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Research article

The Effects of Different Plant Growth Regulators and Nutrient Solutions on Leaf Bud Propagation in Different Cassava Varieties

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Abstract

Cassava mosaic virus is a growing threat to cassava cultivation, and the rapid propagation of disease-free cassava stems offers an alternative approach to producing planting material. This study investigated the effects of different plant growth regulators and nutrient media on leaf bud propagation among different cassava varieties. A 3 x 4 factorial with a randomized complete block design (RCBD) was used with three replications. Three cassava varieties, Kasetsart 50 (KU50), Rayong 9 (RY9), and Huay Bong 60 (HB60) were assigned as factor A, and four different media, i.e., control treatment (distilled water), indole-3-butyric acid (IBA) at 500 mg/L, Semi-Autotrophic Hydroponic (SAH) nutrient solution, and IBA+SAH, were assigned as factor B. Data were recorded for growth and survival traits at 30 days. The results showed that the RY9 variety exhibited superior growth parameters, whereas the KU50 and HB60 varieties showed a higher survival rate. Applying IBA, alone or with SAH, negatively affected cassava plantlet growth and survival rate. On the other hand, applying SAH alone yielded results comparable to the control treatment, significantly differing from the IBA and IBA+SAH treatments. This study also noted that IBA applications enhanced root traits in the RY9 variety but not in other varieties. The HB60 variety outperformed the survival rates and dry shoot weights of RY9 and KU50 under SAH treatment. This finding can be used to select an effective method for leaf bud multiplication in response to the demand for disease-free cassava planting materials.

Keywords: BA; leaf bud cutting; *Manihot esculenta* Crantz; SAH; SLCMD

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a versatile crop with a wide range of industrial applications, including the production of starch, bioethanol, and other bio-based products (Li et al., 2017). Cassava production in Southeast Asia has been steadily increasing due to rising demand, improved varieties, and increased accessibility to land and water (Graziosi et al., 2016). However, this growth has also brought about serious challenges from pests and diseases, particularly cassava mosaic disease (CMD) (Hareesh et al.,

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2023). Cassava mosaic disease is a significant threat to cassava production, caused by various species of cassava mosaic *geminiviruses*. The Sri Lankan cassava mosaic virus (SLCMV) was first reported in Southeast Asia in 2016 and has since been detected in Cambodia and Vietnam (Minato et al., 2019). In Thailand, a survey conducted from October 2018 to July 2019 across five provinces along the Thailand–Cambodia border found CMD prevalence to be 40% in the surveyed area, with the highest disease incidence of 43.08% in Sakaeo province. Severity ranged from mild chlorosis to moderate mosaic, and the virus was primarily transmitted by whiteflies (Saokham et al., 2021).

Management strategies have been employed to combat the disease, with the cultivation of resistant varieties showing considerable promise. Creating disease-free planting materials has been a pivotal strategy to address these challenges in Southeast Asia (Hareesh et al., 2023). Traditional methods of cassava propagation through stem cuttings are known for their slow multiplication rates, typically ranging from 1:5 to 1:10 over 12 months (de Oliveira et al., 2020). However, recent studies have explored alternative methods to improve this rate, such as the leaf bud cutting method. Neves et al. (2020) found that using leaf buds from younger plants, particularly those treated with agrochemicals, can significantly increase the multiplication rate to 1:72. The International Institute of Tropical Agriculture (IITA) has shown that the combination of Semi-Autotrophic Hydroponic (SAH) nutrient solution and the rooting hormone, indole-3-butyric acid (IBA), significantly improved the growth and survival of SAH cassava plantlets (Ogwuche et al., 2018). Although this propagation method provides a substantial number of seedlings, the impact of plant growth regulators and nutrient solutions on leaf bud cuttings is less documented. Previously, Pateña & Barba (1979) demonstrated that a mixture of 1 g of Captan and varying doses of IBA, ranging from 50 to 450 mg/100 mL of water, had no significant influence on the root and shoot growth of leaf bud cuttings. In contrast, Ogwuche et al. (2018) demonstrated that a combined treatment of IBA and nutrients in SAH solution notably improved the development and survival of tissue-cultured cassava plantlets compared to separate treatments. Applying IBA at a concentration of 1,000 mg/L improved the rooting of cassava cuttings (Naranjo & Fallas, 2017). However, Chant & Marden (1959) reported that high concentrations of plant growth regulators had a deleterious effect on the shoot formation of greenwood cuttings in cassava. They recommended using IBA and indole-3-acetic acid (IAA) at 500 mg/L, which is the optimum concentration for cassava stem cuttings, to promote root growth. The possibility exists that greenwood is characterized by a leaf bud cutting containing a piece of wood from a cassava stem (López, 2012).

Despite advancements in cassava propagation techniques, there are few comprehensive studies concerning the effects of plant growth regulators and nutrient solutions on the propagation of leaf bud cuttings. Earlier research, such as the study by Pateña & Barba (1979), did not demonstrate significant results, while more recent findings by Ogwuche et al. (2018) suggest potential benefits. This inconsistency in information highlights a knowledge gap in understanding optimal conditions for enhancing cassava plantlet growth and survival rates. In this study, the effect of plant growth regulators (IBA), nutrient solutions (SAH) and their combination on the sprouting and survival rates of leaf bud cuttings in different cassava varieties was investigated.

It is hypothesized that applying specific combinations of plant growth regulators and nutrient solutions will significantly improve the propagation efficiency of leaf bud cuttings, resulting in higher sprouting rates and enhanced cassava plantlet survival across various cultivars. This research holds substantial significance as it addresses the critical challenge of low multiplication rates in cassava propagation. By identifying effective plant growth regulators and nutrient treatments, the study could lead to the development of more efficient propagation methods, thereby supporting the expansion of cassava production to meet growing industrial demand.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted under field and greenhouse conditions at the Ubon Ratchathani University farm, in Northeastern Thailand (15°7'4.95" N, 104°54'4.95" E, 130 m). The experiment was carried out from December 2022 to June 2023.

2.2 Experimental description

A 3 x 4 factorial design in a randomized complete block design (RCBD) was used in this study. Factor A consisted of three cassava varieties: Kasetsart 50 (KU50), Rayong 9 (RY9), and Huay Bong 60 (HB60), while factor B consisted of four different planting media treatments: a control using distilled water, IBA, SAH, and a combination of IBA and SAH. Each treatment was replicated three times, with each replication comprising 21 leaf bud cuttings. KU50, developed by Kasetsart University from a cross between Rayong 1 and Rayong 90, exhibited high root yield and vigorous plant growth, with wide adaptation. HB60 (Rayong 5 x KU50) exhibited high yields, starch content, and germination rates. It was developed in collaboration between Kasetsart University and the Thai Tapioca Development Institute and was released in 2006 by the Rayong Field Crops Research Center. RY9 (CMR-31-19-23 x OMR29-20-118), it exhibited good growth and exceptional yields, high starch content, and high photosynthetic efficiency (Santanoo et al., 2022). Previous research by Malik et al. (2022) indicated that KU50 and HB60 showed remarkable resistance to Cassava Mosaic Disease (CMD), with fewer affected plants and slower disease progression, leading to higher yields than other varieties. Additionally, Saokham et al. (2021), reported that RY9 had a moderate level of resistance to CMD when compared to other varieties.

To ensure the health and uniformity of the mother stocks, each cassava variety was grown in the field to produce the required leaf buds for the study. Before preparing the seedlings, a diagnostic strip test for the cassava mosaic virus, developed by Thailand's National Center for Genetic Engineering and Biotechnology (BIOTEC) under the National Science and Technology Development Agency (NSTDA), was used to detect the absence of SLCMD in the mother stocks.

The SAH nutrient solution was formulated in three separate stocks. Stock A contained 35.4 g of calcium nitrate ($Ca(NO_3)_2$) dissolved in 15 L of distilled water. Stock B comprised 14.7 g of magnesium sulfate ($MgSO_4$), 4.08 g of potassium monophosphate (KH_2PO_4), 15.5 g of potassium nitrate (KNO_3), and 0.02 g of ferrous sulfate ($FeSO_4$), also diluted to 15 L with distilled water. Subsequently, half a liter of each stock A and B were combined and then supplemented with distilled water to reach a total volume of 2 L. Stock B was stored in an opaque container to prevent the oxidation of iron compounds.

Leaf blades were trimmed to a third of their size from 5-month-old mother stock plants using sterile scissors, and then hydrated in water to prevent desiccation. Leaf bud cuttings were collected from the upper two-thirds of stems, excluding the youngest, smallest buds, as recommended previously (Neves et al., 2020). A sanitized blade cut a "V" notch into the stems. To protect against pests and diseases, the cuttings were submerged for 3 min in a solution containing thiamethoxam (16 g), carbendazim (5.7 g), and metalaxyl (0.76 g) dissolved in 10 L of water (Neves et al., 2020). The treated leaf blade cuttings were planted in 21-cell trays filled with equal parts of peat moss, coconut fiber, and rice hulls.

Leaf bud cuttings were then briefly dipped for 10 s in a 500 mg /L IBA plant growth regulator solution for the treatment of IBA and IBA combined with SAH, as documented by Pateña & Barba (1979) and Javier & Mamicpic (1978). After being planted in the trays, SAH solution was applied to the cells of SAH, and a combination of IBA and SAH treatments was applied with 10 mL in each cell. For the control treatment, 10 mL of distilled water was also added to each cell. The cuttings were placed in a greenhouse equipped with an automated misting system for a 30-day acclimatization period.

2.3 Data collection

After the 30-day acclimatization period in the greenhouse, the survival rate of plants in each tray was recorded. Five plants from each replication were randomly chosen to measure the number of leaves, shoot height, root number, and root length. Then, the shoots and roots of the five selected plants were separated and oven-dried at 60°C for 48 h or until a stable weight was achieved. Dry weights were recorded using an electronic scale. The total dry weight was determined by adding the dry weight of the shoots and roots. The sprouting rate was defined as the ratio of leaf buds that developed shoots, following the method described by Neves et al. (2020). The time taken for the highest shoot germination to occur for each plantlet within a replication was recorded as the sprouting day, and multiplying the germination percentage by the seedling length gave the seedling vigor index (SVI) (Abdul-Baki & Anderson, 1973). The rate at which germination occurred was quantified using an equation defined by Hossain et al. (2010) and Choi et al. (2013), whereby it is calculated as the maximum percentage of germination divided by the duration in days.

2.4 Statistical analysis

The statistical analysis of all parameters was performed using a factorial in an RCBD. Tukey's Honest Significant Difference test was used to separate significant differences between means at a confidence level of 95% using Statistix software (version 10). To visualize the data, R software (version 4.3.1) using Metan Package version 1.18.0 was used to estimate Pearson's correlation for all pairs of traits (Olivoto & Lúcio, 2020). FactorMineR and factoextra packages were employed to create PCA plots that encapsulated the characteristics of each treatment (Kassambara, 2017). The data underwent standardization by adjusting for unit variance. Subsequently, a cos2 correlation circle, representing the quality of trait representation, was produced. The PCA biplot's outcomes were then analyzed to determine the positioning of traits within groups.

3. Results and Discussion

3.1 Effect of plant hormone and nutrient solution on cassava leaf bud multiplication

An analysis of variance showed that cassava varieties (V) significantly influenced all parameters of growth and survival performance (Table 1). The greater performance was

Table 1. Analysis of variance on growth parameters and survival traits of three cassava varieties subjected to four different planting media treatments

	Leaf No. (No. plant^{-1})	Shoot Length (cm)	Root No. (No. plant^{-1})	Root Length (cm)	Shoot Dry Weight (g/plant)	Root Dry Weight (g/plant)	Total Dry Weight (g/plant)	Sprouting Percentage (%)	Days until Germination (days)	Speed of Sprouting	Survival Percentage (%)	SVI
Varieties (V)												
KU50	3.83 b	1.97 b	8.81 c	12.73 a	0.35 c	0.17 b	0.51 c	69.09 a	11.66 b	6.73 b	56.26 a	1,143 a
RY9	4.93 a	2.94 a	17.51 a	13.72 a	0.39 b	0.38 a	0.77 a	63.88 b	8.58 a	9.65 a	42.06 b	1,100 ab
HB60	4.98 a	1.97 b	11.49 b	10.76 b	0.45 a	0.20 b	0.65 b	61.50 b	11.25 b	7.26 b	58.72 a	993 b
F-test	***	***	***	***	***	***	***	***	***	***	***	*
Treatments (T)												
Control	5.60 a	2.79 a	9.00 c	14.33 a	0.56 a	0.29 a	0.85 a	96.83 a	7.11 a	14.15 a	85.18 a	1,660 a
IBA	3.08 c	2.66 a	14.34 b	8.15 c	0.10 c	0.27 a	0.27 c	11.56 c	13.66 b	0.92 d	8.35 c	127c
SAH	5.71 a	2.36 a	9.95 c	15.46 a	0.59 a	0.28 a	0.86 a	100 a	8.33 a	12.54 b	78.83 a	1813 a
IBA+SAH	3.94 b	1.33 b	17.13 a	11.69 b	0.32 b	0.17 b	0.60 b	50.56 b	12.88 b	3.91 c	37.03 b	715 b
F-test	**	**	***	***	***	***	***	***	***	***	***	***
V × T	*	***	***	***	***	***	***	***	*	***	***	***
C.V. (%)	9.65	18.89	10.82	12.21	12.30	19.39	11.32	4.15	9.49	11.77	10.23	11.99

Note: * Significant at p ≤ 0.05; ** significant at p ≤ 0.01; *** significant at p ≤ 0.001; SVI = Seedling Vigor Index; C.V = coefficient of variation. Means in the same column followed by the same lowercase letter are not significantly different based on Tukey's HSD test at the 95% probability level.

observed in RY9 variety in almost parameters, such as shoot length (2.94 cm), number of roots (17.51), root dry weight (0.38g/plant), total dry weight (0.77g/plant), days of germination (8.58 days), and sprouting speed (9.65). It was noted in previous studies that the RY9 variety exhibited good growth performance with good canopy structure, leaf area index, and adaptability to tropical savanna climates (Prammanee et al., 2010; Mahakosee et al., 2019). This variety also showed the ability to fully recover its photosystem II efficiency in the evening, even under drought and high-temperature conditions (Vongcharoen et al., 2019). Potentially, a non-forking cassava variety such as RY9 could achieve greater height, leaf size, and stem vigor when compared to forking varieties such as KU50 and HB60 (TTDI, 2006a,b). Non-forking varieties had a higher leaf area index (LAI) compared to forking varieties at a later growth stage, at 6 months after planting, when photoassimilates were preferentially partitioned to build storage roots (Santanoo et al., 2020). A higher LAI enhanced photosynthetic capacity and may probably lead to better growth and yields (Ewert, 2004). This may explain why RY9 showed better performance in our experiment. Additionally, the RY9 and KU50 varieties showed longer roots (13.72 and 12.73 cm) and higher values on SVI (1100 and 1143) than the HB60 variety (10.76 cm and 993). Previous studies demonstrated that the KU50 and RY9 varieties showed high adaptability and growth performance across environments (Prammanee et al., 2010; Ou et al., 2018; Vongcharoen et al., 2019). A significantly higher sprouting percentage was recorded in KU50 (69.09%) than in RY9 (63.88 %) and HB60 (61.50%). Moreover, KU50 and HB60 had a higher survival percentage than RY9 (56.26%, 58.72%, and 42.06%, respectively). HB60 had a KU50 and RY5 genetic background, and this may be the reason that both varieties showed the same characteristics (Malik et al., 2022). The Thai Tapioca Development Institute reported that the KU50 and HB60 varieties had similar agronomic characteristics with good germination and vigorous plant growth with wide adaptation, but HB60 had a higher fresh root yield of about 7% and a higher root starch content than KU50 (TTDI, 2006a,b). Kengkanna et al. (2019) also reported that cassava varieties exhibited significant differences in shoot and root growth reduction under drought conditions. HB60 had the highest shoot dry weight, followed by KU50 and RY9. Conversely, RY9 and HB60 retained more leaves (compared to KU50) under drought stress. Increased leaf numbers might contribute to the higher shoot dry weight of these varieties. The HB60 cassava variety has been found to have good growth and starch content, making it a suitable choice for cultivation (Prammanee et al., 2010).

Our study also observed significant differences among application treatments (T) or plant growth regulators (IBA) for all parameters (Table 1). Almost all parameters exhibited higher values in the control treatment and SAH nutrient application, such as the number of leaves, root length, shoot dry weight, total dry weight, sprouting percentage, days until germination, survival percentage, and SVI. It was unexpected that the control plants exhibited better growth performance than those subjected to the IBA. According to our observations, it was noted that the leaf bud cuttings were young and not mature enough to withstand the application of IBA at a high concentration of 500 mg/L. Previous reports have suggested that high concentrations of IBA may not be suitable for young tissue and plantlets in cutting seedlings (Polat & Caliskan, 2006; Pacholczak et al., 2012). High concentrations of IBA can lead to toxicity in plant cells, resulting in reduced growth, chlorosis, necrosis, and the subsequent death of the plants (Šípošová et al., 2019). Plant development is adversely affected by high IBA concentrations, which disrupt the natural auxin equilibrium and signaling, leading to morphological abnormalities in leaves, stems, and roots (Mao et al., 2018). Gomes & Scortecci (2021) also reported that high IBA concentrations interfered with the natural auxin signaling pathway by modulating the expression of specific genes involved in plant development. Furthermore, the distilled water (control) and SAH treatments had similar effects on cassava leaf bud cutting multiplication, indicating that the leaf bud technique does not require any special treatment or hormone to induce sprouting. This is consistent with previous research that reported high sprouting rates for leaf bud cuttings from various cassava genotypes. The leaf bud technique offers several advantages over the traditional method of using mature stem cuttings, including higher multiplication rates, shorter production cycles, lower risks of disease transmission, and better adaptation to environmental conditions (Neves et al., 2020).

The SAH nutrient application plant propagation is mostly used in a laboratory-controlled environment (Ogwuche et al., 2018; Pelemo et al., 2019; Olagunju et al., 2021). Ogwuche et al. (2018) suggested that SAH nutrient was an alternative solution for multiplying cassava plantlets derived from tissue culture under laboratory growth conditions. In our study, we applied SAH nutrients to cassava leaf bud planting under normal greenhouse conditions in April. We found that the high temperatures in the greenhouse may not be suitable for cassava leaf bud cuttings, as the temperatures may affect germination and development. A combination of IBA with SAH nutrients did not significantly affect the growth and survival rate of the cassava leaf bud plantlets. This finding contrasts with the results of Ogwuche et al. (2018) who found that the combined application of IBA with SAH resulted in the highest survival rate, 95.8%, compared to individual treatments of 88.2% (IBA) and 93.3% (SAH). The differing results between this study and the prior one may be due to variations in experimental conditions, such as environmental factors, application methods, concentrations of treatments, or genetic differences among the cassava varieties used in the studies.

It was also observed that there was a significant interaction between the cassava varieties (V) and nutrient application treatments (T) in all parameters, indicating different responses among the cassava varieties to different nutrient solutions and plant regulators. Therefore, all parameters were analyzed based on the treatment combination (Table 2). The highest shoot length, root length, sprouting percentage, and SVI for all cassava varieties were obtained from the control treatment and the SAH nutrient application. Moreover, the SAH application provided the highest leaves number per plant for all varieties, while the control treatment performed better for the RY9 and HB60 varieties. This suggested that SAH-treated plants exhibited better growth and development. It was observed that the SAH solution application could be optimized for a selected solution on leaf bud cutting multiplication. For example, SAH application provided the highest leaf production across all varieties. Leaves play a crucial role in photosynthesis and overall plant health. The role of leaves in cassava productivity is further underscored by their high capacity for carbon assimilation and their correlation with root yields (El-Sharkawy, 2006). Furthermore, the control (distilled water) and SAH treatment resulted in faster sprouting in the RY9 than the HB60 and KU50 varieties but produced a lower survival rate than those varieties. In our observations, the leaf bud cuttings of the RY9 variety from mother stocks performed well in terms of agronomic growth, with larger, longer, and more vigorous leaf blades. Consequently, plantlets from RY9 sources retained their petioles for more days than KU50 and HB60 (Figure 1, E-H). López (2012) reported that between 8 and 15 days, the roots of the leaf bud cutting were about 1 cm long, and the petioles had detached after trimming the leaf buds from the mother stock. This might be a reason why RY9 exhibited

Table 2. The combined results of the growth parameters and survival traits of three cassava varieties subjected to four different planting media treatments

Parameters	Planting Media Treatment											
	Control			IBA			SAH			IBA+SAH		
	KU50	RY9	HB60	KU50	RY9	HB60	KU50	RY9	HB60	KU50	RY9	HB60
Leaf no. (no. plant^{-1})	4.93 b-e	6.06 ab	5.80 a-c	1.87 g	3.72 ef	3.67 ef	5.40 a-d	5.40 a-d	6.33 a	3.13 fg	4.56 c-e	4.13 d-f
Shoot length (cm)	2.53 a-c	3.24 ab	2.63 a-c	1.39 d	3.11 ab	0.50 d	2.98 a-c	2.07 a-c	2.96 a-c	1.97 bc	3.35 a	1.79 c
Root no. (no. plant^{-1})	7.73 d	9.07 cd	10.20 cd	7.60 d	26.00 a	9.44 cd	8.87 cd	9.00 cd	12.00 bc	11.07 b-d	26.00 a	14.33 b
Root length (cm)	13.88 ab	14.47 a	14.65 a	8.79 c	12.00 a-c	3.67 d	16.18 a	15.23 a	14.97 a	12.10 a-c	13.19 a-c	9.77 bc
Shoot dry weight (g/plant)	0.43 cd	0.60 b	0.67 ab	0.05 f	0.22 e	0.02 f	0.59 b	0.44 c	0.75 a	0.29 de	0.31 c-e	0.36 c-e
Root dry weight (g/plant)	0.20 cd	0.36 ab	0.30 bc	0.05 ef	0.48 a	0.006 f	0.28 b-d	0.22 b-d	0.31 bc	0.16 de	0.48 a	0.21 cd
Total dry weight (g/plant)	0.63 de	0.96 ab	0.97 ab	0.10 f	0.70 cd	0.02 f	0.87 abc	0.66 c-e	1.06 a	0.45 e	0.79 b-d	0.57 de
Sprouting percentage (%)	93.65 a	100 a	96.83 a	9.27 e	17.46 d	7.94 e	100 a	100 a	100 a	73.45 b	38.09 c	41.27 c
Days until germination (days)	8.67 bc	5.67 a	7.00 ab	14.00 ef	11.00 cd	16.00 f	10.00 c	6.33 ab	8.67 bc	14.00 ef	11.33 c-e	13.33 d-f
Speed of sprouting	10.84 d	17.78 a	13.83 bc	0.66 fg	1.61 fg	0.50 g	10.18 d	15.87 ab	11.57 cd	5.24 e	3.34 ef	3.14 e-g
Survival percentage (%)	85.71 ab	74.60 bc	95.24 a	9.18 e	9.52 e	6.35 e	71.43 bc	71.73 bc	93.65 a	58.73 c	12.70 e	40.00 d
SVI	1,538 a	1,771 a	1,6701 a	85.30 d	264 cd	33.00	1,916 a	1,730a	1,793 a	1,032 b	636 c	447 c

Note: Means in the same row followed by the same lowercase letter are not significantly different based on Tukey's HSD test at the 95% probability level.

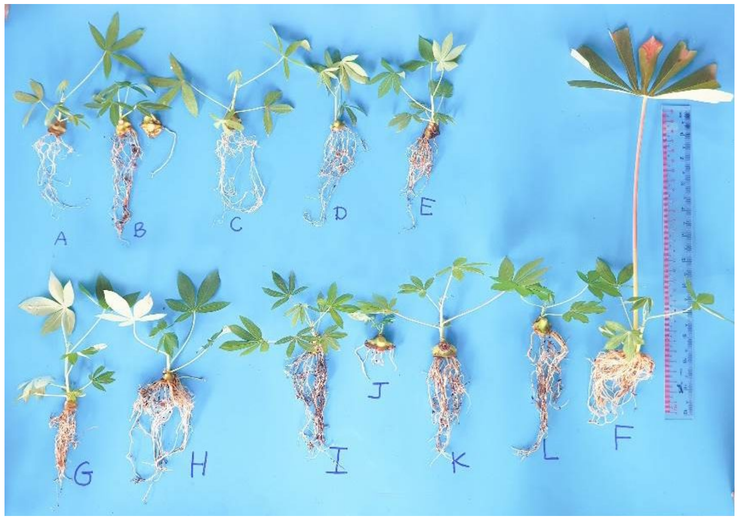

Figure 1. The shoot and root growth of cassava plantlets with different combination treatments under plant regulators and nutrient solution application of cassava leaf bud propagation. A) KU50-Control, B) KU50-IBA, C) KU50-SAH, D) KU50-IBA+SAH, E) RY9-Control, F) RY9-IBA, G) RY9-SAH, H) RY9-IBA+SAH I) HB60-Control, J) HB60-IBA, K) HB60-IBA+SAH, L) HB60-IBA+SAH

faster germination. Applying IBA at a concentration of 500 mg/L to different cassava varieties demonstrated negative effects on all varieties compared to the control treatment. However, it was observed that the RY9 variety outperformed the KU50 and HB60 varieties in many parameters, including shoot length, root number, root length, shoot dry weight, root dry weight, total dry weight, sprouting percentage, and days until germination (Figure 1, E-H). Moreover, SAH application on leaf bud cassava cuttings also had individual effects on each variety. For example, it resulted in a higher weight in HB60 (0.75 g/plant), an average value in KU50 (0.59 g/plant), and a lower weight in RY9 (0.44 g/plant) (Table 2). HB60 also performed at the highest survival percentage (93.65%) followed by RY9 (71.73%) and KU50 (71.43%), respectively. The combination of the auxin IBA with SAH nutrient solution also presented different effects in each variety. Some parameters, such as the sprouting percentage, the survival percentage, and SVI, were higher in KU50 compared to RY9 and HB60. Conversely, root traits (root number, root length, and root dry weight) and shoot length were greater in the RY9. The difference in growth parameters among the cassava cultivars may be attributed to their inherent differences in maturity. A previous study indicated that KU50 reaches optimal harvest maturity around 9 months after planting (MAP), exhibiting the highest starch content at this stage (Hular-Bograd et al., 2011). In contrast, RY9 displays optimal starch content at 10-12 MAP (Prammanee et al., 2010), suggesting a later maturity compared to KU50. HB60, which was assessed for quality traits at 10.5 MAP, falls within a similar maturity range as RY9 (Vichukit et al., 2004). Recent research has also demonstrated that the KU50 variety exhibits superior shoot development and earlier storage root formation at the mature stage of plant development compared to the Hanatee variety at 5 MAP (Chiewchankaset et al., 2022). Although all cultivars were initiated from 5-month-old mother plants in this study, these inherent differences in maturity timelines may have influenced their growth patterns during the experimental period.

3.2 The correlation among measured growth and survival traits

The relationship between growth and survival rates was analyzed using Pearson's correlation coefficient (r) (Figure 2). The results showed that the survival rate was positively correlated with the sprouting rate (r=0.95 p≤0.001), sprouting speed (r=0.86, p≤0.001), dry shoot weight (r=0.86, p≤0.001), number of leaves (r=0.72, p≤0.001), length of root (r=0.68, p≤0.001), and total dry weight (r=0.66, p≤0.001). This indicates that cassava leaf bud seedlings exhibiting fast germination, high sprouting rates, and developing more leaves and roots were indeed crucial for enhancing the survival rate of plantlets. Grossnickle & MacDonald (2017) suggested that several traits enhanced survival rate and promoted growth in crop seedlings. They noted that seedlings with a greater root-system size and stem diameter had a higher chance of avoiding planting stress and enhancing seedling growth. Taller seedlings may have a higher survival rate because they are more likely to have developed a strong root system, which allows them to access water and nutrients more effectively (Pinto et al., 2015). Seedlings with more leaves may have a higher survival rate due to their ability to photosynthesize, providing energy for growth and development (Grossnickle, 2012). This is supported by the finding that leaf traits, particularly the specific leaf area and leaf lifespan, are significant predictors of plant performance (Poorter & Bongers, 2006). Moreover, Muktar et al. (2023) demonstrated that cassava stem cuttings containing at least two nodes exhibited the greatest likelihood of successful propagation and survival when rooted in coco peat or sawdust. The study provided additional information that root length, the number of leaves, and shoot length were critical determinants for the effective propagation of cassava. Neves et al. (2020) found cassava leaf buds from 4–6-month-old mother plants, especially those at the upper stem regions treated with agrochemicals, had a significantly higher sprouting rate and increased plantlet height. This suggests improved survival and growth potential for these cassava plantlets.

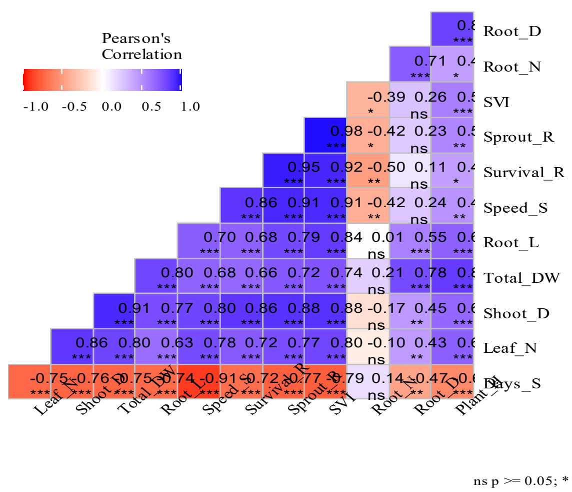

Figure 2. Pearson’s correlation coefficient plot of the growth and survival variables, Root_L = root length (cm), Root_D = root dry weight (g/plant), Plant_H = plantlet height (cm), Shoot_D = shoot dry weight (g/plant), Total_DW = total dry weight (g/plant), Speed_S = speed of sprouting, SVI = seedling vigor index, Days_S = days of reaching the highest sprouting, Survival_R = survival rate (%), and Sprout_R = sprouting rate (%).

3.3 Principal component analysis

The first two components of the PCA analysis explained 88.9 % of the variance in the cassava leaf bud cutting propagation dataset (PCA1: 67.9%; PCA2: 21%) (Figure 3). Quality of representation (cos2) values of plantlet growth and survival variables demonstrated that root number (95%), SVI (95%), sprouting percentage (95%), total dry weight (94%), root dry weight (94%), survival rate (91%), sprouting speed (90%), shoot dry weight (89%), plantlet height (87%), days to reach maximal sprouting (78%), leaf number (77%), and root length (75%), are well represented in PC 1 and PC 2 (Figure 3a). All parameters were strongly associated with the principal components (PCs), indicating they were significant factors in explaining the variation in the growth and propagation of cassava leaf bud cuttings. Correlation analysis of the growth and survival traits (Table 3) showed that shoot dry weight, sprouting percentage, total dry weight, sprouting speed, leaf numbers, root length, shoot length, root dry weight, survival percentage, and SVI were significantly positively correlated to PC 1. The only exception involved the days to reach maximal sprouting, which exhibited a strong negative correlation with PC 1. In our study, it was observed that planting media with faster sprouting rate also achieved higher survival rates and growth parameters (Table 1). This was supported by Schoffel et al. (2022), who reported that faster and higher sprouting rates are advantageous parameters for cassava planting across all seasons. Rapid sprouting and an increased count of nodal sprouts correlated positively with tuber proliferation in cassava. During the sprouting phase, roots emerge from the nodal regions, which serve as the initiation sites for tuber formation (Ntui et al., 2006). Correlation analysis on PC 2 demonstrated that growth traits such as total dry weight, shoot length, root dry weight, and root number were positively correlated, while the survival rate showed a negative correlation. The dominant variables observed in SVI were sprouting percentage, root number, total dry weight, root dry weight, survival rate, sprouting speed, and shoot dry weight (Figure 3b). The PC 1:2 contribution values of plantlet height, day of reaching maximal sprouting, leaf numbers, and root length were below the expected average contribution. Most of the growth and survival traits classified in RY9 variety were in the fourth quadrant, indicating lower values in the number of days to reach maximal sprouting and higher values of other parameters compared to other varieties (Figure 3c). Furthermore, some growth and survival parameters from the control and SAH treatment were positioned in the fourth quadrant, indicating higher values of survival rate, sprouting speed, SVI, sprouting percentage, number of leaves, and root length. In contrast, lower values were observed for the days to reach maximal sprouting and root number compared to IBA and IBA combined with SAH treatments (Figure 3d).

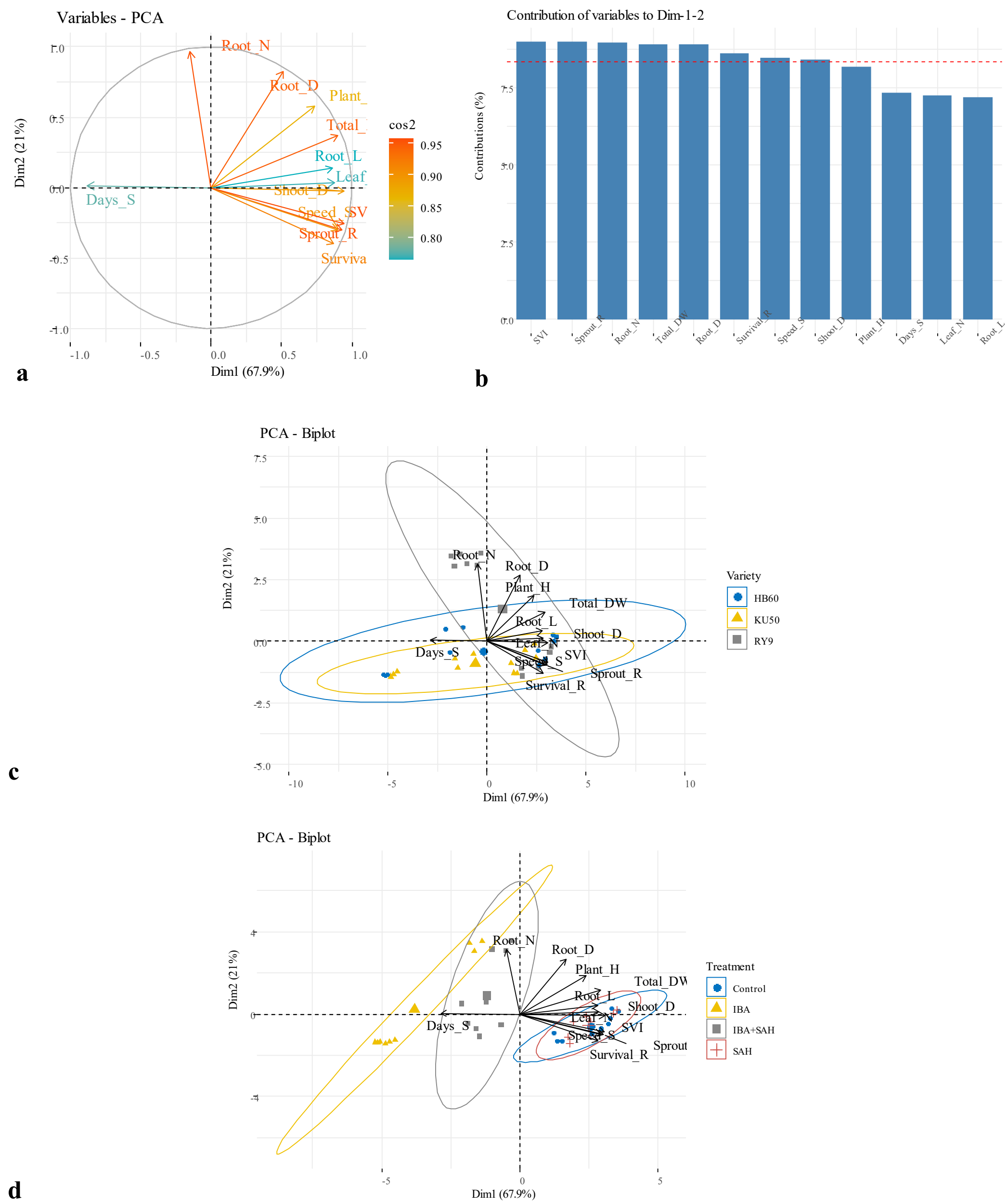

Figure 3. (a) Principal component analysis (PCA) of variables based on the variance in 12 growth and survival traits of the 3 cassava varieties in different media using the leaf bud technique. (b) The most contributing dominant variables to PC1-2. (c) PCA—Biplot of 12 variables for the three different varieties and (d) different media applications Root_L = root length (cm), Root_D = root dry weight (g/plant), Plant_H = plantlet height (cm), Shoot_D = shoot dry weight (g/plant), Total_DW = total dry weight (g/plant), Speed_S = speed of sprouting, SVI = seedling vigor index, Days_S = days of reaching the highest sprouting, Survival_R = survival rate (%), and Sprout_R = sprouting rate (%).

Table 3. Dimension description of cassava plantlets growth and survival parameters to PC 1 and PC 2 at the confidence levels.

Parameters	PC 1 (67.9%)		PC 2 (21%)	
	Correlation	P-Value	Correlation	P-Value
Shoot dry weight (g/plant)	0.945	3.75×10^{-18}	-	-
SVI	0.943	6.41×10^{-18}	-	-
Sprouting percentage (%)	0.929	2.48×10^{-16}	-	-
Total dry weight (g/plant)	0.902	5.82×10^{-14}	0.36	2.76×10^{-2}
Sprouting speed	0.901	6.84×10^{-14}	-	-
Leaf number (no. plant^{-1})	0.877	2.26×10^{-12}	-	-
Survival percentage (%)	0.869	5.77×10^{-12}	-0.40	1.50×10^{-2}
Root length (cm)	0.864	1.05×10^{-11}	-	-
Shoot length (cm)	0.734	3.46×10^{-07}	0.57	2.32×10^{-4}
Root dry weight (g/plant)	0.511	1.42×10^{-03}	0.82	4.52×10^{-10}
Days until germination	-0.883	1.02×10^{-12}	-	-
Root numbers (no. plant^{-1})	-	-	0.96	1.71×10^{-21}

4. Conclusions

This study investigated the impact of various propagation media on root germination and seedling vigor for three different cassava varieties. The findings revealed that the control treatment (distilled water) and SAH nutrient medium provided the most favorable solutions for leaf bud cutting planting for all cassava varieties, particularly for RY9 and HB60, which showed higher values for several growth parameters. Applying IBA at a concentration of 500 mg/L appeared to have a detrimental effect on all cassava varieties, suggesting that its use in leaf bud propagation may not be beneficial. Conversely, the RY9 variety displayed remarkable adaptability, outperforming KU50 and HB60 in shoot length, root number, and dry weight for shoots and roots. The combined application of IBA and SAH nutrient solutions resulted in enhancing certain parameters such as sprouting and survival percentages, particularly in RY9 and HB60. Principal component analysis (PCA) revealed that the first two components accounted for 88.9% of the variance in cassava leaf bud cutting propagation, with the majority of growth and survival traits being significantly associated with these components. The findings of this study suggest that variety selection and careful management of growth conditions, such as the choice of the optimum concentration of plant growth regulators for leaf bud cutting, is crucial for optimizing cassava propagation. Yet, further study on the effects of different concentrations of plant growth regulators on leaf bud cuttings, with a focus on lower concentrations of IBA, is needed.

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6. Conflicts of Interest

The authors declare no conflict of interest.

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