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### Effects of black sun shade nets and humidity levels on cassava growth for minicutting propagation

Nguyen Van Minh<sup>1</sup>, Nguyen Van Loc<sup>2</sup>

<sup>1</sup>Faculty of Agriculture and Forestry, Tay Nguyen University, Dak Lak, 63100, Vietnam <sup>2</sup>Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi, 131000

\*Corresponding author: nguyenvanloc1012@gmail.com

### Abstracts

This research presents the effects of black sun shade nets and humidity levels on cassava growth for mini-cutting propagation. The experiments were split-plots design with three replicates where two net shade covering treatments: one with 100% coverage using black sun shade net and the other with no covering as control were assigned in main plots and three humidity levels: 65-70%, 70-75%, and 75-80% to sub-plots. Data on shoot emergence rate, numbers of shoots per stem cutting, plant height, number of leaves and shoot diameter were measured. The obtained results suggested that the combination of 50% covering with black sun shade nets and maintaining a humidity level of 70-75% is effective for cassava multiplication Our findings may help in further strategy for the application in cassava rapid propagation.

### Key words: cassava, black sun shade nets, humidity levels.

Abbreviations: CMD\_Cassava mosaic disease, DAP\_Days after planting, FAO\_The Food and Agriculture Organization, IMARC\_ International Market Analysis Research and Consulting, SLCMV\_The Sri Lankan Cassava Mosaic Virus.

### Introduction

Cassava (*Manihot esculenta* Crantz) has been recognized as a significant crop of the 21<sup>st</sup> century, as its importance in global agriculture is increasingly prominent, ranking only behind rice and maize (FAO, 2013). Cassava is known for its favorable cultivation characteristics, particularly its high tolerance to drought, relying mainly on rainfall (Burns et al. 2010). Therefore, cassava is often referred to as the "crop of the poor" due to its suitability for cultivation in impoverished regions. In 2020, global cassava production reached 298.8 million tons, an increase of 126.8 million tons compared to 2000, and is estimated to reach 357 million tons by 2028 (IMARC, 2021).

Alongside the impressive growth in cassava production, there are many challenges associated with the development of cassava, with the most prominent being cassava mosaic disease (CMD) caused by the virus (Carmo et al., 2015). The Sri Lankan Cassava Mosaic Virus (SLCMV) strain is identified as the cause of cassava mosaic disease, which spreads through infected cassava planting material and the whitefly vector (Wang et al., 2016; Aimone et al., 2022). In order to promote sustainable growth of cassava, researching and applying technical measures to mitigate the negative impacts of cassava mosaic disease is considered a crucial task in the current context. Among the technical methods, the propagation stage is given emphasis to minimize disease contamination.

Cassava propagation traditionally involves planting lignifiedstem cuttings directly in the field (Howeler and Maung Aye 2014). However, the multiplication rate using this approach is limited to about ten times per year (FAO, 2013; Howeler and Maung Aye, 2014). Prolonged cultivation in field conditions also poses a heightened risk of disease infection. Therefore, efficient methods for cassava propagation and the establishment of a healthy planting system are crucial. Of these, an aeroponic culture system and plant tissue culture rapidly produce a large number of plants for creating a disease-free system (Tokunaga et al., 2019; Mafla et al., 2010; Uke et al., 2021). Nevertheless, large-scale propagation through these methods demands a substantial initial financial investment to establish and maintain facilities, equipment, and a trained technical team. Another propagation technique is the mini-cutting method, which utilizes small stem cuttings of approximately 5 cm in length and is planted in soil. With this approach, the multiplication rate can reach between 60 and 100 times per year (NurulNahar and Tan, 2012). Notably, minicutting propagation provides a cost-effective alternative to the aeroponic system and eliminates the necessity for hydroponic

solutions and specialized equipment, making it a practical choice for farmers. To enhance the effectiveness of minicutting cassava propagation methods, it is crucial to implement technical measures for producing a substantial number of stem cuttings in the initial phase. In this study, we conclude that appropriate covering and moisture levels are essential for the rapid propagation process of cassava.

### Results

### Variance analysis

Covering black sun shade net (C) had a significant effect on shoot emergence rate, plant height, leaf numbers per plant, but not on shoot numbers per stem cutting. Humidity treatment (T) had a significant effect on all traits except shoot diameter. CxT interaction had a significant effect on all traits except shoot numbers per steem cutting and shoot diameter (Table 2).

## The effects of black sun shade nets and humidity levels on the shoot emergence rate

The results in Table 3 indicate that the shoot emergence rate of cassava varies among different formulas. The shoot emergence rate of cassava ranges from 95.1% (CT1) to 98.5% (CT4). Statistical analysis shows that there is a significant difference in germination rate between CT4 and CT1, as well as CT3, at a 95% confidence level. The other combinations do not show a significant difference.

# The effects of black sun shade nets and humidity levels on number of shoots per stem

Similar to the shoot emergence rate, the number of shoots per stem cutting in CT4 also shows a significant difference compared to CT1 and CT3. The number of shoots per stem cutting ranges from 1.26 to 1.53 shoots per stem. Therefore, according to the cassava multiplication method in the plastic net house, it results in higher shoot emergence rates and shoot numbers per stem cutting compared to traditional propagation methods. CT4 demonstrates the highest number of shoots per stem cutting (1.5 shoots per stem cutting) (Table 3).

## The effects of black sun shade nets and humidity levels on plant height

The results in Table 4 show that at the 14 DAP and 21 DAP, almost all formulas did not exhibit statistically significant differences. However, at 28 DAP, the formulas started to show differences in height. Specifically, CT4 had a height of 32.7 cm and CT6 had a height of 32 cm, which differed from CT1 with a height of 28.7 cm and CT3 with a height of 29.0 cm. Similarly, at 35 DAP, only CT4 and CT6 showed height differences compared to the other formulas.

## The effects of black sun shade nets and humidity levels on number of leaves per plant

The actual leaf counts of cassava plants vary after each counting, even within the same formula. Formula CT4: Moisture content of 70-75% and coverage in all counts starting from 14 to 35 days after planting (DAP) consistently yielded the highest results in the following order: 9, 12, 15, and 18 leaves per plant. The statistical analysis shows a significant difference between CT1 and CT5 (at 14 and 35 DAP) at a 95%

level of confidence. No differences have been observed in the other formulas (Table 5).

## The effects of black sun shade nets and humidity levels on stem diameter

The obtianed results showed that the moisture content of 70-75% and coverage has indeed helped in the faster development of stem diameter. In formula CT4: Moisture content of 70-75% (with coverage), the recorded stem diameter of the seedlings at all counting instances, namely 14 DAP, 21 DAP, 28 DAP, and 35 DAP, follows the order of 2.13 mm, 3.03 mm, 4.13 mm, and 4.96 mm, respectively. This difference is statistically significant compared to CT1 and CT5 at a 95% level of confidence (Table 6).

#### Discussion

Many previous studies have indicated that color shade nets as useful materials in agricultural production (Tezcan et al., 2023). Covering the black sun shade net helps regulate the temperature inside the greenhouse, preventing excessive heat and protecting plants from direct sunlight (Kotilainen et al., 2018; Kittas et al., 2019; Verdaguer et al., 2017). It minimizes plant stress, allows for optimal photosynthesis, and promotes healthy growth and development (Kotilainen et al., 2018). In this study, the results showed that covering a black sun shade net had a significant effect on all measured traits except shoot numbers per stem cutting (Table 2). As compared to control, covering black net enhanced cassava growth in mini-cutting propagation.

Soil moisture is an essential to form growth tissue in an active plant (Thitiporn et al., 2020). Adequate soil moisture is indeed crucial for the successful germination of cassava stem cuttings and for promoting healthy root development during the early stages of growth (Yonis et al., 2020). Insufficient moisture can hinder root growth and may lead to weak or stunted plants. The finding of this study indicated that the ideal moisture level falls within the range of 70-75%.

In a combination, the result in this study showed that covering black sun shade net by humidity treatment interaction had significant effects on almost trait. Interestingly, the combination of covering with black sun shade nets and maintaining a humidity level of 70-75% is the most effective for cassava propagation. Our findings may help in further strategy for the application in cassava production

### **Materials and Methods**

### Plant materials

The cassava cultivar HN5 was provided by Agricultural Research Institute of Vietnam. HN5 is resistance to cassava mosaic disease. A 9-month-old cassava stem (stored for 2 months after harvesting to break dormancy) with a 3 cm stem diameter was used to cut into sections, each approximately 10 cm in length, with each section containing 3 dormant buds. These stem cuttings were used for propagation in this experiment.

#### Experimental design

Six plastic net houses with size of  $10m \times 4m \times 3.5m$  and covered with layers of polyethylene film were used for

Table 1. Treatment details. There were two-black sun shade net treatments (C1-C2) and three humidity levels (A1-A3)					
Black sun shade net treatments (C)		Humidity trea	Humidity treatment (T)		
C1	None covering	A1	65-70%		
C2	50% covering	A2	70-75%		
		A3	75-80%		





Figure 1. The net covering treatments :(A)None covering of lack sun shade nets and (B) covering of lack sun shade nets

Table 2. ANOVA analysis for effects of black sun shade nets and humidity levels on measured traits of cassava.

Trait	<i>F</i> -value			
	Black sun shade net	Humidity treatment (T)	C x T interaction	
	treatments (C)			
Shoot emergence rate	8.93*	14.93**	5.5*	
Shoot numbers per stem cutting	0.1 <sup>ns</sup>	4.1*	2.2 <sup>ns</sup>	
Plant height	32.96***	8.04*	12.5*	
Leaf numbers per plant	15.57***	53.61***	6.46*	
Shoot diamter	5.41*	1.15 <sup>ns</sup>	0.26 <sup>ns</sup>	

\*, \*\*, and \*\*\* are significantly different at 0.05, 0,01 and 0,001 probabality, respectively.



Figure 2. Cassava plants under experimental plot.

Table 3. Effects of black sun shade nets and humidity levels on shoot emergence rate and shoot numbers of cassava.

Formula	Shoot emergence rate	Shoot numbers/	
	(%)	stem cutting	
CT1	95.1 <sup>d</sup>	1.3 <sup>c</sup>	
CT2	96.2 <sup>cd</sup>	1.4 <sup>b</sup>	
СТЗ	95.7 <sup>bc</sup>	1.3 <sup>c</sup>	
CT4	98.5°	1.5 <sup>ª</sup>	
CT5	96.7 <sup>c</sup>	1.4 <sup>b</sup>	
СТ6	97.7 <sup>b</sup>	1.4 <sup>b</sup>	

Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

 Table 4. Effects of black sun shade nets and humidity levels on plant height of cassava.

Formula	Plant height at			
	14 DAT	21 DAT	28 DAT	35 DAT
CT1	8.7 <sup>a</sup>	19.3 <sup>c</sup>	28.7 <sup>e</sup>	40.0 <sup>d</sup>
CT2	9.7 <sup>a</sup>	21.7 <sup>abc</sup>	30.3 <sup>d</sup>	41.3 <sup>cd</sup>
СТЗ	9.3 <sup>ª</sup>	20.0 <sup>ab</sup>	29.0 <sup>e</sup>	41.0 <sup>cd</sup>
CT4	12.3 <sup>ª</sup>	23.7 <sup>a</sup>	32.7 <sup>a</sup>	44.7 <sup>a</sup>
CT5	10.3 <sup>ª</sup>	22.3 <sup>ab</sup>	31.3 <sup>c</sup>	42.7 <sup>c</sup>
CT6	11.2 <sup>ª</sup>	23.0 <sup>a</sup>	32.0 <sup>b</sup>	43.7 <sup>b</sup>

Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

Table 5. Effects of black sun shade nets and humidity levels on leaf numbers of cassava.

Formula	Leaf numbe	Leaf numbers at		
	14 DAT	21 DAT	28DAT	35 DAT
CT1	5.7 <sup>e</sup>	8.0 <sup>e</sup>	11.0 <sup>e</sup>	14.0 <sup>d</sup>
CT2	6.7 <sup>d</sup>	9.7 <sup>cd</sup>	12.0 <sup>d</sup>	14.7 <sup>cd</sup>
СТЗ	6.3 <sup>d</sup>	9.0 <sup>d</sup>	11.7 <sup>d</sup>	13.7 <sup>d</sup>
CT4	9.0 <sup>ª</sup>	12.0 <sup>ª</sup>	15.1 <sup>ª</sup>	18.0 <sup>ª</sup>
CT5	7.3 <sup>°</sup>	10.3 <sup>c</sup>	12.7 <sup>c</sup>	15.3 <sup>°</sup>
CT6	8.3 <sup>b</sup>	11.3 <sup>ab</sup>	14.0 <sup>b</sup>	16.7 <sup>b</sup>

Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

Table 6. Effects of black sun shade nets and humidity levels on shoot diameter of cassava.

Formula	Shoot diameter at			
	14 DAT	21 DAT	28 DAT	35 DAT
CT1	1.66 <sup>c</sup>	2.50 <sup>c</sup>	3.66 <sup>d</sup>	4.53 <sup>b</sup>
CT2	<sup>abc</sup> 1.90	2.66 <sup>bc</sup>	<sup>bcd</sup> 3.86	4.66 <sup>b</sup>
СТЗ	1.73 <sup>bc</sup>	2.56 <sup>c</sup>	3.76 <sup>cd</sup>	4.60 <sup>b</sup>
CT4	2.13 <sup>ª</sup>	3.03 <sup>ª</sup>	4.13 <sup>a</sup>	4.96 <sup>a</sup>
CT5	1.96 <sup>ab</sup>	2.73 <sup>bc</sup>	<sup>abc</sup> 4.00	4.73 <sup>ab</sup>
CT6	2.06 <sup>a</sup>	2.86 <sup>ab</sup>	4.06 <sup>ab</sup>	4.76 <sup>ab</sup>

Mean values with different alphabet letters are significantly different at 0.05 probability and vice-versa.

experiments in this research. These plastic net houses were set up in a location that experiences approximately 2,473 hours of bright sunshine each year, with an annual temperature ranging from  $23^{\circ}$ C to  $24^{\circ}$ C and humidity levels typically around 82%. The experiments were Split-plots design with three replicates where two net shade covering treatments:one with 50% coverage using black sun shade net and the other with no covering as control) were assigned in main plot (Figure 1) and three humidity levels: 65-70%, 70-75%, and 75-80% to subplots. A total of six formulas were created using this arrangement method. (Table 1). Each plot (120 x 80 x 40 cm) was filled with sandy soil (sand particles have sizes ranging from 0.25mm to 0.5mm) and controlled for moisture contents (Figure 2). In each plot, cassava cuttings were arranged at a density of 32 cuttings (8 x 4cm)..

### Data collection

The shoot emergence rate and numbers of shoots per stem cutting recorded at 14 days after planting (DAP). Plant height, number of leaves and shoot diameter were measured at 14 DAP, 21 DAP, 28 DAP, and 35 DAP. Plant height was the height of stem from the surface of the soil pot to the top of the main plant stem. Shoot diameter was measured at a 2 cm height.

### 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 $\leq$ 0.05; separating mean values by Turkey's honest significant difference test at P<0.05.

### **Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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