	398.5	323.7	264.2	358.1
Var	15	8007.8***	20156.1***	10847.9***	9619.3***
Var*Year	15	2921.7***	2202.1***	1018.8***	1179.9***
Var* Sett Weight	60	231.4**	354.1***	259.8***	173.7
Var*Year* Sett Weight	60	102.8	286.0**	231.9***	186.8*
Error (c)	300	146.7	164.2	124.2	134.9
CV (%)		65.5	22.3	14.7	16.9
Mean		18.5	57.5	75.9	68.7

*, **, *** = Significant at 5%, 1% and 0.1% probability levels respectively; ns = not significant DF = degree of freedom; CV = Coefficient of variation; MAP = months after planting

Table 4.13: Combined analysis of variance for some of the harvest and post-harvest data measured in yam minisett experiment conducted in two cropping seasons

Source	Df	Number of tubers harvested	Fresh Tuber weight Plot Kg	Fresh Tuber Yield t/ha	Number of SYT tubers	Number of tubers above seed weight	Number of minitubers
Rep	2	36.7	29.9	120.8	1822*	1072	120.4
Year	1	127.1	1065.4*	4265.2*	57014***	134205**	16276.0*
Residual(a)	2	138.46	52.9	210.8	30	168	277.5
Sett weight	4	838.9***	564.37***	2258.3***	2462***	5836***	1418.6**
Year*Sett weight	4	37.2	89.41**	357.8**	853	2411***	628.0*
Residual(b)	16	37.1	14.02	55.6	308	178	201.1
Var	15	1455.4***	531.22***	2125.1***	6919***	8690***	1190.6***
Var*Year	15	121.2***	57.98***	232.5***	2958***	4872***	505.3***
Var* Sett weight	60	28.5*	12.25*	48.9*	440*	266**	239.9*
Var*Year* Sett weight	60	21.2	9.41	37.6	360	228*	218.1
Residual(c)	300	20.7	8.19	32.7	313	163	171.8
CV%		26.1	36.9	36.9	28.8	52.3	92.7
Mean		17.4	7.8	15.5	61.4	24.4	14.1

*, **, *** = Significant at 5%, 1% and 0.1% probability levels respectively; ns = not significant DF = degree of freedom; CV = Coefficient of variation; MAP = months after planting

showed that these improved varieties were more amenable to minisett technique than the landraces. The landraces of *D. rotundata* species: TDr 04-219, Meccakusa, Meccakusa, Alumaco, Obiaturugo, Danacha, Pona and *D. alata* species (TDa 93-36) except TDa 291 had low mean values for early emergence (Table 4.14). Also, the mean values for overall plant emergence were below 70% in most of the landraces. But total emergence was above 80% in most of the improved varieties used in this experiment. At 0.5 MAP, variety TDr 95/18544 had the highest percent plant emergence (PPE) of 30.7, while Danacha had the least overall PPE of 3.7% among the *D. rotundata*. Similarly, variety TDa 291 recorded the highest PPE value of 54.7%. In comparison, TDa 93-36 had the least PPE of 10.5%, a value that is four and five times lower than what was obtained in other *D. alata* cultivars used in this experiment. Variety TDa 98/01176 had the highest overall PPE of 97.7% among *D. alata* varieties, while TDr 95/18544 had the highest overall mean PPE value (93.6%) at 2 MAP among *D. rotundata* varieties used (Table 4.14).

Landraces such as Meccakusa and Meccakusa (Ojuyawo) had overall PPE above 70% at 2 MAP, indicating that these can be successfully used by farmers using the yam minisett technique. Among the improved varieties of *D. rotundata*, TDr 89/02677 TDr 89/02475, TDr 95/19177, TDr 89/02665 and TDr 95/18544, the PPE and number of stands at harvest (which by inference represents the survival rate till harvest) had higher mean values compared to landraces: Obiaturugo, Meccakusa, TDr 04-219, Alumaco and Pona used at SW equal to or above 20 g (Table 4.14).

Although, these landraces: Alumaco (78.5% at 40 g SW); Meccakusa (76.0 and 86.0% at 40 and 50 g SW, respectively; and Obiaturugo (76.0% at 50 g SW) required higher SW for emergence above 70%. The improved varieties, i.e., TDr 95/18544 (10 g SW); TDr 89/02677 (20 g SW); and all the *D. alata* varieties, at 10 g SW, attained at least 70% plant emergence. Although, some of the landraces, e.g., Pona, Danacha, Alumaco, TDr 04-219 (Amula) with low plant emergence across SW used in this experiment, were considered unsuitable for minisett production because of the poor sprout performance recorded in all SW used (Table 4.14).

The overall plant emergence among the *D. alata* varieties used across SW was above 90.0%, indicating that this species responded positively to the minisett technique irrespective of variety (Table 4.14). Sett weights significantly ($p < 0.05$) influence earliness to sprout (PPE at 14 DAP), overall emergence and survival till harvest at 6 MAP. However, SW of 30 g and above were not significantly ($p \leq 0.05$) different, while 10 and 20 g SW were significantly lower ($p < 0.05$) when compared to SW of 30 g and above overall.

Table 4.14: Percent plant emergence as influenced by sett weight and variety interaction in two yam species

Variety	Number of emerged plants at 0.5MAP Sett weights (g)						Number of emerged plants at 2MAP Sett weights (g)					
	10g	20g	30g	40g	50g	Mean	10g	20g	30g	40g	50g	Mean
TDr95/18544	8.5	16.8	39.3	41	47.7	30.7	85.2	89.3	98.5	95.2	100	93.6
TDr89/02677	6.8	21	28.5	32.7	23.5	22.5	78.5	81	91	90.2	96	87.3
TDr95/19177	11.8	16.8	25.2	26.8	36.8	23.5	73.5	79.3	94.3	98.5	94.3	88
TDr89/02665	10.2	12.7	25.2	21.8	16.8	17.3	78.5	89.3	91.8	92.7	81.8	86.8
TDr89/02475	24.3	12.7	31.8	30.2	35.2	26.8	76	75.2	82.7	82.7	79.3	79.2
Meccakusa	3.5	5.2	4.3	14.3	10.2	7.5	60.2	73.5	90.2	89.3	84.3	79.5
Pona	6.8	5.2	6	8.5	6.8	6.7	36.8	38.5	56.8	47.7	37.7	43.5
Obiaturugo	1	8.5	1.8	6.8	7.7	5.2	40.2	55.2	55.2	68.5	76	59
Alumaco	1.8	1.8	5.2	6.8	11	5.3	51.8	56.8	70.2	78.5	63.5	64.2
Danacha	1	1.8	6	4.3	5.2	3.7	41.8	33.5	51.8	34.3	41	40.5
Meccakusa	4.3	3.5	5.2	4.3	9.3	5.3	56.8	70.2	66	76	86	71
TDr 04-219	3.5	12.7	5.2	4.3	2.7	5.7	53.5	41.8	58.5	60.2	64.3	55.7
TDa 93-36	8.5	11	16	11.8	5.2	10.5	85.2	91	95.2	97.7	100	93.8
TDa00/00194	37.7	49.3	46	42.7	41.8	43.5	94.3	100	91	97.7	92.7	95.1
TDa98/01176	51	42.7	47.7	33.5	39.3	42.8	97.7	98.5	98.5	96.8	96.8	97.7
TDa 291	52.7	54.3	56	58.5	51.8	54.7	96.8	98.5	89.3	93.5	98.5	95.3
Mean	14.6	17.3	21.8	21.8	21.9	19.5	69.2	73.2	80.1	81.2	80.8	76.9
SE (±)	4.199	4.049	4.404	4.053	4.172	3.954	4.992	5.341	4.147	4.708	4.817	4.599
CV (%)	115	93.62	80.82	74.37	76.21	81.12	28.85	29.19	20.71	23.19	23.85	23.92

CV = Coefficient of variation; HSD = Tukey's honest significant difference; MAP = months after planting

In the year-based analyses, plant emergence at 1 MAP ($P<0.05$) at 0.5 MAP. Mean values for PE were consistent for all the varieties used in this experiment across years (Table 4.15). Comparison of mean values on emergence and number of stands at harvest by year showed that year 1 mean values for variety were significantly higher ($p \leq 0.05$) than the mean value for the same parameter in year 2 (Table 4.15).

4.5.2 Yield and yield attributes

The mean values for plant survival till harvest, the number of tubers harvested and the fresh tuber yield-FTY (t/ha) are presented in Table 4.16. The overall varietal performances across SW for the number of stands at harvest, number of tubers at harvest and FTY (t/ha) showed that improved varieties of *D. rotundata* (TDr 89/02677, TDr 89/02475, TDr 95/19177, TDr 89/02665 and TDr 95/18544) were amenable to minisett technique. Among the selected landraces of *D. rotundata* used, only Meccakusa, which had a 71% mean value for the stands at harvest, was found suitable for the minisett technique. Varieties such as TDr 95/18544 and TDr 89/02677 produce multiple tubers. Most of the landraces, especially TDr 04-219, Danacha and Pona, had one tuber per stand on the average despite their low mean value for survival (Table 4.16). Among the varieties of *D. alata* varieties used, varieties TDa 291 and TDa 98/01176 had over 90% stand at harvest, indicating that they were more amenable in survival (Table 4.16).

Categories of tubers harvested per factor combination (variety × sett weight) as presented in Table 4.17 showed that the proportion of the harvested tubers produced by 10, 20 and 30 g SW, which on the average corresponded to SYT tubers was 63.7%, 66.9% and 64.3%, respectively. Whereas 20.5%, 13.7% and 13.4% fell into the category of minitubers. The remaining 15.7%, 19.4% and 22.3% of tubers produced by 10, 20 and 30 g SW respectively had weights higher than that of a SYT tuber. Setts weighing 40 and 50 g, respectively, had 57.1% and 55.1% SYT tubers of the total tubers harvested. At the same time, 12.9% and 10.1% were minitubers. The remaining 30.0% and 34.8% of tubers produced by 40 and 50 g SW, respectively, weighed more than SYT. The proportions of actual SYT tuber to ware and minitubers produced among SW used was highest in 20 g SW. The peak value for the number of SYT tubers (66.9) was attained at 20 g SW, after which a steady decline was observed (Table 4.18). The trend was that the proportion of ware yam steadily rose from 15.7% in 10 g SW to 34.8% in 50 g SW across varieties (Table 4.18).

Table 4.15: Overall sprout rate and survival till harvest of two yam species across varied sett weights in two cropping seasons

Variety	Number of emerged plants at 0.5MAP		Number of emerged plants at 1MAP		Number of emerged plants at 2MAP		Number of stands at harvest (6MAP)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
TDr 95/18544	34.3	27.0	85.0	84.6	95.0	92.3	83.7	81.0
TDr 89/02677	31.0	14.0	79.7	73.3	88.3	86.3	77.7	70.7
TDr 95/19177	26.3	20.6	70.3	84.3	81.0	95.0	75.7	85.7
TDr 89/02665	26.7	10.0	76.7	70.0	87.0	86.7	81.7	71.7
TDr 89/02475	45.0	8.7	89.3	43.0	94.0	64.3	91.0	53.3
Meccakusa	7.7	7.3	56.0	59.0	77.3	81.7	71.7	70.7
Pona	8.3	5.0	22.0	27.7	44.0	43.0	46.7	43.0
Obiaturugo	7.0	3.3	42.0	28.3	57.3	60.7	53.7	50.7
Alumaco	5.0	5.7	25.7	34.3	69.3	59.0	76.0	53.7
Danacha	3.0	4.3	5.3	24.7	29.3	51.7	29.3	35.7
Meccakusa	4.3	6.3	23.0	43.0	63.7	78.3	57.3	70.0
TDr 04-219	7.3	4.0	27.3	37.3	50.3	61.0	47.0	50.0
TDa 93-36	2.0	19.0	56.3	75.0	93.3	94.3	84.3	64.7
TDa 00/00194	63.0	24.0	92.7	78.0	95.3	95.0	92.0	83.3
TDa 98/01176	57.0	28.7	91.0	85.0	99.0	96.3	100.0	92.7
TDa 291	85.3	24.0	98.3	84.0	96.0	94.6	97.0	90.7
Mean	25.8	13.2	58.8	58.2	76.2	77.5	72.8	66.7
CV	47.8	49.9	22.3	22.6	16.2	13.7	14.9	19.8
HSD (0.05)	0.6	0.5	37.0	16.7	35.1	13.4	30.9	16.8

MAP = Month After Planting; CV = Coefficient of variation; HSD = Tukey's honest significant difference

Table 4.16: Yield performance in selected yam varieties as influenced by variety × sett weight interaction in the minisett experiment

Variety	Plant stand at harvest (%)						Number of tubers at harvest						Fresh tuber yield (Tonnes per hectare)					
	Sett Weight (g)						Sett Weight (g)						Sett Weight (g)					
	10g	20g	30g	40g	50g	Mean	10g	20g	30g	40g	50g	Mean	10g	20g	30g	40g	50g	Mean
TDa 93-36	59.3	75.2	79.3	82.7	76.0	74.5	12.2	15.8	16.5	17.8	17.0	15.9	14.5	20.0	23.1	22.5	26.5	21.3
TDa 00/00194	79.3	90.2	86.0	88.5	94.3	87.7	19.5	22.7	21.0	21.2	24.2	21.7	21.3	29.4	32.1	38.0	46.8	33.5
TDa 98/01176	96.8	95.2	95.2	99.3	95.2	96.3	29.7	29.2	31.2	38.5	29.8	31.7	20.8	27.8	27.5	39.5	39.8	31.1
TDa 291	86.0	96.0	92.7	95.2	99.3	93.8	26.2	27.8	30.7	29.5	39.5	30.7	14.7	22.6	23.0	25.0	31.2	23.3
TDr 95/18544	60.2	79.3	87.7	91.0	93.5	82.3	13.0	21.8	26.3	27.5	27.0	23.1	7.1	17.5	15.3	22.2	19.1	16.2
TDr 89/02677	60.2	68.5	83.5	75.2	83.5	74.2	14.0	17.3	24.2	20.5	21.3	19.5	8.0	12.3	9.6	13.3	18.0	12.2
TDr 95/19177	66.0	68.5	88.5	87.7	91.8	80.5	13.8	15.5	19.5	24.0	24.5	19.5	9.4	11.9	21.3	28.5	23.6	18.9
TDr 89/02665	51.0	75.2	88.5	83.5	85.2	76.7	10.5	17.3	21.5	22.2	22.8	18.9	6.1	13.6	17.9	20.9	18.2	15.3
TDr 89/02475	52.7	68.5	76.0	76.8	86.8	72.2	10.7	14.7	18.7	18.8	19.8	16.5	6.0	11.5	16.0	17.2	21.7	14.5
TDr 04-219	40.2	44.3	49.3	48.5	60.2	48.5	8.0	9.5	10.0	11.5	15.5	10.9	4.4	5.2	8.1	7.9	9.6	7.0
Alumaco	51.0	59.3	63.5	75.2	75.2	64.8	10.5	12.7	13.0	15.7	17.7	13.9	5.2	7.1	9.8	17.3	14.0	10.7
Danacha	26.8	19.3	41.8	36.0	38.5	32.5	5.2	4.5	9.3	8.2	9.7	7.4	2.8	2.0	4.4	4.6	5.6	3.9
Meccakusa	54.3	63.5	81.0	78.5	78.5	71.2	10.8	13.7	16.5	16.7	17.0	14.9	6.7	12.0	16.9	17.7	17.6	14.2
Meccakusa	48.5	61.8	63.5	68.5	76.0	63.7	10.8	12.8	13.2	14.5	15.5	13.4	3.7	7.4	10.2	12.4	16.6	10.1
Obiaturugo	34.3	46.8	54.3	60.2	65.2	52.2	7.2	10.0	11.8	13.5	14.8	11.5	2.7	6.1	8.5	11.4	12.9	8.3
Pona	34.3	41.8	52.7	49.3	46.0	44.8	6.8	8.3	11.0	10.3	9.2	9.1	3.4	6.1	8.7	9.7	9.5	7.5
Mean	56.3	65.8	74.0	74.8	77.8	69.7	13.1	15.9	18.4	19.4	20.3	17.4	8.6	13.3	15.8	19.3	20.7	15.5
SE (±)	4.6	5.0	4.2	4.4	4.3	4.3	1.6	1.6	1.7	1.9	1.9	1.7	1.5	2.0	1.9	2.4	2.7	2.0
CV (%)	32.7	30.3	22.5	23.4	22.0	24.9	49.9	41.4	37.1	39.1	36.6	38.7	68.2	59.3	48.6	50.0	51.6	52.6

Table 4.17: Variety × sett weight interaction as it influences tuber weight categories obtained at harvest (6 MAP)

Variety	Number SYT tubers (SYT (%))					Number of less than SYT (%)					Number of tubers above SYT (%)				
	10g	20g	30g	40g	50g	10g	20g	30g	40g	50g	10g	20g	30g	40g	50g
TDr 95/18544	75.6	63.6	72.8	59.9	65.7	17.7	20.2	13.8	8.4	5.6	6.6	16.2	13.4	31.6	28.7
Danacha	52.1	58.3	76.3	41.6	73.4	47.9	39.6	22.2	41.2	24.7	0	2.1	1.5	17.2	1.9
Meccakusa	70.7	78.3	80	76.2	66.1	28.2	13.5	12.7	3.4	8	1.1	8.3	7.3	20.4	25.9
TDr 04-219	76.4	80.4	79.8	69.3	66.7	18.8	12.9	11.3	19.6	19.4	4.8	6.7	8.9	11.1	13.9
TDa 93-36	30.3	50	42.2	34.5	36.4	16.5	4.9	6.4	15.8	8.2	53.1	45.1	51.4	49.7	55.4
TDa 00/00194	38.9	41.7	23	35.8	30.6	9.4	5.8	9	6.1	6.3	51.7	52.5	68	58	63.1
TDa 98/01176	34.7	39.7	28.7	30	35.8	23	7.6	17.3	15.6	4.7	42.3	52.7	54	54.3	59.5
TDa 291	37.5	48.2	34.7	43.3	41.9	19.6	9.8	17.2	12	13.2	42.9	42	48.2	44.8	44.9
TDr 89/02677	82.4	69.3	72.2	74.3	57.7	14	20.4	15.8	6.2	6.2	3.6	10.2	12	19.5	36.1
TDr 95/19177	74.2	72.1	72.3	63.2	51.7	17.4	11.4	9.4	7.2	11.7	8.4	16.5	18.3	29.6	36.6
TDr 89/02665	78	71.6	64.7	57.8	67	10.2	14.1	26.4	9.7	8.2	11.7	14.3	9	33.9	24.7
TDr 89/02475	69.6	83.7	70.7	70.8	57.8	26.2	6.5	6.2	9.1	5.7	4.2	9.8	23.1	20.1	36.4
Meccakusa	93.7	70.1	83.9	71.6	59.7	4.4	8.7	3.6	7	10.9	1.9	21.3	12.5	21.4	29.4
Pona	81.9	84.6	67.9	59.9	43	18.1	11.2	16.5	25.3	13	0	4.2	15.6	14.8	44
Obiaturugo	55	82.6	82.3	70.5	73.1	42.2	13.4	10.1	14	3.3	2.8	4.1	7.6	15.5	23.6
Alumaco	68.9	76.3	77.5	55.5	54.6	14.4	19.4	15.8	6.5	12.7	16.7	4.3	6.7	38	32.7
Mean	63.7	66.9	64.3	57.1	55.1	20.5	13.7	13.4	12.9	10.1	15.7	19.4	22.3	30	34.8
CV(%)	29.8	21.8	30.4	26.2	24.3	53.6	59.7	43.6	71.3	54.5	121	90	89.9	48.6	45
SE (±)	4.75	3.65	4.88	3.74	3.34	2.75	2.05	1.46	2.3	1.37	4.74	4.37	5.01	3.64	3.92

Table 4.18: Proportion of tuber categories (%) of yam varieties as influenced by different sett weights in two seasons

Sett weight (g)	Proportion of SYT tubers in the harvested yam tubers		Proportion of ware yam tubers in the harvested yam tubers		Proportion of minitubers in the harvested yam tubers	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
10	56.4	71.1	26.1	5.3	17.0	23.6
20	56.1	77.7	33.2	5.5	10.0	16.7
30	55.5	73.1	38.3	6.4	12.6	20.5
40	44.6	69.7	50.6	9.4	4.0	20.9
50	40.2	70.0	57.6	12.0	2.0	18.0
Mean	50.6	72.3	41.2	7.7	9.1	19.9
SE (±)	0.2	1.9	1.4	4.5	0.5	4.9
CV (%)	24.4	36.0	39.9	9.6	80.0	48.3

CV: Coefficient of variations, SE: Standard error

The respective mean values for SYT, minitubers and tubers above the SYT category were presented in Table 4.17. The year-based analyses were consistent for mean values for percent tuber weight classes yielded by SWs used. The overall mean value for the SYT tuber category in year 1 (50.6) was lower than the value obtained in year 2 (72.3%) (Table 4.19).

Table 4.19 showed that SW influenced the proportion of SYT, minitubers and tubers above seed categories. The ratio of tubers in the SYT category (100−500 g) was higher in 20 and 30 g SW. However, over 50% of tubers from 40 and 50 g SW weighed more than SYT tuber (Table 4.19). The mean yield parameters obtained from the different SWs showed that yield in tonnes per hectare did not increase in the same ratio with SW. The 10 g SW recorded a yield of 8.6 t/ha, but 20 g SW recorded a 13.3 t/ha yield, less than double this value (17.2 t/ha). This disproportionate increase in yield with higher SW continued to a point where there was no significant difference in yield obtained from the increase in SW from 40 to 50 g (Table 4.20).

4.6 Inter-character correlation among selected traits

The correlation coefficients for some of the traits measured for two cropping seasons at IITA are presented in Table 20. Most of the traits showed significant correlation *inter se.* Notably, Fresh tuber yield (t/ha) was positively correlated ($p<0.001$) with early (0.57) and total (0.67) plant emergence, stand at harvest (0.72) and tuber count (0.64). Also, the number of stands at harvest was highly significant (<0.001) and positively correlated with tuber count. The relationship among tuber classes was negative for SYT and Tubers above SYT (-0.84). Also, the number of SYT had a negative correlation with other factors.

4.7 On-farm performances of selected yam varieties and sett weights under minisett technique

The mean values for total plant emergence and survival at 2 and 4 MAP were highly significant ($p <0.05$) for variety and SW. Variety by SW interaction was also highly significant ($p <0.05$) for mean plant emergence at 2 MAP, while it was highly significant ($p <0.05$) for mean plant survival at 4 MAP. The mean value for plant stands at harvest was also highly significant for variety (at $p <0.05$), SW (at $p <0.001$) and SW × variety interaction at $p <0.05$ (Table 4.21). On-farm results on the proportion of emergent plants affirm that breeder lines (improved varieties) were again significantly higher in emergence and survival until harvesting at 6 MAP.

Table 4.19: Mean values of yield and yield-related traits as influenced by sett weights on selected varieties of two yam species

Serial No.	Sett weight. (g)	Yield (t/ha)	Mean tuber weight (g)	Proportion of Seed yam tuber	Proportion of ware yam tuber	Proportion of minitubers
1	10	8.6d	310.0d	63.7ab	15.7b	20.5a
2	20	13.3c	400.0c	66.9a	19.4b	13.7b
3	30	15.8b	440.0bc	64.3ab	22.3b	13.4b
4	40	19.3a	490.0ab	57.1cb	30.0a	12.9b
5	50	20.6a	500.0a	55.1c	34.8a	10.1b

Means followed by the same letter along the same column were not significantly different ($p < 0.05$).

Table 4.20: Correlation coefficient for plant emergence, yield and yield-related traits of sixteen yam varieties evaluated in two seasons at IITA-Ibadan

Traits (%)	Early plant emergence	Total plant emergence	Stands at Harvest	Total tuber count	Fresh Tuber Yield (t/ha)	Seed yam tuber (%)	Tubers **above** SYT (%)	Tubers **below** SYT (%)	Dry Matter content (%)
Early plant emergence	1	0.49***	0.57***	0.52***	0.57***	-0.43***	0.54***	-0.27***	-0.31***
Total plant emergence		1	0.81***	0.68***	0.62***	-0.19***	0.34***	-0.32***	-0.21***
Stands at Harvest			1	0.83***	0.72***	-0.28***	0.45***	-0.37***	-0.25***
Total tuber count				1	0.64***	-0.23***	0.29**	-0.14**	-0.17***
Fresh Tuber Yield (t/ha)					1	-0.51***	0.66***	-0.38***	-0.34***
Seed yam tuber						1	-0.84*	-0.11*	0.27***
Tubers **above** SYT							1	-0.44***	-0.36***
tubers **below** SYT								1	0.2***
Dry Matter content									1

SYT: Seed yam tubers

Table 4.21: Combined analysis of variance for some of the agronomic traits measured in on-farm yam minisett trials

Sources	Degree of Freedom	Proportion of emergent plant at 2MAP (%)	Number of harvested plants per plot	Proportion of tuber weighing above SYT tubers	Proportion of SYT tubers in the harvested yam tubers	Minitubers proportion in the harvested yam tubers	Mean weight of Tubers above SYT (g)	Mean SYT weight (g)	Mean Minituber weight (g)	Fresh tuber weight (g)
Rep	2	112.47	721.82	44.21	873.73	3584.78	0.10	0.01	0.00	9.79
Setwgt	2	3007.29**	2866.50**	623.10*	4572.64	324.12	5.95***	0.00	0.00	615.85*
Error(a)	4	104.72	101.01	80.82	1388.59	971.54	0.10	0.00	0.00	40.54
Variety	9	1104.62***	1153.96***	230.84***	226.88	581.93**	0.90*	0.00	0.00*	127.03***
Setwgt*Var	18	332.00***	275.29**	37.46	269.27*	277.10	0.47	0.00	0.00	23.84**
Error(b)	53	47.77	99.92	41.13	146.26	166.90	0.34	0.04	0.00	8.73
CV%		8.45	14.52	86.85	25.08	35.54	64.66	27.43	51.96	34.99
Mean		81.75	68.86	7.38	48.23	36.38	0.90	0.23	0.06	8.45

*, **, *** = Significant at 5%, 1% and 0.1% probability levels respectively; ns = not significant; DF = degree of freedom; CV = Coefficient of variation; MAP = months after planting

Also, the number of tubers harvested was highly significant for variety and SW but was not significant for their interaction. The proportion of tubers above the SYT category was highly significant ($p < 0.01$) for variety and SW but was not significant for their interaction (Table 4.21).

The tuber weight categories showed varying levels of significance influence by the treatments imposed on the experimental units. The proportion of tubers weighing above SYT tubers was highly significant ($p < 0.001$) concerning variety and SW but not significantly concerning the variety × SW interaction. The proportion of SYT tubers in the harvested tubers was significantly different ($p < 0.05$) for SW, but there was no significant difference base on the variety and variety × SW interaction. Based on the variety, there was a significant difference ($p < 0.05$) in the proportion of minitubers in the harvested tubers. But, based on SW and variety × SW interaction, there was no significant difference. Fresh tuber yield was significant ($p < 0.05$) for variety, SW, but had significant interaction at $p < 0.05$ (Table 4.21). The overall mean value for the proportion of emergent plant (PEP) at 2 MAP across SW in selected variety was highest in TDa 00/00194. At the same time, TDr 95/18544 recorded the highest plant emergent among the *D. rotundata* varieties. The smallest setts (weighing 10 g) of *D. alata* and improved *D. rotundata* varieties gave overall plant emergent well above 70%. The number of surviving plants at 4 MAP recorded a similar trend affirming that SW as low as 10 g can be used in minisett production (Table 4.22).

Mean values of the number of tubers harvested per plot were significantly influenced by variety and SW. The number of tubers increased with an increase in SW. Variety TDa 291 among the *D. alata* varieties used and variety TDr, 95/18544 among the *D. rotundata* varieties, used recorded an average value of 74.7 and 83.3 tubers at harvest from 50 g SW. This is an indication that these varieties form multiple tubers per stand (Table 4.23). About 60% and 70% of tubers harvest from 10 and 30 g SW across varieties had weights greater than or equal to SYT tubers in the on-farm trial. Although 50 and 30 g SW recorded a more uniform yield within seed and minitubers categories due to higher percent sprout when compared to tubers from 10 SW.

Table 4.22: Percent plant emergence and survival till harvest in selected yam varieties tested on-farm

	Plant emergence at_2MAP (%)			Plant stand at_4MAP (%)			Plants harvested (%)		
Variety	10g	30g	50g	10g	30g	50g	10g	30g	50g
Amula	36.7	90.8	79.2	51.7	84.2	77.5	40.0	70.8	71.7
Meccakusa	42.5	80.0	76.7	76.7	86.7	87.5	41.7	59.2	54.2
Pona	58.3	57.5	80.8	55.8	62.5	88.3	38.3	40.0	75.8
TDa 00/00194	95.8	99.2	95.0	96.7	95.0	97.5	78.3	87.5	87.5
TDa 291	85.8	80.8	88.3	90.0	83.3	91.7	80.8	71.7	89.2
TDa 98/01176	77.5	90.8	80.8	88.3	93.3	91.7	61.7	83.3	67.5
TDr 89/02475	79.2	92.5	93.3	85.8	91.7	91.7	55.8	85.0	74.2
TDr 89/02665	70.8	90.8	97.0	68.3	92.5	85.3	58.3	76.7	82.8
TDr 95/18544	89.2	96.7	95.0	90.0	95.0	94.2	75.8	73.3	84.2
TDr 95/19177	65.8	88.3	96.7	79.2	91.7	85.0	47.5	71.7	80.8
CV (%)	26.5	13.0	8.7	18.5	10.6	6.0	26.6	18.4	13.1
Mean	70.2	86.7	88.3	78.3	87.6	89.0	57.8	71.9	76.8
SE (±)	5.9	3.6	2.4	4.6	2.9	1.7	4.9	4.2	3.2
CV (%)	0.3	0.1	0.1	0.2	0.1	0.1	0.3	0.2	0.1

CV = Coefficient of variation; HSD = Tukey's honest significant difference; MAP = months after planting

Table 4.23: Mean yield and seed classes obtained across sett weights among selected yam varieties tested on-farm.

Varieties	Mean number of harvested tubers			Seed yam tubers (SYT) (%)			Tubers less than SYT (%)			Tubers greater than Seed (%)		
	Sett Weight			Sett Weight			Sett Weight			Sett Weight		
	10g	30g	50g	10g	30g	50g	10g	30g	50g	10g	30g	50g
TDr 04-219	16.7	35.3	40.3	37.0	62.0	47.8	53.0	37.4	53.7	10.0	0.6	2.0
Meccakusa	16.7	26.7	24.0	37.0	62.0	47.8	46.0	47.8	32.6	17.0	10.0	19.6
Pona	16.3	17.7	36.0	58.0	77.0	52.0	42.0	17.7	37.1	0.0	5.3	10.9
TDa 00/00194	38.7	43.0	51.3	54.0	64.0	63.6	25.2	23.2	18.2	20.8	12.8	18.2
TDa 291	52.0	52.0	74.7	50.0	60.0	56.8	31.8	44.7	32.0	18.2	5.0	11.3
TDa 98/01176	32.3	41.0	38.3	56.0	60.0	51.6	25.6	25.6	24.4	18.4	14.4	24.0
TDr 89/02475	27.0	52.3	49.7	58.0	60.0	47.4	40.0	47.5	47.4	2.0	7.0	5.3
TDr 89/02665	24.7	38.7	44.7	43.0	70.0	73.1	42.1	30.7	19.4	14.9	1.0	7.5
TDr 95/18544	48.0	58.0	83.3	33.0	65.0	46.7	49.0	37.9	48.4	18.0	3.0	4.9
TDr 95/19177	22.3	33.7	44.7	34.0	58.0	60.2	47.7	32.9	30.2	18.3	9.1	9.6
Mean	29.5	39.8	48.7	46.0	63.8	54.7	40.2	34.5	34.3	13.8	6.8	11.3
SE (±)	3.9	3.7	5.4	3.1	1.7	2.6	2.9	3.1	3.7	2.2	1.4	2.1
CV (%)	41.8	29.4	34.7	21.2	8.6	15.1	22.9	28.6	34.0	50.4	66.0	59.9

CV = Coefficient of variation; HSD = Tukey's honest significant difference

4.7.1 Participatory varietal selection

Varieties TDr 95/19177, Meccakusa and TDa 00/00194 were the most preferred varieties, in that order, according to ranking carried out by farmers organized to harvest and assess harvested tubers (Plate 4.3). The farmers selected these varieties based on observed overall performances across SW. Other criteria considered by farmers and the number and yield of tubers include tuber shape and ease of harvest.

4.8 Related efficiency of the assessed rapid SYT production techniques

The comparative efficiency of assessed rapid SYT tuber production techniques is presented in Table 4.24. The multiplication ratio of 1:1800 in Temporary Immersion Bioreactor System (TIBS) was the highest while the proportion of <1:10 in Yam Minisett Technique (YMT) was the lowest. Aeroponics System (AS) and Vine Cutting Technique (VCT) had intermediate ratios of 1:300 and 1:80, respectively. Disease screening ability in TIBS, AS, VCT and YMT were very high, medium and low. The four SYT tuber (SYT) production techniques could produce tubers while only AS and VCT produced. Only AS produced bulbils, while TIBS and AS produced plantlets. The tubers' weight ranged from less than 5 g in TIBS to less than 2000 g in YMT. The least number of tubers produced was 2 in VCT and YMT, while the highest was 20 in TIBS. The longevity of plants obtained from AS (108 weeks) was highest, while the lowest was in VCT and YMT (24 weeks). Two of the techniques, AS and VCT, could be applied just once, but TIBS could undergo as many as six cycles. High technical know-how was required in TIBS and AS, medium in VCT and low in YMT. The initial capital outlay in TIBS and AS was high, fair in VCT and low in YMT. All four SYT production techniques had a high capacity for varietal selection (Table 4.24).

Plates 4.2: (a) Farmers assessing yam varietal and yield × sett weight performances;
(b) A farmer displaying the seed yam tubers of one of the choice varieties during selection based on their unanimous decisions

Table 4.24 Comparative efficiency of assessed rapid SYT tuber production techniques

Assessments	TIBs	AS	VCT	YMT
Multiplication ratio	1:1800	1:300	1:80	< 1:10
disease screening	Very high	High	Medium	Low
Derivable propagule	1, 4	1, 2, 3, 4	1, 2	1
Tuber weight (g)	< 5	> 500	> 1000 (Field)	< 2000
number of tubers	20	8	2	2
plant longevity (weeks)	8	108	24	24-30
number of cycles	6	1	1	4
technical Know-how	High	High	Medium	Low
cost of set-up	High	High	Fair	Low
Varietal selection	High	High	High	High

Derivable Propagule: 1 = Tuber; 2 = VC; 3 = Bulbil; 4 = Plantlet.
AS = Aeroponics System; TIBS = Temporary Immersion Bioreactor System; VCT = Vine Cutting Technique; YMT = Yam Minisett Technique.

CHAPTER FIVE

DISCUSSION

Technologies for high-ratio production of SYT tubers and yam seedlings are critically low in awareness among farmers. Varietal amenability to these, measured in terms of plant performance, tuber formation and yield as assessed in this study, showed significant variability among varieties tested. This study showed that the knowledge and use of SYT production technologies are ripe among researchers while the same is critically low among farmers contacted. The level of awareness and use of the yam minisett technique as reported in this study agreed with Agbarevo's (2014) findings, which assert that 46.6% of respondents surveyed in a study were aware of the minisett technique. In contrast, only about 22.4% were using this technique. Also, the 44.0% awareness rating for the minisett practice among farmers, as reported in this study, agrees with Agbarevo's (2014) findings.

Lack of information and training cited as reasons for low minisett adoption were also corroborated. However, there is potential for adopting and using research outputs among these farmers because 93.0% of the farmers surveyed belong to a farmer group or association. The low sprouting rate of minisetts earlier stated as the reason for non-usage among farmers contacted was confirmed by the low sprout value of most landraces as obtained in the minisett experiment conducted in this study.

In contrast, the breeder lines were above 70.0% in the sprout performance. Hence, this reason for poor adoption is invalid should farmers adopt improved yam varieties. Findings from the key actors contacted in this study also showed that lack of technical detail, particularly minisett technique, lack of farm inputs (seed in particular) and lack of storage facilities, were given by farmers as challenges. These challenges agree with the findings reported by Okoro (2008). Sanginga (2015) affirms that seed production techniques require deliberate dissemination and refining to make them friendly to farmers. An insignificant number of farmers are aware and practicing some of these techniques, while others are unknown to the farmers.

The SYT production techniques (minisett and vine cutting techniques) are better refined and disseminated using an on-farm dissemination approach. Findings among trained and or contacted yam farmers revealed that the farmers relied more on milking and cutting ware yam into yam sett to sustain yam production. The result obtained also showed the non-usage of any of the SYT production techniques among farmers contacted. This non-usage agrees with the report by Aighewi *et al.* (2015) on seed sources for yam propagation by farmers. As observed during training and demonstration, farmers were interested in these techniques but did not explore them due to a lack of awareness or knowledge. As observed in this study, the number of farmers (58%) patronizing the informal seed market indicates high prospects in the SYT business.

In vitro plant survival, growth in terms of the number of leaves, nodes and tuber initiation were influenced by increased sucrose level, light type, media composition and variety. Regarding the number of leaves and nodes, Yam vine growth was comparatively higher in plants grown under blue LED and white fluorescent light treatments than red LED treatment. This effect of LED on plants agrees with the findings of Bantis *et al.* (2018). However, *in vitro* plants grown under blue LED had higher vine. The plants cultured under red-LED were etiolated, while plants grown under white fluorescent blue LED light had normal growth. Despite etiolation and observed pale green leaf colour, red LED plants showed a higher frequency of tuber formation.

The low plant growth parameters, e.g., the number of leaves, plant height and vigour obtained for the red LED treatment, suggest that this light type is unsuitable for yam growth. Both blue-LED and FL can be used to grow plants when combined with other factors such as High sucrose concentration and JA, enhancing tuber formation. Although the blue LED was less efficient in tuber induction, but it adequately enhances vine formation. Yam could be grown under blue-LED for 8−12 weeks after culture (Phase 1) and then introduced into a red-LED for tuber production.

Exposure to only red-LED light resulted in plant elongation (etiolation), low chlorophyll content, poor vigour and reduced biomass of yam plant cultured *in vitro.* In this study, Yam test-tube plants under blue LED produced a good number of broad leaves in all the varieties tested. This finding agrees with those of Li *et al.* (2013), Hogewoning *et al.* (2010), Hanhong (2001) and Johkan *et al.* (2012) on the

importance of light for leaf expansion which enhances Leaf area and biomass production. The red-LED light was found to be a low irradiation source compared to blue-LED and white fluorescent light for growth and development in yam. Kim *et al.* (2004) reported similar results for *in vitro* multiplication of potatoes. The larger leaf allowed greater light interception, which may have led to a significant increase in biomass.

The production of viable microtubers in TIBS within 12 weeks is an improvement in a similar study undertaken by Cabrera *et al.* (2011). In the minitubers production experiment conducted using a plant bioreactor reported by Cabrera *et al.* (2011), minitubers were harvested after 24 weeks of culture. Whereas, minitubers were harvested 12 weeks after culture in the TIBS experiment conducted in this study. This is an improvement on previous protocol and time taking to produce minitubers using TIBS. In this study, conventional tissue culture (CTC) plantlets recorded good establishment in TIBS and produced viable minitubers. This achievement could be a solution to the acclimatization challenge often encountered in the use of humidified polythene chambers for the hardening of CTC plantlets. The humidified chamber, which causes up to 70.0% loss in CTC, can now be circumvented. The virus-free Tissue culture materials cultured in a vented plastic showed a prospect for use in TIBS plantlets' production compared to CTC test tube plantlets. Hardened TIBS plantlets potted in Screen house and the plantlets introduced into the field were suitable mother plants for Vine cutting and SYT tuber (SYT) generation. Such derived tubers were highly relevant for further seed production using the yam minisett technique.

The use of the Aeroponics System (AS) to grow yam was first attempted and reported at IITA-Ibadan in this course of this study. The explant sources, ease of vine rooting and plant establishment in AS varied with species and cultivars in this study. This corroborated the findings of Hartman *et al.*, 1990 which asserts that plant performance in the AS is also a function of physiological factors related to plant growth. Variations in plant establishment, vine and tuber weights were observed in TDa 00/00194, TDa98/01176, TDr 95/18544and TDr 95/19177 varieties, which showed good response in AS. Whereas survival and general vine growth and yield were poor in TDa 291, Pona, TDr 89/02665 irrespective of explant sources in AS. Varieties TDr 89/02665 and TDa 291 were less suitable for AS culture as the yield of

tubers obtained in these varieties irrespective of origin were lower than the yield obtained in TDa 98/01176 and 95/18544 and TDr 95/19177.

In their findings, Farran and Mingo-Castel (2006) attributed low plant performances in AS to the weak capacity of plants in utilising the low light intensity in the AS greenhouse. They also cited unlimited nitrogen supply as the reason for poor performance in new shoot development and growth in some varieties under AS. In addition to these stated observations, plant performances in AS were also influenced by variety. Before senescence, occasioned by low adaptability, varieties such as Pona, though without new shoot, produced minitubers. The influence of explant sources and observations in AS under this study agreed with Okunade (2011) findings, in which explant source was found to influence the success rate in vine cuttings. The TIBs sourced plants performed better than RVC and DVC plants. Perhaps, TIBs plantlets are from an environment like that of AS. This report agrees with the findings of Kikuno *et al.* (2007), which asserts that the performance of explants sourced from Tissue culture (and TIBS, by extension) is superior to those of other sources. Therefore, TIBs were recommended as the most suitable source of *explants* for AS establishment.

The planting of pre-rooted and non-pre-rooted yam vines of two−three nodal cuttings has been reported to be successful and minitubers of 0.2−2.7 were harvested from the preliminary trial conducted at IITA-Ibadan (Maroya *et al.*, 2014a). Direct planting of acclimatized *in vitro* or TIBS plantlets into the field readily exposes the plants to harsh environmental conditions and field pests and diseases, thereby reducing the success rate of clean plants introduced to the field (Balogun *et al.*, 2014b). The production of bulbils under AS is significant as it increases the chances of securing adequate planting materials for yam production. The ease of plant proliferation and the resultant vine cutting production, particularly in *D. alata* then *D. rotundata* cultivars under AS, can be improved to ensure seed sufficiency.

The low sprout potential among landraces compared to the improved varieties tested under the minisett technique in this study was a pointer to the low adoption of the technique among farmers. Ayankanmi *et al.* (2006) and Okoro (2008) reported low sprouting as the rationale behind the poor adoption. In the formers' report, low sprouting was recorded in the breeder lines used when wood ash was used as a pre-planting treatment. Minisett experiments conducted in three seasons under this study

gave a near contrary result to the above report as all the breeder lines used had above 70% sprout even in sett weights as low as 10 g.

The use of improved varieties, a combination of insecticide and fungicide, against wood ash for the treatment of the setts must have enhanced the overall minisett output in this study. The technologies for SYT (100−300 g) and minitubers (1−99 g) production are critical to establishing a formal seed system and large-scale seed production for enhanced commercialization. Availability of seed tubers in quality and quantity for rapid completion of the breeding cycle is also critical for researchers. Techniques and protocols or approaches for minitubers production as explored using healthy *in-vitro* materials account for over 80% of the yield from other seed production techniques except in the minisett technique as obtained in this study. Contrariwise, the use of minitubers and SYT sorted from bulk field harvest by farmers was perceived as the reason for the yield decline as observed among farmers. Such tubers were often than not the outcome of disease-infested plants.

The use of 100−400g tubers to generate minisett was also a contributory factor to successful minisett sprouts in these trials. This was corroborated in the work of Akoroda *et al.* (2007), which asserted that smaller tubers with a large periderm to cut-surface ratio had more tendency to sprout when compared to the use of large tubers above 1.5 kg with low periderm to cut-surface area. The use of chemicals in the treatment of minisett should be encouraged among farmers because wood ash, which is the usual practice among farmers, has been ineffective, cumbersome and obsolete. Phytotoxicity of wood ash and its low potentials for sett treatment as reported by Asare-Bediako *et al.* (2007) confirms that benomyl (Methyl 1-butyl-carbamoyl)-2-benzimidazole carbamate) the chemical was more effective than wood ash in the treatment of sett before planting. Even though smaller yam tubers were used and treated with chemicals, sprouting was influenced by SW and variety.

The yield and yield-related parameters measured concerning sett weight and variety influenced by species, variety and SW conform to Aighewi *et al.* (2003). Although, some of the farmers who attempted it used sett weights of 50−100 g range. This use of large minisett was advanced as the reason for the low sprouting characteristics of the popular landraces among these farmers. The recycling of these local varieties over several decades with attendant viral and nematode load may have contributed to the low performance of these varieties. Overall yield in tonnes per hectare was significantly higher ($p < 0.05$) among the breeder lines than the landraces.

These yield differences can be attributed to superiority in plant emergence, plant survival and yield across SW and, above all, the genetic variability among the varieties used. Yield differences due to variation in amount and pattern of rainfall were observed. But, this did not affect the sett weight and varietal influence.

Although yam has a general assumption that yield increases with an increase in sett weight, the ratio of sett weight to yield and the multiplication factor in this study decreased with higher SW. Hence, a perpetual rise in SW to attain a reasonable sprout rate and seed tuber yield was uneconomical. Emokaro and Law-Ogbomo (2008) reported that mother seed tuber cost increased above 60% when sett weight is doubled. Optimising the actual sett weight needed in a variety was critical to a profitable SYT business. Therefore, the productivity of the SYT business is limited to the minimum sett weight required to produce the SYT.

The soil analysis obtained from the three locations showed that available soil nitrogen was below the critical value. But no fertiliser was applied to remedy this. Therefore, yield partitioned effect for SW and variety became more appropriate.

The SW of 20 g with 73.2% overall emergence at 2 MAP was most suitable for *D. alata* cultivars and breeder lines of *D. rotundata*. The 30, 40 and 50 g SW were ideal for SYT tuber production in some of the landraces of *D. rotundata,* which could not attain 70% emergence with lower sett weights. The improved varieties had overall plant emergence of 80% and above, suggesting the need for farmers to adopt these improved and released varieties to replace some of the landraces with low sprout emergence and subsequent low yield across SW. Sett weights above 10 and 20 g should be discontinued in *D. alata* and *D. rotundata* breeder lines, respectively, because sett weight above these tends to produce tubers above SYTs.

On-farm participatory trial on SYT production with farmer groups in *Agunrege* Oyo State was carried out based on; (1) the outcome of the survey on the awareness and use of SYT production technologies and; (2) the Outcome of the two-year (on-site) minisett trials. Farmers confirm that planting material after milking and cutting ware yam to generate yam setts is not responsive in landraces like Pona, TDr 04-219 and Danacha. Hence, the need to source alternative seed sources for these varieties.

Approaches such as viral indexing preceding the Breeder seed production, Basic and Quality Declared Seed (QDS) should be encouraged in varieties with less ease of sprouting. Even though it was asserted that the yam minisett technique

(YMT), unlike TIBS and CTC, could not eliminate or be used to produce virus-free plants, positive selection was an alternative. In the findings of Gildemacher *et al.* (2011), positive selection in comparing the seed of known and unknown generations showed that yield is superior in breeder seeds compared with landraces. Farmers who adopt minisett can re-use their seeds for a lengthier generation if a positive selection is conducted by tagging healthy mother plants for seed generation in each of the succeeding planting seasons. This approach agreed with the finding of Aighewi *et al.* (2014) on the positive selection model for eliminating virus-infected plants with high to mild viral incidence and severity as a measure to gradual attainment of disease-free seed for a subsequent establishment.

Although the yam minisett technique existed for decades with several findings reported, this study evaluated some basic principles and provided information lacking in previous works. In the earlier studies conducted by Eke-Okoro *et al.* (2006), Ezulike *et al.* (2006), Ironkwe *et al.* (2008) and Aighewi *et al.* (2014), procedures for SYT tuber production using YMT corroborated the procedures used in this study, were reported. Although, information on the relative performance of the breeder lines known mostly to yam researchers and landraces often referred to as popular market varieties were less reported. Landraces were though reported, but these exhibit low sprout and poor field establishment. Therefore, the YMT could not gain wide acceptance among farmers. To address these challenges, McNamara and Morse (2014) recommended the use of SW as high as 80 g to eradicate the low sprout and poor field establishments associated with most of the landraces among farmers.

This recommended approach was then referred to as the adapted yam minisett technique (AYMT). This weight rarely produces an actual SYT of 100−300 g. Instead, ware yams above 1 kg are mostly the outcome. This adapted minisett technique does not consider the resultant cost of seed per stand, so the minisett was employed. In this study, sett weights as low as 20 g were enough for SYT production, especially when non-dormant seed tubers less than 400 g were used to generate the minisett. The cost of planting material is critical and must be reduced to the barest minimum if the minisett technique must be productive and yield the desired SYT. Previous works recommended that the minisett dust (a mixture of fungicide, insecticide and nematicide) was recommended. This dust is not readily found in the market or outrightly out of circulation.

The combination of bactericides, fungicides and insecticide to produce abroad spectrum mixture for the treatment of minisett was applied in these three cropping seasons in which minisett trials were carried out in this study.

This study provided information on yam varieties' performances across yam growing regions in Nigeria and the elite breeder line used in this study.

The enhancement of sprout performance among varieties while using small setts as low as 10 g will prevent excessive use of tubers from planting materials under the minisett technique. Information on differences observed in varietal responses to this technique in terms of different seed sizes produced per variety and SW will be of good use to farmers to combat the low adoption challenge which characterised the initial transfer of YMT to farmers. Farmers must have likely held on to the less yielding local cultivars or had no access to the improved variety. The latter statement was confirmed in the baseline survey conducted before this study showed that farmers have no access to improved varieties.

Since some of the yam tubers from farmers are laden with nematodes and viruses, sprouting is limited even in large setts. In the discussion sessions held while assessing the harvested tubers from the on-farm trial, farmers stated that the use of smaller SYT and chemical treatment must have been responsible for the performance of actual SW as low as 10−20 g. The first four varieties chosen by farmers at the participatory selection were TDr 95/19177, TDr 95/18544, TDa 00/00194 and TDa 291. This is an indication that farmers are willing to adopt improved varieties. The implication is that YMT will record a better adoption since these varieties are very amenable to the yam minisett technique.

The improved varieties showed higher uniformity in sprouting, while most of the landraces exhibited non-uniformity in sprouting. Again, the low sprout performance of most of the landraces used in this study explains why the technique remains unsuccessful and unpopular among yam growers. The mean value for plant emergence and stands at harvest for year-1, which was superior to that of year-2, was attributed to a too high rainfall that succeeded planting at week one of June in year 2. In June and July, the rainfall amount recorded (367.2 and 324.2 mm) was enough to cause planted setts to become turgid in the soil. Rainfall amounts for the same period in year-1 were 154.95 and 178.55.

The relationship among the SYT production techniques suggests that attaining the full potential of individual techniques was hinged on combining the techniques for

an effective seed production value chain. In relating the efficiency of the high ratio propagation techniques assessed, deductions from results obtained show that selected yam varieties were selective of these techniques. Variations on multiplication ratio, disease screening capability, derivable planting materials, yield and number of tubers varied among the techniques used in this study

Also, exploring the techniques based on their advantages and outputs will reduce seed bottlenecks for yam breeding schemes and commercial seed supply. Harnessing the potentials of these HrPT will increase the availability of SYT in quality and quantity to the end-users at a reduced cost. Plants of TIBS origin can be relied upon as sources of virus-free pre-basic seed since the plants were subject to virus indexing. Such seeds can be planted in a potted sterilised medium to produce disease-free SYTs. As found in this study, AS was selective of explant sources. The optimum potential of AS can only be realized using explants of TIBS origin, as earlier reported. In like manner, Vine cuttings (VCs) excised from AS plants performed optimally in terms of survival, new vine emergence and yield. Vine cuttings excised from potted plants in the screen house and those excised from field plants were less suitable.

Therefore, SYT thus derived can be multiplied through YMT and re-cycled till significant disease incidence with critical severity is noticed. Tubers from AS were nematode-free since the plants were cultured in the air. Using AS tubers to generate yam setts under YMT can reduce the cost and time required to obtaining clean tubers through positive selection in YMT. Yam minisett performance across different minisett used showed good nd repeatable results. This sprout performance even in 10 g sett weight could be attributed to the use of mother-tubers which are less than 200 g. Such seed produces setts with a high ratio of periderm cover. This high ratio of periderm cover is critical to the success of YMT.

The use of chemicals (a mixture of fungicide and insecticide) for sett treatment also influenced sprouting. Also, breeder lines were superior to landraces in overall performance and should be introduced to farmers for enhanced productivity. Optimizing the actual sett weight needed in a variety is critical to a profitable SYT business. Therefore, the productivity of the SYT business is limited to the minimum SW required to produce seed tubers. The enhancement of sprout performance among varieties while using small setts as low as 10 g will prevent excessive use of tubers from planting materials under the minisett technique. Although increased SW gave a

resultant increase in yield, this increase in yield is not proportional to the increase in SW. There is a benchmark beyond which further increase in SW will result in a waste of planting material. Aighewi *et al*., 2020; Lyonga et al., 1973; Emokaro and Law-Ogbomo, 2008, among other studies, affirm the SW and yield relationship. Information on differences observed in varietal responses to this technique in terms of different seed weights produced per variety and SW is of good use to farmers to combat low productivity and low adoption challenges which characterized the initial transfer of YMT to farmers. Also, Since some of the yam tubers from the Landraces were laden with nematodes and viruses, sprouting is limited even in large setts. This was also confirmed during the discussion sessions held with the farmers in the course of the on-farm trial. These reports on the likelihood of diseases (nematode) concerning sprouting and yield quality were corroborated by Claudius-Cole *et al.* (2017).

Moreover, the differences observed among the varieties could also be accounted for by the inherent traits. Alieu *et al*., 2012 related the yields of improved and Landraces to the phenotypic traits of these yam varieties. Varieties with desired traits such as early plant emergence, field establishment, early tuber initiation, short tuber dormancy period, among others, were identified as significant tuber yield-related traits in yam. Therefore, varieties with these traits must be considered for recommendation to farmers since they are likely to have held on to the less yielding Landraces or had no access to the improved variety. The latter was confirmed in this study's baseline survey, which proved that farmers have no access to improved varieties.

SUMMARY AND CONCLUSIONS

Edible yam is a staple food crop for over 300 million people in sub-Saharan Africa (SSA). It has high nutritional value and has been found suitable for the alleviation of hunger in SSA. Yam is a potential source of ethanol and starch for use in industries. Evolving confectionery food and food products will widen the dependence on this food crop. Leveraging on yam to eradicate hunger in SSA requires disseminating improved varieties and developing and distributing more adaptive varieties with higher quality traits for the near future.

Low multiplication–ratio resulting in short supply of SYT and limited distribution of its germplasm across borders are critical challenges. Moreover, SYT tubers' required and actual production gap is critically wide due to a lack of awareness and high-ratio propagation technologies (HrPT), which are beneficial for SYT production in quality and quantity.

The solutions to these challenges of low multiplication rate in yam, with the resultant high seed deficit, were attempted in this study by contacting some of the yam stakeholders (researchers and farmers) to identify the critical limiting factors to SYT tuber production. Selected SYT production techniques were refined or adapted for yam seedlings, minitubers (primary seed tuber) and SYT production. About 58% of the farmers interviewed in this study patronized the informal seed market, indicating a high SYT production and marketing prospect. The development of the protocol for improved minituber production by circumventing acclimatisation will also ease the transfer of genetic resources across the country and regional borders. A combination of red LED, 16-hour photoperiod, 60g/L sucrose and Jasmonic acid significantly enhanced tuber production. Although, the red LED did not support good plantlet growth and chlorophyll development.

On the contrary, the blue LED significantly enhanced shoot growth, as observed in the tissue culture experiment conducted at Ghent University, Belgium. A blue and red LED mixture, higher sucrose concentration, Jasmonic acid and 16-hour photoperiod promoted plantlet growth and tuber initiation, as observed in this study. Higher sucrose levels without NAA critically enhanced minitubers production in the TIBS experiment conducted at IITA, Ibadan, Nigeria. Plantlets of TIBS origin were most suitable for the Aeroponics system (AS). The AS produced a high shoot mass,

which generated about 300 single nodal cutting per plant within three months. Bulbils and tubers were harvested from AS. This technology was adapted from potato seed production and was suitable for seed and seedlings production in yams. Rooted vine seedling, direct vine cuttings and TIBS generated and acclimatised plants adapted to AS, but, of all, TIBS-originated plants were the most suitable explant source for AS.

The AS system offered an alternative source for sustainable seed tuber production in the SYT value chain. Irrespective of explant source, AS was varietal selective hence the recommendation of most adapted yam as reported in this trial. Milking of the fresh tuber from an actively growing two−three times harvest of tubers from growing plants is an advantage in AS. Since AS plants were "grown in the air" from plantlets and vine cuttings originated from virus-free TIBS or tissue culture plants, minisetts generated from tubers thus originated has the potential to reduce the yield gap in yam.

Yam minisett technique (YMT), developed by IITA in conjunction with NRCRI since 1982, recorded a low value for awareness and was used among farmers despite its long year of release and promotion. The low performance of popular landraces such as TDr 04-219, Alumaco and Pona sprouting revealed one reason while YMT has not met the farmers' expectations. Hence, the poor spread and adoption of the technology. The number of tubers harvested per plot was significantly higher in all the breeder lines when compared to the landraces. Varieties such as TDr 95/18544 and TDr 89/02677 produced multiple tubers, while most landraces, mainly TDr 04-219 and Pona, rarely produced numerous tubers. The highest proportion of whole tubers obtained as SYT tubers from the SW used were produced in SW of 10−30 g while 40 and 50 g sett weights were unsuitable for whole seed production. This study, therefore, recommended the use of sett weight of 10−30 g strictly to reduce seed production cost. The use of setts as low as 10 g in *D. alata* varieties and 10−30 g in *D. rotundata* will prevent excess tubers from planting materials under YMT. An anomaly observed in the on-farm trial was that 10 g SW tended to have higher tubers in the above seed class. This anomaly can be attributed to low PEP, which allows for more feeding areas for the survived plants.

The improved adoption and practice of high-ratio propagation technologies for plantlets, minitubers and SYT production are required to bridge the current SYT deficit. A deliberate establishment of a formal seed system for yam encourages some

farmers to specialize in SYT production by providing adequate training, incentives and guide.

This study showed that:

i. Awareness of the benefits of using healthy SYT for ware yam production is required through improved adoption and practice of HrPT for SYT production.

ii. Attaining the required seed status in healthy SYT production is possible by combining two or more technologies. Different users need the YMT, VC, AS, CTC and TIBS to enhance SYT production in quality and quantity.

iii. The HrPT were varietal selective, hence distributing those yam varieties adapted to each of the systems. Dissemination of varieties with good amenability to respective technologies will enhance the adoption and optimisation of the technologies.

iv. Protocols for enhanced tuber production in CTC and TIBs were advantageous in that Acclimatisation can now be circumvented. The enhanced production of viable minituber in CTC and TIBS will influence breeder seed production and release to foundation seed producers in the proposed formal seed system (FSS).

v. Yam and yam explants from different sources were found to adapt adequately to AS. Continuous harvesting of vine cuttings for seedling production in addition to harvested tubers and bulbils were achievements recorded in this study.

6.1 Recommendations

i. Increased sucrose concentration, Jasmonic acid supplemented medium, white light-emitting diode were recommended for the microtuber production under tissue culture techniques

ii. Plants originated from Temporary Immersion Bioreactor Systems were considered most appropriate for use in Aeroponics Systems and are therefore recommended

iii. The use of improved varieties of yam for seedlings and SYT tuber production is most appropriate for the considered seed production technologies

iv. Under good agronomic practices, the use of 20 g sett weights generated from a non-dormant whole tuber (≤ 400 g) for SYT production is most appropriate.

v. Diffusion of breeder lines to farmers and the use of 10−30 g MW in *D.alata* and *D. rotundata* to reduce the quantity and cost of planting materials will enhance the adoption of the minisett technique for SYT tuber production.

6.2 Contributions to the knowledge

i. The definite factors i.e. media composition and light-type responsible for plant growth and microtuber production in Conventional Tissue Culture (CTC) and Temporary Immersion Bioreactor Systems (TIBS) were established.
ii. The use of the Aeroponics System (AS) for yam culture and the identification of the most suitable explants source (TIBS) for use in this system were established.
iii. Appropriate minisett weight and varieties which are most suitable for yam minisett technique were identified.
iv. The need to apply more than one seed yam tuber production technique to achieve the required seed production in quality and quantity was established.`

6.3 Suggestions for further studies

Even though the varieties with good performances were identified across the propagation technologies investigated in this study, uniformity in FTY could not be attained within sett weight. There is a need to assess protocols for uniform SYT tuber production as this is one of the criteria for quality seed production.

The setup costs for some of the technologies assessed were high as most materials were not sourced locally. There is a need to investigate the potentials of locally sourced but cheaper items with some locally available materials for enhanced adoption and sustainability.

Developing operational protocols for establishing sustainable formal seed systems (FSS) in yam and other clonal crops using these technologies will further promote seed availability in the required measure.

It is necessary to carry out molecular and physiological studies to unravel the conditions needed for root and shoot formation in vine cutting and sprout emergence in landraces, particularly in those varieties with good quality traits.

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APPENDICES

Appendix 1: Questionnaire for baseline information on high ratio SYT production techniques

Section A (General background information of respondents)

Name Dr. Mr./Mrs.	
Sex	
Age	
Marital status	
Village/town	
Local govt./District	
State/ Region	
Nationality	
Religion	
Level of Education	
Family size	
Primary occupation	
Secondary occupation	
E-mail address	

Instruction: Please see foot note for Instructions and definitions before filling this section.

Appendix 1. continued

1.	For how long haveyou been working as a SYT expert in your institute	……. Years					
	Existing and developing techniques for SYT production	1. Minisett Tech.	2.Minituber Tech.	3.Vine Cutting Tech.	4.Conventional Tissue culture	5.TIBS	6.AS
2	Indicate your awareness of any of these seed production techniques						
3	Which of these are you practicing?						
4	Which of these techniques are you willing to adopt?						
5	What is the estimated cost of establishing the known technique: $ (1−99); (100−999); (1000−10000); (up to 20,000); (more than 20,000).						
6	Which of the techniques you are aware of are you willing to adopt/practice?						
7	What is the source of your starter stock: 1. Field; 2. Screen house; 3. Tissue culture; 4. Market; 5. Research institute, 6. Seed company; 7. Self; 8. Others						
8	What are your challenges on the technique off interest? (*Number*						

	according to tech.)						
9	What is your recommendation on these techniques? (*Comment as appropriate:* *4 In vitro; 5 Tibs),*	1. …… 2. …… 3 …… …… 4. …… 5. …… 6. ……..					
10	Success rate recorded so far in seed tuber production (Please tick) under the listed techniques	< 25%,	25 %	50%,	75 %,	100 %	Remarks
A	Minisett Tech						
B	Minituber Tech						
C	Vine Cutting Tech						
D	Conventional Tissue culture						
E	TIBS						
F	Aeroponics system						
11	Disease infection/contamination rate (Score between 1−5; 1. No severity, 2 low, 3 moderates,4. high; 5. Very high severity)						
12	What tuber weight (g) is obtainable						

	1−25; 26−50; 51−100; 101−300; >300						
13	Which of these explants do you prefer under *in vitro*		1. Leaf (); 2. Meristem (); 3. Nodal culture ()				
14	What is the Tissue/callus formation rate (Score between 1−5)						
15	What proportion in percent is contaminated: < 25%; 25%; 50%; 75%; >75%						
16	Please state the proportion that develops and grow into a desired plant status: < 25%; 25%; 50%; 75%; >75%						
17	Regeneration period: 1−4 months; 4−8 months, 8−12 months; 12−18 months, 18−24 months						
18	Survival of the regenerate/vine cuttings: < 25%; 25%; 50%; 75%; >75%						
19	Survival at acclimatization/nursery :< 25%; 25%; 50%; 75%; >75% (%)						
20	Survival at transplanting (screen house/Field < 25%; 25%; 50%; 75%; >75%						

21	In 1 year, what weight of tuber will produce from a tuber of 50g under these techniques as practiced by you? 1−10; 11−100; 101−1000; above 1000						
22	Do you know any specialized seed expert or company in your country?	Yes ( ); 2. No ( )					
23	If yes, name them						
24	How healthy are the tubers harvested from your technique	Very healthy ( ); ( ); Not healthy ( )					
25	Does your organization involve the multiplication of healthy seed and distribution of same to end-users and researchers?	Yes ( ), No ( )					
26	If yes, how often? 1. all season; 2. Planting season only; 3. During harvesting only						
27	What quantity of SYT can a farmer get to buy from your institute? 0; 50; 100; 500; 1000; 10000;						
COMMENTS:							

Minitubers sprouted at 14DAH

Appendix 2: Minitubers produced under TIBs at 12 WAC

- Tuber production in TIBs took only 12 WAC.
- Tuber production in TIBs increased with an increase in sucrose level.
- Sprouting of minitubers was early: short (14 days) dormancy period

Minitubers from TIBs sprouted in the pot and develop to produce seed tubers

Appendix 3: Minitubers produced at 12 WAC from vine cutting seedling cultured in a nursery bag under screen house condition

Appendix 4. Fertilizer application rates used under aeroponics system trials

Nutrient	Establishment rate (g)	Vine growth rate (g)	Tuber bulking rate(g)
Ammonium nitrate	136	272.7g	136.0
Calcium nitrate	193.5	195.5g	193.5
Potassium Sulphate	60	60g	60.0
Triple Super Phosphate	65.2	65.2g	130.4
Magnesium Sulphate	98.3	98.3g	98.3
Fetrilon C	5	5	5.0

Appendix 5. Field layout of Minisett experiment conducted in this study

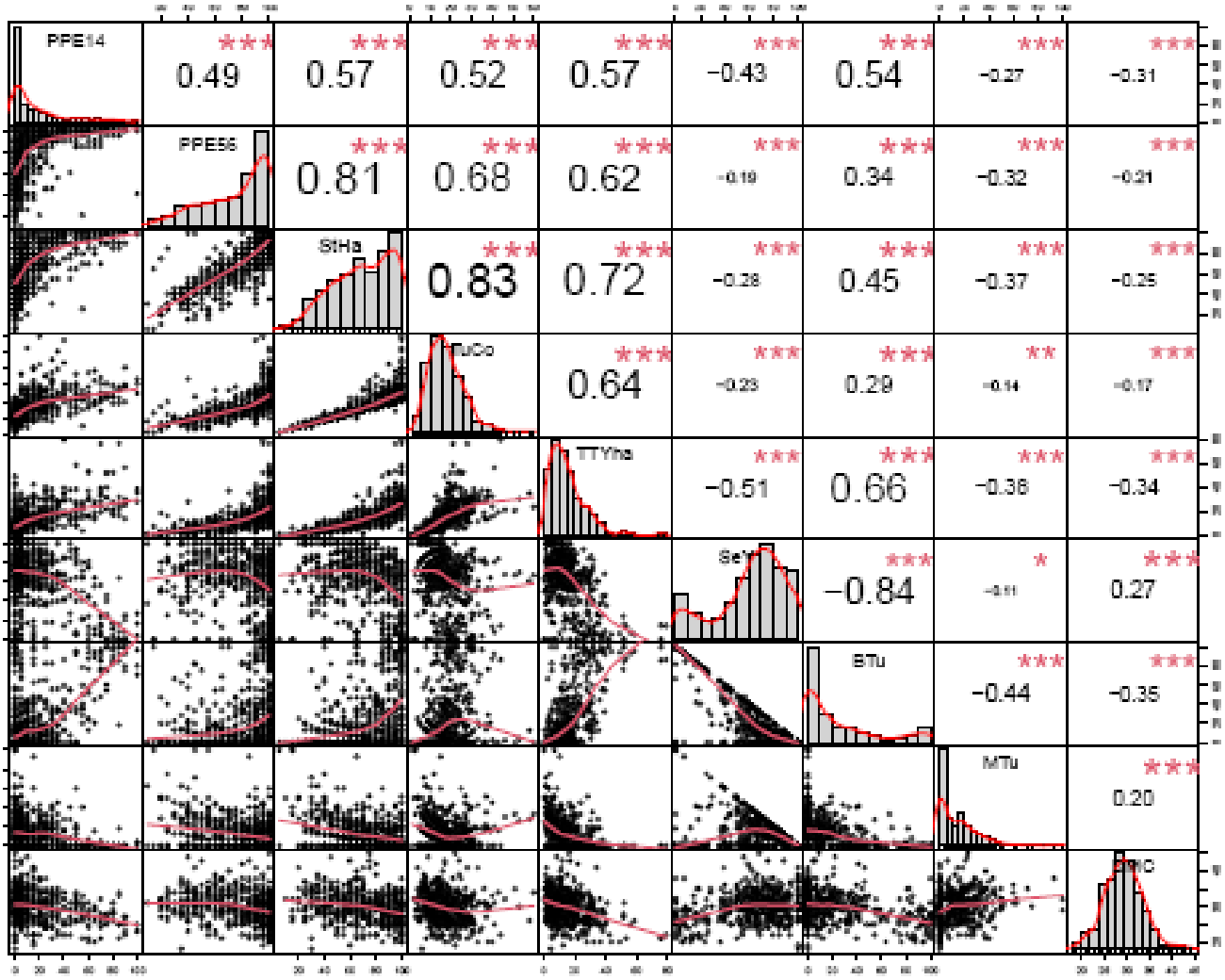
		TDa Varieties		TDr Varieties					
	SW (g)	**Border**							
REP 1	40	00/00194	93-36	89/02475	Danacha	Pona	Meccakusa	89/02665	89/02677
		98/01176	291	Alumaco	Amula	Obiaturugo	95/19177	95/18544	Kikuno
		2m Alley							
	10	00/00194	93-36	89/02475	Danacha	Pona	Meccakusa	89/02665	89/02677
		98/01176	291	Alumaco	Amula	Obiaturugo	95/19177	95/18544	Kikuno
		2m Alley							
	50	00/00194	93-36	89/02475	Danacha	Pona	Meccakusa	89/02665	89/02677
		98/01176	291	Alumaco	Amula	Obiaturugo	95/19177	95/18544	Kikuno
		2m Alley							
	20	00/00194	93-36	89/02475	Danacha	Pona	Meccakusa	89/02665	89/02677
		98/01176	291	Alumaco	Amula	Obiaturugo	95/19177	95/18544	Kikuno
		2m Alley							
	30	00/00194	93-36	89/02475	Danacha	Pona	Meccakusa	89/02665	89/02677
		98/01176	291	Alumaco	Amula	Obiaturugo	95/19177	95/18544	Kikuno
		Border							
REP 2	20	00/00194	291	Alumaco	95/18544	89/02665	95/19177	Amula	Danacha
		93-36	98/01176	Kikuno	Meccakusa	89/02677	89/02475	Pona	Obiaturugo
		2m Alley							
	10	00/00194	291	Alumaco	95/18544	89/02665	95/19177	Amula	Danacha
		93-36	98/01176	Kikuno	Meccakusa	89/02677	89/02475	Pona	Obiaturugo
		2m Alley							
	40	00/00194	291	Alumaco	95/18544	89/02665	95/19177	Amula	Danacha
		93-36	98/01176	Kikuno	Meccakusa	89/02677	89/02475	Pona	Obiaturugo
		2m Alley							
	50	00/00194	291	Alumaco	95/18544	89/02665	95/19177	Amula	Danacha
		93-36	98/01176	Kikuno	Meccakusa	89/02677	89/02475	Pona	Obiaturugo
		2m Alley							
	30	00/00194	291	Alumaco	95/18544	89/02665	95/19177	Amula	Danacha
		93-36	98/01176	Kikuno	Meccakusa	89/02677	89/02475	Pona	Obiaturugo
		Border							
REP 3		98/01176	291	89/02665	Meccakusa	Amula	89/02475	Pona	Alumaco
		93-36	00/00194	Danacha	Kikuno	Obiaturugo	95/18544	95/19177	89/02677
		98/01176	291	89/02665	Meccakusa	Amula	89/02475	Pona	Alumaco
		93-36	00/00194	Danacha	Kikuno	Obiaturugo	95/18544	95/19177	89/02677
		98/01176	291	89/02665	Meccakusa	Amula	89/02475	Pona	Alumaco
		93-36	00/00194	Danacha	Kikuno	Obiaturugo	95/18544	95/19177	89/02677
		98/01176	291	89/02665	Meccakusa	Amula	89/02475	Pona	Alumaco
		93-36	00/00194	Danacha	Kikuno	Obiaturugo	95/18544	95/19177	89/02677
		98/01176	291	89/02665	Meccakusa	Amula	89/02475	Pona	Alumaco
		93-36	00/00194	Danacha	Kikuno	Obiaturugo	95/18544	95/19177	89/02677
		Border							

Appendix 6: Yam Tuber weight classification

yam Tubers	Categories (gram)
Ceremonial yam	Greater than 5000
Ware yam	Greater than 1000
Seed yam (Grade 1)	500−1000
Seed yam (Grade 2)	100−300
Minitubers	> 100
Primary Seed tubers	> 10
Microtubers	>1
Seed yam tuber range for this study	1−500g

Source: Ekanayake and Asiedu (2003),

Appendix 7: Sett weight categories used in YMT trials



Appendix 8: Correlation coefficient for plant emergence, yield and yield related traits of sixteen yam varieties evaluated for two seasons at IITA-Ibadan