PERFORMANCE OF CASSAVA PLANTING MATERIALS PRODUCED USING THE "SEMI – AUTOTROPHIC HYDROPONIC" TECHNOLOGY IN THE LABORATORY AND ACROSS LOCATIONS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Performance of Cassava Planting Materials Produced Using the "Semi Autotrophic Hydroponics" Technology in the Laboratory and Across Locations in the Democratic Republic of Congo

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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Mamy Makumbu Binzunga

This thesis has been submitted for examination with our approval as the university supervisors

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DEDICATION

To my dear husband Louis Phelix Kaja Mukinay and our dear daughter Divine Alexandre Mutombo Kaja for their unwavering love, encouragement, and steadfast support throughout my thesis journey.

In loving memory of my dear late Dad Binzunga André and my dear late Mum Musau Annastasie, whose prayers, willingness, and support paved the way for my Master's degree attainment.

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ACRONYMS AND ABBREVIATION

BASICS	Building on An economically Sustainable Integrated Seed System
Ca	Calcium
CBSD	Cassava Brown Streak Diseases
CBSV	Cassava Brown Streak Viruses
CEC	Cation Exchange Capacity
CMD	Cassava Mosaic Disease
CV	Coefficient of Variation
D.R.CONGO	Democratic Republic of Congo
EC	Electrical Conductivity
IITA	International Institute of Tropical Agriculture
INERA	Institut National pour l'Etude et la Recherche Agronomiques (National Institute of Agronomic Study and Research)
K	Potasium
LSD	Least Significant Difference
MAP	Month After Planting
Mg	Magnesium
N	Nitrogen
P	Phosphorus
PCR	Polymerase Chain Reaction
pH	Potential Hydrogen
RCBD	Randomized Complete Block Design
SAH	Semi - Autotrophic Hydroponics
SENASEM	Service National de Semences (Seed national service)
SNSA	Service National de Statistique Agricole (National Service of agriculture statistics)
SSA	Sub Sahara Africa
TC	Tissue Culture

ABSTRACT

Cassava (*Manihot esculenta* Crantz)) is a crucial food crop in D.R. Congo, sustaining over 70% of the population and serving as a primary income source. However, the cassava seed system faces the challenge of a low propagation rate associated with an extended growing cycle. This research aimed to contribute to the improvement of the cassava seed system in D.R. Congo using stem cuttings provided by SAH technology. Two experiments assessed plantlet performance using two types of substrates. Experiment 1, employing a split-plot design, used four genotypes (IB961089A, MM060083, Nase14, and Albert28) and four single substrates: KlasmannTS3 (K), Vermiculite (V), Local Peat (P), and Sawdust (S). It involved three subculture periods lasting four weeks, with data collection on survival, height, leaf, internode, and cutting numbers. Experiment 2, following a similar design, investigated the performance of three genotypes (IB961089A, IBA070520, and IBA980555) under single substrates (K, V, and P) and their combinations ($K_{25}P_{75}$, $V_{25}P_{75}$, and $V_{10}P_{90}$). The field experiment in Mulungu and Kiliba utilized SAH-derived plantlets employing a 4 x 4 split-plot design. Data were collected on survival, growth parameters, and stem length at different months after planting. Finally, a simple cost analysis was carried out, comparing the production cost of SAH-derived plantlets to that of producing cuttings under the conventional propagation method. Experiment 1 revealed substrate significantly influenced survival rates, surpassing 90% ($p<0.05$), showing the highest number of cuttings of 70.4 in three months, representing a ratio of 1:4 with the genotype MM060083 ($p<0.001$). Experiment 2 showed $K_{25}P_{75}$ did not differ from single KlasmannTS3, with the highest survival rate and an increase of 80.5 cuttings, representing a ratio of 1:4 with IBA961089A. The superior effect of the substrates was attributed to their favorable properties, allowing rapid plantlet growth. Field results indicated that Kiliba recorded a higher survival rate of 81.3% compared to Mulungu's 73.8%. Across locations, MM060083 had the highest survival, exceeding 80%, while Nase14 had the lowest. The check-cutting method demonstrated the highest survival of over 90% compared to SAH-derived plants, but KlasmannTS3 had the highest among the SAH at Kiliba (85.4%). The highest stem length was obtained at Mulungu (17.5 m) compared to Kiliba's 10.5 m at 12 MAPs. Nase14 achieved the highest stem length of 17.7 m per plot, while IBA961089A had the lowest at 9.3 m at 12 MAPs. SAH-derived plants caught up with conventional checks across locations by 12 MAPs. The use of combined substrates, particularly $K_{25}P_{75}$, demonstrated an equal unit cost to that of the conventional mini stem, both amounting to USD 0.07. These findings provide relevant insights into policymakers' decisions to promote efficient cassava propagation method for sustainable agriculture, enhance food security, and promote economic development in D.R. Congo.

Keywords: Cassava, performance, planting material, multiplication rate, substrate, cost analysis, Semi - Autotrophic Hydroponic (SAH).

CHAPTER ONE

INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* Crantz) is a staple food crop belonging to the Euphorbiaceae family (Charrier and Lefevre, 2015). Cassava domestication began 5000–7000 years BC in Brazil (Léotard et al., 2009) (Léotard et al., 2009). Historical evidence shows that the crop originated in Brazil and South America and that Portuguese explorers and traders introduced it to sub-Saharan Africa (SSA) during the 16th century (Henry and Hershey, 2001). Its dissemination through the other regions of SSA was ensured by Africans and its incorporation in agricultural systems has been a great success as it replaced traditional staple foods such as yams and millet (Carter et al., 1997). Cassava crop can adapt to marginal soils and irregular rainfall conditions that can give acceptable yields (Henry and Hershey, 2001). It has demonstrated a strong ability to adapt to climate change, making it a valuable choice when other food crops face challenges (Jarvis et al., 2012; Mupakati et al., 2017; Malik et al., 2020; Pushpalatha and Gangadharan, 2020).

Additionally, cassava is the most important carbohydrate food for millions of people in tropical Africa (Bayata, 2019; Otun et al., 2023 and Scaria et al., 2024). Cassava plays a crucial role in food security and offers economic opportunities for small- farm owners, landless farmers, processors, and traders globally, serving as a gateway for employment and income generation (Spencer and Ezedinma, 2017). Its cultivation contributes significantly to livelihoods in diverse regions, as emphasized by Thresh et al. (1994) and Abass et al.(2013)(Abass et al., 2013)(Abass et al., 2013)(Abass et al., 2013)(Abass et al., 2013)(Abass et al., 2013).

In D.R. Congo, cassava is a vital staple food crop, ensuring food security and supporting the livelihoods of millions. It also serves as a significant source of income for farmers, occupying the foremost position among other food crops. Cassava accounts for over half of the annual crop area and is regularly utilized by more than 70% of the population for its roots and about 80% for its leaves (Mahungu et al.,2022).

1.2 Cassava Production

Cassava crop is currently grown in 40 of the 54 countries and cultivated on 18.7 million hectares in Africa, which makes up almost 75% of the global total (Spencer & Ezedinma, 2017; FAOSTAT, 2022). Cassava root production in the African continent accounts for more than 50% of the global total of 233.8 million metric tons (Tumwegamire et al., 2018). On global production, D.R. Congo occupies the second position of 40,05 million tons per year after Nigeria (FAOSTAT, 2022).

The yield of fresh cassava roots in Africa averages 8.87 t/ha, which is low compared to the yields recorded in Asia of 22.01 t/hha (FAOSTAT, 2022). This is due to several constraints, including poor quality of planting material, suboptimal agronomic practices, poor cultivation, pests, and disease infestations, which together can cause yield losses of up to 50% in Africa (El-sharkawy, 2004; Fermont et al., 2009; Sanginga and Mbabu, 2015).

1.3 Constraints in Cassava Production

In D.R. Congo, low cassava root production stems from various factors. The distribution system for cassava genotype stems, based on community multiplication, lacks sufficient capacity to reach the majority of farmers, despite seed multiplication schemes initiated by breeding programs. Bidiaka et al. (2022) reported that only 15% of planting material needs were met nationwide in 2012, with a demand for improved cuttings estimated at 5 billion linear meters (Ndjadi et al., 2017; Mubalama et al., 2019; Ganza et al., 2019). Additionally, the cassava's lengthy growth cycle combined with its low propagation rate results in inadequate availability of basic seeds in research programs. Consequently, rural smallholders often resort to cultivating disease-vulnerable local varieties yielding less than 8.8 tons per hectare, compared to yields ranging from 35 to 45 t/ha observed in research stations of the National Institute of Agronomic Studies and Research (INERA) (Sanginga and Mbabu, 2015; Mahungu et al., 2022). This situation reflects an imbalance in the seed system, from the production of basic seeds at the cassava breeding level to their deployment to farmers.

On the other hand, the persistent use of poor-quality planting materials contributes significantly to low yields (Wossen et al., 2020; Sanginga and Mbabu, 2015). Wossen et al. (2020) attested that efforts to improve the quality of plant material exchanged in markets or other channels are often hampered by the unique biological and economic characteristics of vegetative propagation. For lack of good materials, farmers plant cuttings that they take from their fields after harvest or from neighboring fields. These cuttings, which are often already affected by diseases and pests, contribute to low yields in future harvests.

Agronomic challenges in cassava cultivation include diseases, pests, soil and nutrient management, and key practices like planting density, site-specific genotype choice, and poor weed management. Challenges also include high perishability, handling costs, transportation issues, and the inconvenient weight and size of the materials (Mulimbi et al., 2012; Hillocks and Maruthi, 2015; Legg et al., 2015; Pallett, 2016; Zeyimo et al., 2019; Mahungu et al., 2022). Diseases and pests spread from one field to another through infected cuttings. Economic diseases, particularly Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD), pose a significant threat, causing annual losses of about US$1 billion and impacting food security (Legg et al., 2014; Hillocks and Maruthi, 2015; Zeyimo et al., 2019). To address these challenges, ongoing research focuses on advancing breeding and genetic improvement, exploring new seed production technologies, and enhancing varietal evaluations, multiplication, conditioning, and distribution to ensure a sustainable supply of quality plant material (Wossen et al., 2020).

1.4 Statement of the Problem

The Cassava seed system in D.R. Congo, as well as worldwide, faces constraints of the low propagation rate of 1:10 when multiplied traditionally by farmes or conventionally, along with the lengthy growing cycle (Santana et al., 2009). These factors contribute to a limited supply of planting materials for improved varieties, thereby impeding the seed system’s ability to meet the increasing demands from farmers and other users. The breeding program faces many challenges as it develops plant varieties, including evaluating genetic materials in multi-local tests and at testing

stations, distributing genetic materials between programs, and distributing them to seed companies and seed growers (IITA, 1990 & Otoo, 1996).

Another challenge is the high production costs of basic seeds, which make certified seeds to be too expensive for end users (Escobar et al., 2006). As long as D.R. Congo's breeding program faces these challenges, any effort deployed won't be effective. Due to the lack of certified cassava seeds, farmers still using degenerate varieties with low yields, limiting their incomes (Sanginga and Mbabu, 2015).

The tissue culture technique can be used to rapidly multiply small amounts of improved seeds and increase stocks for the benefit of Breeding programs, and for all parties involved in the cassava sector (Escobar et al., 2006; Hussain et al., 2012). However, the technique is more expensive than conventional methods of plant propagation (Sahu and Sahu, 2013; Santana et al., 2009).

Semi-Autotrophic Hydroponics (SAH) technology, which was developed by SAHTECHNO Ltd., Argentina (Bentley et al., 2020a), for the production of potato seeds, was adopted for cassava propagation by IITA Cassava breeders for the first time through the International Institute of Tropical Agriculture (IITA) in Nigeria in 2016. The technique focuses on the mass propagation of virus-free plants of tissue culture origin under an organic substrate (Adesanya et al., 2016; Bentley et al., 2020; Thiele et al., 2022). The technique was then adopted in D.R. Congo in 2018 (Kajibwami et al., 2018) and in other countries in Africa as well (Bentley et al., 2020a). The technology is a low-cost novel technic, with a large potential for seedling production in space and over time. It is easy to adapt to improve the multiplication rate in breeding programs and for commercial seed production for clonal crops such as cassava and yam (*Dioscorea spp*.) (Olugboyega et al., 2019, Pelemo et al., 2019; Bentley et al., 2020; Ceballos et al., 2020; Thiele et al., 2022). The benefits of the SAH technology over other propagation techniques are its high multiplication ratio in the laboratory and allows for propagation of true-to-type cassava planting materials (Thiele et al., 2022). However, the main bottleneck is the importation of substrate for planting material production, from Germany, and in some cases, the unit cost per plant becomes unaffordable. The issue of making cassava cuttings for varietal evaluation and for

producing basic seeds of improved varieties is critical to breeding programs as well as to users across the cassava chain in general. Hence, it is necessary to adopt strategies to further reduce production costs and lower the unit cost. Several authors have pointed out that convenient substrates should not only supply the physical, chemical, and biological properties required by plants but also be available, affordable, and sustainable for practical plant production Mayo-Prieto et al., 2020; Jan et al., 2021; (Bhattacharjya et al., 2014; Lin et al., 2017; Barbosa et al., 2022; Kumar and Singh, 2023). Given the financial constraints explained, the use of alternative substrates might be the most feasible low-cost option to optimize the SAH technology.

1.5 Justification

This research addresses critical constraints in the cassava seed system across Africa and in D.R. Congo, specifically focusing on the low propagation rate and extended growing cycle of cassava cuttings (Santana et al., 2009; Mahungu et al., 2022). The evaluation of SAH technology's performance in laboratory and field settings is expected to have far-reaching impacts on various levels (Pelemo et al., 2019; Olugboyega et al., 2019; Ceballos et al., 2020). Researchers will gain insights into the efficacy of SAH technology, advancing scientific understanding of cassava propagation and hydroponics. The cost-benefit analysis will offer economic perspectives for future research and sustainable propagation methods. Policy-makers can leverage these findings to support the adoption of SAH technology, enhancing the efficiency of the cassava seed system. The production of virus-free and true-to-type cassava plantlets using SAH technology is poised to improve food production, security, and economic opportunities. The study's outcomes will inform breeders, entrepreneurs, and stakeholders involved in seed production, optimizing practices, and boosting cassava propagation efficiency. Moreover, economic feasibility insights will benefit stakeholders, including farmers, seed companies, and policymakers, fostering a competitive cassava value chain and creating economic opportunities. The availability of high-quality planting materials is anticipated to increase crop yields, improve farmers' income, and enhance the sustainability of cassava farming in the face of environmental challenges and market demands (Shegro Gerrano et al., 2011; Intens et al., 2013; Ceballos et al., 2020).

1.6 Objectives of the Study

1.6.1 Overall Objective

This study aimed to contribute to the improvement of the cassava seed system in the Democratic Republic of Congo, using planting materials produced through the Semi Autotrophic - Hydroponics technology.

1.6.2 Specific Objectives

i. To assess the performance of selected cassava genotypes plantlets produced under different substrates in the SAH system in the laboratory;
ii. To evaluate the performance of selected cassava genotype plantlets produced using different SAH substrates in the field at different locations;
iii. To assess the cost-benefit of producing the SAH-derived plantlets in comparison to the conventional method of multiplication at the research station level.

1.7 Null Hypothesis of the Study

i. There is no difference among the selected cassava genotypes for growth performance traits under different substrates under the SAH system in the laboratory;
ii. There is no difference among the selected cassava genotypes plantlets produced using different SAH substrates in the field at different locations;
iii. The cost of producing SAH-derived plantlets under the SAH system did not differ from that of using the conventional propagation method.

1.8 Scope of the Study

This study is important for different cassava seed actors including the government, researchers, seed companies, International community, different academicians, professionals, and farmers. It brings new knowledge on the techniques of micro propagation. This will contribute to the spread of sustainably cassava varieties, assist selection efforts in many activities, and reduce the use of degenerate and infected planting materials by growers. This study will also help to support the national policies

that are consistent with food security and poverty reduction for the Congolese population. Cassava is the main crop and staple food for the majority of Congolese. The areas of cultivation still growing and increase in seed demands. These research findings will help significantly to improve sustainably the seed system for high productivity and increasing incomes of farmers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Domestication and Genetics of Cassava

Cassava (*Manihot esculenta* Crantz) was domesticated in the Amazon region (Olsen and Schaal, 2001). Its domestication process involved the selection of size, root, growth ability, number of stems, and the ability for clonal propagation by cuttings (Otoo, 1996). Cassava has a chromosome number of 2n = 36 and is an allotetraploid with a base number of x = 9 (Nassar, 2002). However, it behaves like a diploid during its sexual multiplication (Carvalho and Guerra, 2002). Genetic resources of cassava include improved varieties, genetic stocks, related wild species, and local and introduced landraces (IITA, 1990).

2.2 Ecology, Growth, and Nutrient Requirement of Cassava

Cassava is a plastic plant that can tolerate drought and performs better with an annual rainfall of 600 to 1500mm and temperatures of 25° to 29°C. It can be grown in the tropics between latitudes 30°N and 30°S up to 2000m altitude, and under in vitro technology conditions, it requires 20-30°C temperature and a photoperiod of 12/12 (Alves, 2009). Compared to other crops, cassava tolerates marginal, poorly fertile, and acidic soils better, but is sensitive to soils with a pH above 7.8 (Alves, 2002; El-Sharkawy, 2003; Nassar and Collevatti, 2005). There are several morphological characteristics to distinguish cassava varieties, including height, size of the plant, branching habit, color of stems and petioles, and color of leaves and stems. The cassava plant may be divided into two main parts, as shown in Figure 2.1, the shoot system and the root system. The shoot system develops from axillary buds located on the nodes of the cuttings (IITA, 1990). Shoots consist of leaves and stems together.

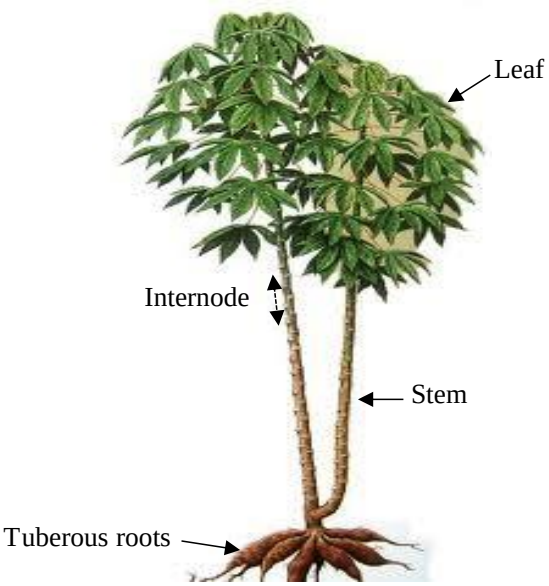


Figure 2.1: General Morphology of the Cassava Plant

Surce: (IITA, 1990)

Cassava stems, vital for vegetative propagation, can reach 4m in height, with dwarf varieties at 1m. Stem cuttings, or lignified parts, serve as "seeds" and are crucial for commercial production. Nodes, where leaves join stems, and internodes, the stem parts between nodes, shape stems (IITA, 1990; Ceballos et al., 1996) .

Environmental factors influence leaves, pivotal for photosynthesis. Light, heat, water, humidity, and nutrition impact plant development. Growth t hinges on node production rate and internode elongation. Conventional propagation relies on field growth parameters-leaf number, node number, and their correlation. Higher leaf numbers denote vigor, increased photosynthesis, and potential yield (Neves et al., 2018). More nodes suggest greater branching potential, aiding stem production for planting. The leaf-to-node ratio varies among genotypes and conditions, affecting growth performance. Optimizing planting material production requires proper agronomic practices such as proper spacing, adequate nutrient management, and disease control. Selecting genotypes with favorable characteristics enhances conventional propagation

efficiency, supporting the dissemination of improved cassava varieties (H. Ceballos et al., 2020).

From the basic soil requirements of crops, the nutritional aspect is among the several factors affecting the optimal growth and productivity (Thomas, 1996; Howeler and Reinhardt., 2014). Essential nutrients for plants 'growth and development can be broadly categorized as macronutrients and micronutrients. Macronutrients include carbon (C), hydrogen (H), oxygen (O_2), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg). Micronutrients include iron, manganese, zinc, boron, molybdenum, chlorine, copper, and nickel (Byju and Suja, 2020).

Cassava is known to grow reasonably well in very acid and low-fertility soils. Still, like other plants, its growth is affected by nutrient supply, and, if some nutrients are not present or are present in inadequate amounts, plant growth and yield will be reduced. In other cases, plant growth decreases because some elements in the soil may be too high, causing either a toxicity or a reduction in the uptake of other essential nutrients (Howeler and Reinhardt, 2014). For example, the first symptoms of nitrogen (N) deficiency are usually slow growth and uniform yellowing of older leaves (Leghari et al., 2016).

2.3Breeding and Multi-local Testing in D.R. Congo

The main objective of the cassava breeding program in D.R. Congo is to develop varieties that ensure food security and fulfill multiple specific uses. This involves breeding new varieties with traits such as high yield, resistance to major diseases and pests, nutritional value, and adaptability to various agroecological conditions across the country (Mahungu et al., 2022). The process of developing cassava varieties follows a classic selection cycle for 6 to 7 years starting from the evaluation of seedlings from controlled and open pollination of a parental diversity, passing through the clonal evaluation, preliminary yield trial, advanced yield trial then a uniform yield trial followed by successive selections.

The promising genotypes selected during this last cycle will be evaluated in different ecologies under farmer management in their fields (Bidiaka et al., 2022). To assess the genotypes for their general and specific adaptation, the national breeding trial must be carried out in the various agro-ecological sites in the country (Mahungu et al., 2022). At this level, the need for planting materials is very high. However, because of the low multiplication rate associated with the long cycle of cassava, the breeding program faces challenges in terms of the time for seed multiplication to undertake the multi-local trials as well as for seed dissemination purposes.

2.4 Seed Multiplication and Efficiency in D.R. Congo

The multiplication scheme used for cassava in D.R. Congo includes, as detailed in Figure 2.2, from the pre-basic (breeder seeds), three hierarchical levels: As discussed by Bidiaka et al. (2022) and Mahungu et al. (2022) basic seeds (Primary), R1-registered seeds (Secondary) and R2-registered seeds (Tertiary) are based on the cascade multiplication principle.

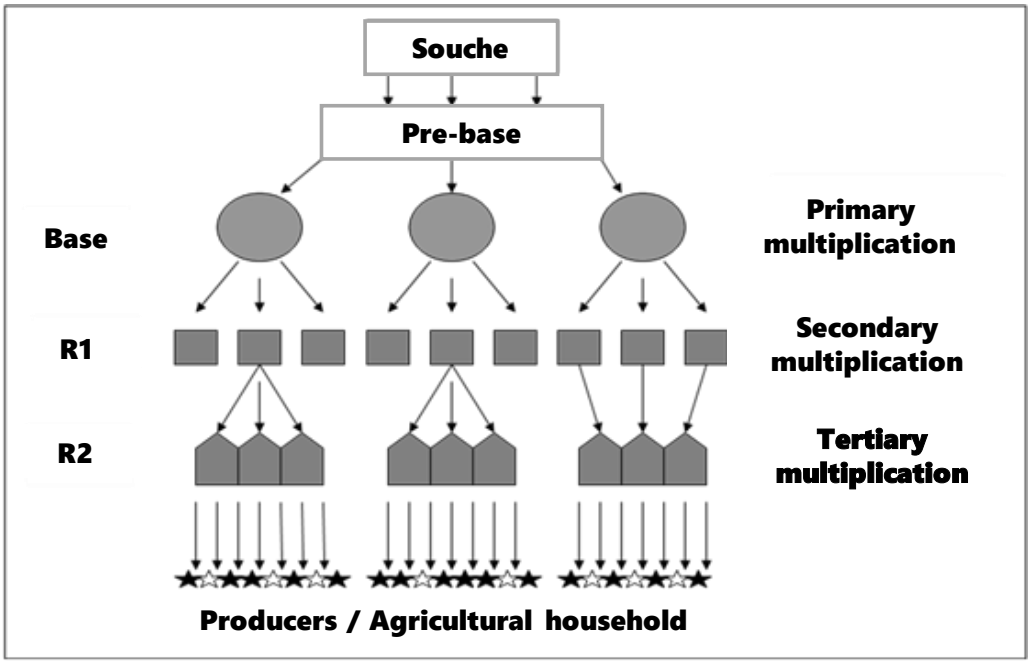


Figure 2.2: Cascade Scheme of Cassava Cuttings Multiplication in D.R. Congo

Source: (Bidiaka et al., 2022)

The goal of this initiative is to rapidly increase the availability of planting materials, moving from breeder seed to R1-registered seed, distributed through organized communities registered and monitored by the National Seed Service (SENASEM). R1 materials are then used to establish fields in the tertiary category (R2), closely involving farmers' associations and farmers' school fields for widespread distribution to farm households. The distribution channels include multi-local trials and specialized agricultural events. In D.R. Congo, the demand for improved cassava cuttings is substantial, estimated at 5 billion meters, with only about 15% of this need covered in 9 The breeding program plays a crucial role in maintaining genetic purity, multiplication, and distribution to meet stem demands and enhance national production, which relies heavily on improved varieties. The breeding program, in collaboration with international partners, has been developing and disseminating improved varieties since the early 2000s. Despite these efforts, challenges persist, particularly in reaching remote and rural areas due to the vast and complex nature of the country. Low adoption rates of improved varieties are observed, with farmers often unable to access these varieties.

The slow multiplication rate and a lengthy cycle of varieties developed in research stations contribute to delayed adoption by farmers (Mahungu et al., 2022); (Bidiaka et al., 2022). To address these challenges, the SAH technology has been recently integrated into maintenance and multiplication schemes. This technology offers advantages in boosting the cassava seed system sustainably, providing a potential solution to the constraints of low multiplication rates and extended cycle durations. The incorporation of SAH technology is expected to expedite the flow of pre-basic seeds, ensuring a continuous and efficient seed multiplication process. This innovative approach aims to enhance the adoption of improved varieties and contribute to the overall success and impact of the breeding program in meeting the diverse needs of cassava farmers across the country (Bidiaka et al., 2022; Mahungu et al., 2022).

2.5 Hydroponic Growing Techniques and Basic Components

Hydroponics is a method of growing plants without soil, where plants are either suspended directly in the aqueous solution or grown in a soil-free medium in controlled

conditions of light, temperature, and photoperiod (Gebicke, 1945; Jensen, 2013). In hydroponics, plants are provided with nutrient-rich solutions in a water solvent, which the plants conventionally obtain from the soil in traditional farming. The main objective of hydroponics is to supply the ideal nutritional environment for optimum plant performance, further optimized by controlling the climate (Gebicke, 1945; Maucieri et al., 2019; Kumar and Singh, 2023). Hydroponics is a promising technological solution to the problems faced by current agricultural systems in underdeveloped countries. Several authors have reported many advantages of growing plants in a hydroponics system, including less space and water, an extended growing season, increasing yields, fast-growing more crop cycles, higher plant density, less disease and pest pressures (Morgan & Peckenpaugh, 2004; Rehman, 2015; Saaid et al., 2015; Treftz and Omaye, 2016; Suryathi and Delly Resiani, 2017). The advantages of hydroponics cultivation have increased its popularity drastically in a short period of time leading to an increase in experimentation and research (Putra and Yuliando, 2015; Horibe, 2018; Cifuentes-Torres et al., 2021). With the help of this technique, the demand and supply gap can be filled by providing high yields and better quality also consistency can be maintained.

According to various authers, the basic components for a hydroponics system include:

Substrate: a good growing medium is recognized by its ability to provide sufficient anchorage for the plant, serve as a reservoir for nutrients and water, at the same time allow oxygen to diffuse to the roots and gas exchange between the roots and the atmosphere outside the root medium (Regan, 2014; Maucieri et al., 2019; Dunn et al., 2021) . The physical characteristics of a growing medium are determined by the components used and the proportions in which they are blended (Sahin, 2006; Sabatino, 2020) pointed out that apart from the chemical, physical, and biological properties required, a good substrate also needs to be available, affordable, and sustainable for practical plant production. Growing media components are either organic or inorganic **.**Organic components include, but are not limited to: sawdust, peat, coconut coir, etc. Inorganic components include, but are not limited to perlite, pumice, vermiculite, sand, hydrogel, etc (Regan, 2014).

In most hydroponic systems, growers use different types of media to help lower cost, at the same time obtaining crop yields as expected. Most of researches related to hydroponics have been focused on vegetable crops. But hydroponics application has been used on clonal propagated crops, in its various forms (Zhao et al., 2021; A. Rose et al., 2024; Rajendran et al., 2024). For clonal propagated crops, the hydroponics technique commonly uses inert substrates where plantlets (cuttings) are grown with nutrient solution supplying all necessary nutrient components for the optimal growth conditions. Thus, the cutting propagation through hydroponics technology enables consistent multiplication of quality plants that are genetically identical to the parent plant, for use in breeding and other research applications as well as seed production for commercial purposes over the year (Bentley et al., 2020; IITA-BASICS., 2021).

Water: Efficient water conservation is achieved in hydroponics depending on the specific type of hydroponic system in use. Through water, nutrients are provided to the root according to the crop and stage of plant (Jan et al., 2021; Naresh et al., 2024).

Nutrients: under hydroponics conditions, plants are cultivated in nutrients enriched water, without the use of soil. Authors pointed out that the management of nutrient solutions is the cornerstone for a successful hydroponic system (Santiago-Aviles & Light, 2018; Sato et al., 2006). The hydroponic nutrient solution is required to supply the plant roots with water, oxygen, and essential mineral elements in soluble form. Seventeen elements are required for the proper growth of plants grouped into macronutrients and minor nutrients (Sato et al., 2006). Nutrients play a key role in the quality and productivity of vegetables and fruits. Thus, the balanced application of nutrients is vital in determining the quality of the product. Usually, soilless media requires a higher concentration of nutrients than soil media (Khan et al., 2020).

Electrical conductivity (EC): EC reading measures the ability of soil water to carry an electrical current and is an indication of the amount of nutrients available for crops to take up. In soilless culture, the various media used may exhibit varied EC values based on the admixtures and source from which the media are procured. Desirable EC for general purpose growing media is between 1.0-2.0 mmhos/cm (Bunt, 2012; Abad et al., 2002).

pH: pH is a determination of how acidic or basic a substance or solution is. Besides the physical properties of the media, chemical properties such as pH is an important parameter for the optimum growth of soilless crops. Recommended pH ranges for soilless media vary depending on crop species (Othman et al., 2019). Klougart (1983) reported that pH directly affects nutrient availability in the rhizosphere and nutrient uptake by plants and macronutrients such as N, K, Ca, Mg etc. are highly available at pH 6.0 - 6.5.

Light: Light is an important factor that influences growth of a plant by affecting photosynthesis, photorespiration, and photoperiodism. If the light intensity is diminished, photosynthesis slows down and affects the growth. Light plays a second role in photoperiodism, which is the response of plant during the day-night cycle. In order to attain good growth of plants, there should be sunshine of desired quantity and intensity (Zanon et al., 1990).

Temperature: Temperature affects plant growth either by increasing or decreasing the rate of different plant process as photosynthesis, respiration, and transpiration. The maximum activity is obtained between 21^{o}-27^{o}C day temperatures under greenhouse for most of the vegetables (Kawasaki and Yoneda, 2019).

Carbon dioxide (CO_2): CO_2 concentration is directly proportional to the rate of photosynthesis. An increase in the CO_2 concentration increases the rate at which carbon is incorporated into carbohydrates in the light-independent reaction, and so the rate of photosynthesis increases until limited by another factor (Boretti and Florentine, 2019).

Relative humidity: plant growth is correlated positively to the relative humidity inside. Normal plant growth will occur at relative humidity of 25-80 %, while too high relative humidity is also harmful to plants because most pathogenic spores germinate at high relative humidity (Xu et al., 2016).

2.6 Propagation of Cassava

Cassava is commonly propagated vegetatively from stem cuttings. Seeds are reserved for the plant breeding process for the development of new varieties and other purposes (IITA, 1990; Mahungu et al., 2022). High-quality cassava cuttings for planting are often in short supply due to low multiplication ratio on the order of 1:10 (Otoo, 1996).

This represents one of the main causes of the slow breeding process in cassava programs (Chavarriaga-Aguirre et al., 2016). In addition, cassava cuttings when infected, constitute an effective means for the spread of diseases and pests in new fields and in free areas. Diseases are mainly Cassava Mosaic Disease (CMD), Cassava Brown Streak Disease (CBSD), Cassava Bacterial Blight, and Cassava root necrosis can lead to huge yield losses in the fields (Legg et al., 2015; Rey and Vanderschuren, 2017; Zeyimo, et al., 2019a; Zeyimo et al., 2019b). Due to physiological, biotical, and technical constraints, research uses improved approaches to increase the quantity of material with good phytosanitary quality to ensure a sustainable supply to the users. These techniques are also beneficial for breeders in that they significantly increase the quantity of material of new genotypes for evaluations and bring new varieties to end users. Using disease-free, mature, true-to-type planting materials is recommended to start multiplication.(Ceballos et al., 2020; Malik et al., 2020).

2.6.1 **Conventional Propagation**

Conventional propagation of cassava is the easiest and most widely used method which involves the use of the woody parts of the stem. However, it has the disadvantage of having a low multiplication rate per year compared to sexually propagated crops (Otoo, 1999; Santana et al., 2009; de Oliveira et al., 2012). Thus, improved conventional techniques have been developed to rapidly increase the quantities of cuttings. These techniques are beneficial for breeding programs for germplasm evaluation in stations, multilocal trials, and multiplication of seeds to ensure a wide distribution of the improved varieties (Otoo, 1996; Ceballos et al., 2020).

2.6.2 **Tissue Culture Technique**

Plant tissue culture for micro propagation involves growing plants in sterile conditions, starting from shoot initiation, then root initiation in the laboratory before acclimatization in the greenhouse for field establishment (Kartha et al., 1974; Villaluz and Acedo, 2008). Tissue culture materials are cultured from plant tissue and organs such as meristem tip, anther, buds, etc. (George et al., 2007). But most of the cassava tissue culture plantlets are meristem tip derived. Using the totipotency capacity of cells, tissues, and organs are multiplied and regenerated into a whole plant. Thus, tissue culture materials are genetically the same as the genotype of origin (García-Gonzáles et al., 2010; Su et al., 2021).

Different authors have mentioned the advantages of tissue culture are the rapid production of healthy, uniform, and better-quality planting material throughout the year under aseptic conditions and sheltered from climatic hazards (Villaluz and Acedo, 2008; (Santana et al., 2009; Sahu and Sahu, 2013; Oseni et al., 2018). Also, other authors have demonstrated the considerable contribution of tissue culture in cassava breeding programs. In that, it allows for overcoming challenges the crop improvement, evaluation of the genetic materials, the multiplication, and the distribution of cuttings of improved varieties to beneficiaries (Vasil et al., 1979; Rego and Faria, 2001; Chavarriaga-aguirre et al., 2016; Tazeb, 2017). However, the weakness is that technology is expensive, especially for developing countries, although labor is affordable (Hussain et al., 2012; Santana et al., 2009). The technology has proven its effectiveness in supporting breeding programs. However, low-cost alternatives have been developed for weaning and hardening micro-propagated cassava plants during field establishment. These innovations hold the potential to accelerate breeding efforts and ultimately enhance farmers' productivity.

2.6.3 **Mini Cutting Technique**

Mini stem cuttings are small cuts of cassava stem having one or more nodes depending on the part of the stem from which the cutting was taken. The mini stem technique aims to increase the multiplication rate between two successive generations of vegetative multiplication from 1:60 to 100 (Otoo, 1996). A mature cassava stem can

generate three types of mini stems including the apical part with at least 6 nodes, the semi-apical part with 4 to 6 nodes, and the hardwood portion with one or two nodes (Figure 2.3).

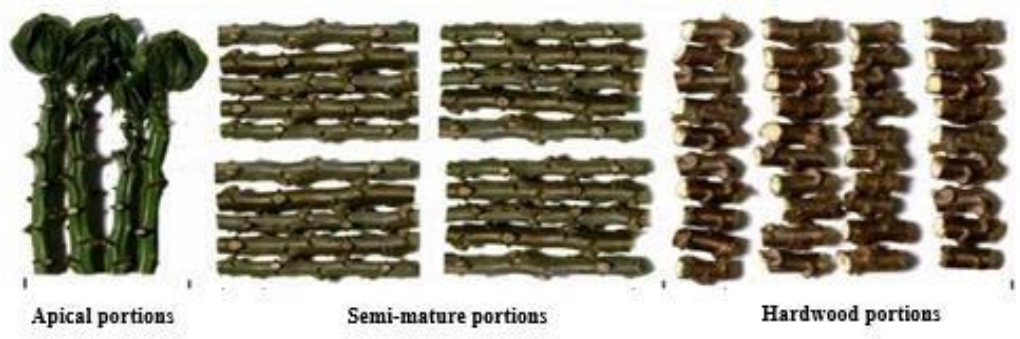


Figure 2.3: Mini Stem Cutting Parts of a Cassava Plant

Source: (IITA, 1997)

The mini stems can be directly planted in the field or pre-sprouted in pots, tubs, on beds, or using polyethylene bags filled with garden soil (Otoo, 1996). To ensure pest-free stakes, a mixture of insecticide and fungicide is sprayed before planting.

In a wood park, research recommends specific cultural techniques and production standards such as isolation, reduced spacing, purification, fertilization, phyto-sanitizing, etc. (Mahungu et al., 2022). These practices allow for the multiplication of plants that are free from diseases and the maintenance of varietal identity (Abdullahi et al., 2014; Mahungu et al., 2022). The multiplication ratio depends on the type of stems and the spacing used (1 m x 0.5 m, 1 m x 0.75 m, and 1 m x 1 m), without aiming for root production (Mahungu et al., 2022).

2.6.4 **Semi–Autotrophic Hydroponics (SAH)**

The SAH technique consists of growing virus-free planting materials of tissue culture origin without natural soil under controlled crop environmental conditions (Adesanya et al., 2016; Adetoro et al., 2020). Usually, the plant cuttings are placed in an organic substrate contained in plastic boxes (Plate 2.a) and then maintained with a nutrient-riche solution which promotes roots to grow down and the dry soil on top discourages

damp-off and other diseases caused by excess moisture in the growth room (Adetoro et al., 2020). The source materials from tissue – culture are rapidly multiplied from mother plantlets within two to three weeks to generate four to five cycles of plantlets in trays. Plantlets are grown under controlled conditions of temperature, light, and photoperiod in a growth room (Plate 2.b), and the ready plantlets are transplanted directly to the field after 2 to 3 months (Olugboyega et al., 2019; Pelemo et al., 2019).

(**a**) (**b**)

Plate 2.1: Development of Cassava Plantlets under the SAH System: One-Week-Old Cuttings in KlasmannTS3 Substrate (a) and Plantlets Growing in a Controlled 60 m^2 Growth Room (b) at IITA Kalambo

The SAH has the advantage of doubling the number of planting materials, with the possibility of going from 2.500 to 40.000 seedlings in 2 months (Ceballos et al., 2020), corresponding to a ratio of 1: 16. Furthermore, the SAH technology facility does not require a large space for set up. For example, the cassava SAH facility at the Olusegun Obasanjo Research Campus of IITA in D.R. Congo has the potential to produce million plants cumulatively per year within an area of 60 m^2 (Figure 2.4b), corresponding to 100 ha of field area with 1 x 1 m standard spacing.

Given the challenges faced with tissue culture (high cost and time-consuming), the SAH is proving to be an affordable technology with the undeniable ability to increase the healthy cassava seed quantity in a reasonable period. Thus shortening the time required for seed multiplication. For example, the cassava breeding program at IITA in Nigeria and that of D.R. Congo have integrated the technology into the multiplication scheme as well as into the varietal maintenance scheme. This is to make

available at a reasonable time, large quantities of seeds while maintaining varietal purity as much as possible to guarantee high production (Mahungu et al., 2022).

The advantages offered by the SAH are indisputable in DRC throughout the cassava value chain. For example, as part of the ongoing 145 Territory Development Program (PDL-145T), SAH technology is essential for boosting local cassava production across the country. With this technology, a total quantity of 600,000 linear meters is being produced, within a period of two years. It is estimated that this quantity will cover more than 300 ha of the first seed fields. It would not be possible to achieve such results using the conventional methods, let alone the traditional method, where an initial field seed would require more than three years to produce the same amount of planting materials.

The most common substrate used under SAH technology refers to the KlasmannTS3 produced in Europe by the Klasmann Deilmann company. KlasmannTS3 which is one among different packages according to specific growth purposes is used for starting seeds and growing plants. The main raw material for development and the production of Klasmann's growing media are white and black peat. This is supplemented with other organic and mineral raw materials, including wood fiber, green compost, and coconut fiber (Klasmann-Deilmann, 2019).

This substrate is among the best commercial substrates worldwide. Several authors have reported success in survival and plant growth under Klasmann compared to other substrates for crops such as Yam (*Dioscorea spp*.), Lettuce *(Lactuca Sativa L.)*, Brassica *(Brassica olerecea* var. *capitate*, and *Brassica olerecea* var. *botrytis)*, Marigold (*Tagetes* L.), Globe amaranth (*Gomphrena globosa* L.) (Manios et al., 1987; Balalic, 2004; Mišković et al., 2009; Adesanya et al., 2016; Olugboyega et al., 2019; Maślanka and Magdziarz, 2017; Zeljković et al., 2021**)**. In cassava, Kajibwami et al., (2018) reported a plantlet recovery rate of 80 % in the laboratory within 2 weeks using KlasmannTS3, with 70% -100% field survival, depending on site conditions. Similarly, in Nigeria, a summary was reported on the laboratory survival of 93.8% of cassava cuttings and better plant growth with the highest plantlet height of 6.7cm under KlasmannTS3 (Adesanya et al., 2016a).

Despite the undeniable success reported on Klasmann's substrate in cassava specifically, the main bottleneck is the importation of substrate from Germany for planting material production, which makes the unit cost to be unaffordable in some cases and limits the technology's expansion.

Several authors pointed out that a convenient substrate should not only supply the physical, chemical, and biological properties required by plants but also be available, affordable, and sustainable for practical plant production. Thus, the use of alternative substrates may be the most feasible and low-cost option for optimizing SAH technology. Several studies have reported the use of different media in hydroponics production such as Vermiculite and local affordable materials such as Sawdust (Lin et al., 2017; Mayo-Prieto et al., 2020; Barbosa et al., 2022; Ferrarezi et al., 2022)(Lin et al., 2017)(Lin et al., 2017)(Lin et al., 2017)(Lin et al., 2017). Vermiculite is so lightweight, so it is suggested mainly for starting seeds and cuttings, and is reported to be used alone or can be mixed with other materials (Hydroponics media and Guide, 2021).

On the other hand, previous authors' summaries reported, it was observed limited findings regarding the performance of the cassava SAH-derived plants in the laboratory as well as under field conditions, which is the final destination for stem production. Such plantlets produced under a controlled environment have small juvenile leaves with reduced photosynthesis capacity and malfunctioning stomata. Their performance to respond under natural growing conditions can be affected; because plants in their natural environment are in interact with many bacteria, fungi, and temperature.

2.7 Performance of Cassava Genotypes under Field Conditions

Evaluation of cassava genotypes under field conditions is a crucial aspect that provides insights into the adaptability, productivity, and overall suitability of different cassava varieties in real-world agricultural settings (Hershey, 1987 and Adetoro et al., 2021). This evaluation involves the systematic assessment of various growth parameters, including but not limited to plant height, stem production, leaf and node characteristics, and overall plant vigor.

The height of cassava plants is an essential indicator of their growth potential. Taller plants often signify robust growth and can contribute to higher biomass production (IITA, 1990). The evaluation of plant height allows researchers and farmers to identify varieties that exhibit desirable stature for various purposes. The number and quality of stems produced by cassava plants are critical factors influencing the potential for vegetative propagation and subsequent planting material availability (Otoo, 1996). A higher stem production is generally favorable for sustainable cassava cultivation. Assessing leaf or node-related traits, such as their number, provides valuable information on the plant's overall health and its ability to convert sunlight into energy. In the case of a seed field, numerous leaves and nodes contribute to the overall growth and subsequent higher yields of planting material (Otoo, 1996). The overall vigor and adaptability of cassava genotypes to specific environmental conditions are assessed through field evaluations. This includes observing how well the plants respond to variations in temperature, soil types, and water availability, and providing valuable data for recommending suitable varieties for diverse agro ecological zones (Otoo, 1996; Adetoro et al., 2021).

2.8 Performance of Genotypes under Conventional Methods of Propagation

Traditional propagation methods of cassava face limitations in obtaining healthy and high-yield planting material due to factors such as cutting length and other agricultural practices. Conventional approaches, like using lengthy stems, have been supplemented with methods such as mini-cutting, aiming to increase material yield from a small starting quantity (Otoo, 1996). Studies by (Bidiaka et al., 2022) demonstrated that from 10.000 units of mini-cuttings planted in one hectare, the expected production was about 10,000 m matching the outcomes of using longer stems of 20 cm to 30 cm. Despite the advantage of increasing quantity, conventional methods may not guarantee disease-free materials and are also time-consuming (Escobar et al., 2006 and Feyisa, 2021). In contrast, SAH technology appears more attractive as it enables the production of planting materials under controlled conditions, optimizing space and time efficiency (Oseni et al., 2018; Bentley et al., 2020; Ceballos et al., 2020; Kumar & Singh, 2023).

2.9 Field Performance of Genotypes Produced Using the SAH System

The SAH system holds significant promise in transforming cassava seed production, boasting advantages such as accelerated multiplication, disease-free propagation, resource efficiency, and year-round production. Limited findings exist on the field evaluation of SAH-derived plantlets. Preliminary evaluations conducted by (Kajibwami et al., 2018) indicate a positive field emergence rate of above 80%. The scarcity in field-specific data highlights the need for more comprehensive studies to enhance our understanding of the performance and adaptability of SAH-derived plantlets in real-world conditions. These evaluations are necessary to assess the system's real-world adaptability, validate promising preliminary findings, identify potential challenges and effective solutions, and conduct a comparative analysis with conventional methods. The transition from controlled environments to diverse field conditions is a pivotal step in unlocking the full potential of SAH, contributing to the advancement of cassava seed systems.

2.10 Cost Analysis of Using SAH Technology

The cost-benefit analysis of adopting SAH technology for producing cassava planting materials involves analyzing the costs of implementing and maintaining the system, as well as evaluating the potential benefits. Implementing SAH requires investment in infrastructure, equipment, labor, and inputs. On the other hand, the potential benefits of SAH include increased yield, improved crop quality, reduced disease risk, and enhanced sustainability (Bentley et al., 2020; IITA-BASICS, 2021) . While there are initial costs, the benefits make SAH a viable option for cassava production. However, specific studies are necessary to assess its economic feasibility in different contexts.

As discussed by Bentley et al., (2020) and IITA-BASICS, (2021), the cost of producing cassava planting materials under the SAH system is influenced by several key factors:

a) Initial investment: The upfront costs associated with implementing SAH technology, including infrastructure setup, equipment purchase, and system installation, significantly impact the unit cost.

b) Operational expenses: Ongoing costs such as energy consumption, water usage, nutrient solutions, substrate procurement, and maintenance contribute to the overall expenses and affect the unit cost.

c) Labor costs: The expenses related to labor, including skilled personnel for system operation, maintenance, and plant care, are important factors influencing the unit cost.

d) Market prices: The prevailing market prices for cassava planting materials and the demand-supply dynamics play a crucial role in determining the potential returns and profitability of SAH-derived products. Depending on the inputs used and the product obtained, the unit price of an SAH-derived plantlet will differ from crop to crop. In the case of a cassava plantlet derived from SAH, the unit cost is USD 0.1 (Bentley et al., 2020b). In contrast to the conventional field method, which incurs a unit cost of USD 0.07 for producing planting material, the unit cost for SAH-derived material is relatively high. This cost disparity can be attributed to the factors discussed above.

e) Potential returns: The anticipated yield and quality of cassava planting materials produced using SAH technology directly affect the potential returns and, consequently, the cost-benefit ratio.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Performance of Selected Cassava Genotype Plantlets Produced under Different Substrates in the SAH System

This study involved the SAH - plantlets production and evaluation under laboratory conditions. Two laboratory experiments were carried out. Experiment 1 was carried out from October to December 2021, while Experiment 2 was carried out from November 2021 to January 2022.

3.2 Study Location

The laboratory experiments were carried out at the SAH laboratory of the Olusegun Obasanjo Research Campus of the International Institute of Tropical Agriculture (IITA) in Kalambo, in South Kivu province of D.R. Congo (S 2°23’50’’, E 28°50'42", and 1,488 m.a.s.l).

3.2.1 Experiment 1: Assessment of the Performance of Cassava Genotype Plantlets Produced under Single Substrates

3.2.1.1 Source and Description of Study Materials

Four improved genotypes were used in this study, comprising two introduced genotypes (IBA961089A and MM060083) under evaluation at the IITA Kalambo station and two released genotypes (Nase14 and Albert28) grown by farmers (

Table 3.1). The genotypes were selected for their fast recovery from cutting in the laboratory, fast growth, wide adaptability in the field, and high-yielding traits. All genotypes used were cassava mosaic disease-resistant. Each of the four genotypes originated from 4-week-old mother plantlets produced from tissue culture plantlets using the common substrate (KlasmannTS3).

Table 3.1: General Information on Cassava Genotypes Used in the Study on under Single Substrates the Performance of Planting Materials

Genotype	Pedigree	Institution /Country of origin	Branching habit
Nase14	MM96/4271	IITA/ Uganda	Branched
Albert28	Local landrace	Tanzania	Straight
IBA961089A	M94/0461 x 90/01559	IITA/ Nigeria	Straight
MM060083	MM96/4271 x 90/01778	IITA/ Nigeria	Straight

Source: https://www.cassavabase.org.2022

3.2.1.2 Substrate preparation

Four substrates were used for performance evaluation (Plate 3.1). KlasmannTS3, which is a reference substrate in SAH cassava plantlet production and imported from Germany, was compared to vermiculite (imported from Kenya) and two other D.R.CONGO local materials, including local peat and sawdust. Local peat was collected from a farm at Bukavu (an undeveloped land, usually temporarily flooded with water and covered with a thin layer of vegetation) (S 2°40' 42", E 28° 46' 58", and 1934 m m.a.s.l). The local peat was then sterilized at 121 °C for 15 minutes, cooled down for 24 hours, and then used as a substrate. Sawdust of fine texture of wood residue collected from the CAPA carpentry workshop in Bukavu town (S 2° 30' 5", E 28°51' 10" and 1501m m.a.s.l). For each substrate, 500 ml was put into a transparent light box of 15 cm x 15 cm x 9 cm.

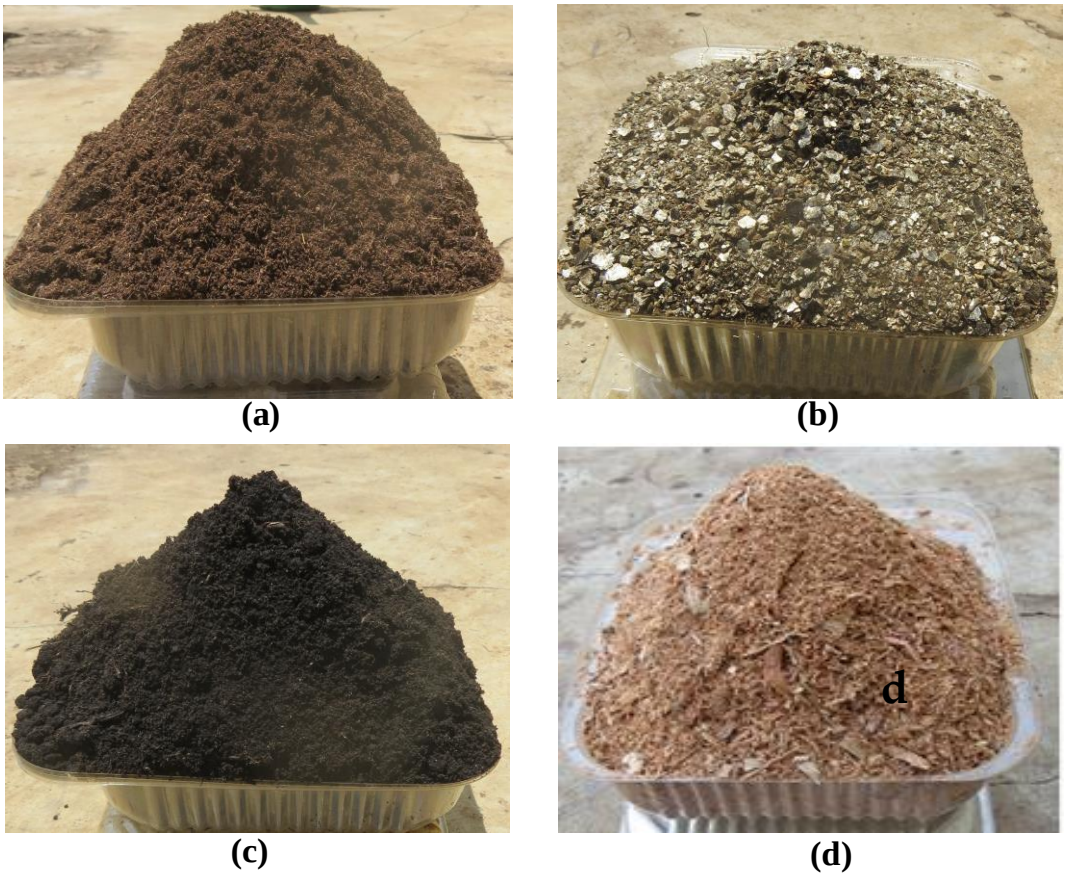


Plate 3.1: Single Substrates Used in the Evaluation of the Performance of Cassava Plantlet Production under the SAH System: KlasmannTS3 (a); Vermiculite (b); Local Peat (c); and Sawdust (d).

3.2.1.3 Experimental Design

The experimental design was a split plot based on a randomized complete block design (RCBD) replicated five times (Figure 3.). The main plot consisted of four of genotypes: IBA961089A, MM060083, Albert28, and Nase14. The subplot involved four levels of substrates: KlasmannTS3, vermiculite, local peat, and sawdust.

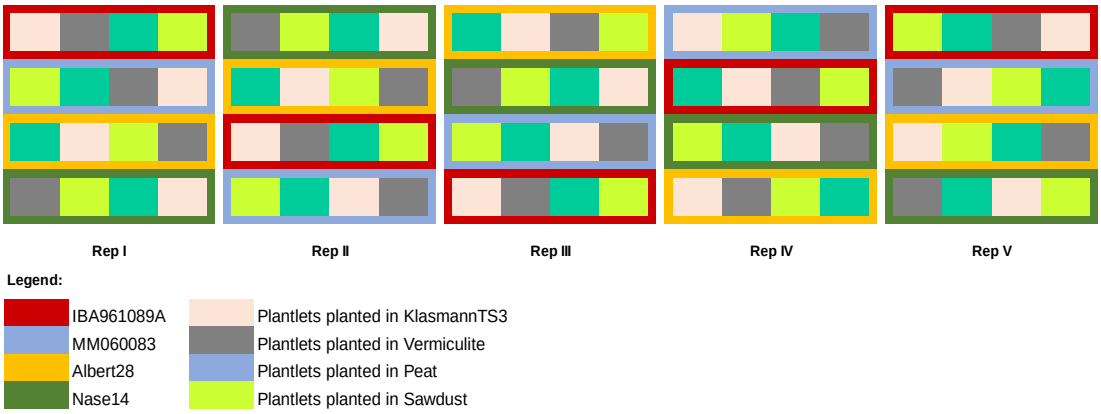


Figure 3.2: A Split-Plot Experiment, Arranged in a Randomized Complete Block layout, was Used in the Laboratory to Evaluate the Performance of SAH Plantlets of Four Cassava Genotypes under Four Single Substrates

3.2.1.4 Sub-Culture Cutting Production

Plate 3. illustrates the hierarchical transplanting and sub-culturing processes in this study. The flow chart depicts the sequential steps of transplanting the plantlets from one subculture period to the next. Each genotype (IBA961089A, MM060083, Albert28, and Nase14) was propagated on different substrates (KlasmannTS3, vermiculite, local peat, and sawdust), and the cuttings were transplanted and propagated through three consecutive subculture periods.

The SAH system involved planting young nodal cuttings into transparent light boxes containing different substrates and watering them with a nutrient solution. The experiment comprised three subsequent subculture periods, each lasting four weeks, starting from the mother plantlets. Subculture one involved planting cuttings obtained from the mother plantlets. In Subculture two, all cuttings produced by a genotype's plantlets at the end of Subculture one were transplanted and grown into the corresponding four substrates. Subculture three was established by transplanting all cuttings produced from a specific genotype and substrate in Subcultures one and two into the respective four substrates.

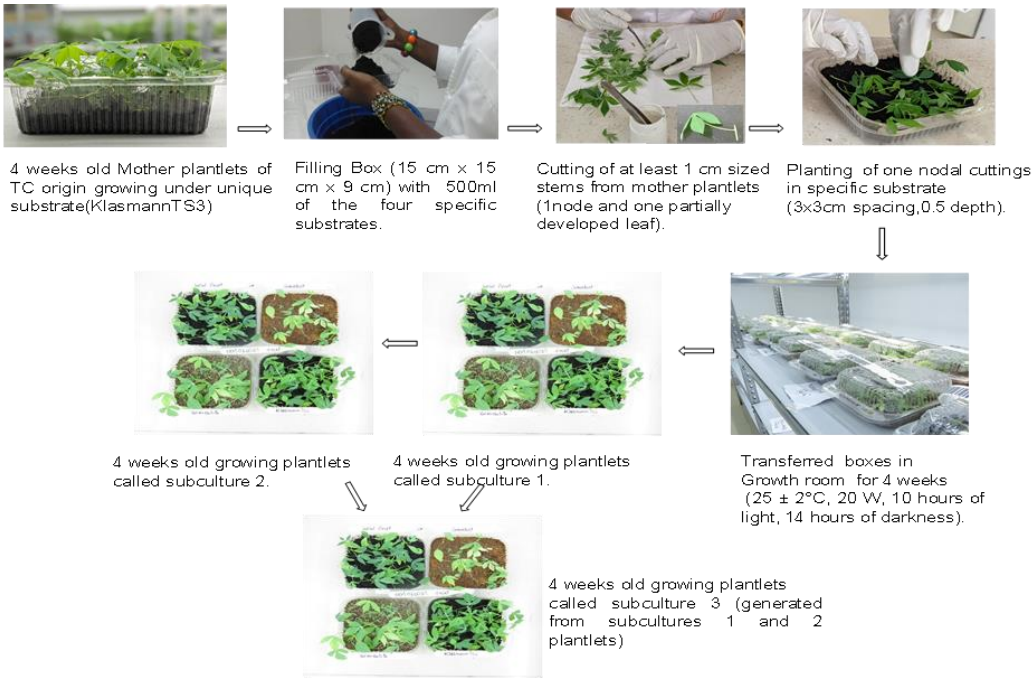


Plate 3.2: Subculture Process for Cassava Cutting Propagation under Four Substrates in the SAH System, Starting from Tissue Culture-Origin Mother Plantlets

Each subculture lasted for four weeks

The stem propagation process was carried out step by step, according to the protocol detailed by Adetoro et al. (2020). Each transplanted cutting was approximatively 1 cm in length. Transplanting of the cuttings during all the subculture periods involved inserting approximatively 0.5 cm-long cutting portion with one node and one partially developed leaf into the corresponding four substrates (Plate 3.a). During subculture one, each plot received twenty cuttings from the respective four genotypes of mother plantlets, which were transplanted at regular intervals of 3 cm by 3 cm. For Subculture two, when the cuttings obtained from the plantlets of Subculture one exceeded 20, additional boxes with the specific substrate were used. For Subculture three, when the cuttings obtained from the combination of Subculture one and Subculture two exceeded 20 cuttings (i.e., to be transplanted in a box with 3 cm x 3 cm spacing), additional boxes with the required substrate were also used.

Nutrient solution (NS) was prepared (2.6 g/41), using Miracle-Gro all-purpose water solution, and the boxes containing 500 ml of substrate were watered with 100 ml of NS at planting time and once a week throughout the subculture duration. Plantlets were grown in a controlled environment at 25 ± 2°C, 20 W of light, and a photoperiod of 10 hours of light and 14 hours of darkness per day in a growth chamber. The lids of the SAH boxes in the growth room were kept closed to reduce transpiration rate during growth (Plate 3.b). The height and leaf count of the plantlets generating cuttings showed variations based on the specific genotypes and substrates employed. These plantlets had shoots ranging from 4 to 13 cm in height and were characterized by 3 to 8 expanded leaves.

(a) **(b)**

Plate 3.3: Stages of Cassava Plantlet Development under SAH System: One Nodal Stem Cutting Ready for Transplanting (a) and Plantlet Growth in Tansparent-Light Boxes (b)

3.2.1.5 Substrate Analysis

The pH and the electrical conductivity (EC) of the substrates were determined using the electrometric method (Bray and Kurtz, 1945). The total nitrogen was determined using the Kjeldahl digestion method (Simard and Zizka, 1994). The exchangeable cations (potassium, calcium, and magnesium) and cationic exchange capacity (CEC) were determined using the ammonium acetate extraction method (Howeler and

Reinhardt., 2014) The available phosphorus was determined using the Bray 1 method (Bray and Kurtz, 1945).

The four substrates used had the following chemical characteristics (Table 3.2). Local peat was the most acidic substrate. KlasmannTS3 had the highest Ca, P, and EC. Local peat had the highest N content, representing 1.8 times the N content in KlasmannTS3. Local peat also has a higher CEC, but almost nothing in exchangeable Mg. Vermiculite had the highest exchangeable K and Mg but was low in EC, CEC, and nutrient solution (NS) per box. Sawdust was low in exchangeable K and P. For the same volume (500 ml), the weights of the local peat and vermiculite averaged 200 and 205 g, respectively, and they were high compared to the weights of KlasmannTS3 (135 g) and sawdust (92 g). On the same volume (500 ml), local peat and vermiculite weight averaged 200 and 205 g, respectively, which were higher than average weight of KlasmannTS3 (135 g) and sawdust (92 g). This affected the amount of NS delivered to the substrates at the time of planting as well as at the end of every week throughout the subculture periods. Thus, the NS received by local peat and vermiculite was approximately 0.5 ml g^{-1}, which was lower than the 0.7 and 1.1 ml g^{-1} received by KlasmannTS3 and Sawdust, respectively.

Table 3.2: Chemical Characteristics and Nutrient Concentration of the Four Substrates Used to Produce Cassava Plantlets under the SAH System at IITA Kalambo, D.R.Congo, in 2021

Substrate	Weigth of 500 ml(g)	pH (H2O)	Total N (gKg-1)	Exch.K (gKg-1)	Exch.Ca (gKg-1)	Exch. Mg (gKg-1)	P (gKg-1)	CEC (cmolKg-1)	EC (µS/cm)	NS(ml)per g of substrate
Klasmann TS3	135	5.86	7.8 (1.05)	1.2 (0.16)	51.7 (6.98)	2.9 (0.39)	0.8 (0.11)	57.8	247.1	0.74
Vemiculite	200	5.23	0.5 (0.10)	20.4 (4.08)	46.6 (9.32)	124.8 (24.96)	0.4 (0.08)	6.3	8.6	0.5
Local peat	205	3.74	13.8 (2.83)	2.4 (0.49)	20.9 (4.28)	0.0 (0.00)	0.6 (0.12)	71.9	91.4	0.49
Sawdust	92	5.19	1.4 (0.13)	0.2 0.02)	37.1 (3.41)	0.7 (0.06)	0.3 (0.03)	25	73.2	1.09

Values in parentheses are the total nutrient quantities (g) in 500 ml of substrate used per box to produce the plantlets. They were calculated using substrate weight (2nd column of the table) and the corresponding nutrient concentration. NS: nutrient solution.

3.2.1.6 Data collection

Data were collected at the time of cutting (before cuttings of plantlets), which was 4 weeks after transplanting of each subculture period for all 5 replications. The survival rate was collected per plot (genotype x substrate) and was calculated as a percentage of surviving plantlets in each box during the observation period compared to the number of cuttings initially transplanted in the subculture period.

$$\textbf{Survival rate}\ (\%) = \frac{\text{Number of survivng plantlets}}{\text{Number of cuttings transplanted}}\ \text{x}\ 100$$

Growth parameters, including height (cm), leaf number, and internode number, were recorded from five randomly selected plantlets of each genotype growing on a specific substrate in different subculture periods. Height was measured from the base to the newly emerging leaf of the plantlets using a measuring tape. Cuttings were counted for each genotype growing on a specific substrate in different subculture periods. The total number of cuttings was calculated as the sum of the cuttings obtained after the three subculture periods in 12 weeks in each treatment (genotype x substrate).

3.2.1.7 Data analysis

The data were analyzed for each subculture period using the statistical analysis software R version 4.2.1 (R Core Team, 2023). A two-way analysis of variance (ANOVA) was used as the statistical analysis. Genotype, substrate, and their interactions were considered fixed effects. When the interaction between genotype and substrate was significant, further one-way ANOVA analysis was performed for substrates within each genotype. Alternatively, if the interaction effects were found not to be significant, the predicted means of the genotypes and substrates were considered. In cases where significant differences were observed among treatment means, the Fisher's Least Significant Difference (LSD) test set at $p < 0.05$ was used for all parameters considered. There were no plantlets under the sawdust substrate in subculture three.

3.2.2 Experiment 2: Performance of Selected Cassava Genotype Plantlets Produced under Single and Combined Substrates

The experiment was set up based on the preliminary observations made in Experiment 1. At the early plantlet stage in Experiment 1, plantlets produced in KlasmannTS3 seemed to perform better in growth parameters. Conversely, positive effects on plantlet growth were also observed in those cultivated on local peat and vermiculite substrates. This Experiment investigated the effect of combining KlasmannTS3, Vermiculite, and/ or Local peat on the cassava growth and propagation performance under the SAH system.

3.2.2.1 Plant Material and Source of Material for Propagation

Three genotypes were used in this experiment, namely IBA961089A, IBA70520, and IBA980505 which were obtained from IITA Kalambo station. IBA961089A was the consistent genotype across experiments, whereas the other two genotypes varied from those used in the initial experiment. As in Experiment 1, genotypes were selected for their fast recovery from cutting in the laboratory, fast growth, wide adaptability in the field, and high-yielding traits. All genotypes are cassava mosaic disease-resistant. Table 3.3 presents the pedigree, country of origin, characteristics, and status. As in Experiment 1, each of the three genotypes originated from 4-week-old mother plantlets produced from tissue culture plantlets using the common substrate (KlasmannTS3). IBA961089A was used consistently across experiments due to a lack of materials for other genotypes.

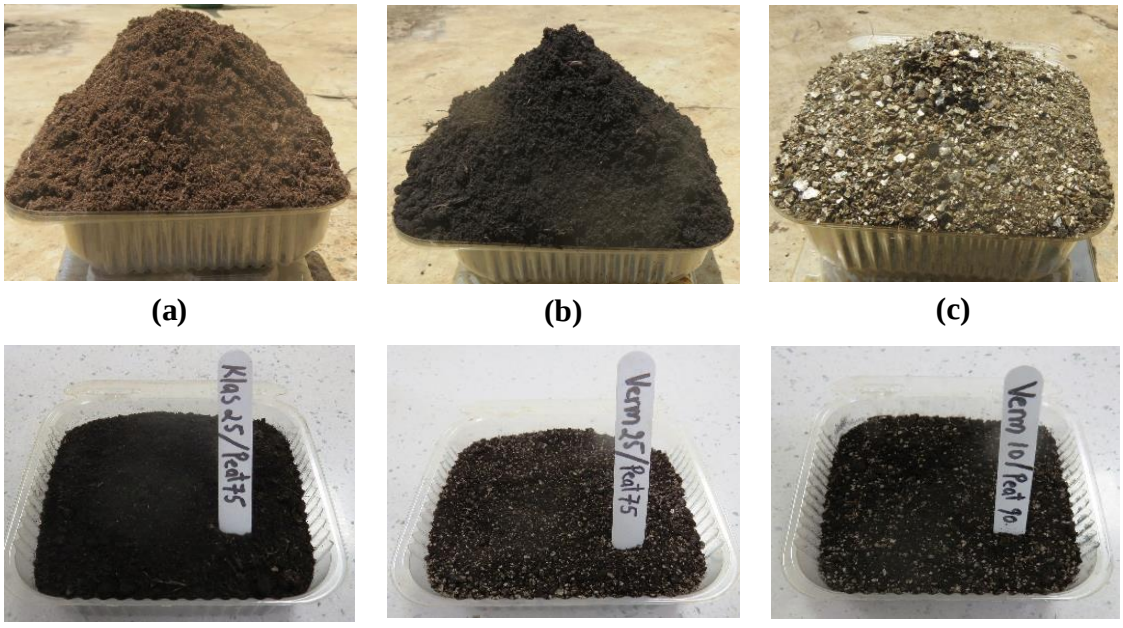
Table 3.3: General Information on Cassava Genotypes Used in the Study on the Performance of Planting Materials under Single and Combined Substrates

Genotype	Pedigree	Institution /Country of origin	Branching habit
IBA961089A	M94/0461 x 90/01559	IITA/ Nigeria	Straight
IBA70520	90/01560 x M100/0645	IITA/ Nigeria	Straight
IBA980505	M150/0721 x IBA98075	IITA/ Nigeria	Straight

Source: https://www.cassavabase.org.2023

3.2.2.2 Substrate Preparation

This experiment tested plantlet performance under single and combined substrates (Plate 3.). The substrates were: (i)KlasmannTS3, (ii) Vermiculite, (iii) local peat, (iv) combination of KlasmannTS3 and local peat at respective rates of 25% and 75% of the total volume ($K_{25}P_{75}$), (v) combination of Vermiculite and local peat at respective rate of 10% and 90% of the total volume ($V_{10}P_{90}$) and (vi) combination of Vermiculite and local peat at respective rate of 25% and 75% of the total volume ($V_{25}P_{75}$). The local peat used was prepared as described in experiment 1. These proportions were preselected from a variety of combinations using the three single substrates, as they demonstrated the best performance in terms of survival and growth during a one-month test.



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Plate 3.4: Six Substrates Used in the Evaluation of the Performance of Cassava Plantlet Production under the SAH System: KlasmannTS3 (a); Vermiculite (b); Local Peat (c); $K_{25}P_{75}$ (d); $V_{25}P75$ (e) and $V_{10}P_{90}$ (f).

3.2.2.3 Substrate Analysis

Substrate samples were taken for analysis at the soil laboratory at Olusegun Obasanjo Research Campus of IITA in D.R.CONGO, as in experiment 1. Table 3.4 presents the characteristics, revealing significant differences among substrates, whether in single or combined forms. The variations in combined substrates can be attributed to the proportion levels of the combination. Local peat displayed the highest acidity (pH range: 3.7 to 4.1). When substituting 10% or 25% of local peat with Vermiculite or KlasmannTS3, acidity levels remained comparable to local peat. Local peat exhibited the highest nitrogen (N) content (13.8g kg-1), surpassing KlasmannTS3 (7.8g kg-1) by 1.8 times. Blended substrates ($V_{10}P_{90}$, $V_{25}P_{75}$, and $K_{25}P_{75}$) demonstrated significantly higher nitrogen levels, both in concentration and total amount per box, compared to single KlasmannTS3 and Vermiculite. Exchangeable potassium (K) was notably higher in Vermiculite (20.4g kg-1) and local peat (2.4g kg-1) compared to KlasmannTS3 (1.2g kg-1) and Sawdust (0.2g kg-1). Blended substrates exhibited elevated K content. Vermiculite's exchangeable magnesium (Mg) was markedly high (125g kg-1), leading to blended substrates with increased Mg content when substituting 10% or 25% of local peat. KlasmannTS3 had lower total calcium (Ca) levels (7g in 500 ml) but had the highest exchangeable Ca content. Phosphorus (P) content was consistently low among single substrates. The nutritive solution added during plantlet production was lower for local peat, Vermiculite, and blended substrates (0.5ml g-1) compared to KlasmannTS3 (0.7ml g-1). Vermiculite showed lower cation exchange capacity (CEC) and electrical conductivity compared to other substrates.

Table 3.4: Chemical Characteristics and Nutrient Concentration of the Six Substrates Used to Produce Cassava plantlets under the SAH System at IITA Kalambo, D.R.Congo, in 2021

Substrate	Weigth of 500 ml(g)	pH (H2O)	Total N (gKg-1)	Exch.K (gKg-1)	Exch.Ca (gKg-1)	Exch. Mg (gKg-1)	P (gKg-1)	CEC (cmolKg-1)	EC (µS/cm)	NS(ml)per g of substrate
Single substrate										
Klasmann TS3	135	5.86	7.8 (1.05)	1.2 (0.16)	51.7 (6.98)	2.9 (0.39)	0.8 (0.11)	57.8	247.1	0.74
Vemiculite	200	5.23	0.5 (0.10)	20.4 (4.08)	46.6 (9.32)	124.8 (24.96)	0.4 (0.08)	6.3	8.6	0.5
Local peat	205	3.74	13.8 (2.83)	2.4 (0.49)	20.9 (4.28)	0.0 (0.00)	0.6 (0.12)	71.9	91.4	0.49
Blended substrate										
$\mathbf{K_{25}P_{75}}$	3.74	187	12.3 (2.30)	2.1 (0.39)	28.6 (5.35)	0.7 (0.13)	0.7 (0.13)	68.4	130.3	0.53
$\mathbf{V_{25}P_{75}}$	4.07	204	10.5 (2.14)	6.9 (1.41)	27.3 (5.57)	31.2 (6.36)	0.6 (0.12)	55.5	70.7	0.49
$\mathbf{V_{10}P_{90}}$	3.69	204	12.5 (2.55)	4.2 (0.86)	23.5 (4.79)	12.5 (2.55)	0.6 (0.12)	65.4	83.1	0.49

Values in parentheses are the total nutrient quantities (g) in 500 ml of substrate used per box to produce the plantlets. They were calculated using substrate weight (2^{nd} column of the table) and the corresponding nutrient concentration. NS: nutrient solution.

3.2.2.4 Experimental Design

Experiment 2 was laid out in a split-plot design, based on a randomized complete block design with four replicates (Figure 3.). The three cassava genotypes were used as the main factor, and the six substrates as sub-factor.

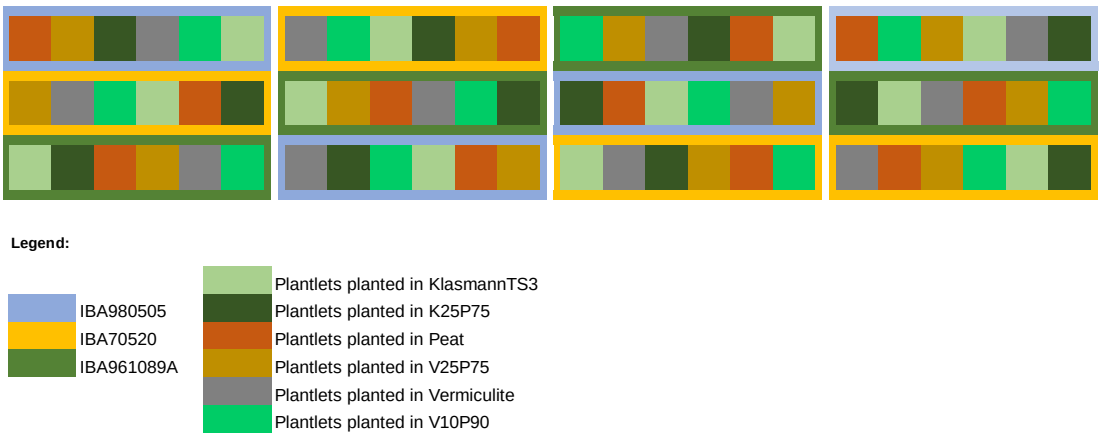


Figure 3.3: A Split-Plot Experiment, Arranged in a Randomized Complete Block Layout, Was Used in the Laboratory to Evalu ate the Performance of SAH Plantlets of Three Cassava Genotypes under Six Substrates

3.2.2.5 Sub-Culture Cutting Production

The genotype cuttings were produced during three subsequent subculture periods of four-week duration each, step by step as described in Experience one. Mother plantlets for respective genotypes were also produced in the same way as described in experiment 1. Substrate and nutritive solution were used in the same way and with the same amount and frequency as in Experiment one. Cuttings had the same size as in Experiment one, and they were transplanted in the same space.

3.2.2.6 Data Collection and Analysis

Data were collected on survival, plantlet height, leaf number, internode number, and the number of cuttings in each subculture period as described in Experiment one (Section 3.1.2.6). The same was true for data analysis, the effect of genotype and substrate as well as their interaction were assessed, using a two-way ANOVA using

the statistical analysis software R (R Core Team, 2023). Tests of significance were also reported at the 0.05, 0.01, and 0.001 levels. In cases where significant differences were observed among treatment means, the Fisher's Least Significant Difference (LSD) test set at $p < 0.05$ was used for all parameters considered.

3.3 Performance Evaluation of the Selected Cassava Genotypes Plantlets Produced under SAH System in the Field

The field experiment was conducted to compare the performance of the 1st generation of SAH-derived plants to those obtained from mini cuttings of one node as one of the known conventional methods of cassava propagation**.** The SAH-derived plantlets transferred in the field were those produced in KlasmannTS3, Vermiculite, and local peat.

3.3.1 **Site of Study**

The field experiment was conducted from December 2021 to June 2022 in two locations in the South Kivu province: INERA Mulungu Research Station (E 28°47'13", S 2°20'0") in the territory of Kabare and Kiliba (E 29°10'10", S 3°13'4") located in the territory of Uvira (Figure 3.). The sites were selected based on elevation, rainfall, and temperature. Altitudes differed notably: Mulungu at 1699.7m, Kiliba at 849.7m, almost half of Mulling's elevation.

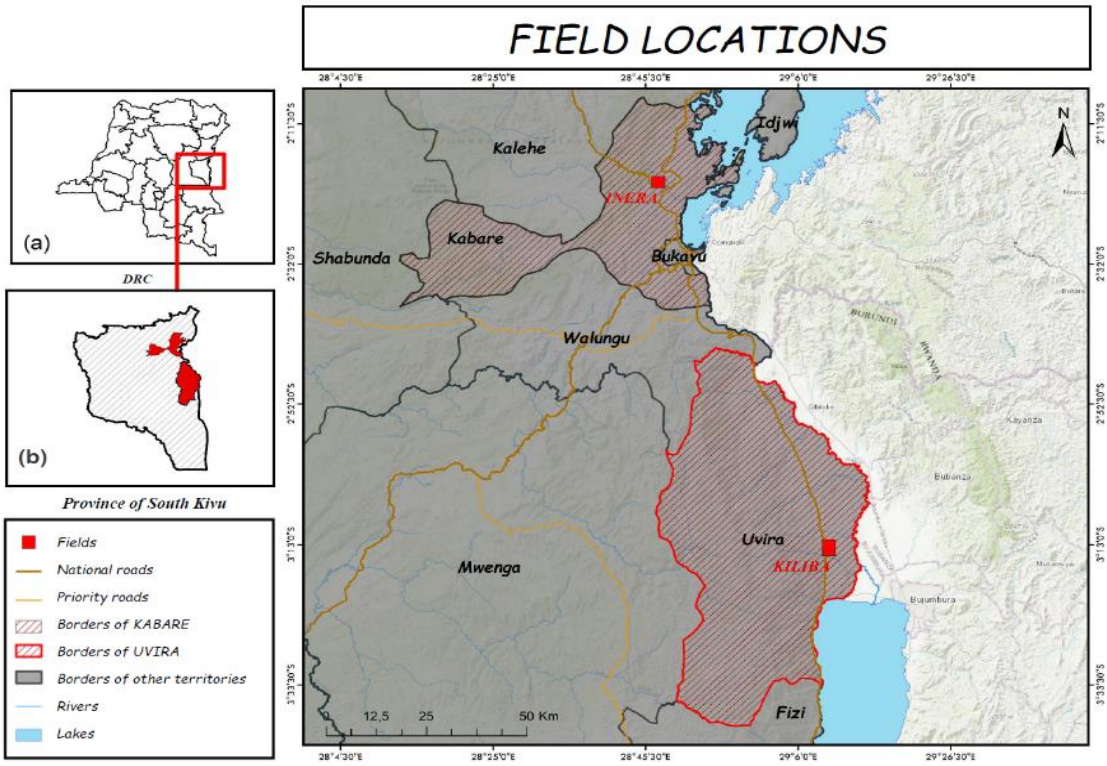


Figure 3.4: Map Showing Study Locations (INERA Mulungu and Kiliba) in the South Kivu Province of D.R. Congo (

Source: (***http://www.rgc.cd, 2022***).

3.3.2 **Site Characteristics**

Monthly rainfall and temperature data were collected from the weather station offices at both Mulungu and Kiliba sites, with data retrieved from the INERA weather station (Appendix 1). The amount of precipitation received during the growing seasons, varied between sites, with Mulungu receiving more rainfall than the Kiliba site. The annual rainfall difference between sites was 39.90 mm, while the annual temperature difference between sites was 8.37 C^0. The Mulungu site had better rainfall distribution compared to the Kiliba site during the growing sampled months. The temperature varied also across sites, with Kiliba having higher values than of Mulungu. This trend was not in line with the amount of rainfall across the sites.

Before field establishment, a composite soil samples from 5 random sampling spots per field were collected with a soil auger at 0-30cm topsoil and brought to the same IITA soil laboratory for analyses. Soil samples were then analyzed for physical and chemical (Table 3.5). The soil pHs were medium and almost similar across sites. Soil total nitrogen (N) was higher in Mulungu than in Kiliba. Exchangeable phosphorous (P) and exchangeable potassium (K),) were higher in Kiliba than in the Mulungu site. The soil texture classification varies also across these fields, with sandy clay at Kiliba, then clay –loam at INERA Mulungu.

Table 3.5: Chemical and Physical Characteristics of the Two Experimental Locations

Characteristic	Unit	Inera Mulungu	Kiliba	Nutrient requirement	Source
Chemical					
pH (H_2O)		6.14	6.74	4.0 to 7.0	Howeler (1996)
Nitrogen	(%)	0.29	0.1	<5	Byju (2006)
Exchangeable P	(ppm)	20.85	103.94	4 to 15	Howeler (1996)
Exchangeable K	(cmol/kg)	0.23	1.13	0.15 to 0.25	Howeler (1996)
Particle size					
Sand	(%)	4.5	74.1		
Clay	(%)	73.5	20		
Silt	(%)	17.9	5.9		
Textural class		Clay-loam	Sandy-clay	Loam, sandy loam	Byju (2006)

Source: IITA Kalambo station, 2021

3.3.3 **Plant Material**

Only the SAH technology-derived plantlets from experiment 1 were taken to the field. Genotypes were the four improved used for producing plantlets in Experiment 1, comprising of two introduced genotypes (IBA961089A and MM060083) and under evaluation at the IITA Kalambo station, and two released genotypes (Nase14 and Albert28) grown by farmers.

3.3.4 **Experimental Design**

The experiment was set using a 4 x 4 split plot design based on Randomized Complete Block Design in three replications at both sites with the four genotypes as the main factor and four methods under which plant materials were produced (KlasmannTS3, Vermiculite, Local peat, and Mini cutting) as a sub-factor in three replications (Figure 3.5). The SAH derived –plantlet performance was compared to the mini cutting - derived plantlets as a control of propagation methods. The plot sizes were 2m x 2.5m each and the plant spacing of 0.5m giving a total plant population of 10 per plot. Each plot was set 3m apart within replication and 2.5m apart between replications giving a field size of 42 m x 13.5m. The first and last rows and the first and last plants within the middle row of each plot were considered as border plants. Additional plants were initially planted at the plot's outset and subsequently transplanted to replace plantlets that failed to sprout or were affected by cutworms during the first month after planting.

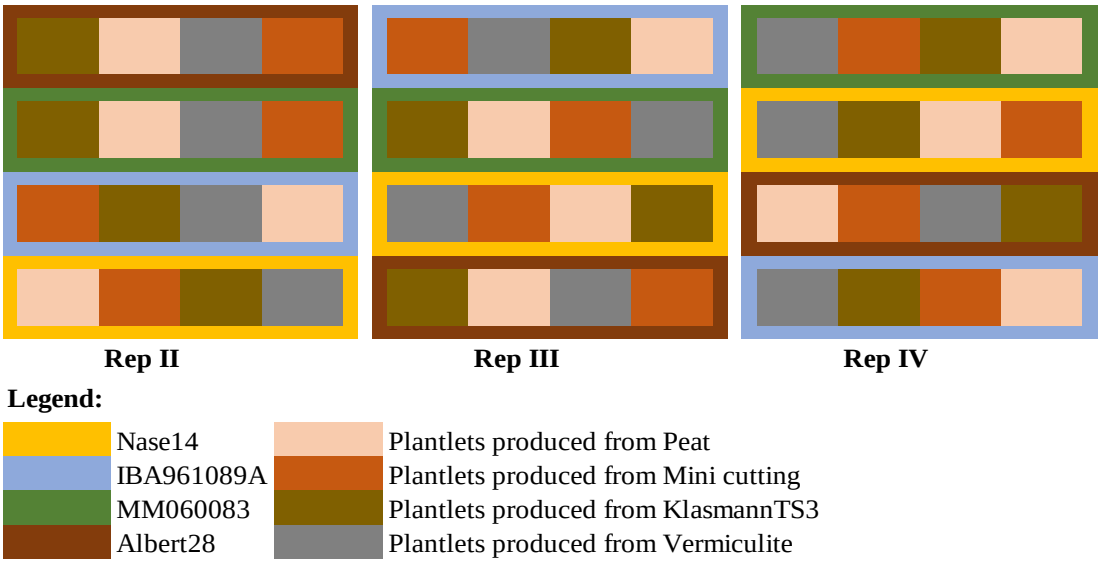


Figure 3.5: A Split-Plot Experimental Design, Arranged In A Randomized Complete Block Layout, Used in the Field to Evaluate the Performance of SAH-Derived Plantlets from Four Cassava Genotypes Produced Using Four Propagation Method

3.3.5 **Field Preparation**

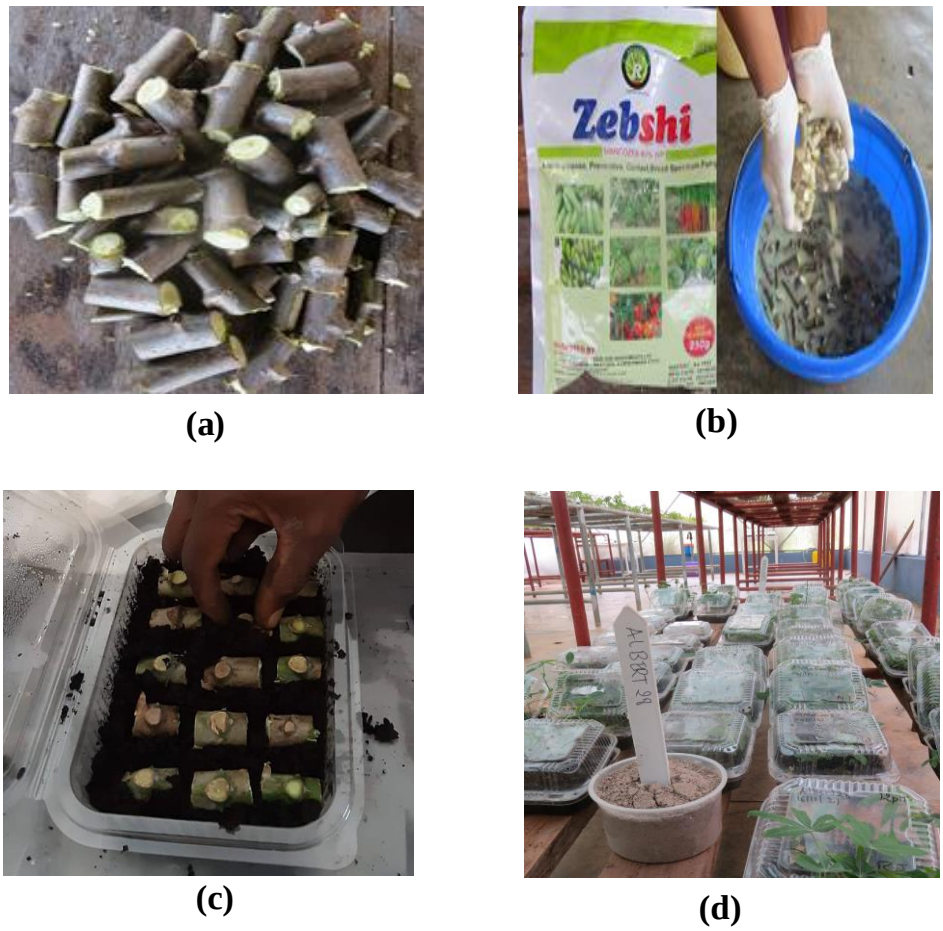
The land plowing was done using disc plows mounted on tractors and harrowed once, followed by hand harrowing with casual workers' hoes. Then the entire fields were set in planting beds as plots with corresponding sizes as explained above, and holes of 5cm deep were dug in advance to receive plantlets the same day. Weed control of the trial plots was done by hand using worker's hoes (twice a month at Mulungu, and once a month at Kiliba).

3.3.6 **Plantlet Preparation for Field Experiment**

A total of 2,416 SAH-derived plantlets were obtained from the four genotypes produced under three substrates (KlasmannTS3, Vermiculite, and local peat) and were ready for field transplanting. Two weeks before field establishment, these plantlets were indexed for CBSD virus at IITA Virology laboratory for virus status confirmation, using polymerase chain reaction (PCR). Young leaf samples were collected from three randomly selected plantlets in each of the 12 treatments across the three subcultures. Observations were based on the presence or absence of virus strain in plant leaves sampled (Appendix 2). Plantlets underwent a two-week acclimatization period outdoors (Plate 3.), facilitating robust field establishment.

Plate 3.5: Two Weeks Acclimatized SAH Plantlets Before Field Transplanting

Meanwhile, mini stem cuttings of one nodal of the same four genotypes were prepared and germinated for field establishment. They were taken from hardwood portions (Plate 3.a) of stems collected at the INERA Mulungu Cassava Program isolated seed field. The one-nodal cuttings were disinfected (Plate 3.b) against fungus using MancoZeb 80% WP fungicide (13.5 g in 4 liters of water) for 5 minutes then planted horizontally side by side at 2 cm deep and keeping node up in SAH transparent light boxes filled with garden soil (PlatePlate 3.c) and covered with perforated lid with 2 holes for air circulation, while watering 3 times a week. The boxes were then transferred to the IITA greenhouse for 4 weeks' germination to reach a physiological state of 5 to 7 leaves (PlatePlate 3.d)



(a) **(b)**

(c) **(d)**

Plate 3.6: Process of Using one Nodal Cutting as a Check in the Study (Conventional Method): Nodal Cutting Preparation (a), Fungicide Disinfection (b), Planting (c), Growth of One Nodal Stem-Cuttings in the Greenhouse (d) at IITA/Kalambo

3.3.7 **Transplanting Procedure and Field Maintenance**

In each site, 360 plantlets were transplanted, comprising 30 plantlets per treatment (i.e., genotype x substrate) distributed as 10 plantlets per plot in three replicates. This resulted in a total of 720 plantlets transplanted across both locations. In parallel, 240 plantlets derived from mini cuttings were introduced as checks for all four genotypes across both Mulungu and Kiliba locations The field transplanting was done before the onset of the rains period according to the following step-by-step of the standard operating procedures developed at IITA (Adetoro et al., 2020 and IITA-BASICS., 2021):

Step 1: In each plot, 10 holes were dug at a depth of 5 cm to receive the plantlets (Plate 3.a).

Step2: The dried substrate boxes containing plantlets were watered for moisture to allow plantlet removal without root damage (Plate 3.b and c);

Step 3: Once the block substrates were removed from the SAH boxes, the plantlets were gently extracted one by one for direct transplanting into 5 cm deep holes in the moist soil. Supplementary watering followed to prevent root stress (Plate 3.d-f);

Step 4: According to the water requirements, when it did not rain, water was applied twice daily using a watering can—once in the early morning and once at sunset—during the first month of growth. Approximately 10 liters of water per plot were applied at the Mulungu location, and 20 liters per plot at the Kiliba location. However, on the day of planting, water was applied regardless of whether it rained, to reduce plant stress.

The trial plots and spacing were kept weed-free by hand weeding twice a month at both Mulungu and Kiliba during the plant growth period. No nitrogen fertilizer was applied to promote plant growth.

Plate 3.7: Steps for Cassava Plantlet Transplanting in the Field: Digging Holes for Plantlet Placement (a); Moistening Substrate Block with Water (b); Removing Substrate Block with Plantlets from Boxes (c); Plantlet Extraction For Individual Planting (d); Direct Transplanting of Plantlets (e); Watering after Transplanting (f)

3.3.8 **Data collection**

Data on the agronomic traits, namely plant survival rate (%), plant height (cm), leaf number per plant (No), node number per plant (No), Stem number per plot (No), and stem length per plot (m) were recorded. Survival rate was observed once at 1 month after transplanting on each plot. The plant growth parameters such as plant height, leaf, and node number were recorded at 3 MAPs and thereafter at 6, and 12 MAPs using six plants selected as samples over the ten planted within the plot. Stem length was measured twice, at 6 and 12 MAPs, because a SAH field dedicated to stem production can be harvested early or late without removing the plant stumps. Plant height (vertical height) was measured from the ground to the top of the canopy of the tallest plant using

a measuring tape. Leaves and nodes were manually counted on the tallest plant from the base. Thereafter mean per treatment (genotype x propagation type) was computed. The estimation of the number of stems consisted of multiplying the number of plants standing in a plot by the average number of stems that a plant had, taking into account the branches on the plants. The stem length estimation was calculated per plot as a product of the average plant height and the total stem number obtained in a plot.

3.3.9 **Data Analysis**

The survival rate was calculated as a percentage of the number of plantlets alive during the observation period, compared to the initial number transplanted in a plot. The average values for height, leaf, node, and stem numbers were calculated per treatment. The statistical analysis software R version 4.2.1 (R Core Team, 2023)(R Core Team, 2023) was used for data analysis. All the data were subjected to the analysis of variance (ANOVA) for testing the single effect of location, genotype, propagation type and their interactions on the performance. Tests of significance were reported at the 0.05, 0.01, and 0.001 levels. The Fisher's Least Significant Difference (LSD) test set at $p < 0.05$ was used for mean comparison for all parameters.

3.4 Cost Analysis of Producing Cassava Plantlets under the SAH System

The cost analysis was done based on the budget of the study and fixed costs. For the cost analysis, the prevailing market price for inputs during experiment time was used. The total number of cassava plantlets produced, that is output was calculated for each substrate. All the costs were calculated using US Dollars as a common denominator. The two key concepts used for the cost analysis were the following:

a) Costs of producing cassava plantlets under different substrates.
b) The unit cost of producing a cassava plantlet under different substrates.

The cost analysis was performed for Experiment 2, which utilized both types of substrates. The total cost of plantlets' production was established by the addition of the cost of each input used. The inputs were general consumables, fertilizer, human

labor, and substrates (Table 3.6), and, as in use at the SAH laboratory at IITA/ Kalambo station in D.R. Congo.

Table 3.6: List of Materials and Equipment Used for Cutting Propagation in the SAH Laboratory at IITA Kalambo, D.RCongo, in 2021

Item	Description	Source
Chemicals (Miracle Grow)	Salts, Micronutrients and Acids. Used as a substitute of the licensed nutrient solution Previously used at IITA.	IITA / Nigeria
Substrate		
Klasmann TS3 (**K**)	Made from dried and processed sphagnum peat moss. Served for growing plantlets	IITA / Nigeria
Vermiculite (**V**)	Medium size, made from heat expanded mica. Used mainly for starting seeds and cuttings.	Kenya
Local peat (**P**)	Organic material from periodically flooded land. Served for growing plantlets	Farm/ DR Congo
Sawdust	Fine particles of wood residues. Served for growing plantlets	Carpentry/DR Congo
$\mathbf{K}_{25}\mathbf{P}_{75}$	Made from a combination of 25% imported K and 75% of P. Served for growing plantlets	_
$\mathbf{V}_{25}\mathbf{P}_{75}$	Made from a combination of 25% imported V and 75% P. Served for growing plantlets	_
$\mathbf{V}_{10}\mathbf{P}_{90}$	Made from a combination of 10% Imported V and 90% P. Served for growing plantlets	_
Consumables		
Box	Plastic transparent –light. Substrate containers used for growing plantlets.	General consumable and laboratory merchant/supplier
Tissue paper	Used for cleaning surfaces and support while cutting plantlets in the cutting area.	
Hand gloves	Used for biosecurity and biosafety measure when handling boxes and plantlets	
Permanent marker	Used for labelling boxes to aid traceability	
Morning Fresh	Liquid detergent used for sanitization purposes in the laboratory.	
Detergent	For sanitization purposes in the laboratory	
Handle and scalpel blades	Used for handling and cutting plantlets during subsequent subculture periods.	
Human worker	Casuals worked for subsequent cuttings, Substrate and nutrient preparation, Cutting and growth rooms monitoring, data collecting assistance	IITA/ DR Congo

3.4.1 **Cost Analysis of Producing Cassava Plantlets under Different Substrates**

The total cost of each substrate was obtained based on the substrate weight used, by adding all the subtotal costs of each substrate which encompassed each input utilized during the production process.

The unit cost of producing one plantlet, was obtained by dividing the total production cost of a specific substrate per the total quantity of plantlets produced with the four genotypes after 3 subsequent subculture periods (three months). Then the SAH unit cost of each substrate was compared to that of producing a 25 cm length of conventional stem collected from the INERA station (Table 3.7).

Table 3.7: Unit Cost for Producing a 25 cm Conventional Cassava Stem under Field Conditions after 12 Months at INERA Stations in 2022, with a Planting Density of 1 m x 1 m.

Item	Unit	Quantity	Unit cost (USD)	Total cost (USD)
Agricultural Input				
Acquisition of cassava basic stems	Meter	2500	0.04	100.00
Acquisition of fertilizer 10 kg bag (NPK)	Bag	30	14.00	420.00
Sub - total 1				**520.00**
Pre-cultural works				
Land boundary	Casual	5	3.00	15.00
Stump removal/Collection	Casual	25	3.00	75.00
Plowing	Hectar	1	200.00	200.00
Harrowing	Hectar	1	100.00	100.00
Sub - total 2				**390.00**
Crop work				
Picketing,	Casual	6	3.00	18.00
Stem preparation	Casual	6	3.00	18.00
Planting	Casual	34	3.00	102.00
Fertilizer spreading	Casual	40	3.00	120.00
Weeding	Casual	204	3.00	612.00
Plant sanitation	Casual	10	3.00	30.00
Maintenance of field	Casual	24	3.00	72.00
Field guard (2 months)	Day	60	3.00	180.00
Sub - total 3				**1152.00**
Control				
Inspection by seed service (SENASEM)	Round	3	100.00	300.00
Supervision	Round	6	25.00	150.00
Sub - total 4				**450.00**
Harvesting				
Stem cutting	Human	25	3.00	75.00
Transportation	Human	50	3.00	150.00
Sub - total 5				**225.00**
Total				**2737.00**
Unpredicted (5%)				**136.85**
GREAT TOTAL				**2873.85**
Unit production cost				
Stem length	Meter			10000.00
Unit production cost (1 m length)	USD			0.29
Unit production cost (25 cm length)	USD			**0.07**

Source: (INERA, 2022)

CHAPTER FOUR

RESULTS

4.1 Performance of Cassava Genotype Plantlets Produced under Different Substrates in the SAH System

4.1.1 Performance of Cassava Plantlets under Single Substrates

The results of the ANOVA analysis, as presented in Table 4.1, revealed that there was a significant difference among the genotypes ($p<0.005$) for survival in subcultures two and three only. Additionally, genotypes significantly ($p<0.001$) differed for all the growth parameters, including height, node, leaf, and cutting numbers, across subcultures, except for leaf number in subculture three and the number of cuttings in subculture one. Furthermore, the substrate had a significant effect ($p<0.001$) on all the mentioned parameters across subcultures, except for leaf number in subculture 3. Lastly, genotype x substrate interaction had a significant effect ($p<0.001$) on all the growth parameters observed, except on height in subculture three and the number of cuttings in subcultures two and three. This interaction did not have any significant effect on plant survival across subcultures.

Table 4.1: ANOVA for Genotype, Substrate, and Interaction Effects on Survival Rate and Growth Performance of Cassava Plantlets Across Three Subculture Periods in the SAH System

		Survival rate(%)				Plantlet height (cm)			
Source of variation	**df**	**Subculture 1**	**Subculture 2**	**df**	**Subculture 3**	**Subculture 1**	**Subculture 2**	**df**	**Subculture 3**
Genotype (G)	3	175.78	588.11*	3	317.17*	15.151***	34.768***	3	5.144*
Substrate (S)	3	1670.83***	430.75*	2	1470.61**	126.186***	182.483***	2	61.542***
G X S	9	75	113.28	6	53.65	8.825***	9.824***	6	2.961
Ea	12	113.07	33.11	12	330.39	0.488	0.88	12	1.412
Eb	48	50	134.83	32	446.12	0.745	0.667	32	1.453
		Internod number (No)				**Leaf number (No)**			
Source of variation	**df**	**Subculture 1**	**Subculture 2**	**df**	**Subculture 3**	**Subculture 1**	**Subculture 2**	**df**	**Subculture 3**
Genotype (G)	3	4.9693***	3.953***	3	5.091**	3.999***	1.208*	3	2.098
Substrate (S)	3	26.072***	51.565***	2	16.539***	31.072***	27.879***	2	0.105
G X S	9	2.465***	1.855***	6	0.370*	3.582***	2.167***	6	11.373***
Ea	12	0.223	0.279	12	0.733	0.120	0.300	12	1.135
Eb	48	0.107	0.317	32	0.132	0.152	0.303	32	0.682
		Number of cuuting (No)							
Source of variation	**df**	**Subculture 1**	**Subculture 2**	**df**	**Subculture 3**	**df**	**Total subcultures**		
Genotype (G)	3	3.033	16.95*	3	158.35***	3	337.3**		
Substrate (S)	3	66.833***	354.88***	2	2461.11***	3	5416.5***		
G X S	9	3	3.47	6	49.21***	9	67.6*		
Ea	12	4.523	4.35	12	8.45	12	40		
Eb	48	2	4.56	32	12.02	48	31.9		

Values presented are mean squares

Significant codes: * 0,05; **0,01; ***0.001.

4.1.1.1 Survival

Survival rates of cassava genotypes were found to differ significantly ($p<0.05$) only in subcultures two and three (Figure 4.1B and 4.C). The highest survival rate was consistently observed in Nase14 with means above 89%. The lowest survival rate was observed in Albert28 in subculture two, and in IBA961089A and MM060083 in subculture three, with rates not exceeding 83%. Substrate significantly ($p<0.001$) affected the survival rate of plantlets across subcultures. In subculture one, KlasmannTS3 exhibited the highest survival rate (92%), whereas Vermiculite showed the lowest rate (69.8%) (Figure 4.1d). In subculture two, KlasmannTS3, Sawdust, and local peat demonstrated the highest survival rates, with values of 93.6%, 86.4%, and 86.3%, respectively. Notably, local peat and vermiculite did not significantly differ in terms of their effect plantlet survival rate (Figure 4.1e). In subculture three, plantlets had the highest survival (92.8%) on KlasmannTS3, and lowest (75.8%) on Vermiculite (Figure 4.1F). No significant interaction between genotype and substrate was observed in any of the subcultures.

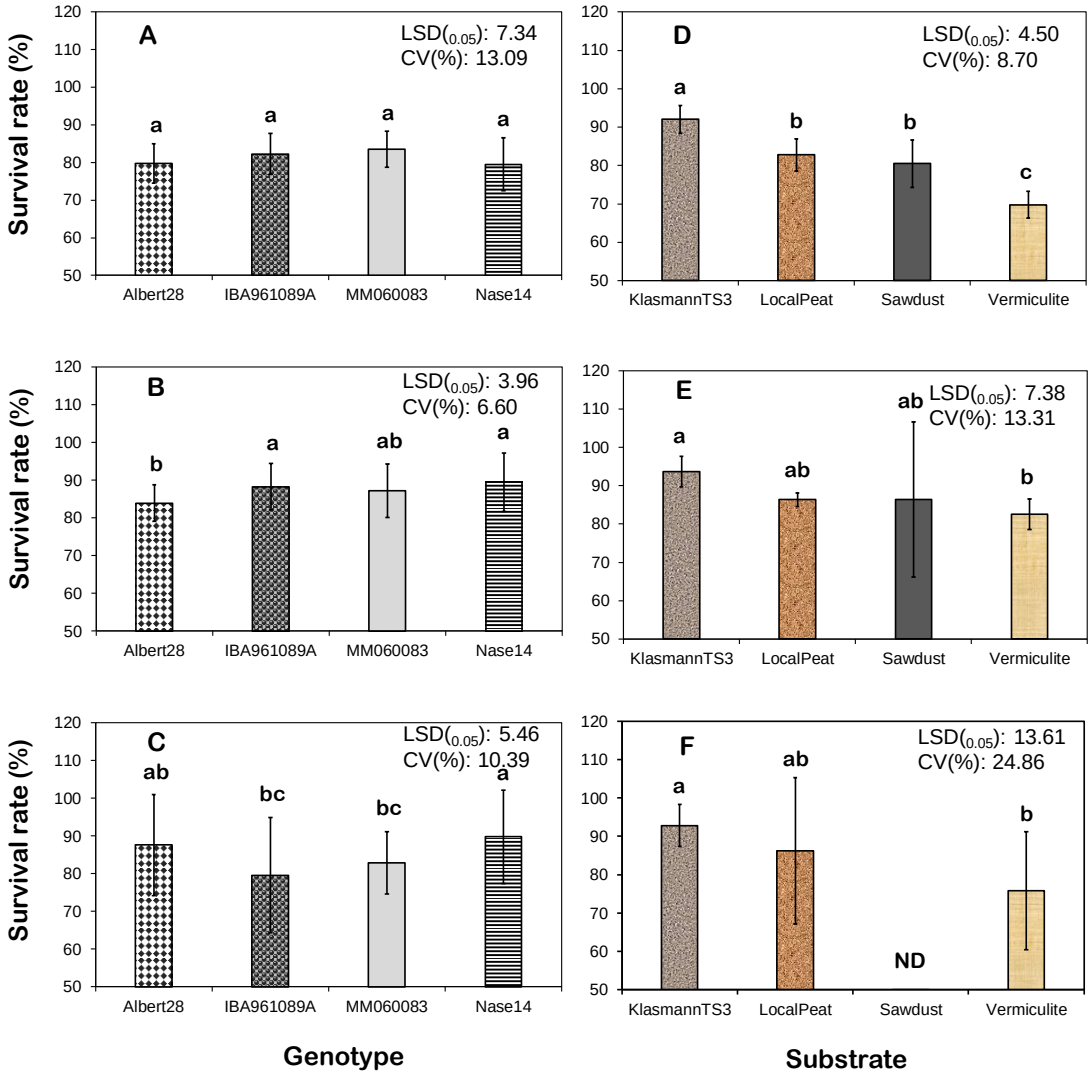


Figure 4.1: Survival Rate (%) of Four Cassava Genotypes and Four Substrates Across Three Subculture Periods under the SAH System. A, B, and C Represent Genotypes at Subcultures One, Two, and Three, Respectively, while D, E, and F Represent Substrates at the Corresponding Subculture Periods. Means within Graph Followed by the Same Lowercase Letter are Not Significantly Different by the LSD Test ($p<0.05$). ND: No Data

4.1.1.2 Plant Height

Plantlet height significantly ($p<0.001$) differed among cassava genotypes in all subcultures. MM060083 consistently had the tallest plantlets, measuring 7.6 cm in

subculture one, 7.2 cm in subculture two, and 6.0 cm in subculture three. The lowest plantlet heights were consistently observed in Albert28, measuring 5.5 cm in subculture one, 4.3 cm in subculture two, and 4.9 cm in subculture three. Furthermore, plantlet height was significantly ($p<0.001$) affected by the substrates. The tallest plantlets were obtained under KlasmannTS3 at the ends of Subculture one (9.8 cm), Subculture two (10.0 cm), and Subculture three (7.4 cm). Conversely, the lowest plantlet heights were recorded in Sawdust at the end of Subcultures one (3.7 cm) and 2 (2.9 cm), and in local peat (3.9 cm) in Subculture three.

The interaction between genotypes and substrates had a significant ($p<0.001$) influence on the height of cassava plantlets in both subculture one and subculture two, but no significant interaction was observed in subculture three (Figure 4.2). Across all genotypes, KlasmannTS3 consistently resulted in the highest heights, while Sawdust consistently resulted in the lowest heights. For example, in subculture one, MM060083 grown in KlasmannTS3 had the highest mean height (13.2 cm). This was similar to the results obtained in subculture two, where MM060083 grown in KlasmannTS3 had the highest mean height (13.1cm). Based on the result, MM060083 grown in KlasmannTS3 had the highest increase in height, while Albert28 had the lowest increase in height when grown in Sawdust substrate in both subcultures one and two.

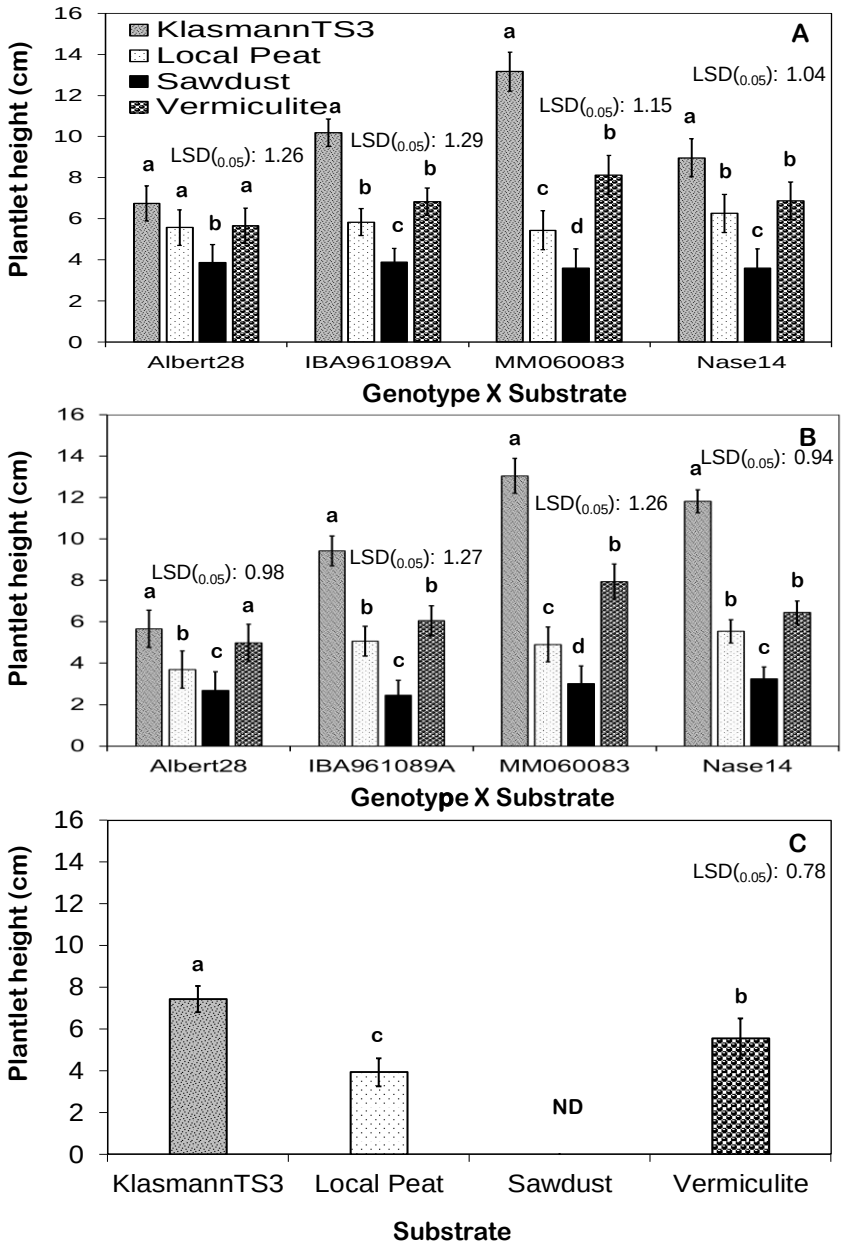


Figure 4.2: Height (cm) of Cassava Plantlets Grown under the SAH System. A and B Represent the Interaction of Genotype with the Substrate at Subcultures One and Two, Respectively; C Represents the Substrate Effect at Subculture Three. Means within the Graph Followed by the Same Lowercase Letter are Not Significantly Different by the LSD Test ($p<0.05$). ND: No Data.

4.1.1.3 Leaf Number

Results on leaf number are presented in Table 4.2. Leaf number significantly ($p<0.001$) differed among cassava genotypes throughout subcultures one and two, while no significant difference occurred in subculture three. MM060083 had a significantly higher leaf number of 5.0 in subculture one and 4.8 in subculture two. The lowest number of leaves was produced under Albert 28 in subculture one (3.9). However, this pattern changed in subculture two, where leaf numbers for IBA961089A, Albert28, and Nase14 were relatively lower, all at 4.3, compared to MM060083, which still maintained the highest leaf number of 4.8.

Similarly, leaf number was significantly ($p<0.001$) influenced by the substrate in subcultures one and two. Plantlets grown under KlasmannTS3 recorded a higher leaf number of 6.2 in subculture one, which remained consistent at 6.0 in subculture two. During the same subculture periods, leaf numbers of plantlets grown in vermiculite (4.0 and 4.1) and local peat (4.1 and 4.4) did not show significant differences, but they were significantly higher than the lowest observed in sawdust (3.3 and 3.2).

There was a significant ($p<0.001$) interaction effect of genotype and substrate on leaf number in all three subcultures. In general, the cassava plantlets performed better under the KlasmannTS3 substrate, producing more leaves, but the increase differed among genotypes. In Subculture one, the highest leaf number was observed with the genotype MM060083 grown under the KlasmannTS3 substrate at 8.3 leaves. The lowest leaf numbers were observed with sawdust across all the genotypes, with values ranging from 3.20 leaves to 3.32 leaves. A similar contrast was observed in subculture two, where the highest leaf numbers were observed with MM060083 grown under KlasmannTS3 at 7.8 leaves. The lowest leaf numbers were observed with all the genotypes grown under sawdust, ranging from 3.08 leaves to 3.32 leaves. The best interactions in subcultures one and two were observed with MM060083 grown under the KlasmannTS3 substrate, as this consistently resulted in the highest increase in leaf numbers (8.3 and 7.8, respectively). All genotypes grown in Sawdust consistently had the lowest leaf numbers, ranging from 3.1 to 3.3.

Table 4.2: Leaf Number per Plantlet (No) for Sixteen Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods

Genotype	Substrate		Leaf number (No)		
			Subculture 1	Subculture 2	Subculture 3
IBA961089A	KlasmannTS3		6.68 ± 0.08[a]	5.69 ± 0.11[a]	5.13 ± 0.27[a]
	Vermiculite		3.96 ± 0.12[b]	4.08 ± 0.09[b]	4.05 ± 0.36[a]
	Local peat		4.12 ± 0.15[b]	4.42 0.09[b]	3.94 ± 0.39[a]
	Sawdust		3.20 ± 0.19[c]	3.24 ± 0.09[c]	(-)
		LSD($_{0.05}$)	0.39	0.81	
Albert28	KlasmannTS3		4.48 ± 0.08[a]	5.36 ± 0.12[a]	5.18 ± 0.18[a]
	Vermiculite		4.16 ± 0.12[ab]	4.29± 0.10[bc]	3.61 ± 0.24[a]
	Local peat		3.72 ± 0.15[bc]	4.44 ± 0.10[b]	3.81 ± 0.39[a]
	Sawdust		3.32 ± 0.25[c]	3.09 ± 0.09[d]	(-)
		LSD($_{0.05}$)	0.53	0.89	
MM060083	KlasmannTS3		8.32 ± 0.26[a]	7.82 ± 0.16[a]	5.42 ± 0.19[a]
	Vermiculite		3.88 ± 0.27[bc]	3.88 ±0.08[bc]	3.96 ± 0.37[a]
	Local peat		4.32 ± 0.15[b]	4.40 ± 0.09[b]	4.02 ± 0.0.33[a]
	Sawdust		3.32 ± 0.15[c]	3.08 ± 0.08[c]	(-)
		LSD($_{0.05}$)	0.72	0.67	
Nase14	KlasmannTS3		5.24 ± 0.16[a]	5.21 ± 0.11[a]	5.02 ± 0.34[a]
	Vermiculite		4.04 ± 0.10[b]	4.32 ± 0.10[b]	3.76 ± 0.12[a]
	Local peat		4.12 ± 0.24[b]	4.24± 0.11[b]	3.64 ± 0.17[a]
	Sawdust		3.32 ± 0.08[c]	3.32 ± 0.09[c]	(-)
		LSD($_{0.05}$)	0.45	0.64	
		Mean	4.39	4.39	4.29
		CV(%)	8.89	12.42	11.46

Mean followed by the same lowercase letter in the same column are not statistically different by LSD significance test. (-): There was no Sawdust data in Subculture three because cuttings to be transplanted could not be obtained in Subculture two. Values are presented as mean ± standard error.

4.1.1.4 Internode Number

There was a significant ($p<0.001$) difference among genotypes for internode number (

Table 4.3). In sub-culture 1, plantlets of IBA961089A (4.2) and MM060083 (4.1) had a significantly higher number of internodes compared to Nase14 (3.5) and Albert28 (3.2), with no significant difference between the latter two. However, in sub-cultures 2 and 3, only plantlets of MM060083 had a higher number of internodes (4.1 and 4.5 in respective subcultures), while there was no significant difference between plantlets of IBA961089A, Albert28, and Nase14, with means ranging from 3.1 to 3.5 across both subcultures.

Furthermore, the number of internodes showed a significant ($p<0.001$) difference among substrates in all three subcultures. Plantlets produced under KlasmannTS3 had a higher number of internodes in all the three subcultures (5.3, 5.6, and 4.7, for respective subcultures). There was no significant difference in internode numbers between those produced in vermiculite and local peat, with means ranging from 3.0 to 3.2 for both subcultures. Sawdust resulted in the lowest number of internodes in sub-cultures 1 and 2 (2.6 and 1.7, respectively).

The interaction between genotypes and substrates had a significant influence on the internode number of cassava plantlets ($p<0.001$). A high number of internodes were observed in plantlets of all the genotypes grown in KlasmannTS3; however, the increase observed differed among genotypes. In subculture one, the highest internode numbers were observed with the genotypes MM060083 (6.6) and IBA961089A (6.2) grown under the KlasmannTS3 substrate. Similarly, in subculture two, the highest increase in the number of internodes was observed in plantlets of MM060083 grown under KlasmannTS3 substrate (7.5). In subculture three, the highest increase in internode number was observed with the genotype MM060083 (5.7) grown under the KlasmannTS3 substrate. The lowest internode numbers were observed in all the genotypes grown under Sawdust, with values ranging from 1.5 to 2.7.

Table 4.3: Internode Number per Plantlet (No) for Sixteen Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods

		Internode number (No)		
Genotype	**Substrate**	**Subculture 1**	**Subculture 2**	**Subculture 3**
IBA961089A	KlasmannTS3	6.16 ± 0.21[a]	5.45 ± 0.29[a]	4.84 ± 0.29[a]
	Vermiculite	3.12 ± 0.14[c]	2.74 ± 040[b]	2.84 ± 0.29[b]
	Local peat	4.92 ± 0.17[b]	3.44 ± 0.15[b]	2.76 ± 0.37[b]
	Sawdust	2.68 ± 0.19[c]	1.53 ± 0.19[c]	(-)
	LSD($_{0.05}$)	0.4	0.8	0.55
Albert28	KlasmannTS3	3.76a ± 0.18	4.56a ± 0.19	4.02a ± 0.22
	Vermiculite	3.20 ± 0.23[b]	3.05 ± 0.22[b]	2.88 ± 0.34[b]
	Local peat	3.20 ± 0.19[b]	3.05 ± 0.20[b]	2.60 ± 0.21[b]
	Sawdust	2.48 ± 0.19[c]	1.76 ± 0.17[c]	(-)
	LSD($_{0.05}$)	0.35	0.7	0.52
MM060083	KlasmannTS3	6.60 ± 0.31[a]	7.48 ± 0.14[a]	5.72 ± 0.08[a]
	Vermiculite	3.48 ± 0.14[b]	3.17 ± 0.33[b]	3.84 ±0.25[b]
	Local peat	3.44 ± 0.10[b]	3.57 ± 0.19[b]	3.88 ± 0.29[b]
	Sawdust	2.68 ± 0.12[c]	2.04 ± 0.26[c]	(-)
	LSD($_{0.05}$)	0.5	0.73	0.53
Nase14	KlasmannTS3	4.64 ± 0.07[a]	4.85 ± 0.35[a]	4.12 ± 0.27[a]
	Vermiculite	3.44 ± 0.15[b]	3.21 ± 0.19[b]	3.16 ±0.13[b]
	Local peat	3.20 ± 0.23[b]	3.05 ± 0.21[b]	2.88 ± 0.15[b]
	Sawdust	2.52 ± 0.10[c]	1.57 ± 0.27[c]	(-)
	LSD($_{0.05}$)	0.48	0.85	0.52
	Mean	3.72	3.41	3.63
	CV(%)	8.79	16.53	10.02

Means within a column followed by the same lowercase letter are not significantly different by the LSD test ($p< 0.05$). (-) There was no sawdust data in subculture three because cuttings to be transplanted could not be obtained in subculture two. Values are presented as mean ± standard error.

4.1.1.5 Number of cuttings

The number of cuttings significantly ($p<0.001$) differed among the cassava genotypes across subcultures, as well as in the overall subcultures. MM060083 had the highest

number of cuttings in subculture two (8.7) and subculture three (16.3), whereas Albert28 and Nase14 recorded the lowest numbers. Specifically, both Albert28 and Nase14 had 6.7 and 6.9 cuttings in subculture two, and 10.3 and 10.4 cuttings in subculture three, respectively. At the end of subsequent subcultures, the genotype MM060083 produced the highest total number of cuttings at 41.7, which represented a propagation ratio of 1:2 from the initial 20 cuttings in subculture one. In comparison, the genotypes IBA961089A, Nase14, and Albert28 produced 36.9, 33.3, and 32.9 cuttings, respectively. MM060083 demonstrated the most substantial increase, reaching 209%, compared to 185%, 166%, and 165% for IBA961089A, Nase14, and Albert28, respectively, from the initial count of 20 cuttings.

Similarly, substrate significantly ($p<0.001$) influenced the number of cassava cuttings across subcultures and the total number of subcultures. KlasmannTS3 consistently produced the highest number of cuttings in all subcultures, with 18.4 in subculture one, followed by 13.1 in subculture two, and a further increase to 27.1 in subculture three. On the other hand, the lowest number of cuttings was observed under vermiculite in subculture one (14.0) and under sawdust in subculture two (2.8). In subculture three, the lowest number of cuttings was obtained under vermiculite (11.1) and local peat (11.7). At the end of the three subcultures, KlasmannTS3 had the highest mean number of cuttings at 58.4, with a ratio of 1:3. On the other hand, local peat and vermiculite produced 35.5 and 32.0 cuttings, respectively, corresponding to ratios of 1:2. Compared to the other substrates, KlasmannTS3 showed a remarkable increase of 292% in the number of cuttings obtained from the initial amount (20). In contrast, local peat and vermiculite had an increase of only 178% and 160%, respectively. Sawdust had the lowest mean number of cuttings at 19, which was 5% less than the initial (20) number of cuttings.

The interaction between genotype and substrate significantly ($p<0.001$) influenced the number of cuttings in subculture three and the total across all three subcultures (Table 4.4). High numbers of cuttings were observed for all genotypes under KlasmannTS3 substrate. However, the highest number of cuttings occurred with MM060083 in KlasmannTS3, reaching 37.2 in subculture three and 70.4 overall. Conversely, the lowest number of cuttings in subculture three was found in Albert28 and Nase14 when

grown in vermiculite and local peat, yielding 9.0 and 9.8, respectively. Similarly, the lowest overall number of cuttings was observed for all genotypes in Sawdust, ranging from 18.0 to 20.8.

Table 4.4: Number of Cuttings (No) per Box for Sixteen Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods and Their Overall

			Number of cuttings (No)			
Genotype	**Substrate**		**Subculture 1**	**Subculture 2**	**Subculture 3**	**Total Subcultures**
IBA961089A	Klasmann TS3		18.20 ± 0.66^{a}	12.40 ± 0.46^{a}	25.00 ± 0.63^{a}	55.60 ± 1.11^{a}
	Vermiculite		14.60 ± 0.75^{a}	8.00 ± 0.15^{a}	12.80 ± 0.45^{bc}	35.40 ± 0.42^{bc}
	Local peat		17.00 ± 0.95^{a}	8.00 ± 0.32^{a}	13.20 ± 0.61^{b}	38.20 ± 0.82^{b}
	Sawdust		16.00 ± 0.45^{a}	2.20 ± 0.27^{a}	(-)	18.20 ± 0.22^{d}
		LSD$_{(0.05)}$			7.98	9.04
Albert28	Klasmann TS3		17.80 ± 0.58^{a}	12.40 ± 0.43^{a}	22.20 ± 0.51^{a}	52.40 ± 0.92^{a}
	Vermiculite		14.40 ± 0.68^{a}	6.00 ± 0.10^{a}	9.80± 0.40^{b}	30.20± 0.52^{b}
	Local peat		15.80 ± 0.97^{a}	5.60 ± 0.47^{a}	9.00 ± 0.93^{b}	30.40 ± 0.34^{b}
	Sawdust		15.80 ± 0.92^{a}	2.60 ± 0.23^{a}	(-)	18.40 ± 0.13^{c}
		LSD$_{(0.05)}$			2.84	6.19
MM060083	Klasmann TS3		18.40 ± 060^{a}	14.80 ± 0.41^{a}	37.20 ± 0.41^{a}	70.40 ± 1.19^{a}
	Vermiculite		13.60 ± 0.51^{a}	7.60 ± 0.33^{a}	12.60 ± 0.69^{c}	33.80 ± 0.99^{c}
	Local peat		17.20 ± 0.66^{a}	9.20 ± 0.27^{a}	15.40 ± 0.44^{b}	41.80 ± 0.73^{b}
	Sawdust		17.60 ± 0.75^{a}	3.20 ± 0.14^{a}	(-)	20.80 ± 0.25^{d}
		LSD$_{(0.05)}$			4.34	10.57
Nase14	Klasmann TS3		19.20 ± 0.37^{a}	12.40 ± 0.44^{a}	23.60 ± 0.87^{a}	55.20 ± 1.17^{a}
	Vermiculite		13.20 ± 0.73^{a}	6.00 ± 0.10^{a}	9.20 ± 0.16^{b}	28.40 ± 0.28^{c}
	Local peat		16.20 ± 0.80^{a}	6.20 ± 0.43^{a}	9.00 ± 0.58^{b}	31.40 ± 0.38^{b}
	Sawdust		15.00 ± 1.14^{a}	3.00 ± 0.31^{a}	(-)	18.00 ± 0.46^{d}
		LSD$_{(0.05)}$			5.58	6.57
		Mean	16.25	7.48	12.44	36.16
		CV(%)	8.70	28.58	22.81	15.62

Means within a column followed by the same lowercase letter are not significantly different by the LSD significance test ($p<0.05$). (-) There was no sawdust data in Subculture three because cuttings to be transplanted could not be obtained in Subculture two. Values are presented as mean ± standard error.

4.1.2 **Performance of Cassava under Single and Combined Substrates**

The results of the analysis of variance (Table 4.5) revealed that there was a significant ($p<0.001$) difference among the genotypes on survival in subcultures two and three,

then on all growth parameters namely: height, internode, leaf, and cutting numbers across sub culture periods, except on survival rate and number of cuttings in subculture one. Furthermore, the substrate showed a significant effect ($p<0.001$) on all the mentioned parameters across subcultures. Lastly, the interaction of genotype x substrate had a significant effect ($p<0.001$) on all the growth parameters mentioned above, except on survival across subcultures.

Table 4.5: ANOVA for Genotype, Substrate and Their Interaction Effect on Survival Rate and Growth Performance Traits of Cassava Plantlets Across Three Subculture Periods under the SAH System

		Survival rate (%)			Plantlet height (cm)		
Source of variation	**df**	**Subculture 1**	**Subculture2**	**Subculture 3**	**Subculture 1**	**Subculture2**	**Subculture 3**
Genotype (G)	2	151.39	343.57*	210.4 *	9.194***	22.636 ***	20.682***
Substrate (S)	5	1884.72***	1548.8***	1209.2***	34.639***	53.905 ***	59.677***
G X S	10	109.72	47.98	47.98	1.419 **	1.072 *	1.234 ns
Ea	6	51.39	75.37	25.92	0.507	0.773	0.591
Eb	45	55.46	68.01	58.52	0.454	0.447	0.767
		Internode number (No)			**Leaf number (No)**		
Source of variation	**df**	**Subculture 1**	**Subculture2**	**Subculture 3**	**Subculture 1**	**Subculture2**	**Subculture 3**
Genotype (G)	2	3.483***	11.369***	9.317***	0.299*	2.119***	1.789*
Substrate (S)	5	12.097***	14.434***	13.026***	8.045***	5.890***	4.138***
G X S	10	0.997***	1.070***	1.060***	0.402***	1.606***	1.390***
Ea	6	0.069	0.244	0.144	0.045	0.053	0.184
Eb	45	0.178	0.131	0.168	0.1	0.147	0.141
		Number of cuuting (No)					
Source of variation	**df**	**Subculture 1**	**Subculture2**	**Subculture 3**	**Total subcultures**		
Genotype (G)	2	7.389	66.67*	115.68**	392.68*		
Substrate (S)	5	82.456***	11.96***	698.86***	2668.52***		
G X S	10	5.456**	349.7***	78.61***	244.58***		
Ea	6	1.537	11.96	8.74	42.7		
Eb	45	12.99	33.67	7.12	24.74		

Values presented are mean squares.

Significant codes: * 0,05; **0,01; ***0.001.

4.1.2.1 Survival

The survival rate of cassava plantlets showed a significant difference among the genotypes in subcultures two and three (Figure 4.3B-C). IBA961089A had consistently the highest rate of above 80%, while IBA70520 had consistently the lowest. Plantlet survival rates significantly ($p<0.005$) differed also among substrates

in all subculture periods (Figure 4.3D-F). Among the single substrates, KlasmannTS3 had the highest plantlet survival rate. The survival rate of plantlets produced in Vermiculite did not differ significantly from those produced in local peat and were the lowest. The combination of KlasmannTS3 and local peat ($K_{25}P_{75}$) resulted in a similar survival rate as that of KlasmannTS3 but was significantly higher than that of local peat in all sub-cultures. The combinations of Vermiculite and local peat ($V_{10}P_{90}$ and $V_{25}P_{75}$) increased the survival rates as compared to Vermiculite or local peat used alone. The interaction between genotype and substrate did not significantly affect the survival rate.

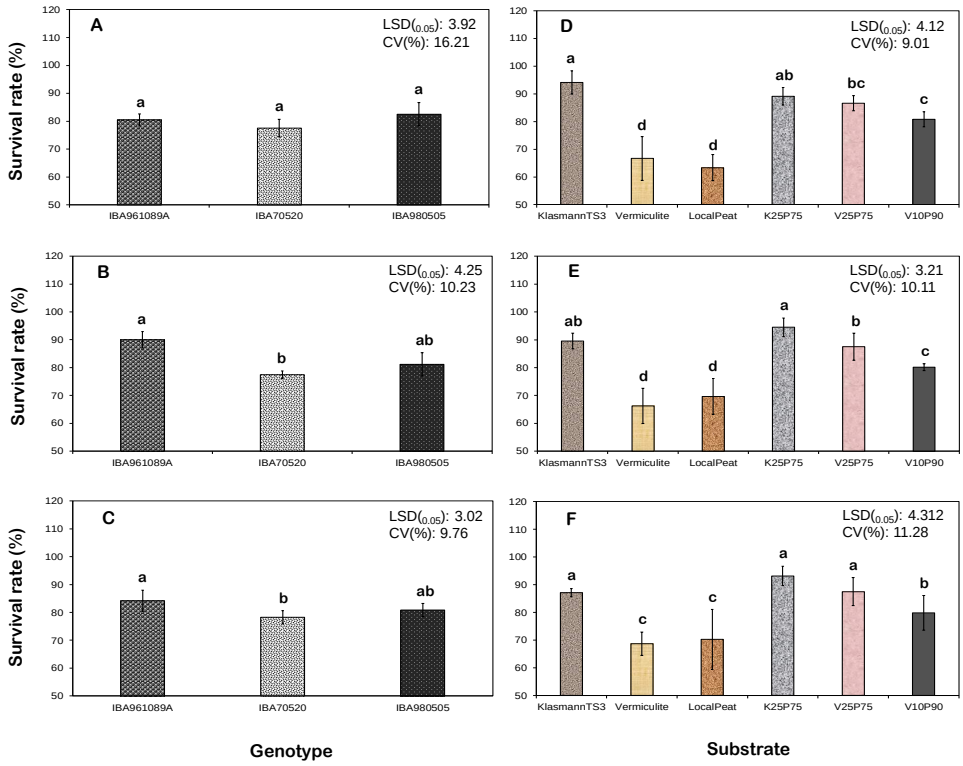


Figure 4.3: Survival Rate (%) of Three Cassava Genotypes and Six Substrates Across Three Subculture Periods under the SAH System

Key: A, B, and C represent genotypes at subcultures one, two, and three, respectively, while D, E, and F represent substrates at the corresponding subculture periods. Means within graph followed by the same lowercase letter are not significantly different by the LSD test ($p<0.05$).

4.1.2.2 Plant Height

Significant differences ($p<0.001$) in plantlet height were observed among genotypes in all subculture periods. Irrespective of the subculture, IBA961089A produced the tallest plantlets. There was no significant difference in height between IBA70520 and IBA980505 at the end of sub-cultures 1 and 2. However, at the end of subculture three (Figure 4.4C), IBA980505 produced significantly shorter plantlets.

Substrate significantly influenced plantlet height ($p<0.001$) in all subcultures. Plantlets grown in KlasmannTS3 were significantly taller than those grown in other single substrates, with average heights ranging between 8.8 cm and 9.5 cm compared to 4.3 cm to 5.6 cm for other single substrates at the respective periods. A combination of KlasmannTS3 and local peat ($K_{25}P_{75}$) produced plantlets as tall as those produced in KlasmannTS3 in subculture one (8.3 cm vs 8.8 cm). Plantlets grown in Vermiculite were taller than those grown in local peat in subcultures two and three, and when 10% and 25% of the local peat were replaced by Vermiculite ($V_{10}P_{90}$ and $V_{25}P_{75}$). However, the plantlets produced in these combinations ($V_{10}P_{90}$ and $V_{25}P_{75}$) were as tall as those grown in local peat, which produced the shortest plantlets. A combination of Vermiculite and local peat produced plantlets shorter than those produced in KlasmannTS3.

The interaction of genotype with substrate had a significant ($p<0.001$) effect on plantlet height in subcultures one and two, but not in subculture three (Figure 4.4A-B). Overall, KlasmannTS3 and its combination with local peat ($K_{25}P_{75}$) led to the highest means of height, but the increase differed among genotypes and across subcultures. In subculture 1, the highest height resulted in IBA961089A under KlasmannTS3 (10.4cm) and $K_{25}P_{75}$ (9.2). In subculture two, IBA961089A consistently had the tallest plantlets only under KlasmannTS3 at 11.2cm. Among the combined substrates, $K_{25}P_{75}$ led the highest with the same genotype (IBA961089A) at 8.4cm. The poorest genotype-substrate interaction for plantlet height was consistently observed across all genotypes grown under Vermiculite, local peat, $V_{25}P_{75}$, and $V_{10}P_{90}$ substrates, with values ranging between 3 cm and 5 cm.

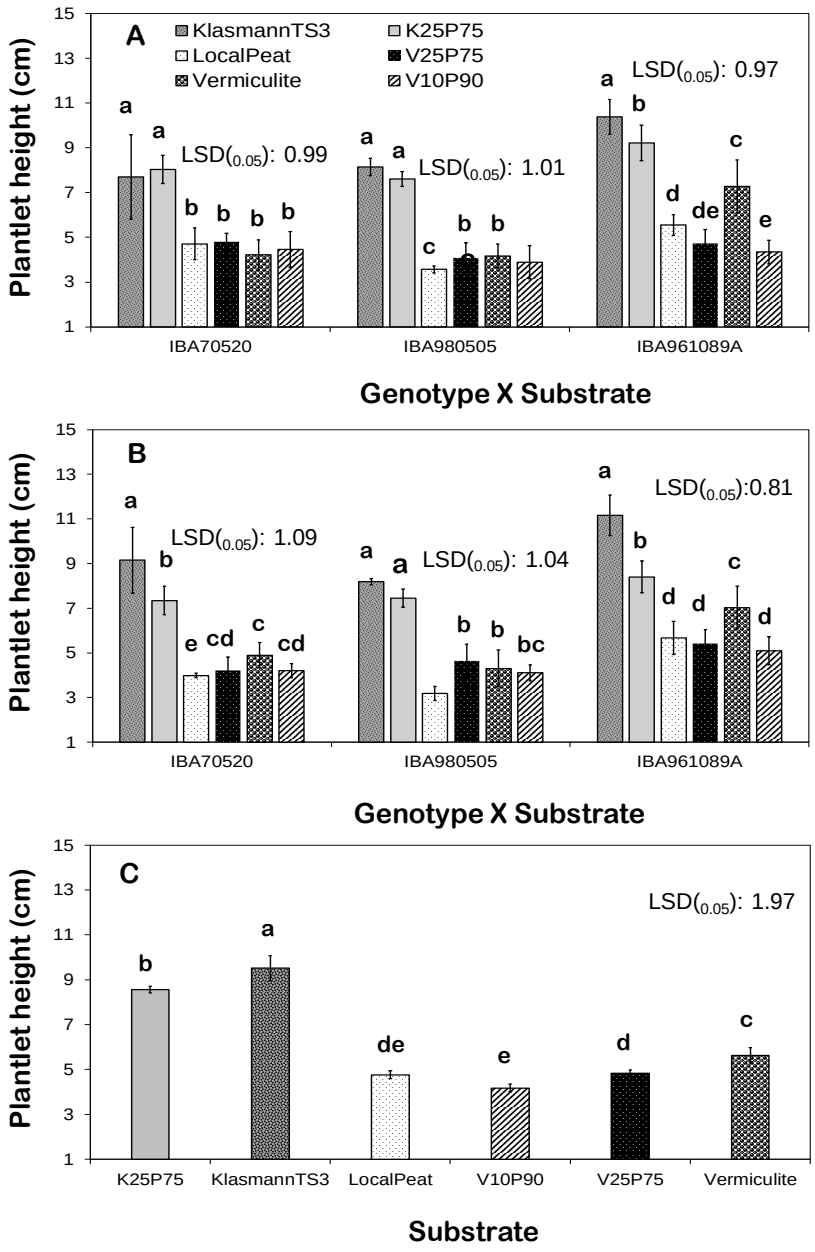


Figure 4.4: Height (cm) of Cassava Plantlets Grown under the SAH System. A and B Interaction of Genotype with Substrate at Subcultures One and Two, Respectively; C Substrate Effect at Subculture Three. Means within the Graph Followed by the Same Lowercase Letter are Not Significantly Different by the LSD Test ($p<0.05$).

4.1.3 **Leaf Number**

Significant ($p<0.001$) differences in leaf number were observed among cassava genotypes in all subcultures. The plantlets of IBA961089A had significantly higher leaf numbers than those of IBA980505 and IBA70520 in sub-culture 1, and both IBA961089A and IBA980505 exhibiting higher leaf numbers than IBA70520 in subcultures two and three.

Substrate also had a significant ($p<0.001$) effect on leaf number in all subcultures, with KlasmannTS3 producing plantlets with higher leaf numbers than Vermiculite or local peat, and the latter two substrates yielding similar leaf numbers. Combinations of Vermiculite and local peat ($V_{10}P_{90}$ and $V_{25}P_{75}$), increased leaf numbers compared to each substrate alone, but only the combination of KlasmannTS3 and local peat produced plantlets with leaf numbers similar to those of KlasmannTS3.

There was a significant ($p<0.001$) interaction between genotype and substrate regarding leaf number in all the subcultures (Table 4.6). Overall, KlasmannTS3 and its combination with Local peat ($K_{25}P_{75}$) led to higher means of leaf number, however, the increase differed among genotypes. The highest leaf number was consistently obtained with IBA961089A grown under the two substrates in all subculture periods.

Table 4.6: Leaf Number per Plantlet (No) for Heighten Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods

		Leaf number (No)		
Genotype	**Substrate**	**Subculture 1**	**Subculture 2**	**Subculture 3**
	KlasmannTS3	5.28 ± 0.18^{a}	6.08 ± 0.20^{a}	5.60 ± 0.28^{a}
	Vermiculite	3.35 ± 0.10^{c}	3.75 ± 0.17^{d}	4.25 ± 0.23^{bc}
	LocalPeat	$3.80 \pm 0.08b^{c}$	3.70 ± 0.17^{cd}	3.65 ± 0.22^{c}
IBA961089A	K25P75	5.15 ± 0.10^{ab}	5.05 ± 0.36^{ab}	4.45 ± 0.24^{b}
	V25P75	3.95 ± 0.31^{bc}	3.85 ± 0.13^{cd}	3.80 ± 0.22^{c}
	V10P90	4.10 ± 0.13^{b}	4.30 ± 0.21^{bc}	3.95 ± 0.23^{bc}
	$LSD_{(0.05)}$	1.09	1.05	0.64
	KlasmannTS3	4.53 ± 0.08^{ab}	3.95 ± 0.15^{ab}	3.81 ± 0.22^{bc}
	Vermiculite	3.60 ± 0.08^{c}	3.65 ± 0.10^{bc}	3.35 ± 0.21^{c}
IBA70520	LocalPeat	3.20 ± 0.05^{c}	3.55 ± 0.15^{bc}	3.40 ± 0.22^{bc}
	K25P75	5.55 ± 0.13^{a}	4.68 ± 0.09^{a}	4.50 ± 0.24^{a}
	V25P75	4.05 ± 0.22^{ab}	4.15 ± 0.24^{ab}	4.10 ± 0.23^{ab}
	V10P90	3.40 ± 0.08^{c}	3.65 ± 0.22^{bc}	3.80 ± 0.23^{ab}
	$LSD_{(0.05)}$	0.90	1.02	0.89
	KlasmannTS3	4.88 ± 0.26^{ab}	4.58 ± 0.24^{bc}	4.75 ± 0.24^{b}
IBA980505	Vermiculite	3.18 ± 0.10^{de}	3.50 ± 0.17^{de}	3.55 ± 0.21^{cd}
	LocalPeat	3.50 ± 0.24^{d}	3.95 ± 0.22^{d}	4.00 ± 0.23^{bc}
	K25P75	5.40 ± 0.08^{a}	6.33 ± 0.05^{a}	6.10 ± 0.28^{a}
	V25P75	4.45 ± 0.13^{bc}	4.95 ± 0.15^{b}	4.23 ± 0.24^{b}
	V10P90	3.30 ± 0.06^{de}	3.40 ± 0.14^{def}	3.25 ± 0.20^{d}
	$LSD_{(0.05)}$	0.90	0.79	0.66
	Mean	**4.1**	**4.3**	**4.1**
	$CV_{(\%)}$	**20.2**	**21**	**19.4**

Means within a column followed by the same lowercase letter are not significantly different by the LSD test (0.05). Values are presented as mean ± standard error.

4.1.3.1 Internode Number

A significant difference ($p<0.001$) was observed among genotypes in terms of the number of internodes in plantlets (

Table 4.7). Specifically, plantlets of IBA961089A had a significantly higher number of internodes compared to the other genotypes, while the internode number of IBA70520 plantlets did not differ significantly from that of IBA980505.

Substrate had a highly significant ($p<0.001$) effect on internode number across all three sub-cultures. Plantlets produced in KlasmannTS3 consistently had a higher number of internodes compared to those produced in other single substrates or combinations of Vermiculite and local peat. The internode number in the combination of KlasmannTS3 and local peat ($K_{25}P_{75}$) was slightly lower than that in Klasmann TS3, but the difference was not significant, in contrast to the results for leaf number. Meanwhile, the internode number in Vermiculite, local peat, and in the combinations of the two substrates did not differ significantly with respect to leaf number.

There was a significant interaction ($p<0.001$) between genotype and substrate in relation to the internode number at the end of each subculture period. Overall, KlasmannTS3 led to a high mean internode number, but the increase was specific to the genotype. The highest mean internode number was recorded consistently with IBA961089A under the KlasmannTS3 across subculture periods (6.7, 7.2, and 6.9). This contrasted a little bit with IBA980505, which had a high number under both KlasmannTS3 and $K_{25}P_{75}$ in subcultures 1 and 3. The lowest mean internode numbers were recorded, practically with all genotypes grown under Vermiculite, local peat, and their combinations across subculture periods.

Table 4.7: Internode Number per Plantlet (No) for Eighteen Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods

Genotype	Substrate	Internode number (No)		
		Subculture 1	Subculture 2	Subculture 3
IBA961089A	KlasmannTS3	6.72 ± 0.40^{a}	7.21 ± 0.40^{a}	6.85 ± 0.47^{a}
	Vermiculite	3.12± 0.18^{d}	3.10 ± 0.13^{d}	2.70 ± 0.21^{d}
	LocalPeat	4.05 ± 0.15^{c}	3.30 ± 0.33^{cd}	3.20± 0.16^{d}
	K25P75	3.95 ± 0.37^{b}	5.05 ± 0.15^{b}	5.10 ± 0.19^{b}
	V25P75	3.10 ± 0.26^{d}	3.95 ± 0.15^{c}	4.00 ± 0.20^{c}
	V10P90	3.10 ± 0.17^{d}	3.95 ± 010^{c}	4.20 ± 0.16^{c}
	$LSD_{(0.05)}$	0.86	0.68	0.79
IBA70520	KlasmannTS3	4.45 ± 0.15^{a}	4.10 ± 0.26^{a}	4.45 ± 0.10^{a}
	Vermiculite	2.95 ± 0.22^{bc}	2.10 ± 0.10^{c}	2.00 ±0.12^{e}
	LocalPeat	3.15 ± 0.13^{b}	2.60 ± 0.24^{bc}	3.25 ± 0.10^{c}
	K25P75	3.95 ± 0.15^{b}	3.95 ± 0.30^{a}	3.90 ± 0.10^{b}
	V25P75	3.20 ± 0.08^{b}	3.10 ± 0.13^{b}	3.20 ± 0.14^{c}
	V10P90	2.90 ± 0.17^{bc}	2.85 ± 0.13^{b}	2.75 ± 0.17^{d}
	$LSD_{(0.05)}$	0.51	0.54	0.39
IBA980505	KlasmannTS3	5.10 ± 0.19^{a}	5.18 ± 0.13^{a}	4.65 ± 0.33^{a}
	Vermiculite	3.10 ± 0.17^{bc}	3.00 ± 0.12^{c}	2.75 ± 0.25^{bc}
	LocalPeat	2.85 ±0.13^{c}	2.15 ± 0.05^{e}	2.20 ± 0.08^{c}
	K25P75	4.80 ± 0.06^{a}	4.15 ±0.13^{b}	4.05 ± 0.22^{a}
	V25P75	3.40 ± 0.08^{b}	3.35 ±0.10^{c}	3.25 ± 0.05^{b}
	V10P90	2.85 ± 0.17^{c}	2.60 ± 0.14^{d}	2.70 ± 0.21^{bc}
	$LSD_{(0.05)}$	0.46	0.37	0.61
	Mean	**3.77**	**3.65**	**3.62**
	$CV_{(\%)}$	**29.23**	**24.69**	**23.33**

Means within a column followed by the same lowercase letter are not significantly different by the LSD test (0.05). Values are presented as mean ± standard error.

4.1.3.2 Number of Cuttings

There was observed a significant ($p<0.01$) difference among cassava genotypes for the number of cuttings obtained in subcultures two and three, as well as the total recorded. IBA961089A consistently yielded a higher number of cuttings, with a total of 53.4 cuttings. On the other hand, IBA70520 and IBA980505 consistently had similar

means, showing no significant difference from each other, with a total of 45.6 and 47.9 cuttings, respectively.

The number of cuttings highly differed ($p<0.001$) among substrates throughout all subculture periods. Overall, the number of cutting increased significantly under the combined substrates, compared to single ones, except for KlasmannTS3. $K_{25}P_{75}$ consistently led to the highest mean cuttings, with a total mean of 68.3 cuttings, which did not differ significantly from 66.6 cuttings under KlasmannTS3. From the starting number (20 cuttings), both $K_{25}P_{75}$ and KlasmannTS3 had the highest increase at 341.5% and 333%, representing a ratio of 1:3. On the other hand, $V_{25}P_{75}$ had high mean numbers compared to Vermiculite and Local peat, particularly for the total means, while $V_{10}P_{90}$ had a high number compared to local peat, and did not differ from Vermiculite. Both combinations differed significantly throughout subcultures, with $V_{25}P_{75}$ having higher mean numbers than $V1_0P_{90}$. The fewest cuttings were obtained under Vermiculite and local peat, totaling 36.8 and 35.2, respectively. These represented increases of 184% and 176% from the starting number of 20 cuttings.

There was a significant ($p<0.001$) genotype x substrate interaction for the number of cuttings throughout subculture periods and their total (Table 4.8). Overall, the highest number of cuttings were obtained under $K_{25}P_{75}$, which were similar to those from KlasmannTS3. However, the increases observed significantly differed among genotypes. The highest increases in cuttings were obtained with IBA961089A produced under $K_{25}P_{75}$, as well as KlasmannTS3, with a total of 80.5 and 83.3 cuttings, respectively. Considering the starting number of 20 cuttings, the average increase in the number of cuttings obtained with IBA961089A under $K2_5P_{75}$ was 402%, which was similar to 416.5% (Ratio of 1:4) under KlasmannTS3. The lowest numbers of cuttings were obtained with all the genotypes grown under Local peat and Vermiculite, with some particularity in IBA961089A x local peat interaction.

Table 4.8: Number of Cuttings (No) per Box for Eighteen Cassava Genotype-Substrate Interactions under the SAH System over Three Subculture Periods and Their Overall

		Number of cuttings (No)			
Genotype	**Substrate**	**Subculture 1**	**Subculture 2**	**Subculture 3**	**Total subcultures**
	KlasmannTS3	20.00 ± 0.06^{a}	25.50 ± 0.71^{ab}	37.75 ± 1.22^{a}	83.25 ± 1.35^{a}
	Vermiculite	13.00 ± 0.62^{e}	11.00 ± 0.71^{e}	6.50 ± 0.89^{f}	30.50 ± 0.09^{ef}
	LocalPeat	11.50 ± 0.53^{f}	6.50 ± 0.71^{f}	14.00 ± 0.89^{de}	32.00 ± 1.05^{e}
IBA961089A	K25P75	19.00 ± 0.45^{ab}	27.00 ± 1.05^{a}	34.50 ± 1.08^{ab}	80.50 ± 1.32^{ab}
	V25P75	17.00 ± 0.45^{cd}	15.00 ± 0.71^{c}	16.50 ± 0.87^{c}	48.50 ± 0.87^{c}
	V10P90	18.00 ± 0.05^{bc}	13.50 ± 0.72^{cd}	14.25 ± 0.86^{cd}	45.75 ± 1.09^{cd}
	$LSD_{(0.05)}$	1.40	1.63	2.27	2.81
	KlasmannTS3	17.50 ± 0.82^{b}	17.50 ± 0.71^{ab}	24.25 ± 1.07^{a}	59.25 ± 1.33^{b}
	Vermiculite	12.50 ± 0.71^{ef}	12.50 ± 0.71^{c}	12.00 ± 0.87^{e}	37.00 ± 1.05^{d}
IBA70520	LocalPeat	13.00 ± 0.71^{e}	8.50 ± 0.71^{de}	14.00 ± 0.80^{d}	35.50 ± 1.05^{e}
	K25P75	19.00 ± 0.45^{a}	19.00 ± 0.72^{a}	24.00 ± 0.88^{ab}	62.00 ± 1.02^{a}
	V25P75	17.00 ± 0.45^{bc}	12.00 ± 0.70^{c}	16.00 ± 0.89^{c}	45.00 ± 1.02^{c}
	V10P90	14.00 ± 1.00^{d}	9.00 ± 0.70^{d}	11.50 ± 0.83^{ef}	34.50 ± 1.02^{ef}
	$LSD_{(0.05)}$	0.89	1.66	1.68	1.44
	KlasmannTS3	17.50 ± 1.00^{bc}	18.50 ± 1.05^{ab}	21.25 ± 1.05^{ab}	57.25 ± 1.25^{b}
IBA980505	Vermiculite	14.50 ± 0.71^{e}	17.00 ± 1.04^{c}	11.50 ± 0.50^{ef}	43.00 ± 1.07^{d}
	LocalPeat	13.50 ± 1.09^{ef}	10.00 ± 1.20^{e}	14.50 ± 0.51^{cd}	38.00 ± 1.07^{ef}
	K25P75	19.00 ± 0.45^{a}	20.00 ± 0.71^{a}	23.50 ± 0.87^{a}	62.50 ± 10.7^{a}
	V25P75	18.00 ± 0.76^{ab}	13.00 ± 0.71^{d}	16.50 ± 0.87^{c}	47.50 ± 1.07^{c}
	V10P90	16.50 ± 0.82^{cd}	10.00 ± 0.71^{e}	12.50 ± 0.87^{de}	39.00 ± 1.03^{e}
	$LSD_{(0.05)}$	1.62	1.68	2.32	2.26
	Mean	**16.03**	**21.86**	**25.08**	**25.08**
	$CV_{(\%)}$	**17.46**	**25.09**	**27.51**	**26.51**

Means within a column followed by the same lowercase letter are not significantly different by LSD test (0.05). Values are presented as mean ± standard error.

4.2 Performance of Selected Cassava Genotype Plants Produced Using SAH Technology in Different Locations

4.2.1 Analysis of Variance for Survival and Agronomic Traits of Four Cassava Genotypes Produced from Three SAH Substrates, Compared with Mini Cuttings of the Same Genotypes Across Two Locations at Different MAPs

The ANOVA analysis revealed that all the single factors namely Location, Genotype, and Propagation method had significant ($p<0.001$) effects on all the agronomic traits (survival, height, leaf and node per plant, stem number, and stem length per plot)

evaluated across months (Table 4.9). Furthermore, genotype alone had a highly significant influence ($p<0.001$) on all the traits. The same was true for the propagation method which also influenced significantly ($p<0.001$) the performance of the traits from 3 to 12 MAPs, except for height for which the effect was limited to 6 MAPs.

Significant interactions were also observed for some traits evaluated. The two-level interaction of location x genotype had a significant ($p<0.001$) effect only on height from 3 to 6 MAPs, then on stem length per plot at 6 and 12 MAPs. Similarly, the interaction of location and propagation method had a significant ($p<0.001$) effect on the same parameters (height) at 3 and 6 MAPs, and then on stem length per plot at 6 MAPs as well. On the other hand, the interaction of genotype and propagation method had a significant effect on height at 3 and 6 MAPs. And also on leaf, node, and stem numbers, as well as stem length across their respective sampled MAPs. Any of the mentioned interactions had a significant effect on the survival of plants. There was no observed significant interaction for all three interactions - levels of location x genotype x propagation method on all the traits evaluated.

Table 4.9: ANOVA for Location, Genotype, Propagation Method, and their Interaction Effect on Survival Rate and Growth Performance of Cassava Plants Across Different MAPs.

Source of variation		Survival rate (%)	Height (cm)			Leaf number (No)		
	df	1MAP	3MAP	6MAP	12MAP	3MAP	6MAP	12MAP
Location	1	1350***	2205.1***	28740***	0.147*	55.37***	69.87***	626.7***
Genotype	3	314***	1426.8***	2731***	3.71***	57.64***	77.87***	268.2***
Propagation type	3	4625***	2865.2***	18741***	0.167	190.24***	191.36***	6.6*
Location:Genotype	3	108	164.9***	604***	0.011	2.9	0.71	0
Location:Propagation type	3	64	135.6***	675***	0.031	1.65	1.29	0
Genotype:Propagation type	9	72	148.2***	461***	0.069	9.96***	6.88***	11.4***
Location:Genotype:Propagation typ	9	30	9.6	56	0.011	0.58	0.61	0
Residuals	64	59	5.1	29	0.076	1.41	1.38	2.4

Source of variation		Node number (No)			Stem number per plot (N_0)	
	df	3MAP	6MAP	12MAP	6MAP	12MAP
Location	1	55.37***	66.28***	1143.95***	10.38*	131.6 ***
Genotype	3	57.64***	76.69***	263.8***	12.10***	94.77***
Propagation type	3	190.24***	196.95***	6.8*	69.38***	47.03***
Location:Genotype	3	2.9	1.07	0	4.33	4.06
Location:Propagation type	3	1.65		0	3.71	0.8
Genotype:Propagation type	9	9.96***	6.73***	11.5***	6.62**	15.01***
Location:Genotype:Propagation typ	9	0.58	0.66	0	1.84	1.05
Residuals	64	1.41	1.34	2.3	1.97	3.97

Source of variation		Stem lenght per plot (m)	
	df	6MAP	12MAP
Location	1	180.46***	1176.6***
Genotype	3	36.93***	289.6***
Propagation type	3	268.90***	594.00***
Location:Genotype	3	10.64*	50.00**
Location:Propagation type	3	26.95***	18.3
Genotype:Propagation type	9	18.46***	59.60***
Location:Genotype:Propagation typ	9	4.81	7
Residuals	64	2.87	9

Values presented are the mean squares.

Significant codes: * 0,05; **0,01; ***0.001. MAP: month after planting.

4.2.1.1 Effect of Location, Genotype, and Propagation Method and Their 2 and 3 Interaction Levels on the Survival and Growth Performance of Cassava Plants Across Different MAPs

4.2.1.2 Survival

Results on plant survival rate are presented in Figure 4.5. Over all, hihest survival rate among genotypes or propagation methods was recorded at Kiliba as opposed to Mulungu. Among genotypes, MM060083 had the highest survival rate exceeding 80% across locations, while lowest was obtained in Nase14, particularly at Inera Mulungu location,of less than 70%.

Plants obtained from the check mini cutting showed the highest survival rate, particularly at Kiliba, exceeding 90%, compared to those produced from SAH substrates. However, among SAH substrates, plants produced from KlasmannTS3 had

consistently, higest survival rate, while those obtained from local peat and vermiculite di not differ significantly across location.

No significant interactions were observed for plant survival rate.

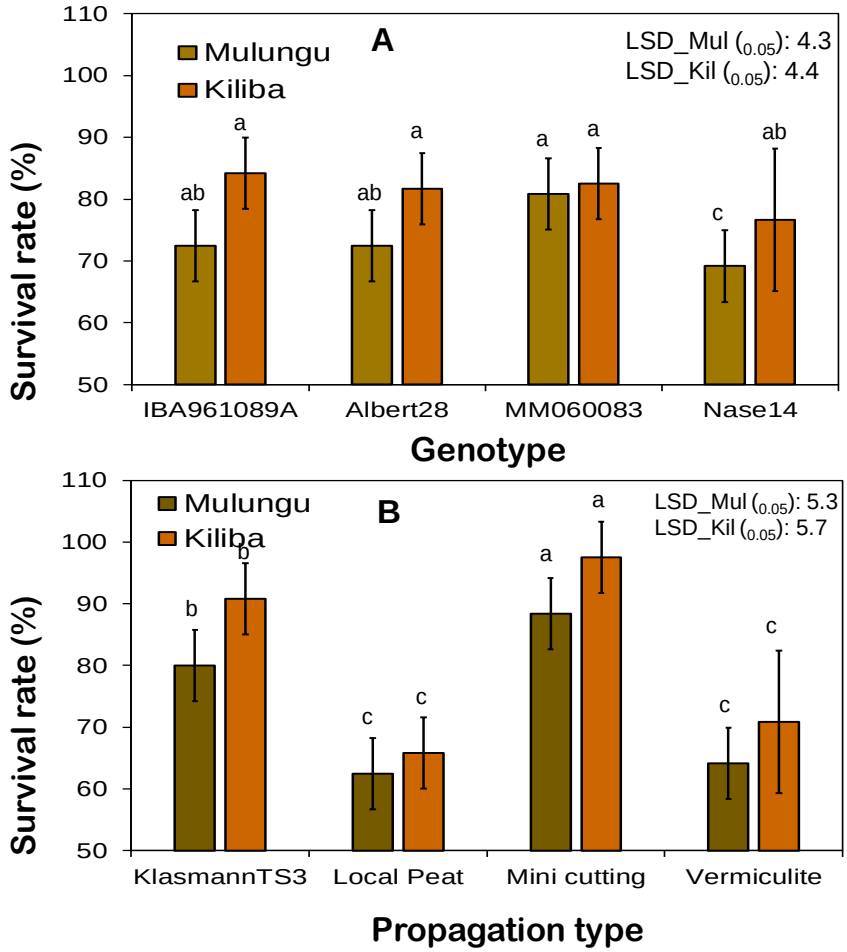


Figure 4.5: Survival Rate (%) of Cassava Plantlets Across Two Locations One Month After Planting: Genotype (A); Propagation Type (B). Means within a Graph Followed by the Same Lowercase Letter are Not Significantly Different by the LSD Test ($p < 0.05$).

4.2.1.3 Leaf, Node, and Stem Numbers

Results of the effects of single factors are presented in Table 4.10. Highest number of leaves per plant were obtained consistently at Mulungu, which represented 1.1 times the number of leaves of Kiliba at 12 MAPs. Significant ($p<0.001$) difference was also

observed among genotypes where Nase14 recorded consistently the highest, with 41.7 leaves at 12 MAPs. The lowest leaf number was recorded consistently on IBA961089A, with 33.5 leaves at the respective month. The mini cutting method had highest leaf number at 3 and 6 MAPs (17.6 and 22.6 respectively), compared to all SAH substrate methods. Among SAH methods, KlasmannTS3 and Vermiculite did not differ significantly at the same months, while local peat had the lowest consistently. However, at 12 MAPs, all SAH methods catch up the check mini cutting method, showing no significant difference among methods, with mean varying between 36.9 and 38.1.

For node number, the highest means were consistently obtained at Mulungu, representing 1.2 times the number at Kiliba, at 12 MAPs. Nase14 recorded consistently the highest node number, with 58.7 at 12 MAPs, while IBA961089A had the lowest at 3 and 6 MAPs. The check mini cutting method had highest number of nodes, with 54.3 at 12 MAPs. Among SAH methods, KlasmannTS3 and Vermiculite had the highest, showing no significant difference, with means of 46.4 and 46.8, respectively. Local peat recorded consistently the lowest numbers, with 44.2 at 12 MAPs.

For stem number, Mulungu had consistently highest numbers, compared to Kiliba across MAPs, representing 1.3 times at 12 MAPs. Nase14 had the highest number, with 11.5 stem per plot, while IBA961089A had the lowest, with 6.7 stem per plot at the same month. The mini-cutting check consistently showed the highest stem numbers across all sampled months, with 10.9 stem per plot at 12 MAPs. KlasmannTS3 had the highest at 6 MAPs, compare to Vermiculite, and Local peat, which did not differ significantly. However, at 12 MAPs, all the SAH matched, but still low compared to the check.

Table 4.10: Effect of Location, Genotype, and Propagation Type on Leaf and Node Numbers (No) per Plant, as well as Stem Number per Plot, at Different Months after Planting

			Leaf number (No)			Node number (No)			Stem number per plot (No)	
Factor			**3 MAP**	**6MAP**	**12MAP**	**3 MAP**	**6MAP**	**12MAP**	**6MAP**	**12MAP**
Location	Mulungu		14.87 ± 0.06^{a}	19.58 ± 0.06^{a}	39.63 ± 0.0.07^{a}	18.37 ± 0.06^{a}	24.58 ± 0.06^{a}	48.70 ± 0.16^{a}	5.69 ± 0.06^{a}	10.09 ± 0.06^{a}
	Kiliba		13.36 ± 0.07^{b}	17.88 ± 0.07^{b}	34.82 ± 0.07^{b}	16.89 ± 0.07^{b}	22.92 ± 0.07^{b}	47.20 ± 0.17^{b}	5.04 ± 0.04^{b}	7.74 ± 0.06^{b}
		LSD(0.05)	0.48	0.48	0.63	0.48	0.47	0.32	0.57	0.61
Genotype	IBA961089A		12.47 ± 0.09^{c}	17.45 ± 0.10^{b}	33.54 ± 0.13^{c}	15.97 ± 0.09^{c}	22.46 ± 0.10^{c}	43.90 ± 0.10^{b}	4.46 ± 0.05^{b}	6.73 ± 0.09^{c}
	Albert28		14.02 ± 0.10^{b}	18.14 ± 0.12^{b}	37.21 ± 0.13^{b}	17.52 ± 0.10^{b}	23.14 ± 0.12^{b}	44.60 ± 0.12^{b}	5.32 ± 0.08^{ab}	9.07 ± 0.10^{b}
	MM060083		13.76 ± 0.11^{b}	17.93 ± 0.11^{b}	37.06 ± 0.13^{b}	17.26 ± 0.11^{b}	23.01 ± 0.11^{bc}	44.60 ± 0.13^{b}	5.48 ± 0.09^{ab}	8.35 ± 0.11^{b}
	Nase14		16.20 ± 0.18^{a}	21.40 ± 0.17^{a}	41.70 ± 0.13^{a}	19.70 ± 0.18^{a}	26.39 ±0.17^{a}	58.70 ± 0.38^{a}	6.19 ± 0.13^{a}	11.51 ± 0.14^{a}
		LSD(0.05)	0.69	0.68	0.89	0.69	0.67	0.88	0.81	0.91
Propagation type	KlasmannTS3		13.96 ± 0.08^{b}	18.27 ± 0.06^{b}	38.10 ± 0.15^{a}	17.46 ± 0.08^{b}	23.05 ± 0.06^{b}	46.40 ± 0.19^{b}	6.09 ± 0.07^{b}	8.23 ± 0.08^{b}
	Vermiculite		14.160 ± 0.09^{b}	18.43 ± 0.08^{b}	37.41 ± 0.21^{ab}	17.66 ± 0.09^{b}	23.43 ± 0.08^{b}	46.80 ± 0.21^{b}	4.25 ± 0.04^{c}	8.73 ± 0.15^{b}
	Local peat		10.73 ± 0.05^{c}	15.80 ± 0.07^{c}	36.90 ± 0.17^{ab}	14.23 ± 0.05^{c}	20.83 ± 0.07^{c}	44.20 ± 0.20^{c}	3.71 ± 0.04^{c}	7.77 ± 0.11^{b}
	Mini cutting (Check)		17.62 ± 0.13^{a}	22.60 ± 0.14^{a}	37.11 ± 0.18^{ab}	21.12 ± 0.13^{a}	27.69 ± 0.14^{a}	54.30 ± 0.49^{a}	7.41 ± 0.11^{a}	10.93 ± 0.14^{a}
		LSD(0.05)	0.69	0.68	0.89	0.69	0.67	0.88	0.81	0.91
		Mean	**14.11**	**18.73**	**37.38**	**17.61**	**23.75**	**47.95**	**5.36**	**8.91**
		CV(%)	**22.96**	**17.66**	**11.33**	**18.40**	**13.98**	**16.73**	**21.96**	**25.84**

Means within a column followed by the same lowercase letter are not significantly different by the LSD significance test (0.05). Values are presented as mean ± standard error.

No significant interactions were observed for leaf, node, and stem numbers at the 2-levels of location x genotype or location x propagation method across all the months. However, a significant ($p<0.001$) interaction was noted for the genotype x propagation method on the mentioned parameters (Table 4.11).

Overall, interactions of all genotypes with the mini-cutting methods resulted in a high number of leaves. Notably, highest leaf number per plant was obtained under Nase14 x mini-cutting at 3 and 6 MAPs (21.9 and 27.4 leaves, respectively). At 12 MAPs, highest number of leaves was obtained in the interaction of Nase14 with Mini cutting, KlasmannTS3 and Vermiculite (41.7; 41.0, and 43.6 leaves respectively). Consistently, the lowest leaf numbers were obtained under the interaction of all genotypes with the local peat at 3 and 6 MAPs. However, at 12 MAPs, lowest number of leaves were obtained under the interactions of IBA961089A with Vermiculite, local peat, and mini-cutting (31.0; 33.2, and 33.2 leaves respectively).

For node number, similar trend was observed, but only the interaction of Nase14 x mini cutting yielded the highest number of nodes across months, with 73.8 at 12 MAPs. The lowest node numbers were obtained under the interactions of IBA961089A with Mini cutting, Albert28 x local peat, and MM060083 x local peat, with mean values not exceeding 41 nodes at 12 MAPs.

A high stem number per plot was observed in the interaction of all genotypes with mini-cutting (check) across all months, with the highest number recorded for Nase14. Notably, at 12 MAPs, this interaction had the maximum number of stems, reaching 15.3 stems per plot. Conversely, a consistently poor interaction was noted with IBA961089A x local peat, resulting in 5.2 stems per plot at 12 MAPs. No significant interactions were observed for location x genotype x propagation method across the sampled months for the mentioned parameters.

Table 4.11: Effect of Sixteen Genotype-Propagation Method Interactions on Leaf and Node Numbers (No) per Plant, as Well as Stem Number per Plot, at Different Months after Planting

		Leaf number (No)			Node number (No)			Stem number per plot (No)	
Genotype	**Propagation method**	**3 MAP**	**6 MAP**	**12 MAP**	**3 MAP**	**6 MAP**	**12 MAP**	**6 MAP**	**12 MAP**
IBA961089A	KlasmannTS3	12.30 ± 0.13^{b}	17.20 ± 0.05^{bc}	33.22 ± 0.67^{b}	15.80 ± 0.12^{b}	22.30 ± 0.05^{bc}	43.70 ± 0.05^{bc}	5.27 ± 0.10^{a}	7.40 ± 0.05a
	Vermiculite	11.80 ± 0.13^{bc}	17.80 ± 0.09^{b}	31.00 ± 0.71^{c}	15.20 ± 0.05^{bc}	22.80 ± 0.06^{b}	44.30 ± 0.08^{b}	3.94 ± 0.08^{c}	6.83 ± 0.10^{ab}
	Local peat	10.80 ± 0.14^{d}	14.80 ± 0.07^{d}	33.20 ± 0.69^{b}	14.30 ± 0.04^{d}	19.80 ± 0.09^{d}	41.20 ± 0.06^{d}	3.50 ± 0.10^{d}	5.17 ± 0.10^{c}
	Mini cutting (Check)	15.00 ± 0.12^{a}	20.00 ± 0.08^{a}	36.00 ± 0.91^{a}	18.50 ± 0.04^{a}	25.00 ± 0.08^{a}	46.40 ± 0.03^{a}	5.14 ± 0.09^{ab}	7.52 ± 0.10^{a}
	$LSD_{(0.05)}$	0.67	0.71	1.20	0.65	0.92	1.23	0.40	1.53
Albert28	KlasmannTS3	13.80 ± 0.14^{c}	17.80 ± 0.06^{b}	37.70 ± 0.67^{b}	17.30 ± 0.06^{b}	22.90 ± 0.06^{b}	44.30 ± 0.11^{b}	6.17 ± 0.07^{ab}	8.78 ± 0.11^{b}
	Vermiculite	14.40 ± 0.12^{b}	17.70 ± 0.03^{b}	37.50 ± 0.63^{bc}	17.90 ± 0.04^{b}	22.7 ± 0.03^{bc}	44.10 ± 0.09^{b}	4.85 ± 0.07^{c}	8.60 ± 0.05^{bc}
	Local peat	10.90 ± 0.12^{d}	15.40 ± 0.07^{c}	37.90 ± 0.60^{a}	14.40 ± 0.07^{c}	20.40 ± 0.07^{d}	41.80 ± 0.06^{c}	3.70 ± 0.03^{d}	8.50 ± 0.14^{bc}
	Mini cutting (Check)	17.00 ± 0.15^{a}	21.60 ± 0.06^{a}	35.70 ± 0.60^{b}	20.50 ± 0.05^{a}	26.60 ± 0.06^{a}	48.00 ± 0.07^{a}	6.58 ± 0.04^{a}	10.40 ± 0.10^{a}
	$LSD_{(0.05)}$	0.49	0.10	0.38	0.63	0.95	1.38	0.60	1.03
MM060083	KlasmannTS3	13.60 ± 0.12^{c}	17.60 ± 0.06^{b}	37.70 ± 0.58^{a}	17.10 ± 0.05^{bc}	22.60 ± 0.06^{b}	44.10 ± 0.06^{b}	6.47 ± 0.04^{ab}	8.57 ± 0.10^{b}
	Vermiculite	14.50 ± 0.13^{b}	17.30 ± 0.06^{bc}	36.80 ± 0.57^{ab}	18.00 ± 0.05^{b}	22.30 ± 0.06^{bc}	43.70 ± 0.06^{bc}	3.96 ± 0.05^{c}	6.5 ± 0.07^{d}
	Local peat	10.40 ± 0.14^{d}	15.40 ± 0.05^{d}	35.90 ± 0.51^{bc}	13.90 ± 0.08^{d}	20.40 ± 0.05^{d}	41.80 ± 0.05^{d}	3.93 ± 0.06^{c}	7.87 ± 0.09^{c}
	Mini cutting (Check)	16.50 ± 0.14^{a}	21.40 0.04^{a}	37.80 ± 0.49^{a}	20.00 ± 0.06^{a}	26.70 ± 0.04^{a}	49.00 ± 0.04^{a}	7.57 ± 0.03^{a}	10.50 ± 0.08^{a}
	$LSD_{(0.05)}$	0.32	1.06	1.08	0.95	1.02	1.37	1.11	1.20
Nase14	KlasmannTS3	16.10 ± 0.14^{b}	19.50 ± 0.06^{bc}	41.00 ± 0.45^{bc}	19.60 ± 0.05^{b}	24.50 ± 0.06^{bc}	53.80 ± 0.06^{c}	6.47 ± 0.02^{b}	8.17 ± 0.10^{d}
	Vermiculite	16.00 ± 0.12^{bc}	20.80 ± 0.060^{b}	43.60 ± 0.45^{a}	19.5 ± 0.04^{b}	25.80 ± 0.07^{b}	55.20 ± 0.07^{b}	4.23 ± 0.03^{c}	13.00 ± 0.10^{b}
	Local peat	10.80 ± 0.12^{d}	17.80 ± 0.05^{d}	40.50 ± 0.45^{d}	14.30 ± 0.07^{c}	22.80 ± 0.05^{d}	52.10 ± 0.05^{d}	3.73 ± 0.04^{d}	9.53 ± 0.09^{c}
	Mini cutting (Check)	21.90 ±0.12^{a}	27.40 ± 0.05^{a}	41.70 ± 0.43^{b}	25.40 ± 0.04^{a}	32.40 ± 0.05^{a}	73.80 ± 0.05^{a}	10.30 ± 0.05^{a}	15.30 ± 0.08^{a}
	$LSD_{(0.05)}$	0.86	1.65	0.70	0.81	1.31	1.38	0.49	1.3
	Mean	**14.11**	**18.73**	**37.38**	**17.61**	**23.75**	**47.95**	**5.36**	**8.91**
	$CV_{(\%)}$	**22.96**	**17.66**	**11.33**	**18.40**	**13.98**	**16.73**	**21.96**	**25.84**

Means that a column followed by the same lowercase letter are not significantly different by LSD significance test (0.05). Values are presented as mean ± standard error.

4.2.1.4 Height and Stem Length

Table 4.12 presents the effects of individual factors. Mulungu consistently exhibited tall plant heights, reaching 169 cm at 12 MAPs. Genotype MM060083 consistently displayed the tallest height (162 cm at 120 MAP), while IBA961089A consistently had the lowest height (135 cm in the same month). The mini-cutting method achieved the highest heights at 3 and 6 MAPs (57.4 cm and 132.2 cm, respectively) compared to all SAH substrate methods. Among the SAH substrates, KlasmannTS3 and Vermiculite showed no significant difference at 6 MAP, while local peat consistently resulted in the lowest heights. However, at 12 MAPs, all SAH substrate methods matched the check mini-cutting method, with no significant difference among them, with mean heights varying between 123.4 cm and 189.5 cm.

For stem length, Mulungu consistently had the highest with ,17.5 m, compared to Kiliba's 10.5 Lm at 12 MAPs. Nase14 had the highest mean across months, with 17.7 m at 12 MAPs. However, at early 6 MAPs, this genotype was matched by Albert28 and MM060083, showing not significant difference among them. IBA961089A consistently had the lowest stem length, with 9.3 m at 12 MAPs. Mini cutting consistently, surpassed all the SAH substrates methods in stem length, with 21.2 m, which represented 1.7, 1.6, and 2.2 times, the quantity obtained under KlasmannTS3, Vermiculite, and Local peat, respectively. Among the SAH methods, KlasmannTS3 had highest stem length at 6 MAPs (5.4 m). However, at 12 MAPs, this substrate was matched by vermiculite, showing not significant difference. Local peat consistently resulted in the lowest stem length.

Table 4.12: Effect of Single Factors (Location, Genotype, and Propagation Method) on Plant Height (cm) and stem Length (m) per Plot, at Different Months after Planting

Factor		Height (cm)			Stem length per plot (m)	
		3MAP	6MAP	12MAP	6MAP	12MAP
Location	Mulungu	46.24 ± 0.30[a]	110.23 ± 0.68[a]	169.03 ± 0.71[a]	6.78 ± 0.10[a]	17.50 ± 0.16[a]
	Kiliba	36.66 ± 0.23[b]	75.62 ± 0.48[b]	131.42 ± 0.48[b]	4.04 ± 0.05[b]	10.50 ± 0.11[b]
	$LSD_{(0.05)}$	0.92	2.19	2.17	0.69	1.22
Genotype	IBA961089A	31.21 ± 0.34[d]	78.68 ± 0.89c	134.98 ± 0.89d	3.63 ± 0.07[b]	9.31 ± 0.16[c]
	Albert28	45.42 ± 0.54[b]	100.14 ± 1.43[a]	156.44 ± 1.43b	5.57 ± 0.14[a]	14.58 ± 0.26[b]
	MM060083	48.98 ± 0.63[a]	102.00 ± 0.16[a]	162.30 ± 1.77a	6.02 ± 0.19[a]	14.39 ±0.33[b]
	Nase14	40.22 ± 0.45[c]	90.88 ± 1.34[b]	147.18 ± 1.34c	6.43 ± 0.23[a]	17.71 ± 0.35[a]
	$LSD_{(0.05)}$	1.3	3.09	3.08	0.98	1.73
Propagation type	KlasmannTS3	36.76 ± 0.29[c]	86.12 ± 0.79[b]	143.42 ± 0.89b	5.38 ± 0.10[b]	12.08 ± 0.18[b]
	Vermiculite	38.86 ± 0.39[b]	87.21 ± 0.88[b]	144.51 ± 0.98b	3.69 ± 0.05[c]	12.87 ± 0.26[b]
	Local peat	32.78 ± 0.25[d]	66.13 ± 0.58[c]	123.43 ± 0.69c	2.47 ± 0.03[c]	9.84 ± 0.18[c]
	Mini cutting (Check)	57.39 ±0.63[a]	132.24 ± 1.36[a]	189.54 ± 1.45[a]	10.10 ± 0.22[a]	21.21 ± 0.36[a]
	$LSD_{(0.05)}$	1.3	3.09	3.08	0.98	1.73
	Mean	**41.45**	**92.92**	**150.22**	**5.41**	**14.00**
	CV(%)	**22.96**	**25.52**	**23.00**	**25.94**	**22.90**

Means within a column followed by the same lowercase letter are not significantly different by LSD significance test (0.05). Values are presented as mean ± standard error.

Significant ($p<0.001$) interaction of location x genotype was observed on height at 3 and 6 MAPs and on stem length at all its sampled months (Table 4.13). The highest height was consistently observed with MM060083, reaching 57.1 cm at 3 MAPs and 125.9 cm at 6 MAPs when grown at Mulungu. In contrast, the lowest interaction was noted with IBA961089A, particularly when grown at Kiliba, having 25.7 cm at 3 MAPs and 65.4 cm at 6 MAPs. In terms of stem length, the interactions of Mulungu with Nase14 and MM060083 had the highest, peaking at 8.6 m and 7.7 m at 6 MAPs, then 21.2 m and 19.7 m at 12 MAPs. Lowest stem length resulted in the interaction of Kiliba with IBA961089A with 2.9 m at 6 MAPs and 7.5 m at 12 MAPs.

Table 4.13: Effect Of Eight Location - Genotype Interactions on Plant Height (cm) and Stem Length (m) per Plot, at Different Months after Planting

		Height (cm)			Stem length per plot (m)	
Location	**Genotype**	**3 MAP**	**6MAP**	**12MAP**	**6MAP**	**12MAP**
Mulungu	IBA961089A	36.67 ± 0.68^{d}	91.70 ± 0.50^{d}	148.00a ± 1.56^{a}	4.34 ± 0.13^{d}	11.10 ± 0.27^{c}
	Albert28	47.70 ± 1.22^{b}	113.50 ± 3.04^{ab}	170.00 ± 3.04^{a}	6.49 ± 0.35^{bc}	17.90 ± 0.58^{b}
	MM060083	57.10 ± 1.21^{a}	125.90 ± 2.97^{a}	193.00a ± 2.97^{a}	7.72 ± 0.48^{ab}	19.70 ± 0.60^{ab}
	Nase14	43.51 ± 1.04^{c}	108.60 ± 2.54^{c}	165.00a ± 2.54^{a}	8.57 ± 0.53^{a}	21.20 ± 0.70^{a}
	$LSD_{(0.05)}$	2.49	3.22		1.25	2.2
Kiliba	IBA961089A	25.68 ± 0.23^{d}	65.41 ± 1.25^{d}	122.00 ± 1.25^{a}	2.91 ± 0.11^{c}	7.49 ± 0.31^{d}
	Albert28	43.22 ± 0.95^{a}	86.10 ± 2.23^{a}	142.00 ± 2.23^{a}	4.64 ± 0.19^{a}	11.30 ± 0.29^{b}
	MM060083	40.79 ± 0.93^{ab}	77.72 ± 1.88^{b}	132.00 ± 1.88^{a}	4.33 ± 0.18^{ab}	9.04 ± 0.32^{c}
	Nase14	36.08 ± 0.69^{c}	73.22 ± 1.98^{bc}	130.00 ± 1.98^{a}	4.28 ± 0.29^{b}	14.20 ± 0.60^{a}
	$LSD_{(0.05)}$	3.57	4.5		1.29	1.42
	Mean	**41.45**	**92.92**	**150.22**	**5.41**	**14.00**
	$CV_{(\%)}$	**22.96**	**25.52**	**23.00**	**25.94**	**22.90**

Means within a column followed by the same lowercase letter are not significantly different by LSD significance test (0.05). Values are presented as mean ± standard error.

Additionally**,** significant ($p<0.001$) interaction of location with propagation method was observed on plant height at 3 and 6 MAPs, whereas not at 12 MAPs. This interaction also had significant effect on stem length at 6 MAPs (

Table 4.14). The highest height was consistently observed under Mini cutting- derived plants grown at Mulungu, reaching 65.3 cm at 3 MAPs and 155 cm at 6 MAPs. The lowest height was obtained under local peat –originated plants, grown at Kiliba, at 30.7 cm at 3 MAPs and 55.9 cm at 6 MAPs. Highest increase in stem length was obtained under the interaction between Mulungu and Mini cutting method (12.9 m), while lowest resulted in Kiliba x Local peat interaction (2.1 m).

Table 4.14: Effect of Eight Location - Propagation Method Interactions on Plant Height (cm) and Stem Length (m) per Plot, at Different Months after Planting

Location	Propagation method	Height (cm)			Stem quantity per plot(m)	
		3 MAP	6MAP	12MAP	6MAP	12MAP
Mulungu	KlasmannTS3	41.40 ± 0.40^{bc}	103.00 ± 0.79^{b}	162.00 ± 1.26^{a}	6.96 ± 0.18^{b}	15.30 ± 0.24^{a}
	Vermiculite	43.30 ± 0.83^{b}	106.00 ± 0.78^{b}	16500 ± 1.09^{a}	4.44 ± 0.10^{c}	16.20 ± 0.51^{a}
	Local peat	34.90 ± 0.48^{d}	76.30 ± 0.82^{c}	135.00 ± 1.16^{a}	2.85 ± 0.06^{d}	12.50 ± 0.34^{a}
	Mini cutting (Check)	65.30 ± 1.06^{a}	155.00 ± 2.28^{a}	214.00 ± 2.52^{a}	12.90 ± 0.49^{a}	25.90 ± 0.71^{a}
	$LSD_{(0.05)}$	3.07	3.02		1.48	
Kiliba	KlasmannTS3	32.10 ± 0.46^{c}	69.20 ± 0.47^{b}	125.00 ± 0.47^{a}	3.80 ± 0.08^{b}	8.83 ± 0.20^{a}
	Vermiculite	34.40 ± 0.51^{b}	67.90 ± 0.42^{b}	124.00 ± 0.47^{a}	2.93 ± 0.07^{c}	9.52 ± 0.36^{a}
	Local peat	30.70 ± 0.49^{d}	55.90 ± 0.75^{c}	112.00 ± 0.72^{a}	2.10 ± 0.05^{cd}	7.16 ± 0.20^{a}
	Mini cutting (Check)	49.50 ± 1.10^{a}	109 ±1.53^{a}	165.00 ± 1.52^{a}	7.33 ± 0.20^{a}	16.50 ± 0.50^{a}
	$LSD_{(0.05)}$	1.38	2.72		0.84	
	Mean	**41.45**	**92.92**	**150.22**	**5.41**	**14.00**
	$CV_{(\%)}$	**22.96**	**25.52**	**23.00**	**25.94**	**22.90**

Means within a column followed by the same lowercase letter are not significantly different by LSD significance test (0.05). Values are presented as mean ± standard error.

Finally, there was observed significant interaction of genotype with propagation method on height at 3 and 6 MAPs, and on stem length at 6 and 12 MAPs (

Table 4.15). Overall, interactions of all genotypes produced under the mini-cutting methods resulted in a high height. Notably, highest height resulted in the interaction of MM060083 and Albert28 with mini cutting at 3 MAPs (68.0 cm and 66 cm, respectively), then at 6 MAPs (148 cm and 149 cm, respectively). Lowest heights were obtained in IBA961089A produced under Local peat, with mean of 25.2 cm at 3 MAPs, and 54.8 cm at 6 MAPs. No significant interaction was observed at 12 MAPs for the mentioned parameter. For stem length, high mean values were obtained with all genotypes obtained from Mini cutting check. But highest was under Nase14 x Mini cutting interaction, with 28.9 m at 12 MAPs. Lowest interaction was IBA961089A with local peat substrate, at 5.9 m at 12 MAPs.

Table 4.15: Effect of Sixteen Genotype - Propagation Method Interactions on Plant Height (cm) and Stem Length (m) per Plot, at Different Months after Planting

		Height (cm)			Stem lenght (m)	
Genotype	**Propagation method**	**3 MAP**	**6MAP**	**12MAP**	**6MAP**	**12MAP**
IBA961089A	KlasmannTS3	31.10 ± 0.51^{b}	79.20 ± 2.70^{bc}	135.00 ± 2.70^{a}	4.22 ± 0.21^{ab}	10.20 ± 0.63^{ab}
	Vermiculite	30.30 ± 0.23^{bc}	80.80 ± 2.65^{b}	137.00 ± 2.65^{a}	3.20 ± 0.15^{c}	9.43 ± 0.65^{bc}
	Local peat	25.20 ± 0.31^{d}	54.80 ± 1.80^{d}	111.00 ± 1.80^{a}	1.98 ± 0.17^{d}	5.88 ± 0.44^{d}
	Mini cutting (Check)	38.30 ± 0.43^{a}	99.90 ± 2.63^{a}	156.00 ± 2.63^{a}	5.11 ± 0.21^{a}	11.80 ± 0.55^{a}
	$LSD_{(0.05)}$	1.08	1.82		0.89	1.60
Albert28	KlasmannTS3	41.20 ± 0.09^{b}	90.80 ± 2.50^{b}	147.00 ± 2.50^{a}	5.66 ± 0.35^{b}	13.00 ± 0.50^{b}
	Vermiculite	40.20 ± 0.15^{b}	89.20 ± 2.52^{bc}	146.00 ± 2.52^{a}	4.34c ± 0.20^{c}	12.70 ± 0.91^{bc}
	Local peat	33.90 ± 0.08^{c}	71.20 ± 1.22^{d}	128.00 ± 1.22^{a}	2.59 ± 0.08^{d}	10.90 ± 0.60^{d}
	Mini cutting (Check)	66.40 ± 0.48^{a}	149.00 ± 4.65^{a}	206.00 ± 2.65^{a}	9.68 ± 0.64^{a}	21.60 ± 1.19^{a}
	$LSD_{(0.05)}$	1.35	1.97		0.72	0.76
MM060083	KlasmannTS3	37.70 ± 0.43^{d}	92.90 ± 2.22^{b}	153.00 ± 3.33^{a}	6.17 ± 0.52^{b}	13.60 ± 0.99^{b}
	Vermiculite	49.80 ± 0.15^{b}	90.60 ± 2.10^{c}	151.00 ± 3.20^{a}	3.31 ± 0.14^{c}	10.30 ± 0.92^{cd}
	Local peat	40.30 ± 0.13^{c}	76.70 ± 2.48^{d}	137.00 ± 2.57^{a}	3.00 ± 0.11^{c}	11.20 ± 0.80^{c}
	Mini cutting (Check)	68.00 ± 0.06^{a}	148.00 ± 6.23^{a}	208.00 ± 3.30^{a}	11.60 ± 0.88^{a}	22.50 ± 1.51^{a}
	$LSD_{(0.05)}$	2.31	1.99		0.81	0.90
Nase14	KlasmannTS3	37.00 ± 0.06^{b}	81.60 ± 3.15^{c}	138.00 ± 1.6^{8a}	5.46 ± 0.42^{b}	19.00 ± 0.95^{b}
	Vermiculite	35.20 ± 0.23^{c}	88.20 ± 3.88^{b}	145.00 ± 2.88^{a}	3.90 ± 0.32^{c}	11.50 ± 0.65^{c}
	Local peat	31.80 ± 0.21^{d}	61.70 ± 2.10^{d}	118.00 ± 2.10^{a}	2.33 ± 0.13^{d}	11.40 ± 0.65^{c}
	Mini cutting (Check)	56.90 ± 0.16^{a}	132.00 ± 3.95^{a}	188.00 ± 2.95^{a}	14.00 ± 0.90^{a}	28.90 ± 0.77^{a}
	$LSD_{(0.05)}$	1.63	1.66		0.94	1.12
	Mean	**41.45**	**92.92**	**150.22**	**5.41**	**14.00**
	$CV_{(\%)}$	**22.96**	**25.52**	**23.00**	**25.94**	**22.90**

Means within a column followed by the same lowercase letter are not significantly different by LSD significance test (0.05). Values are presented as mean ± standard error.

4.3 Cost of Producing Cassava Plantlets under SAH System

The inputs equivalent to producing cassava plantlets under single and combined substrates are shown in Table 4.16. The total input for plantlet production, based on total substrate weight, was USD 407.25. Overall, high input costs were observed for substrate sourcing, which represented 30.61% of the total cost. The lowest input cost was observed for fertilizer, representing 1.45% of the total cost. Moreover, the imported substrates (KlasmannTS3 and Vermiculite) incurred higher purchase costs compared to locally sourced peat. Specifically, KlasmannTS3 was the most expensive

substrate, accounting for 20.41% of the total substrate purchase cost, while local peat was the least expensive, with a purchase cost of 0.27%.

However, the combination of these substrates reduced the purchase cost. When Klasmann TS3 was combined with 75% local peat ($K_{25}P_{75}$), the purchase cost was reduced to 1.91%, representing a cost difference of 18.51%. On the other hand, when Vermiculite was combined with local peat, purchase costs were reduced to 0.69% for $V_{25}P_{75}$ and 0.41% for $V_{10}P_{90}$, representing cost differences of 6.21% and 6.50%, respectively. Local peat, when used alone, had a relatively low production cost. Based on the calculations, it was observed that the $K_{25}P_{75}$ blend was the most effective in reducing purchase costs compared to other combinations.

Table 4.16: Production Costs of Items Used for Cassava Propagation under Single Substrates and Their Combinations during 3-Month Subculture Periods in the SAH System, Compared to the Conventional Method, in D.R. Congo, 2021

Category	Item	Unit	Quantity	Unit cost (USD)	Total cost (USD)
A. Substrate	KlasmannTS3	Kg	4.86	12.90	62.69
	Vermiculite	Kg	5.40	1.00	5.40
	Local peat	Kg	7.38	0.15	1.11
	K25P75	Kg	6.73	0.89	5.99
	V25P75	Kg	6.89	0.15	1.01
	V10P90	Kg	7.18	0.13	0.91
	Sub-total 1				**77.11**
B. Freighting of substrate	KlasmannTS3	Kg	4.86	4.21	20.46
	Vermiculite	Kg	5.40	4.21	22.73
	Local peat	Kg	7.38	0.00	0.00
	K25P75	Kg	6.73	0.26	1.77
	V25P75	Kg	6.89	0.26	1.81
	V10P90	Kg	7.18	0.11	0.76
	Sub-total 2				**47.53**
C. Total substrate (A +B)	KlasmannTS3	Kg	4.86		83.15
	Vermiculite	Kg	5.40		28.13
	Local peat	Kg	7.38		1.11
	K25P75	Kg	6.73		7.76
	V25P75	Kg	6.89		2.82
	V10P90	Kg	7.18		1.66
	Sub-total 3				**124.65**
D. General consumable	Box	No	216.00	0.45	97.20
	Tissue paper	Pack	9.00	1.00	9.00
	Hand gloves	Pack	1.00	2.50	2.50
	Permanent marker	Pack	0.50	4.60	2.30
	Morning Fresh	No	1.00	0.50	0.50
	Detergent	liter	1.20	3.50	4.20
	Scalpel blades	Pack	1.00	5.00	5.00
	Sub-total 4				**120.70**
E. Fertilizer	Miracle-Gro				
	Sub-total 5	g	182.52	0.03	**5.89**
F. Labor	Human				
	Sub-total 6		2 persons x 26 days x 3 months	1.00	**156.00**
G. Total SAH cost (Sub- tot3+ Sub- tot4 +Sub- tot5 + Sub-tot6)					**407.24**

The utilization of combined substrates results in a reduction of the unit cost, with $K_{25}P_{75}$ emerging as the most cost-effective option, having the lowest unit cost for producing a cassava plantlet (

Table 4.16). Notably, $K_{25}P_{75}$ demonstrates an equal unit cost to that of the conventional mini stem, both amounting to USD 0.07. The other two combinations also contributed to a reduction in unit cost compared to using single substrates, although the costs remain comparatively higher than the conventional stem.

Table 4.16: Unit Costs of Producing Cassava Plantlets under Three Single Substrates and Their Combinations During 3-Month Subculture Periods in the SAH System, Compared to the Conventional Propagation, in D.R. Congo, 2021

Item/Description	Substrate/ propagation method							
	Unit	KlasmannTS3	Vermiculite	Local peat	K25P75	V25P75	V10P90	Stem
Status		**Imported**	**Imported**	**Local**	**Combined**	**Combined**	**Combined**	**Conventional method**
Substrate purchasse Cost	(USD)	62.69	5.40	1.11	5.99	1.01	0.91	
Substrate freighting cost	(USD)	20.46	22.73	0.00	1.77	1.81	0.76	
General consumable cost	(USD)	20.12	20.12	20.12	20.12	20.12	20.12	
Fertilizer cost	(USD)	0.98	0.98	0.98	0.98	0.98	0.98	
Labor cost	(USD)	26.00	26.00	26.00	26.00	26.00	26.00	
Total production cost	(USD)	130.25	75.24	48.21	54.87	49.93	48.76	
TOTAL	**(USD)**			**407.25**				**2873.85**
Unit production cost	(USD)	**0.16**	**0.17**	**0.11**	**0.07**	**0.09**	**0.10**	**0.07**

CHAPTER FIVE

DISCUSSION

5.1 Performance of Cassava Plantlets Produced under Different Substrates in the SAH System

5.1.1 Performance of Cassava Plantlets Produced under Single Substrates

Survival

The survival rate of cassava plantlets was primarily influenced by the substrate used rather than genotype and substrate interactions. KlasmannTS3 consistently resulted in the highest survival rates, aligning with previous research on cassava (Adesanya et al., 2016; Kajibwami et al., 2018), who achieved the highest. Another study focusing on pineapple (*Ananas comosus*) grown in KlasmannTS3 reported the highest survival rates compared to other substrates, highlighting the positive effect of the SAH substrate (Olagunju et al., 2021). Conversely, vermiculite exhibited poor survival rates due to its physical properties, such as lightness and inconsistent particle sizes, which may lead to weak root support and hindered development (Spomer et al., 1997; Maucieri et al., 2019; Khan et al., 2020), . Despite vermiculite's high water-holding capacity, it may lack the balance between water and root aeration crucial for plant growth, potentially resulting in excessive moisture within the root zone (Khan et al., 2020; Shewa et al., 2020).

Growth

Significant variations were observed among genotypes for all the traits suggesting, genetic variability. MM060083 exhibited exceptional growth characteristics. Genotypes had better grown when grown under KlasmannTS3, known for its suitability and superior properties for cassava propagation (Howeler and Reinhardt, 2014).The lower weight of KlasmannTS3 compared to other substrates of the same volume ensured an adequate supply of nutrient solution to meet the plantlets' requirements. Nutrient management is crucial for successful hydroponic systems, as

emphasized by various studies (Sato et al., 2006; Santiago-Aviles and Light, 2018; Khan et al., 2020). Previous studies on various crops, including cassava (*Manihot esculenta),* yam (*Dioscorea spp*.), lettuce (*Lactuca sativa L.),* brassica (*Brassica olerecea),* and marigold (*Tagetes L*.), have demonstrated rapid growth and favorable performance when cultivated under KlasmannTS3 substrate, as reported by previous studies (Balalic, 2004; Mišković et al., 2009; Adesanya et al., 2016; Maślanka and Magdziarz, 2017; Olugboyega et al., 2019). In contrast, sawdust consistently showed poorer performance. Sawdust's limited nutrient levels likely hinder plantlet growth, particularly in nitrogen (N), phosphorus (P), and potassium (K), crucial for cassava (Byju and Suja, 2020). Several authors emphasized the significance of adequate nutrients for plant growth and development, with the composition of the growth medium being responsible for half of the success in promoting plant growth (Furuta, 1970; Shand, 2007; Ezui et al., 2017). The observed poor plantlet growth under sawdust is consistent with Garner, (2014) and Sanchez et al. (2021), who reported a reduction in plant growth because of low nutrient content in the growth medium. On the other hand, sawdust's gradual nutrient release, typical of wood-based substrates, might lead to reduced cassava plantlet performance (Pennington et al., 2009; Media and Guide, 2021).

Genotypes responded differently to various substrates, emphasizing a significant genotype-substrate interaction. MM060083, grown under KlasmannTS3, consistently showed optimal growth, possibly due to complementary characteristics.

Propagation

Regardless of genotype, KlasmannTS3 substrate facilitated a higher propagation rate, attributed to its rapid plantlet growth and optimal chemical properties. The exceptional properties of the KlasmannTS3, such as optimal pH and high electrical conductivity, significantly contribute to the propagation process. Several authors reported that EC is a vital indicator of nutrient availability for plants, and the medium's suitability is contingent on pH and EC, which are important parameters for optimum growth of soilless crops (Asaduzzaman et al., 2015; Saaid et al., 2015; Khan et al., 2020; Jan et al., 2021; Fussy and Papenbrock, 2022). This combination of properties likely

contributed to faster and more vigorous plantlet growth, leading to taller plants with increased leaf and internode numbers. As a consequence, KlasmannTS3 facilitates the production of a higher number of stem cuttings.

Local peat, despite its higher nitrogen content that could lead to better plant growth (Mburu et al., 2011; Masinde and Agong, 2012), did not perform as well as KlasmannTS3 due to potential antagonistic effects and higher acidity, hindering plant growth (Furuta et al., 1970). Additionally, the dense structure of local peat caused rapid substrate drying, potentially harming plantlet growth. Significant genotype - substrate interactions were observed, with MM060083 grown under KlasmannTS3 resulting in the highest number of cuttings, highlighting complementary characteristics.

Several authors revealed that the nitrogen supplied to the soil had a significant effect on plant growth of *Solanum spp.* and *Zea mays L*. (Mburu et al., 2011; Masinde and Agong, 2012). In this study, despite having a higher nitrogen content, local peat did not perform better compared to KlasmannTS3. This is because nitrogen alone is not sufficient, and the excess nitrogen found in local peat, beyond what is suitable for cassava (Khan et al., 2020), could lead to antagonistic effects (Furuta et al., 1970).

The higher acidity observed in local peat could have also hindered plant growth since pH is a crucial factor in substrate selection (Thomas, 1996; Massignam et al., 2009). Furthermore, pH directly affects nutrient availability in the rhizosphere and nutrient uptake by plants, with macronutrients like nitrogen, potassium, calcium, and magnesium being highly available at a pH of 6.0-6.5 (Sanchez et al., 2021).

Moreover, the local peat received a lesser amount of nutrient solution compared to KlasmannTS3 due to differences in weight. Furthermore, the dense structure of the local peat caused rapid substrate drying between watering intervals, resulting in drainage and cutting off the oxygen supply, which could potentially harm the growth of plantlets. These findings align with those of several authors, who emphasized the importance of a hydroponic medium providing good structure and stability, high water holding capacity, and sufficient root aeration for successful plant growth (Jones, 1982; Michel, 2010; Santiago-Aviles and Light, 2018; Shewa et al., 2020). Significant

genotype x substrate interaction was observed, where highest number of cuttings was obtained under MM060083 grown under KlasmannTS3, likely because of their complementary characteristics.

5.1.2 Performance of Cassava Plantlets Produced under Combined Substrates

Survival

The survival rate of cassava plantlets was predominantly influenced by the substrate used rather than the genotype or genotype-substrate interactions. The combination of KlasmannTS3 and local peat ($K_{25}P_{75}$) resulted in the highest survival rates, comparable to KlasmannTS3 alone. This suggests that KlasmannTS3's superior physical and chemical properties influenced the survival rate similarly in both scenarios. Conversely, the combinations of vermiculite and local peat (V_{25}P75 and $V_{10}P_{90}$) increased survival rates compared to their individual uses. Similar findings from previous studies support the idea that combining specific substrates enhances plant survival (Suarez et al., 2020; Abishay et al., 2023).

Growth

As observed in experiment 1, plantlet growth performance was genotype-dependent across SAH subcultures. IBA961089A consistently had better performance in terms of plantlet growth and the number of cuttings. This suggested that genotype IBA961089A possesses genetic traits that contribute to the development of superior growth characteristics in the SAH system.

The use of combined substrates generally improved plantlet performance compared to single substrates. $K_{25}P_{75}$ resulted in the highest performance comparable to single KlasmannTS3. This increase in performance resulted in the 25% contribution of KlasmannTS3 improving the mixture's properties. On the other hand, the combination of vermiculite and local peat ($V_{25}P_{75}$ and $V_{10}P_{90}$) increased plantlets' performance compared to vermiculite and local peat used alone. These findings are consistent with previous research indicating the benefits of combining substrates to enhance plant

performance (Al-Ajmi et al., 2009; Aklibasinda et al., 2011; Wisdom et al., 2017; Zeljković et al., 2021; Hunlawmawmi and Deven 2022).

A significant genotype - substrate interaction was observed. IBA961089A consistently had superior growth under $K_{25}P_{75}$, which was similar under KlasmannTS3. Conversely, the lowest growth performance was consistently observed across all genotypes when grown on a combination of local peat and vermiculite substrates. These outcomes suggest that the compatibility between IBA961089A and $K_{25}P_{75}$ or KlasmannTS3 significantly influenced the observed growth performance.

Propagation

Cutting multiplication rates under blended substrates were higher than under single substrates, regardless of genotype. KlasmannTS3 and the combination with local peat ($K_{25}P_{75}$) produced more cuttings, attributed to faster plantlet growth, which determines whether or not the stem can be used as a cutting. However, these increases were lower than reported in previous studies (Pelemo et al., 2019; Ceballos et al., 2020), possibly due to various factors such as crop, substrate, lighting, and temperature. Nitrogen content in substrates did not consistently correlate with plantlet growth or cutting production, particularly when comparing local peat to KlasmannTS3, despite the fact that nitrogen is an essential constituent of chlorophyll for plant growth (Croft et al., 2017; Li et al., 2018; Parkinson and Allen, 1975; Li et al., 2018).

Similarly, vermiculite had the highest exchangeable K and Mg contents, it produced fewer cuttings than KlasmannTS3 and $K_{25}P_{75,}$ which had a low content of the two exchangeable cations. Although basal nutrients in the substrates should feed the plantlets, the external weekly nutrient supplement played an important role. However, there is an unclear relationship between plantlet growth and the individual physical or chemical characteristics of the substrate. Differences among substrates may also have resulted from other characteristics or other nutrients not analyzed in this study. For example, KlasmannTS3 may contain other nutrients or hormones that contribute to promoting plant growth. Authors reported that commercial formulations can contain a relative amount of fertilizers or hormones to promote plant growth (Baudoin et al., 2013; Savvas and Gruda, 2018; Khalaj et al., 2022; Bar-tal et al., 2019). The best

interaction was observed between IBA961089A and $K_{25}P_{75}$ or KlasmannTS3, resulting in higher cutting numbers, while the combination of local peat and vermiculite consistently showed the lowest numbers of cuttings.

5.2 Performance of Selected Cassava Genotype Plantlets Produced Using SAH Substrates in Different Locations

Survival

The study found that the genotype MM060083, as well as the mini-cutting-originated plants, had the highest survival rate across locations, with the highest observed at Kiliba. Conversely, Nase14 had consistently the lowest survival rate, particularly at Mulungu. Kiliba's high survival rate aligns with findings from previous studies (Egesi et al., 2007; Tumuhimbise et al., 2014), underscoring cassava's adaptability to thrive in drought-prone areas under marginal conditions, where other crops may struggle. Moreover, Kiliba’s weather conditions (limited rainfall) could have benefited the tender plantlets at planting time and during the first month.

However, Kiliba's success should be interpreted with caution due to a notable challenge at Mulungu. Shortly after transplantation, Mulungu experienced a cutworm attack that particularly affected laboratory-sourced plantlets, damaging the young and tender stems at the collar level. As the plants matured and the stems hardened, the impact of the attack diminished.

The observed differences in survival rates among genotypes suggest inherent variations in their adaptability and resilience. MM060083's highest survival rate may indicate favorable traits or resistance to environmental stressors, while Nase14's lower survival rate might imply susceptibility to specific field challenges. These results align with the genetic characteristics of cassava; despite the plant's generally broad adaptability to diverse environmental conditions, many genotypes demonstrate limited adaptability (Tumuhimbise et al., 2014; Bakare et al., 2022).

On the other hand, mini-cuttings exhibited the highest survival rate, particularly at Kiliba, suggesting favorable weather conditions that could have benefited the tender

plantlets at planting time and during the first month. Moreover, mini-cutting-derived plants had the highest survival rate compared to all SAH-derived plants, attributed to their prior acclimatization compared to laboratory-derived plants. However, among the SAH methods, KlasmannTS3-derived plants showed higher survival rates than those from vermiculite and local peat, suggesting potential hormonal benefits from KlasmannTS3 that aided in better plant establishment. The differences in the physical structures of vermiculite and local peat may have hindered root establishment after transplanting.

Growth and stem length

The location significantly influenced all growth parameters and stem length, with the highest obtained at Mulungu. Nase14 consistently exhibited the highest number of leaves, nodes, and stems, while IBA96108A displayed the lowest, particularly at the Mulungu location. MM060083 consistently had the tallest plants, particularly at Mulungu, while IBA961089A consistently had the shortest plants. Nase14 also consistently exhibited higher stem length across locations, but the highest was observed at Mulungu, while IBA961089A had the lowest. On the other hand, tallness in MM060083 did not correlate with higher leaf or node numbers, consistent with findings on other cassava genotypes where plant height showed no significant correlation with leaf or node count along the stem (Egesi et al., 2007). Conversely, the higher leaf and node numbers observed in Nase14 align with its stem morphology, which is characterized by short internodes. On the other hand, the highest stem number observed under Nase14 corresponds to instances where secondary stems sprout around the main stem, influenced by the node capacity. Despite uniform growth conditions within a location, genotypes responded differently. Genetic diversity, which is influenced by factors such as heritability, could have contributed to the observed differences in plant traits (Ceballos et al., 2020).

The study found that the Mini cutting-derived plants consistently exhibited superior growth traits and stem length across locations; however, the highest was obtained at Mulungu. However, SAH methods caught up with Mini cuttings at 12 months after planting in terms of stem length.

Among the significant interactions observed, Mulungu * Nase14, Mulungu * Mini Cutting, and Nase14* Mini Cutting exhibited the highest stem lengths. This could be a result of compatible genetic traits or favorable growth conditions created by each of the factors. Conversely, the lowest stem lengths were recorded in interactions involving Kiliba *IBA961089A, Kiliba *local peat, and IBA961089A *local peat. This suggests challenges or limitations, potentially due to incompatible genetic characteristics or unfavorable conditions.

The superior agronomic performance at Mulungu indicates the substantial impact of location characteristics on cassava genotype performance. Variations in soil composition, nutrient levels (especially nitrogen), temperature, rainfall, and altitude between Mulungu and Kiliba played a pivotal role in shaping the growth of cassava plants (IITA, 1990; Spencer and Ezedinma, 2017). Mulungu's soil texture and higher nitrogen levels, essential for early-stage plant growth, contributed to faster and more robust development (Fageria and Moreira, 2011). Additionally, Mulungu received significantly higher rainfall compared to Kiliba, up to 6 MAPs, which is crucial for optimal plant growth (Silva and Uchida, 2000). Recent improvements in rainfall at Kiliba over the past six months have also positively impacted growth performance. Location trials in plant breeding programs are vital for accurate genotype evaluation and selection, acknowledging the inherent heterogeneity across environments, which enhances the reliability of genotype assessments (Egesi et al., 2007; Tumuhimbise et al., 2014; Bakare et al., 2022). Despite tallness in MM060083, it did not correlate with higher leaf or node numbers, consistent with findings that plant height shows no significant correlation with leaf or node count along the stem (Egesi et al., 2007) Conversely, the higher leaf and node numbers observed in Nase14 align with its stem morphology, which is characterized by short internodes. The highest stem number in Nase14 corresponds to secondary stems sprouting around the main stem, influenced by node capacity.

5.3 Cost of Producing Cassava Plantlets under Different Substrates in Comparison to the Conventional Cutting Method

The cost analysis revealed that imported substrates such as KlasmannTS3 and Vermiculite used alone incurred higher production and unit costs compared to locally sourced substrates like local peat in the SAH system. The additional expenses associated with imported substrates, including shipping, customs duties, and handling charges, contributed to these cost differences. Conversely, local peat, being readily available and requiring minimal processing, was more affordable due to the elimination of importation costs and simpler preparation processes. These findings align with previous research (Al-Ajmi et al., 2009), which emphasized the high production costs associated with imported substrates compared to locally sourced ones.

Moreover, single SAH substrates, whether imported or locally sourced, resulted in higher production and unit costs compared to the conventional mini-cutting method. The unit costs of single substrates ranged from USD 0.11 to USD 0.17, representing two times the unit cost of mini-cutting (USD 0.007). This difference in costs can be attributed to the controlled conditions and expensive inputs required in the SAH system, whereas the conventional method, with uncontrolled conditions, reduces costs and yields a higher number of cuttings.

The unit costs obtained under SAH substrates in this study differed from with Bentley et al. (2020), who reported a lower unit cost of USD 0.1 for SAH plantlets. These variations could stem from differences in local conditions, substrate sourcing, production processes, or other specific parameters considered in each study. Additionally, variations in cost structures, methodologies, and timeframes could contribute to the differences in results.

Furthermore, using combined substrates led to a reduction in both production and unit costs compared to using them individually. The most significant reduction in production costs was observed with the $K_{25}P_{75}$ combination, where substituting 75% of expensive imported KlasmannTS3 with locally sourced and low-cost local peat resulted in a considerable cost reduction of 18.51%. This combination achieved an

equivalent unit cost to that of the conventional mini stem, both amounting to USD 0.07. Similarly, combinations like $V_{25}P_{75}$ and $V_{10}P_{90}$ also exhibited cost reductions compared to using vermiculite alone, proportional to the percentage of substrate substitution in the combination. These findings support the cost-effectiveness of combining substrates, especially when utilizing locally sourced media, as affirmed by Al-Ajmi et al. (2009). The strategic combination of $K_{25}P_{75}$ not only reduced costs but also resulted in a high number of plantlets.

Additionally, the quantity of plantlets produced influenced the unit cost, as seen when comparing imported substrates like KlasmannTS3 and Vermiculite. Despite incurring higher production costs, KlasmannTS3 resulted in a greater number of plantlets, contributing to a lower unit cost compared to vermiculite.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research evaluated the effect of substrates on genotype performance under the SAH system in the laboratory. Findings revealed that genotypes performed differently depending on the substrate. The highest survival rates and cutting numbers were obtained with the $K_{25}P_{75}$ blend and single KlasmannTS3 substrate, particularly for the MM060083 and IBA961089A genotypes, achieving a propagation ratio of 1:4 in three months.

Through a field experiment evaluating the performance of SAH-derived plantlets in comparison to conventional mini-cuttings, this study revealed that the highest survival rate was observed at the Kiliba location, particularly with the MM060083 genotype obtained using the mini-cutting method. The greatest stem length was recorded at INERA Mulungu, in the Nase14 genotype, also produced by the mini-cutting method. However, all the SAH-derived plants eventually caught up with the performance of the conventional method over time across location, indicating their potential adaptability, efficiency, and scalability.

Finally, a basic cost analysis revealed that blended substrates had lower unit costs compared to all single substrates. Specifically, the $K_{25}P_{75}$ blend was the most cost-effective, matching the conventional stem unit cost of USD 0.07. This demonstrates the advantage of combining substrates and using locally sourced materials.

6.2 Recommendations

a. K_{25}P75 should be adopted as the best-performing substrate combination. Policies should support its use for efficient cassava-plantlet production. The seed system should incorporate Klasmann with local peat (K_{25}P75) as a standard substrate for producing high-quality planting materials.
b. The seed system should incorporate KlasmannTS3 as a standard substrate recommended for high-quality planting materials under field conditions.

Further research should focus on identifying the specific genetic traits responsible for Nase14's exceptional stem length. Farmers are encouraged to use KlasmannTS3 as a substrate for cassava planting material production, contributing to enhanced crop establishment and yield, and to select suitable locations for stem production.

c. Policymakers should support the use of $K_{25}P_{75}$ as a cost-effective substrate for planting material production. Research should also focus on developing other cost-effective combinations using local substrates.

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APPENDICES

Appendix I: Monthly Temperature (°C) and Rainfall (mm) Data during Cassava Plant Growth at Mulungu and Kiliba Locations, in 2022

	Temperature (^{o}C)		Rainfall (mm)	
Month	**Mulungu**	**Kiliba**	**Mulungu**	**Kiliba**
January	20.75	28.14	177.84	145.25
February	21.49	30.11	199.06	165.37
March	21.21	29.12	233.16	197.69
April	20.57	29.12	228.02	192.82
May	19.99	28.14	172.75	140.43
June	19.94	28.14	113.77	90.54
July	20.61	30.11	58.09	65.74
August	21.8	30.11	73.74	75.58
September	22.16	30.11	131.02	133.56
October	21.47	29.12	260.27	274.35
November	20.68	29.12	279.23	284.03
December	20.3	30.11	275.48	276.34

Source, INERA Mulungu, 2021-2022

Appendix II: Detection (Presence/Absence) of CBSV in the Selected Genotypes Grown under Different Substrates during the 3 Subcultures under the SAH System before Field Transplanting

IITA Transforming African Agriculture	**IITA-Kalambo PLANT PATHOLOGY LABORATORY REPORT**	**Date: 08-12-2022**
Project requestor: PICAGL	**Report to:** Binzunga Mamy	**Contact:** Tel +243 994 717 417 E-mail: C.Casinga@cgiar.org / **N°:** 003

SUBCULTURE	VARIETY X SUBSTRATE	CODE	DYE	Cq (ΔRn)	VIRUS STRAIN	DECISION
1	MM060083 VERMICULITE	1 MV24	No fluorescence	No Cq	No Virus	No Infection
1	Albert28 VERMICULITE	1 AV25	No fluorescence	No Cq	No Virus	No Infection
1	IBA961089A VERMICULITE	1 9V26	No fluorescence	No Cq	No Virus	No Infection
1	NASE14 VERMICULITE	1 NV36	No fluorescence	No Cq	No Virus	No Infection
1	MM060083 KLASMANNTS3	1 MK28	No fluorescence	No Cq	No Virus	No Infection
1	Albert28 KLASMANNTS3	1 AK27	No fluorescence	No Cq	No Virus	No Infection
1	IBA961089A KLASMANNTS3	1 9K34	No fluorescence	No Cq	No Virus	No Infection
1	NASE14 KLASMANNTS3	1 NK33	No fluorescence	No Cq	No Virus	No Infection
1	MM060083 LOCAL PEAT	1 MP31	No fluorescence	No Cq	No Virus	No Infection
1	ALBERT28 LOCAL PEAT	1 AP29	No fluorescence	No Cq	No Virus	No Infection
1	IBA961089A LOCAL PEAT	1 9P32	No fluorescence	No Cq	No Virus	No Infection
1	NASE14 LOCAL PEAT	1 NP30	No fluorescence	No Cq	No Virus	No Infection
2	MM060083 VERMICULITE	2 MV35	No fluorescence	No Cq	No Virus	No Infection

2	Albert28 VERMICULITE	2 AP8	No fluorescence	No Cq	No Virus	No Infection
2	IBA961089A VERMICULITE	2 9V6	No fluorescence	No Cq	No Virus	No Infection
2	NASE14 VERMICULITE	2 NV1	No fluorescence	No Cq	No Virus	No Infection
2	MM060083 KLASMANNTS3	2 MK7	No fluorescence	No Cq	No Virus	No Infection
2	Albert28 KLASMANNTS3	2 AK10	No fluorescence	No Cq	No Virus	No Infection
2	IBA961089A KLASMANNTS3	2 9K2	No fluorescence	No Cq	No Virus	No Infection
2	NASE14 KLASMANNTS3	2 NK3	No fluorescence	No Cq	No Virus	No Infection
2	MM060083 LOCAL PEAT	2 MP11	No fluorescence	No Cq	No Virus	No Infection
2	Albert28 LOCAL PEAT	2 AV5	No fluorescence	No Cq	No Virus	No Infection
2	IBA961089A LOCAL PEAT	2 AP9	No fluorescence	No Cq	No Virus	No Infection
2	NASE14 LOCAL PEAT	2 NP4	No fluorescence	No Cq	No Virus	No Infection
3	MM060083 VERMICULITE	3 MV37	No fluorescence	No Cq	No Virus	No Infection
3	Albert28 VERMICULITE	3 AV17	No fluorescence	No Cq	No Virus	No Infection
3	IBA961089A VERMICULITE	3 9V16	No fluorescence	No Cq	No Virus	No Infection
3	NASE14 VERMICULITE	3 NV21	No fluorescence	No Cq	No Virus	No Infection
3	MM060083 KLASMANNTS3	3 MK15	No fluorescence	No Cq	No Virus	No Infection
3	Albert28 KLASMANNTS3	3 AK14	No fluorescence	No Cq	No Virus	No Infection
3	IBA961089A KLASMANNTS3	3 9K20	No fluorescence	No Cq	No Virus	No Infection
3	NASE14 KLASMANNTS3	3 NP13	No fluorescence	No Cq	No Virus	No Infection
3	MM060083 LOCAL PEAT	3 MV39	No fluorescence	No Cq	No Virus	No Infection
3	Albert28 LOCAL PEAT	3 AP19	No fluorescence	No Cq	No Virus	No Infection

3	IBA961089A LOCAL PEAT	3 9P22	No fluorescence	No Cq	No Virus	No Infection
3	NASE14 LOCAL PEAT	3 NK18	No fluorescence	No Cq	No Virus	No Infection

PRIMERS: Specific primers [CBSV CP-F: GCCAACTARAACTCGAAGTCCATT & CBSV CP-R: TTCAGTTGTTTAAGCAGTTCGTTCA and UCBSV CP-Frev: AGATYAAGAARACDTTCAAGCCTCCAA & UCBSV CP-R: AATTACATCAGGRGTTAGRTTCCCTT] targeting capsid proteins of CBSV and UCBSV viruses were used to amplify the amplicons in order to be used as a standard template in absolute quantification assays.

CONCLUSION: Finally, the results of the Real-Time PCR test show that the treatments (genotypes x Substrates) under multiplication in the SAH laboratory are free of diseases.

Appendix III: Cutworms Damaging Young Stem of Cassava Plantlets after Transplanting under Field at Mulungu Location

Appendix IV: List of Publications and Conference Presentation

4.1. Peer-reviewed Article

Binzunga M. M, Kintche K, Mouritala S, Kajibwami A, Aggrey BN. Performances of plantlets from selected cassava (Manihot esculenta Crantz) genotypes under Semi - Autotrophic Hydroponics (SAH) using different substrates. Jagst. 2023;22(6):66–89. https://doi.org/10.4314/jagst.v23i6.5.

4.2. Under Review Article

Mamy Makumbu Binzunga, Kintche Kokou, Sikirou Mouritala, Adetoro Najimu, Dieng Ibnou, Kajibwami Angelique, Jacob Mignouna, and Aggrey Bernard Nyende. An alternative Semi-Autotropic Hydroponics (SAH) substrate for cassava rapid propagation in the Democratic Republic of Congo: A first study case. Mamy Makumbu Binzunga, Kintche Kokou, Sikirou Mouritala, Adetoro Najimu, Dieng Ibnou, Kajibwami Angelique, Jacob Mignouna, and Aggrey Bernard Nyende (Submited to *PLOS ONE journal*, January 2024).

4.3. Conference Participant

The 17th JKUAT scientific , technological and industrialization conference. From 23 to 24 March 2023, in Kenya.