ORIGINAL RESEARCH ARTICLE

Performances of plantlets from selected cassava (Manihot esculenta Crantz) genotypes under Semi - Autotrophic Hydroponics (SAH) using different substrates

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ABSTRACT

The cassava seed system faces challenges due to limited seed stock caused by a slow propagation rate and a lengthy growth period. Conventional methods lacking phytosanitary guarantees further compound these issues. To address these limitations, the Semi-Autotrophic Hydroponics (SAH) technology was adopted for the rapid mass propagation of healthy, disease-free cassava plants. This research aimed to evaluate the performance of cassava planting materials using different substrates for stem-cutting multiplication in the laboratory at the IITA Kalambo research station in DR Congo. The experiment followed a split-plot design of five replications of three consecutive sub-culture periods lasting four weeks each. Four different genotypes: IB961089A, MM060083, Nase14, and Albert28 as the main plots, with four different substrates: KlasmannTS3, Vermiculite, Local Peat, and Sawdust as the sub-plots, were laid out. Cuttings were placed in 500-ml substrate-filled boxes and watered weekly with a 100-ml Miracle-Gro solution. Data were collected on survival, height, leaf and internode numbers, and cutting numbers at the end of each subculture period. The data were analysed using ANOVA in R software. The Fisher's Least Significant Difference (LSD) test was utilised to separate means when significant differences among treatments were present ($p < 0.05$). The results showed that survival was primarily influenced by the substrate used, with KlasmannTS3 demonstrating the highest rate, exceeding 90%. Significant differences ($p < 0.05$) among genotypes for survival rate and performance parameters mentioned above ($p < 0.001$) were observed. Similarly, there were significant differences ($p < 0.001$) among substrates for survival rate and performance parameters. Furthermore, the interaction between genotype and substrate significantly ($p < 0.001$) affected performance parameters. MM060083 performed the best across all traits. KlasmannTS3 was the superior substrate and had the highest average cutting number regardless of genotype, with a notable increase of 292% from 20 to 58.4 cuttings, representing a ratio of 1:3 within three months. Sawdust had the lowest multiplication rate, with a 5% decrease. The

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superior performance of KlasmannTS3 was attributed to its rapid growth and favourable properties. The interaction between MM060083 and KlasmannTS3 consistently showed the highest number of cuttings (70.4). Sawdust consistently showed poor growth performance, regardless of genotype. The study concludes that the SAH offers the potential for rapid multiplication of disease-free cassava planting materials in reduced space and time.

Keywords: Cassava genotypes, plantlets, growth performance, stem cuttings, multiplication, substrate, Semi- Autotrophic Hydroponics (SAH).

1. 0. Introduction

Cassava (*Manihot esculenta* Crantz) is the third most important tropical food crop, which is grown in 40 of the 53 countries in Sub-Saharan Africa and accounts for 61% of global production(FAOSTAT, 2020; Spencer and Ezedinma, 2017). In DR Congo, cassava accounts for more than half of the annual crop area, and regularly, more than 70% of the population consumes its roots and about 80% of the population consumes its leaves (Mahungu et al., 2014).With production of more than 40 million metric tonnes per year, DR Congo is the second-largest cassava producer after Nigeria. This constitutes a significant source of income for farmers compared to other food crops(FAOSTAT, 2020;Mahungu et al., 2022). The last decade since the year 2000 has witnessed research programmes on cassava in DR Congo grow through various organisations' contributions, including national and international institutes, with the participation of community-based organisations (CBOs), federated farmer associations, and village-level farmer groups. These platforms have promoted the development of disease-resistant and high-yielding varieties with farmers' preferred traits(Rusike et al., 2014). This has resulted in an annual increase in production from 14.93 million metric tonnes in 2002 to 15.02 million metric tonnes in 2011 (Minagrider, 2013).

Several authors reported slow dissemination of improved cassava varieties across the country per year, occasioned by a lack of planting materials (Ganza *et al.*, 2019;Mubalama *et al.*, 2019;Ndjadi *et al.*, 2017). The main reason for the unavailability of planting materials is the slow propagation rate of cassava and its relatively longer cropping cycle compared to cereals. This is associated with a high production cost of basic seeds, high perishability, high handling, and transportation costs, as well as the inconvenient weight and size of the materials (Escobar*et al.*, 2006). Alternatively, the tissue culture technique (TC) for mass propagation of disease-free plantlets (Ceballos & Hershey, 2017) is also expensive at $1.00 per plantlet(Bentley et al., 2020).The high cost of producing basic seeds creates challenges for users who cannot afford to purchase certified seeds (Escobar et al., 2006). The traditional methods of seed propagation contribute to the spread of pests and diseases (Baudoin *et al.*, 2013; Santana *et al.*, 2009).Thus, farmers continue to cultivate cassava varieties using poor-quality planting materials, which contribute to the spread of diseases and result in low yields of less than 8.8 tonnes per hectare and lower incomes (Mbwika et al., 1999;Sanginga and Mbabu, 2015; Scott, 2021). The Semi-Autotrophic Hydroponics (SAH) technology, which was developed by SAHTECHNO Ltd., Argentina (Bentley et al., 2020), for the production of potato seeds, was adopted in 2016 for cassava propagation by the International Institute of Tropical Agriculture (IITA) in Nigeria. The

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technique focuses on the mass propagation of virus-free plants of tissue culture origin under an organic substrate (Thiele et al., 2022); (Adesanya et al., 2016).The technique was then introduced in the DR Congo in 2018 (Kajibwami et al., 2018)and in other countries in Africa as well (Bentley et al., 2020). The technology is a low-cost novel technique with a large potential for seedling production in space and over time with a $ 0.10 cost per plantlet (Bentley et al., 2020). Furthermore, the SAH is easy to adapt to improve the stem multiplication rate in breeding programmes and for commercial seed production for clonal crops such as cassava and yam (*Dioscorea spp.*) (Thiele *et al.*, 2022; Bentley *et al.*, 2020;Ceballos et al., 2020;(Olugboyega et al., 2019)(Pelemo et al., 2019)). The benefits of the SAH technology over other techniques are its high multiplication ratio and the ability to propagate true-to-type and pathogen-free cassava plantlets (Thiele et al., 2022). Costs for producing cassava planting materials are also lower when using SAH as compared to tissue culture (Bentley et al., 2020). However, the good commercial substrate (KlasmannTS3) used in the SAH is imported. The use of different media has been largely reported in hydroponics production, such as vermiculite and sawdust (Barbosa et al., 2022)Mayo-Prieto *et al.*, 2020; Lin *et al.*, 2017;Kumar and Singh, 2023; (Bhattacharjya et al., 2014)Jan *et al.*, 2021). On the other hand, the use of affordable substrates has been recognised as one of the most common options for lowering production costs(Sachin *et al.*, 2020) . Low-cost substrates can be used by breeders, particularly in the National Programs, as well as small-scale entrepreneurs in African conditions. Consequently, this study aimed to assess the growth performance and stem-cutting multiplication rates of different cassava genotypes produced using different substrates under laboratory conditions.

2.0. Materials and methods

2.1. Study location

The experiment was carried out between October and December 2021 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 DR Congo (S 2°23’50’’, E 28°50'42'', and 1,488 m.a.s.l).

2.2. Source and description of study materials

Each of the four genotypes originated from 4-week-old mother plantlets produced from tissue culture plantlets using the common substrate (KlasmannTS3).

Four improved genotypes were used in this study, comprising two introduced clones (IBA961089A and MM060083) under evaluation at the IITA Kalambo station and two released varieties (Nase 14 and Albert 28) grown by farmers. 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.

2.3. Substrate preparation

Four different substrates were used for performance evaluation (Figure 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 DR Congo local materials, such

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as local peat and sawdust. Local peat was collected from the 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). Local peat was then sterilised 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 of volume was put into a transparent-light box of 15 cm x 15 cm x 9 cm.

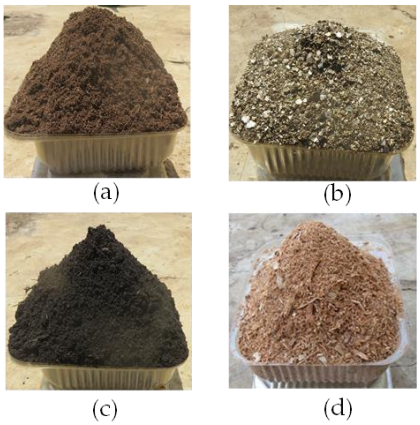

Figure 1. Four substrates used in the evaluation of the performance of cassava plantlet production under the SAH system: (a) KlasmannTS3; (b) Vermiculite; (c) Local peat; and (d) Sawdust.

2.4. Experimental design

The experimental design was a split plot based on a randomised complete block design (RCBD) replicated five times (Figure 2). The main plot consisted of four levels of genotypes: IBA961089A, MM060083, Albert28, and Nase14. The subplot involved four levels of substrates: KlasmannTS3, vermiculite, local peat, and sawdust.

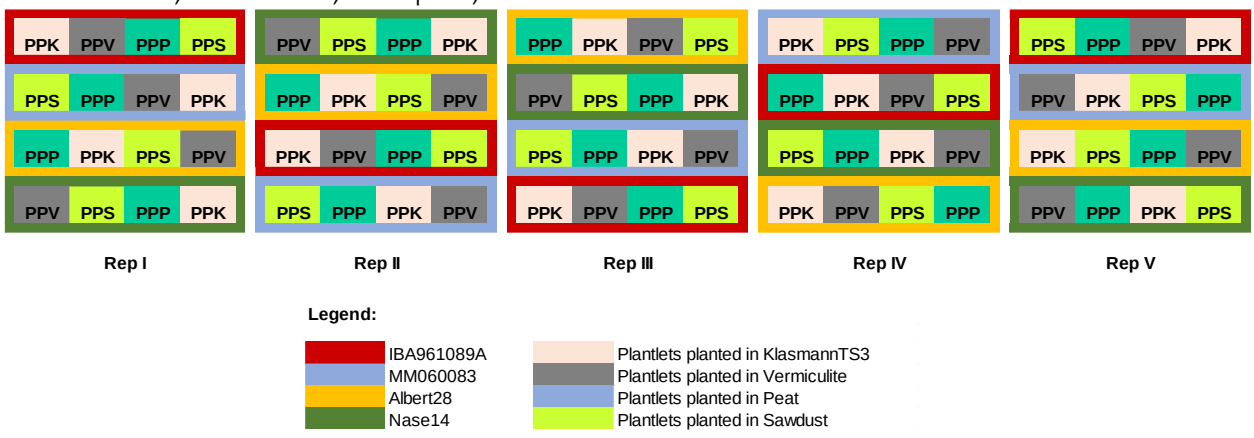

Figure 2. Split-plot experimental design layout, employing a randomized complete block design in five replicates with genotype as main factor and substrate as the sub-factor.

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2.5. Sub-culture cutting production

Figure 3 illustrates the hierarchical transplanting and subculturing processes in our study. The flow chart depicts the sequential steps of transplanting the plantlets from one subculture period to the next, following the experimental design described in Section 2.4. Each genotype (IBA961089A, MM060083, Albert28, and Nase14) was propagated on different substrates (Klasmann TS3, 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 (section 2.2). Subculture 1 involved planting cuttings obtained from the mother plantlets. In Subculture 2, all cuttings produced by a genotype's plantlets at the end of Subculture 1 were transplanted and grown into the corresponding four substrates. Subculture 3 was established by transplanting all cuttings produced from a specific genotype and substrate in Subcultures 1 and 2 into the respective four substrates.

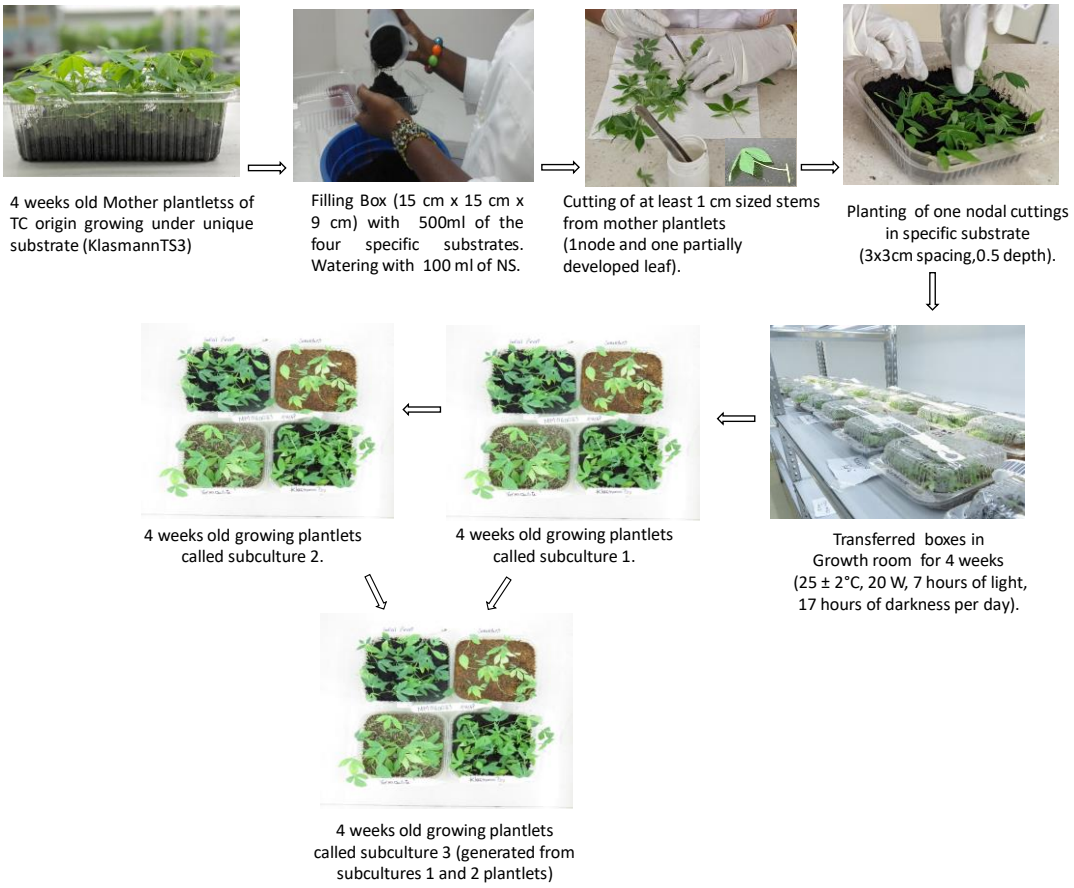

Figure 3. Subculture process for cassava cutting propagation under four substrates in the SAH System, starting from mother plantlets of tissue culture origin. Each subculture lasted four weeks.

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Each transplanted cutting was at least 1 cm in length. Transplanting of the cuttings during all the subculture periods involved inserting a 0.5 cm-long cutting portion with one node and one partially developed leaf into the corresponding four substrates (Figure 4a). During subculture 1, 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 2, when the cuttings obtained from the plantlets of Subculture 1 exceeded 20, additional boxes with the specific substrate were used. For Subculture 3, when the cuttings obtained from the combination of Subculture 1 and Subculture 2 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 7 hours of light and 17 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 (Figure 4b). 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 characterised by 3 to 8 expanded leaves.

(a) (b)

Figure 4. (a) One nodal stem cutting of cassava used for planting, (b) Cassava plantlets (4 weeks old) growing under the SAH system for subsequent subcultures in the laboratory.

2.6. Substrate analysis

The pH and the electrical conductivity (EC) of the substrates were determined using the electrometric method (Bray & Kurtz, 1945). The total nitrogen was determined using the Kjeldahl digestion method(Simard & 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 & Kurtz, 1945).

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The nutrient analysis of substrates revealed that they were highly different from each other (Table 1). 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 the 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 averaged 200 and 205 g, respectively, which were higher than 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 1. Chemical characteristics and nutrient concentration analysis of the four substrates used to produce the plantlets under the SAH.

Substrate	Weight of 500 ml (g)	pH (H_2O)	Total N (g kg^{-1})	Exch. K (g kg^{-1})	Exch. Ca (g kg^{-1})	Exch. Mg (g kg^{-1})	Av. P (g kg^{-1})	CEC (cmol kg_{-1})	EC (µS cm_{-1})	NS (ml) per g of substrate
KlasmannTS3	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
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
Vermiculite	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
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 parenthesis 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.

2.6.1 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.

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

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

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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).

2.6.2 Data analysis

The data were analysed for each subculture period using the statistical analysis software R version 4.2.1 (R Core Team, 2022)(R Core Team, 2022). A two-way analysis of variance (ANOVA) was used as the statistical analysis. Tests of significance were reported at the 0.05, 0.01, and 0.001 levels. Genotype, substrate, and their interactions were considered fixed effects. When the interaction between genotype and substrate was significant, further one-way ONOVA 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 3.

3.0. Results

3.1. Plantlet survival rate

Survival rates of cassava genotypes were found to differ significantly ($p<0.05$) only in subculture 2 (Table 2A). The highest survival rate was observed in Nase14 (89.5%) and in IBA961089A (88.3%). Conversely, the lowest survival rate was observed in Albert 28 (83.9%). Substrate significantly ($p<0.001$) affected the survival rate of plantlets across subcultures (Table 2B). In subculture 1, KlasmannTS3 exhibited the highest survival rate (92%), whereas Vermiculite showed the lowest rate (69.8%). In subculture 2, 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 plantlet survival rates. In subculture 3, KlasmannTS3 maintained the highest survival rate (92.8%), while Vermiculite still recorded the lowest (75.8%). No significant interaction between genotype and substrate was observed in any of the subcultures.

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Table 2. Mean survival rate (%) of four cassava genotypes and four substrates at the end of three subculture periods under SAH system.

			Survival rate (%)		
			Subculture 1	Subculture 2	Subculture 3
	Genotype				
	IBA961089A		82.25[a]	88.30[a]	79.59[a]
	Albert28		79.75[a]	83.91[b]	87.63[a]
A	MM060083		83.50[a]	87.19[ab]	82.89[a]
	Nase14		79.50[a]	89.51[a]	89.78[a]
		Mean	81.25	87.22	84.97
		$LSD_{(0.05)}$	-7.34	3.96	-14.46
		CV(%)	13.09	6.60	21.39
		ANOVA	NS	*	NS
	Substrate				
	KlasmannTS3		92.00[a]	93.63[a]	92.84[a]
B	Vermiculite		69.75[c]	82.53[b]	75.83[a]
	Local Peat		82.75[b]	86.34[ab]	86.25[ab]
	Sawdust		80.50[b]	86.39[a]	(-)
		Mean	81.25	87.22	84.97
		$LSD_{(0.05)}$	4.50	7.38	13.61
		CV(%)	8.70	13.31	24.86
		ANOVA	***	*	*

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 3 because cuttings to be transplanted could not be obtained in Subculture 2. Significant codes: NS: No significant; * 0.05; ** 0.01; *** 0.001.

3.2. Cassava genotype performance under different substrates in three SAH subcultures

3.2.1. Plantlet height

Plantlet height was significantly ($p<0.001$) influenced by the cassava genotype in all subcultures. The genotype MM060083 consistently had the tallest plantlets, measuring 7.6 cm in subculture 1, 7.2 cm in subculture 2, and 6.0 cm in subculture 3. The lowest plantlet heights were consistently observed in the genotype Albert 28, measuring 5.5 cm in subculture 1, 4.3 cm in subculture 2, and 4.9 cm in subculture 3. Furthermore, plantlet height significantly ($p<0.001$) differed among the substrates. The tallest plantlets were observed in KlasmannTS3 at the ends of Subculture 1 (9.8 cm), Subculture 2 (10.0 cm), and Subculture 3 (7.4 cm). Conversely, the lowest plantlet heights were recorded in Sawdust at the end of Subcultures 1 (3.7 cm) and 2

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(2.9 cm), and in local peat (3.9 cm) in Subculture 3, as Sawdust was no longer used. The interaction between genotypes and substrates had a significant ($p<0.001$) effect on the height of cassava plantlets in both subculture 1 and subculture 2, but no significant interaction was observed in subculture 3 (Figure 5). Across all genotypes, KlasmannTS3 consistently resulted in the highest heights, while Sawdust consistently resulted in the lowest heights. For example, in subculture 1, MM060083 grown in KlasmannTS3 had the highest mean height (13.2 cm). This was similar to the results obtained in subculture 2, where MM060083 grown in KlasmannTS3 had the highest mean height (13.1cm). Based on the result, the genotype 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 1 and 2.

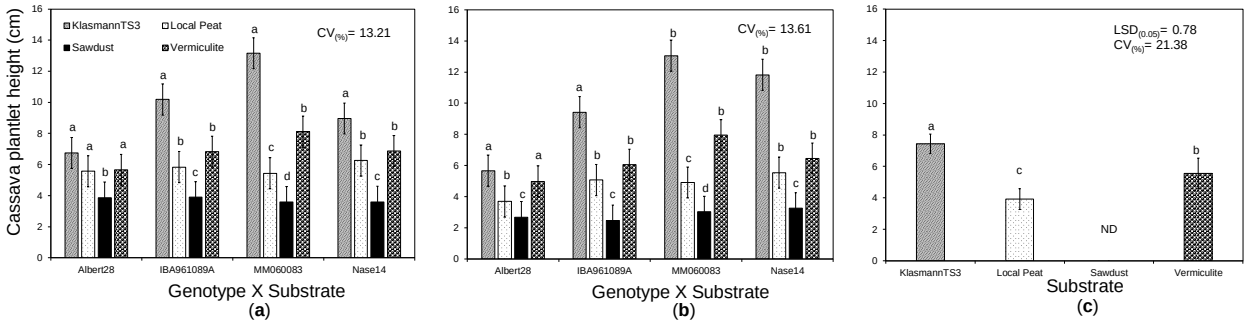

Figure 5. Effect of genotype, substrate, and their interaction on height (cm) of cassava plantlets grown under the SAH system: (a) interaction at the end of subculture 1; (b) interaction at the end of subculture 2; and (c) substrate at the end of subculture 3. Means within the graph and genotype followed by the same lowercase letter are not significantly different by the LSD significance test ($p<0.05$).

3.2.2. Leaf number

Leaf number was significantly ($p<0.001$) influenced by cassava genotype throughout subcultures 1 and 2, while no significant difference occurred in subculture 3. MM060083 had a significantly higher leaf number of 5.0 in subculture 1 and 4.8 in subculture 2. The lowest number of leaves was produced under Albert 28 in subculture 1 (3.9). However, this pattern changed in subculture 2, 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 1 and 2. Plantlets grown under KlasmannTS3 had a higher leaf number of 6.2 in subculture 1, which remained consistent at 6.0 in subculture 2. 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 (Table 3). In Subculture 1, the highest leaf number was observed with the genotype MM060083 grown under the KlasmannTS3 substrate at 8.3 leaves. The lowest leaf

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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 2, 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 1 and 2 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.

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Table 3. Mean number of leaves obtained among the four cassava genotypes x four substrate interactions under the SAH system at the three subculture periods.

			Leaf number (No)		
Genotype	Substrate		Subculture 1	Subculture 2	Subculture 3
IBA961089A	KlasmannTS3		6.68a	5.70a	5.13a
	Vermiculite		3.96b	4.08b	4.05a
	Local peat		4.12b	4.42b	3.94a
	Sawdust		3.20c	3.24c	(-)
		LSD(0.05)	0.39	0.81	
		ANOVA	***	***	
Albert28	KlasmannTS3		4.48a	5.36a	5.18a
	Vermiculite		4.16ab	4.29b	3.61a
	Local peat		3.72bc	4.44b	3.81a
	Sawdust		3.32c	3.09c	(-)
		LSD(0.05)	0.53	0.89	-
		ANOVA	**	**	-
MM060083	KlasmannTS3		8.32a	7.82a	5.42a
	Vermiculite		3.88bc	3.88b	3.96a
	Local peat		4.32b	4.40b	4.02a
	Sawdust		3.32c	3.08c	(-)
		LSD(0.05)	0.72	0.67	-
		ANOVA	***	***	-
Nase14	KlasmannTS3		5.24a	5.21a	5.02a
	Vermiculite		4.04b	4.32b	3.76a
	Local peat		4.12b	4.24b	3.64a
	Sawdust		3.32c	3.32c	(-)
		LSD(0.05)	0.45	0.64	-
		ANOVA	***	***	-
		Mean	4.39	4.43	4.29
		CV (%)	8.89	12.42	11.46
		Genotype (G)	***	*	NS
		Substrate (S)	***	***	***
		ANOVA G X S	***	***	NS

Means within a column followed by the same lowercase letter are not significantly different by the Fisher's Least Significant Difference test (LSD) ($p<0.05$). (-) There was no sawdust data in subculture three because cuttings to be transplanted could not be obtained in subculture two. Significant codes: NS: No significant; * 0.05; ** 0.01; *** 0.001.

3.2.3. Internode number

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

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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 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 effect 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 1, the highest internode numbers were observed with the genotypes MM060083 (6.6) and IBA961089A (6.2) grown under the KlasmannTS3 substrate. Similarly, in subculture 2, the highest increase in the number of internodes was observed in plantlets of MM060083 grown under KlasmannTS3 substrate (7.5). In subculture 3, 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.

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Table 4.Mean number of internodes obtained among the four cassava genotype x four substrate interactions under the SAH system at the three subculture periods.

			Internode number (No)		
Genotype (G)	Substrate (S)		Subculture 1	Subculture 2	Subculture 3
IBA961089A	KlasmannTS3		6.16[a]	5.45[a]	4.84[a]
	Vermiculite		3.12[c]	2.74[b]	2.84[b]
	Local peat		4.92[b]	3.44[b]	2.76[b]
	Sawdust		2.68[c]	1.53[c]	(-)
		$LSD_{(0.05)}$	0.45	0.80	0.55
		ANOVA	***	***	***
Albert28	KlasmannTS3		3.76[a]	4.56[a]	4.02[a]
	Vermiculite		3.20[b]	3.05[b]	2.88[b]
	Local peat		3.20[b]	3.05[b]	2.60[b]
	Sawdust		2.48[c]	1.76[c]	(-)
		$LSD_{(0.05)}$	0.35	0.70	0.52
		ANOVA	***	***	***
MM060083	KlasmannTS3		6.60[a]	7.48[a]	5.72[a]
	Vermiculite		3.48[b]	3.17[b]	3.84[b]
	Local peat		3.44[b]	3.57[b]	3.88[b]
	Sawdust		2.68[c]	2.04[c]	(-)
		$LSD_{(0.05)}$	0.50	0.73	0.53
		ANOVA	***	***	***
Nase14	KlasmannTS3		4.64[a]	4.85[a]	4.12[a]
	Vermiculite		3.44[b]	3.21[b]	3.16[b]
	Local peat		3.20[b]	3.05[b]	2.88[b]
	Sawdust		2.52[c]	1.57[c]	(-)
		$LSD_{(0.05)}$	0.48	0.85	0.52
		ANOVA	***	***	**
		Mean	3.72	3.41	3.63
		CV (%)	8.79	16.53	10.02
		Genotype (G)	***	***	**
		Substrate (S)	***	***	***
		ANOVA G X S	***	***	*

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 3 because cuttings to be transplanted could not be obtained in subculture 2. Significant codes: * 0.05; ** 0.01; *** 0.001

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3.2.4. Number of cuttings

The number of cuttings was significantly ($p<0.001$) influenced by the cassava genotype in both subcultures 2 and 3, as well as in the overall subcultures. MM060083 had the highest number of cuttings in subculture 2 (8.7) and subculture 3 (16.3), whereas Albert28 and Nase14 recorded the lowest numbers. Specifically, both Albert28 and Nase14 had 6.7 and 6.9 cuttings in subculture 2, and 10.3 and 10.4 cuttings in subculture 3, 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 1. 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 ($p<0.001$) affects 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 1, followed by 13.1 in subculture 2, and a further increase to 27.1 in subculture 3. On the other hand, the lowest number of cuttings was observed under vermiculite in subculture 1 (14.0) and under sawdust in subculture 2 (2.8). In subculture 3, 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 3 and the total of the three subcultures (Table 5). Subcultures 1 to 2. The highest cutting numbers were obtained with all the genotypes grown in KlasmannTS3 (from 52.4 to 70.4), and the lowest in Sawdust (from 18 to 20.8). However, the increase in cuttings varied across the genotypes. The highest number of cuttings in subculture 3 was obtained with MM060083 grown in KlasmannTS3 (37.2), while the lowest was observed with vermiculite and local peat for Albert28 and Nase 14 (9.0 to 9.8). For the total number of subcultures, the highest mean number of cuttings was again obtained with MM060083grown under KlasmannTS3 (70.4), while the lowest was observed consistently with Sawdust for all genotypes (18.0 to 20.8). Based on the results, it's clear that MM060083 grown under KlasmannTS3 consistently produced the highest number of cuttings, while Sawdust consistently produced the lowest, particularly with Albert28 and Nase14.

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Table 5.Mean number of cuttings obtained among the four cassava genotypes x four substrate interactions under the SAH system at the three subculture periods.

Genotype (G)	Substrate (S)		Number of cuttings (No) Subculture 1	Subculture 2	Subculture 3	Total Subcultures
IBA961089A	KlasmannTS3		18.20[a]	12.40[a]	25.00[a]	55.60[a]
	Vermiculite		14.60[a]	8.00[a]	12.80[b]	35.40[b]
	Local peat		17.00[a]	8.00[a]	13.20[b]	38.20[b]
	Sawdust		16.00[a]	2.20[a]	(-)	18.20[c]
		$LSD_{(0.05)}$	-	-	7.98	9.04
		ANOVA	-	-	*	***
Albert28	KlasmannTS3		17.80[a]	12.40[a]	22.20[a]	52.40[a]
	Vermiculite		14.40[a]	6.00[a]	9.80[b]	30.20[b]
	Local peat		15.80[a]	5.60[a]	9.00[b]	30.40[b]
	Sawdust		15.80[a]	2.60[a]	(-)	18.40[c]
		$LSD_{(0.05)}$	-	-	2.84	6.19
		ANOVA	-	-	***	***
MM060083	KlasmannTS3		18.40[a]	14.80[a]	37.20[a]	70.40[a]
	Vermiculite		13.60[a]	7.60[a]	12.60[b]	33.80[b]
	Local peat		17.20[a]	9.20[a]	15.40[b]	41.80[b]
	Sawdust		17.60[a]	3.20[a]	(-)	20.80[c]
		$LSD_{(0.05)}$	-	-	4.34	10.57
		ANOVA	-	-	***	***
Nase14	KlasmannTS3		19.20[a]	12.40[a]	23.60[a]	55.20[a]
	Vermiculite		13.20[a]	6.00[a]	9.20[b]	28.40[b]
	Local peat		16.20[a]	6.20[a]	9.00[b]	31.40[b]
	Sawdust		15.00[a]	3.00[a]	(-)	18.00[d]
		$LSD_{(0.05)}$	-	-	5.58	6.57
		ANOVA	-	-	***	***
		Mean	16.25	7.48	16.58	36.16
		$CV_{(\%)}$	8.70	28.58	22.81	15.62
		Genotype (G)	NS	*	***	**
		Substrate (S)	***	***	***	***
		ANOVA G X S	NS	NS	*	*

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 3 because cuttings to be transplanted could not be obtained in Subculture 2. Significant codes: NS: No

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significant; * 0.05; ** 0.01; *** 0.001. (Thiele *et al.*, 2022)

4.0. Discussion

The survival rate of cassava plantlets was primarily influenced by the substrate used rather than genotype and substrate interactions. In each subculture, KlasmannTS3 substrate consistently led to the highest survival rates. This finding aligns with previous research by (Adesanya et al., 2016), who achieved a 93.8% laboratory survival rate using the same substrate. Another study focusing on pineapple (*Ananas comosus*) plantlets grown in KlasmannTS3 also reported superior survival rates compared to other substrates, highlighting the positive impact of the SAH substrate(Olagunju et al., 2021) . The survival rates obtained with KlasmannTS3 in the present study surpassed the 80% survival rate reported by a previous study utilizing the same substrate (Kajibwami et al., 2018).This discrepancy in survival rates may be attributed to variations in genotypes and growth conditions. The poor survival of plantlets under vermiculite is in line with its physical properties, as reported by several authors. Its lightness can cause weak root support, causing instability and vulnerability to environmental stresses. Moreover, its inconsistent particle sizes can hinder robust development (Spomer *et al*., 1997; Khan *et al*., 2020). While vermiculite has a high water-holding capacity, it may lack the balance between water and root aeration essential for plant growth. Moreover, its high water-holding capacity can reduce air-filled space, hampering root respiration and overall plant health (Khan *et al*., 2020;Shewa *et al.*, 2020). In this study, uniform weekly watering was applied to all substrates, including vermiculite. This might have resulted in excessive moisture within the root zone, particularly when compared to substrates with lower water-holding capacities.

Significant variations observed for all the traits tested among genotypes across subcultures indicate the existence of variability among the tested genotypes. Specifically, these findings imply that the genotype MM060083 possesses genetic attributes that contribute to its exceptional growth characteristics within the SAH system. In terms of substrates, the tallest plantlets and higher leaf and internode numbers were obtained under KlasmannTS3 across different subcultures. The physical and chemical properties of KlasmannTS3 (Table 1) were found to be suitable for cassava propagation, as indicated by (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 byseveralstudies(Khan et al., 2020; Santiago-Aviles and Light, 2018; Sato *et al.*, 2006). Various crops, including cassava (*Manihot esculenta)*, yam (*Dioscorea spp*.), lettuce (*Lactuca Sativa L.)*, brassica (*Brassica olererecea)*, and marigold (*TagetesL*.), have demonstrated rapid growth and favorable performance when cultivated in Klasmann substrate, as reported by previous studies (Adesanya *et al.*, 2016; Balalic, 2004; Maślanka & Magdziarz, 2017; Mišković et al., 2009; Olugboyega et al., 2019). In contrast, sawdust consistently showed poorer performance, resulting in lower mean heights and leaf and internode numbers compared to other substrates. The limited nutrient levels of sawdust (Table 1) likely contribute to its hindered plantlet growth. The reduced growth of plantlets in sawdust is partially in line with its insufficient nitrogen (N), phosphorus (P), and potassium (K) levels, which are essential for cassava (Byju and Suja, 2020).

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Furuta (1970) emphasised 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. Nitrogen is vital for cassava, particularly in stem and leaf development, promoting early growth during the growing season. Conversely, P deficiency limits plant growth. Additionally, K is crucial for overall plant development (Shand, 2007; Ezui *et al.*, 2017). The observed poor plantlet growth under sawdust is consistent with (Sanchez et al., 2021) and (Garner, 2014), 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. This characteristic can hinder overall growth, especially in a 4-week timeframe, as slow nutrient release may not meet rapid growth demands (Media and Guide, 2021; Pennington et al., 2009) Regardless of the cassava genotype, a higher propagation rate was observed under the KlasmannTS3 substrate. KlasmannTS3 exhibited the greatest increase in cutting numbers over twelve weeks, indicating a favourable propagation rate. The exceptional attributes of the KlasmannTS3 substrate, such as its rapid plantlet growth, optimal pH, high electrical conductivity (EC)(Table 1), 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 (Khan *et al.*, 2020; Jan *et al.*, 2021; Asaduzzaman *et al.*, 2015; Fussy and Papenbrock, 2022; Saaid *et al.*, 2015). This unique combination of properties 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 across successive subculture periods.

Several authors revealed that the nitrogen supplied on soil had a significant effect on plant growth of nightshades (*Solanum spp.*) and maize (*Zea mays L.*) (Masinde and Agong, 2012,Mburu, Lenga and Mburu, 2011). In the present study, despite having higher nitrogen content (Table 1), 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 (Massignam et al., 2009). Studies indicated that a satisfactory pH range for plant growth media is between 5.5 and 6.5 (Thomas, 1996). 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 (Table 1). 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 emphasised the importance of a hydroponic medium providing good structure and stability, high water holding capacity, and sufficient root aeration for successful plant growth (Michel, 2010; Jones, 1982; Santiago-Aviles and Light, 2018; Shewa *et al.*, 2020)

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The interaction between genotype and substrate was found to be significant, indicating that the choice of substrate influenced each genotype differently. Genotype MM060083 combined with KlasmannTS3 consistently showed good performance in terms of the number of cuttings obtained, likely because of its overall favourable growth characteristics. Conversely, the combination of sawdust substrate with any genotype consistently showed the poorest growth performance. Overall, the results indicate that the choice of substrate, particularly KlasmannTS3, plays a crucial role in the survival, growth, and propagation of cassava plantlets. It provides optimal conditions for nutrient availability and water retention. Genotype MM060083 demonstrated superior performance across various growth parameters, further highlighting the influence of genotype on the success of the SAH system. These findings contribute to a better understanding of substrate-genotype interactions and provide valuable insights for improving cassava propagation and SAH technique.

5.0. Conclusion and recommendation

In conclusion, the Semi-Automatic Hydroponic (SAH) technology offers a distinct advantage compared to traditional propagation methods by enabling the rapid production of large quantities of planting materials. The choice of substrate significantly impacted the performance of cassava plantlets in the SAH system, while the genotype had a lesser impact. KlasmannTS3 demonstrated superior performance compared to sawdust, vermiculite, and local peat. The fast growth of plantlets in KlasmannTS3, characterised by taller plant height, higher leaf numbers, and more developed internodes, contributed to its success. On the other hand, substrates such as sawdust, vermiculite, and local peat exhibited poorer performance in terms of growth parameters and multiplication rates.

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6.3 Declaration of interest

None

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6.4 Conflict of interest

None.

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