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Effects of Different Growing Media on Propagation of the Cassava Mosaic Disease-Resistant HN5 Variety in Tunnel Greenhouse

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ABSTRACT

Researching rapid multiplication techniques for mosaic-resistant cassava in tunnel greenhouses is crucial for ensuring food security and sustainable agriculture. Our previous findings indicated that a combination of 50% shading and 70–75% humidity significantly enhanced the formation of shoots and growth of cassava seedlings in a tunnel greenhouse. The current study examines the impact of different substrate compositions on cassava propagation. The experiments were designed as split plots with three replicates and four formulas including CTRL: 100% sand with a thickness of 40 cm; CT-1: 100% sand with a thickness of 30 cm; CT-2: 60% sand and 40% gravel with a thickness of 40 cm; CT-3: 70% sand and 30% gravel with a thickness of 40 cm; and CT-4: 50% sand and 50% gravel with a thickness of 40 cm. The results demonstrated that CT-3 yielded the highest shoot formation rate and growth of cassava seedlings, suggesting that the combination of sand (70%) and gravel (30%) at a thickness of 40 cm provide the most favorable conditions for cassava propagation in tunnel greenhouse conditions.

Keywords: cassava, propagation, mosaic disease, growing media, sand and gravel.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a crucial staple crop in many tropical and subtropical regions worldwide. Originating from South America, cassava has become a significant source of carbohydrates and industrial raw materials in Africa, Asia, and Latin America (Fatima et al., 2023). Its ability to thrive in poor soils and tolerate drought conditions makes it a reliable food security crop in many developing countries (FAO, 2020). In Vietnam, cassava has grown in importance over recent years, both as a food source and as a vital input for the biofuel and starch industries. The country ranks among the top cassava producers globally, with large-scale cultivation primarily concentrated in the Central Highland and Southern regions (Ministry of Agriculture and Rural Development, 2022). This expansion has been driven by government policies promoting crop diversification and rural development, as well as the increasing demand for cassava-derived products in both domestic and international markets (Kim et al., 2015).

However, cassava production faces significant challenges due to diseases, particularly cassava mosaic disease (CMD). CMD is caused by several species of geminiviruses, and it severely impacts cassava yield and quality (Legg et al., 2014). Infected plants exhibit symptoms such as mosaic patterns on leaves, stunted growth, and reduced tuber production (Bock and Woods, 1983). CMD has been a major concern in Africa for decades, but in recent years, it has also become a critical issue in Asia, including Vietnam (FAO, 2016). The rapid spread of CMD is facilitated by whitefly vectors and the use of infected planting material (Fargette et al., 1990). Effective management strategies are urgently needed to control the disease and ensure sustainable cassava production. Among the strategies being explored, the development and propagation of mosaic-resistant cassava varieties are of paramount importance (Patil and Fauquet, 2009). This approach not only curbs the spread of the disease but also improves the overall resilience and productivity of cassava crops (Thresh and Cooter, 2005).

Various methods are employed for cassava propagation, including traditional stem cuttings, tissue culture, and the use of controlled environments like tunnel greenhouses. Traditional stem cuttings are the most common method due to their simplicity and low cost (Hillocks and Thres, 2002). However, this method has limitations, such as the potential spread of diseases and pests, and the relatively slow multiplication rate (Ospina and Ceballos, 2002). Tissue culture techniques offer a more sterile and rapid means of propagation but require sophisticated laboratory facilities and technical expertise (Roca and Mroginski, 1991). In contrast, tunnel greenhouses provide a middle ground, offering a controlled environment that enhances plant growth and reduces disease incidence (Pandey et al., 2023). The advantages of tunnel greenhouse propagation include improved microclimate control, which optimizes temperature, humidity, and light conditions, leading to faster and healthier plant development (Hartmann and Kester, 1975). The composition of the growth media also plays a crucial role in the success of cassava propagation, particularly in controlled environments like tunnel greenhouses. The proper growth media not only supports the plant physically but also affects water retention, aeration, and nutrient availability, all of which are critical for shoot formation and growth of cassava seedlings (Sousa et al., 2024). In the previous study, our study found that with the growth media containing 100% of sand 40 cm thick, 98.5% of cassava stem cutting produces shoots, and the average shoot per cutting is 1.5 (Nguyen and Nguyen, 2024). Nevertheless, the long-term propagation makes the sand substrate become compacted and reduces air permeability, which inhibits the growth of cassava cuttings. Furthermore, the discovery of other cheaper substrates may replace part of the sand without impacting the efficiency of cassava propagation in the long-term.

For this reason, the aim of this study is to investigate the effects of different ratios of gravel contained in propagation media on the formation of shoots on cassava stem cutting and some growing parameters of cassava seedlings after 14, 21, 28, and 35 days of planting. The obtained results might assist in optimizing the propagation techniques, ultimately contributing to the sustainability and productivity of cassava cultivation in regions affected by CMD.

MATERIALS AND METHODS

Cassava cutting preparation

The 10-month old cassava cuttings used in this study were created from the HN5 variety, which is resistant to cassava mosaic disease, and was supplied by the Agricultural Research Institute of Vietnam. All the cutting of cassava was obtained from the filed condition through multi-steps, including field cultivation, seedling transplantation, fertilization, irrigation, and other standard procedures, which were conducted in accordance with the methods outlined in the Vietnamese National Standards QCVN-01-61-2011.

Experiment design

The experiments were conducted in the tunnel greenhouse located at Tay Nguyen University, which experiences approximately 2.473 hours of bright sunshine each year, with an annual temperature ranging from 23 °C to 25 °C, humidity around 82%, and dimensions of 4 meters in width, 10 meters in length, and 3.5 meters in height. The experiments were split plots designed with three replicates with four formulas of different substrate compositions and ratios, including Control (CTRL): 100% sand, 40 cm thickness; formula 1 (CT-1): 100% sand, 30 cm thickness; formula 2 (CT-2): 60% sand and 40% gravel, 40cm thickness; formula 3 (CT-3): 70% sand 30% gravel, 40 thickness; and formula 4: 50% sand 50% gravel, 40 cm thickness. Each plot (120×80×40 cm) was filled with sand (sizes ranging from 0.25–0.5 mm) and gravel (sizes ranging from 6–8 mm). In each plot, cassava cuttings were arranged of 32 at a density cuttings (8×4).

Data collection

The shoot emergence rate and number of shoots per stem cutting were recorded at 14 days after planting (DAP). Plant height, number of leaves, and shoot diameter were measured at 14, 21, 28, and 35 DAP. Plant height (cm) is the distance from the media surface to the highest position of the leaf. Number of leaves was counted of all full-grown leaf attached to the plant. Shoot diameter is measured by the calipers at the middle position of the shoot. The shoot emergence rate (%), number of shoots per cutting, and number of leaves per shoot were calculated using the following formulas:

$$The\ shoot\ emergency\ rate\ (\%) = \\ = \frac{Number\ of\ cuttings\ emerging\ shoots}{Total\ of\ cuttings\ usage} \times 100 \quad (1)$$

$$The\ number\ of\ shoot\ per\ cutting\ = \\ = \frac{Number\ of\ \text{shoot emergency}}{Number\ of\ cutting\ emerging\ shoots} \quad (2)$$

$$The\ number\ per \frac{leaves}{shoot} = \\ = \frac{Number\ of leaves}{Number\ of\ shoots} \quad (3)$$

Statistical analysis

The Statistical Tool for Agricultural Research (STAR) software, version 2.0.1 (2014), was used to conduct an analysis of variance (ANOVA) at P ≤ 0.05, separating mean values by Turkey's honest significant difference test at P < 0.05.

RESULTS

The effects of different growth media on shoot emergence rate and number of shoot per cassava stem cutting

The rate of shoot emergence and number of formed shoots per cassava stem cutting is an important factor impacting the quantity of cassava seedlings and the efficiency of the propagation process (Mbise et al., 2024). The results showed that changes in substrate composition and ratio impacted slightly on shoot production of cassava cuttings after 14 days after planting (DAP). The evidence is all of the cutting cultivated on tested media reached a high rate of shoot emergence, over 96%. Among these, the highest number of cuttings producing shoots was observed in the CT-3 formula with 98.4%, which was similar to that on control but significantly higher (P < 0.05) than those of CT-1, CT-2, and CT-4 formulas with 96.5, 97.4, and 95.7%, respectively. In addition, the cuttings of the CT-3 formula produced 1.6 shoots per cutting on average, statistically higher than those of control and other tested formulas, which reached from 1.3 to 1.5 shoots/cutting (Table 1). This suggests that gravels might be an appropriate substrate for media of cassava propagation in tunnel greenhouse.

Table 1. Effects of growing media on shoot formation of the cassava mosaic disease-resistant HN5 variety in tunnel greenhouse

Formula	Shoot emergence rate	No. shoot/stem cutting
CTRL	98.3 ± 0.2[a]	1.4 ± 0.1[b]
CT-1	96.5 ± 0.3[c]	1.3 ± 0.1[b]
CT-2	97.4 ± 0.4[b]	1.4 ± 0.1[b]
CT-3	98.4 ± 0.3[a]	1.6 ± 0.1[a]
CT-4	95.7 ± 0.7[c]	1.3 ± 0.1[b]

Note: The data are mean and standard deviation from 3 repetitions. In the same column, different letters show a significant difference of means (Tukey test, P < 0.05).

Different growth media impact on number of leaves per cutting

To find out whether different compositions and ratios of growing media affect on leaf development of formed shoots, the number of leaves per stem cutting across different treatments (CTRL, CT-1, CT-2, CT-3, and CT-4) was evaluated at 14, 21, 28 and 35 DAP.

The data in Table 2 indicated that the shoots of the control group (CTRL) exhibited the strongest production of leaves with 9 leaves/cutting at 14 DAP, whereas there was an insignificant difference of those among CT-1, CT-2, and CT-3, whose were from 7 to 8.7 leaves/cutting. The lowest number of leaves/cutting was observed in CT-4 formula with 6 leaves/cutting.

Notably, from 21 till 35 DAP, the number of leaves/cutting of CT-3 reached equally to that of the CTRL; both formulas had over 17 leaves/cutting at 35 DAP, significantly higher than those of the rest. The second position belonged to CT-1 and CT2 with over 15 leaves/cutting while the cutting of CT-4 exhibited the lowest number of leaves, demonstrating that CT-3 was the most effective treatment, leading to the highest number of leaves per stem cutting by the end of the study period.

Effects of media substrate composition on shoot height

At 14 DAP, there was no significant difference between the shoot height of CTRL and CT-3 formulas with 11.8 and 12 cm, respectively. Following the shoots of CT-2 with 10 cm in height, the shortest shoots were observed in CT-1 and

Table 2. Effects of growing media on the number of leaves per cutting of the cassava mosaic disease-resistant HN5 variety in tunnel greenhouse

No. leaf per stem cutting				
Formula	14 DAP	21 DAP	28 DAP	35 DAP
CTRL	9.0 ± 0.5[a]	12.0 ± 0.4[a]	14.8 ± 1.2[a]	17.3 ± 0.6[a]
CT-1	7.0 ± 1.1[b]	8.7 ± 0.2[b]	11.7 ± 1.2[b]	15.3 ± 1.2[b]
CT-2	8.2 ± 1.1[b]	10.3 ± 0.5[b]	13.1 ± 1.1[b]	15.7 ± 0.6[b]
CT-3	8.7 ± 0.6[b]	12.2 ± 0.6[a]	15.3 ± 1.1[a]	17.7 ± 1.2[a]
CT-4	6.2 ± 0.6[b]	9.0 ± 1.7[b]	11.3 ± 1.5[b]	13.2 ± 0.8[b]

Note: The data are mean and standard deviation from 3 repetitions. In the same column, different letters show a significant difference of means (Tukey test, $P < 0.05$).

CT-4 formulas, whose shoot heights were 8 cm. The increase in height of shoots of CTRL and CT-3 continued to remain at a high rate; evidence was that the shoot heights of these formulas at 28 and 35 DAP were 2.7 and 3.8 folds, respectively, higher than those at 14 DAP. The highest shoot heights were over 44 cm at 35 DAP in the CT3 and CTRL formulas (Table 3). The high gravel ratio in the media of CT-2 and CT-4 formulas likely inhibited the development of shoots with 35.2 and 30.3 cm in height, respectively at 35 DAP. Additionally, the reduction of sand thickness also significantly affects on shoot heights of the CT-1 formula compared to that of the CTRL, with 32 cm against 44.6 cm at 35 DAP. The results suggest that CT-3 again has positive effects on the development of cassava shoots during propagation in tunnel greenhouse conditions.

Effects of media substrate composition on shoot diameter

Stem diameter is one of the most important factors in evaluating the cassava shoot development during propagation. In this study, the stem diameter of shoots in different formulas was measured at 14, 21, 28, and 35 DAP. Similarly, the data in Table 4 showed that the shoots produced by the cuttings from CTLR and CT-3 formulas had the biggest diameter compared to the rest at 35 DAP with 4.5 and 5.0 mm, respectively even though there was no significant difference in shoot diameter at 14 DAP when the shoot diameter of all tested formulas was around 1.8 mm, indicating the long – term effects of media composition on cassava stem development during the propagation.

Comparison of shoot formation and development of the cutting cultivated in growing media contained in trays and spread on soil surface

During the cassava propagation in growing media spreading on the soil surface, the results suggested that the emerging shoots may be infected cause of soil-born microbes. To prevent the infection of cassava shoots, experiments on the growing media-containing trays were conducted. In these tests, all the cassava cuttings were planted in the growing media selected from CTRL and CT-3 formulas contained in the trays whose location was 1m higher than the soil surface. At 14

Table 3. Effects of growing media on shoot height of the cassava mosaic disease-resistant HN5 variety in tunnel greenhouse

Shoot height (cm)				
Formula	14 DAP	21 DAP	28 DAP	35 DAP
CTRL	11.8 ± 0.7[a]	22.3 ± 0.6[a]	31.7 ± 2.1[a]	41.7 ± 4.0[a]
CT-1	8.7 ± 1.2[b]	20.3 ± 0.7[b]	26.0 ± 3.5[bc]	32.2 ± 5.1[b]
CT-2	10.0 ± 1.0[b]	20.7 ± 0.6[b]	27.4 ± 3.6[b]	35.2 ± 4.4[b]
CT-3	12.0 ±0.4[a]	21.3 ± 0.6[a]	32.3 ± 3.5[a]	42.9 ± 3.5[a]
CT-4	8.7 ± 0.6[b]	19.0 ± 0.6[bc]	25.3 ± 3.8[c]	30.3 ± 5.9[c]

Note: The data are mean and standard deviation from 3 repetitions. In the same column, different letters show a significant difference of means (Tukey test, $P < 0.05$).

Table 4. Effects of growing media on shoot stem diameter of the cassava mosaic disease-resistant HN5 variety in tunnel greenhouse

Shoot diameter (mm)				
Formula	14 DAP	21 DAP	28 DAP	35 DAP
CTRL	1.8 ± 0.4[a]	2.5 ± 0.1[c]	3.6 ± 0.2[b]	4.5 ± 0.5[a]
CT-1	1.8 ± 0.6[a]	2.7 ± 0.1[b]	3.5 ± 0.3[b]	3.8 ± 0.4[b]
CT-2	1.8 ± 0.4[a]	2.9 ± 0.1[a]	3.6 ± 0.2[a]	4.4 ± 0.3[ab]
CT-3	1.8 ± 0.4[a]	2.9 ± 0.1[a]	4.2 ± 0.2[a]	5.6 ± 0.5[a]
CT-4	1.7 ± 0.3[a]	2.6 ± 0.1[bc]	3.4 ± 0.2[c]	3.7 ± 0.3[c]

Note: The data are mean and standard deviation from 3 repetitions. In the same column, different letters show a significant difference of means (Tukey test, $P < 0.05$).

DAP, the rate of shoot emergence and number of shoots/cutting were measured, and other developing parameters were estimated at 35 DAP.

Interestingly, there is no significant difference between the rate of shoot emergence in the experiments. Propagating the cutting on growing media contained in trays also showed a high formation of the shoot with around 98% (Table 5). Surprisingly, the number of shoots/cutting cultivating in trays increased significantly to 2.4 shoots against 1.6 shoots in CT-3 grown on the soil surface, whereas those of CTRL formula slightly reduced.

DISCUSSION

Previous research has demonstrated that substrate physical properties, such as aeration, drainage, and nutrient availability, play vital roles in plant growth and development (Smith et al. 2018). Sand and gravel, due to their coarse nature, improve drainage and aeration within the substrate. This is essential for root respiration and the prevention of waterlogging, which can lead to root diseases (Jones et al. 2017). In our study, the effects of different ratios between sand and gravel on cassava shoot formation and development were investigated.

Our data showed that the incorporation of sand and gravel at the ratio of 70:30 with 40 cm of thickness in the growing medium of cassava propagation in tunnel greenhouse conditions significantly influences the rate of shoot formation in cassava cuttings. Cuttings planted in substrates with a proper proportion of sand and gravel exhibited a faster shoot emergence. This finding aligns with the results of Li et al. (2020), who reported that improved aeration and drainage conditions in sand-based substrates promote faster root development and subsequent shoot emergence. Furthermore, the gravel in the media may improve soil aeration by creating spaces that allow air to flow freely around the plant roots. This is particularly beneficial in compacted soils, which can restrict airflow and lead to anaerobic conditions detrimental to root health (Ben-Noah et al., 2028). The inclusion of gravel in the substrate helps prevent this by maintaining soil porosity and allowing oxygen to reach the roots effectively (Nektarios et al., 2011; Ignatieva et al., 2011). However, a high proportion of gravel can lead to rapid water loss and insufficient moisture availability for plant roots (Goodman and Ennos, 1999) that affects seedling development. This can explain why the ratio of gravel increased to 40% (CT-2) and 50% (CT-4) in the media negatively impacts shoot formation.

Table 5. Effects of growing media container on shoot formation of the cassava mosaic disease-resistant HN5 variety in tunnel greenhouse

Rate of shoot emergence (%)			Number of shoots/cutting	
Parameter	Soil surface	Tray	Soil surface	Tray
CTRL	98.4 ± 0.4[a]	97.7 ± 0.4[a]	1.5 ± 0.08[a]	1.3 ± 0.08[b]
CT-3	98.4 ± 0.2[a]	99.0 ± 0.9a	1.6 ± 0.09[b]	2.4 ± 0.06[a]

Note: The data are mean and standard deviation from 3 repetitions. With each parameter in the same row, different letters show a significant difference of means (Tukey test, $P < 0.05$).

In a study focusing on the use of gravel in green roof substrates, it was found that substrates containing gravel showed improved growth rates in plants compared to those without. This was attributed to better water management and enhanced aeration provided by the gravel, which helped maintain an optimal balance of moisture and air in the substrate (Williams et al., 2010). This might lead to promote shoot formation per cutting of CT-3, which contained an appropriate ratio of gravel compared to other formulas.

The enhanced drainage properties by well-mixing sand and gravel likely prevent the accumulation of excess water, reducing the risk of root rot and other water-related stresses that can inhibit leaf development. According to Green et al. (2021), substrates with improved physical properties support healthier root systems, which in turn sustain better leaf growth, possibly leading to an increase in the number of leaves/cutting observed in the shoots from CT-3.

The improved root health and enhanced aeration provided by the optimal proportion of sand and gravel substrates contribute significantly to overall plant vigor and growth (Watson et al., 2014). The correlation between substrate aeration and shoot height has been documented in other studies, such as those by Thompson et al. (2016), indicating that well-aerated substrates support taller shoot growth due to better nutrient uptake and root development, especially with seedlings. Similarly, Roberts and Johnson (2015) reported that plants grown in well-drained substrates exhibit thicker and more vigorous shoots, attributed to better root health and nutrient availability.

Interestingly, the data in Table 5 showed that there was a significant difference in the number of shoots per cutting between CT-3 in the container (2.4 shoots) and on the soil surface (1.6 shoots), indicating the position of growth media might impact on shoot formation of cassava cuttings in the tunnel greenhouse condition. In this study, the trays containing the CTRL and CT-3 media were 1 meter higher than the soil surface, likely leading to reaching a higher light intensity. Among abiotic factors, light plays a prominent role in agricultural production (Yang et al., 2014) since its indispensable for photosynthesis and biomass production (Wang et al., 2013). In the same line, the work on strawberry showed that the number, total dry mass, and total fresh mass of the seedlings increased with the increase in light intensity (Xu and Hernández, 2020). Increasing light intensity also positively affected on rape seedling height, stem diameter, root length, leaf area, and dry weight via promoting photosynthesis (Yao et al., 2017).

CONCLUSION

In conclusion, our research highlights the significant impact of substrate composition on the propagation of mosaic-resistant cassava in tunnel greenhouses. The study found that a substrate with 70% sand and 30% gravel, at a thickness of 40 cm, yielded the highest rates of shoot formation and seedling growth. These findings underscore the importance of optimizing substrate conditions to enhance cassava propagation efficiency.

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