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Leaf morphology characterization and propagation of *Cnidoscolus aconitifolius* (Redonda cultivar) using different stem cutting lengths in the tropical ecosystem

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Abstract. *Gustiar F, Lakitan B, Muda SA, Ria RP, Simamora IA. 2024. Leaf morphology characterization and propagation of* Cnidoscolus aconitifolius *(Redonda cultivar) using different stem cutting lengths in the tropical ecosystem. Biodiversitas 25: 2836-2844.* Chaya (*Cnidoscolus aconitifolius* (Mill.) I.M.Johnst.) is a kind of perennial vegetable known to originate from Mexico and thrive in the tropical climate of Asia, with different leaf morphology as well as several cultivars, including Redonda cv. Therefore, this study aims to assess the leaf morphology of Chaya Redonda cv. and its growth on different stem cutting lengths of 20 cm, 25 cm, and 30 cm, respectively. All treatments were repeated four times and each replication contained five plant units, while collected data included characteristics in the form of leaf length and width, area, growth rate, and yield. Based on observation through a dimensional method, the Chaya Redonda cv. leaf had a square size with the bottom and top width identical to the ratio value of length and width consistently approaching 1.0 and remaining constant across the development process. The results showed that leaf length (L_2) x bottom width (W_1) regression had the highest accuracy level for determining undamaged chaya leaf area with a coefficient of determination (R^2) value of 0.9839. The potential of cuttings with a length of 30 cm was confirmed to have high growth capacity, which also produced a positive effect on leaf number and area, as well as canopy area and fresh leaf weight.

Keywords: Leaf area, leaf shape, planting material, Redonda cultivar

Abbreviations: DAP: Days After Planting; SPAD: Index indicating the greenness level of leaves; WAP: Week After Planting

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius* (Mill.) I.M.Johnst.) is a perennial vegetable plant from southern Mexico and Guatemala. Several cultivars exist, including Redonda cv. (Montero-Astúa et al. 2023). Due to the similarity of the agroecosystem with the area of origin, this plant can thrive in Indonesia (Gustiar et al. 2023a,b). Chaya belongs to the Euphorbiceae family, the *Cnidoscolus* genus with shrub characteristics, and is closely related to the *Manihot* genus. In the origin country, it is known as a tree amaranth plant, and its leaf can be harvested across the year to fulfill the food needs of the people (Manzanilla and Knerr 2020). Chaya contains all required essential amino acids and is rich in vitamin A, vitamin C, calcium, potassium, and iron, as well as antioxidants (Ebel et al. 2019; Gobena et al. 2023). Meanwhile, this plant contains toxic compounds alongside the high nutritional content. Therefore, it cannot be eaten directly and requires special measures before consumption, such as an initial ripening process through boiling (Lennox and John 2018).

A minimum of four cultivars, such as 'Chayamansa', 'Redonda', 'Estrella', and 'Picuda' are recognized in Yucatan and Guatemala in addition to wild species (Montero-Astúa et al. 2023). The two chaya varieties often grown in Indonesia include the Picuda cultivar, which morphologically has a fingered leaf similar to cassava, and Redonda, comprising a wider leaf with a different shape (Gobena et al. 2023; Gustiar et al. 2023c). Leaf organ traits are reported to affect plant development and biomass as they are key to photosynthesis (Abraha et al. 2024). The leaves are essential for many physiological processes, such as photorespiration, transpiration, and temperature regulation. Therefore, leaf size can also affect plant fitness and stress response (Karamat et al. 2021). Leaf size is also an essential morphological feature in plants as an agricultural commodity, where leaves are the main vegetative organs produced for consumption. Chaya is a type of plant whose leaves are used as vegetables. Studies related to the morphology of Redonda chaya leaves are still scarce.

Chaya Redonda cv. has been cloned by the people of the Yucatan Peninsula using stem cuttings (Munguía-Rosas et al. 2019). Chaya propagation is fascinating because this type of plant has infertile seeds that cannot be used to produce new individuals. Consequently, clonal propagation is attempted vegetatively through stem cuttings, making the fixation of selected traits faster in maintenance (Solís-Montero et al. 2020). Furthermore, Muda et al. (2022) reported that propagating plants using stem cuttings improves plant growth and yields comparing apical cuttings. The ability of stem cuttings to regenerate varies depending on the plant species; internal and external factors influence propagation through branch cuttings (Zargar and Kumar 2018). The maturity stage and length of planting material have been reported to affect the early growth of stem cuttings of the *C. aconitifolius* picuda cultivar (Gustiar et al. 2023c). The same method has not been studied in the propagation of the Chaya Redonda cv., which has different stem characteristics.

Research on the morphological characteristics and growth performance of Chaya Redonda cv. through stem cuttings has not been widely conducted. Both aspects are very important for Chaya Redonda cv. as a leafy vegetable plant propagated through vegetative propagation. Therefore, the study aims to characterize and evaluate leaf morphological organs as genetic diversity for further development both taxonomically and in the field of vegetable cultivation and to evaluate the vegetative plant growths through the propagation of stem cuttings at different lengths.

MATERIALS AND METHODS

Biomaterial and procedures study

This study was conducted in the Ogan Ilir area (104°46'44'' E; 3°01'35'' S) in South Sumatera, Indonesia. The research site is classified as a lowland tropical ecosystem characterized by high rainfall and humidity (Figure 1). Chaya plant material used in this study was from the Redonda cultivar with the Latin name *C. aconitifolius* subsp. *aconitifolius* Breckon, which has been domesticated. These stem cuttings were obtained from central lateral branches with a medium maturity level and relatively uniform diameter to minimize effects different from the treatment. The characteristics of the Chaya Redonda cv. can be seen in Figure 2.

The planting medium comprised a 2:1 (v/v) mixture of soil and manure. The polybag used was 15 × 20 cm in size. In each polybag, one cutting is planted to a depth of 7 cm. A randomized block design was applied with three treatment levels for the length of stem cuttings, including 30 cm, 25 cm, and 20 cm; each treatment contains five plants with four repetitions, leading to a total of 60 plant units.

Leaf area estimation

The morphology of the Chaya Redonda cv. leaf with three visible lobes is slightly similar to the Chaya Fikuda leaf type, comprising a minimum of five main lobes. This study was carried out by observing more than 200 leaves of various sizes, including the smallest and largest. During the observation process, midrib and leaf length, as well as upper and lower leaf width were measured, then used as area predictors. The area was calculated with the smartphone software application known as Easy Leaf Area (Easlon and Bloom 2014) based on an estimation conducted using power regression models and zero intercepts linear regression (Gustiar et al. 2023b).

Data collection and analysis

Initial growth was observed during the study, and data collection was carried out at the age of 70 Days After Planting (DAP). Additionally, the percentage level of stem cuttings' ability to grow was calculated based on planting material length. All growth characteristics measured included shoot length, number of branches and leaves, canopy area, as well as SPAD value. The percentage of growth ability was determined through the ratio of the cuttings that develop the root and shoot to the total number of planted cuttings. The canopy area was measured using the digital camera image method (Easlon and Bloom 2014), and SPAD value was calculated with the Konica Minolta SPAD-502, while the dry and fresh weight of leaf and stem, total leaf area, and stem diameter were collected at harvest. Therefore, to obtain dry weight, each plant organ was dried in an oven at 100°C for 24 hours.

The effects of treatments were examined with Analysis of Variance (ANOVA). Moreover, differences between treatments were determined through the Least Significant Difference (LSD) test at $P<0.05$ using the statistical analysis software R-Studio for Windows 10. The simple regression test was performed using Microsoft Excel for Windows 10 to determine the strength level of the relationship between all collected data.

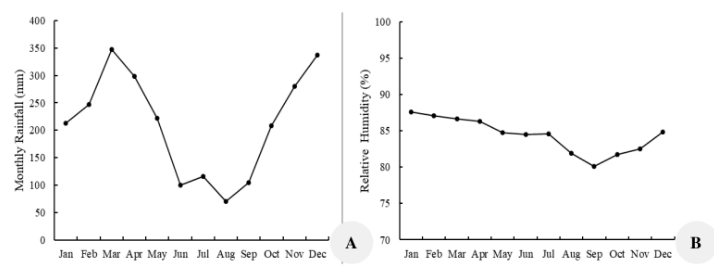

Figure 1. A. Monthly rainfall and B. Average relative humidity at the research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia 2024)

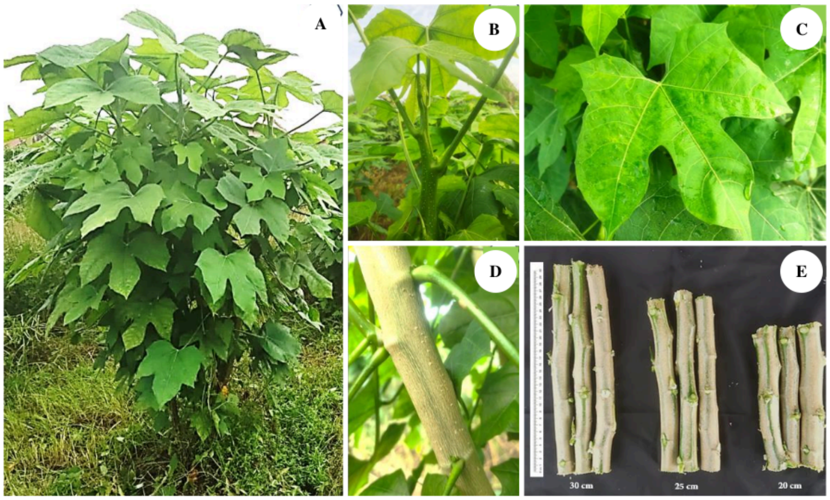

Figure 2. A. Chaya Redonda cv.; B. Young shoot; C. Leaf; D. Stem and E. Planting material

RESULTS AND DISCUSSION

Chaya redonda cv. leaf shape

Chaya Redonda cv. leaf is found with five lobes, but the left and right-side lobes are fused, leading to the appearance of only three main lobes. By observing through a dimensional method, the leaf shows a square size with similar bottom and top width. This can also be seen in the average leaf length and width, which are nearly the same size as presented in Figure 3. Leaf has a palmate shape with three to five lobes and is petiolated, identical to *Jatropha curcas* L. (de Oliveira et al. 2016). The Chaya Redonda cv. leaf shape is unlike the commonly cultivated chaya leaf, chaya fikuda, which has been confirmed to have 5 main lobes with a symmetrical shape, with loose serrated lobe edges, especially in the apical of the lobe (Gustiar et al. 2023b). Leaf shape, anatomy, orientation, and many other leaf traits determine plant growth and nutrient transport and absorption. Moreover, leaf morphology is strongly correlated to plant water status (Ding et al. 2020). Therefore, it is not surprising that morphological indices have been used to measure physiological differences in species or specific conditions in such environments (Yu et al. 2020).

Leaf length and width, as quantitative characters for morphometric analysis, are versatile tools for describing species and assessing morphological variations (Chuanromanee et al. 2019; Lestari et al. 2021). In this study, we divided the quantitative characters of the Chaya Redonda cv. leaf into four, namely midrib length, leaf length, top width, and bottom width (Figure 2.A). Our findings revealed the leaf midrib length ratio (L1/L2) and leaf blade width ratio (W1/W2) of Chaya Redonda cv. remained constant during growth. These results not only demonstrated the regularity of leaf shape but also supported the predictor determination. Moreover, several related morphological traits were found to be useful in predicting Leaf Area (LA), a method similar to that used for Luffa acutangula plants (Lakitan et al. 2022a). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies, such as *Amorphophallus muelleri* Blume (Nurshanti et al. 2022) and *Citrus sinensis* (Mill.) Pers., 1806 (Muda et al. 2023). The wide applicability of these findings can inspire and intrigue botanists, researchers, and scientists in plant morphology and agriculture.

Leaf shape, a key aspect of our research, can be accurately represented by comparing length and width. A value of less than 1 indicates a wide shape, while more than 1 signifies an elongated shape. Our observations consistently show that the value of the length and width ratio is always close to 1.0, confirming that the shape has square dimensions with the same length and width. Furthermore, the ratio of midrib length to leaf blade width (L1/WB) and leaf length to width (L2/WB) remained unchanged during the enlargement process, reinforcing the constant leaf shape of *C. aconitifolius* ssp. Aconitifolius Breckon, as presented in Figure 4. This finding has practical implications, as it adds to the body of knowledge and can be used to understand variations in the leaf length/width ratio, which we found depend on the plant species and growing environment (Liu et al. 2020; Yu et al. 2020).

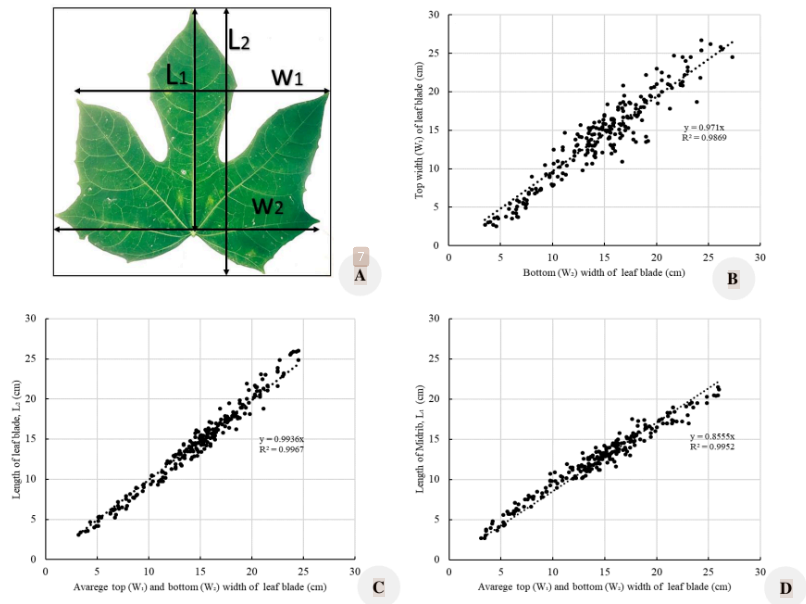

Figure 3. A. Chaya leaf dimensions; B. Linear regression between size leaf traits, top (W_1)-bottom (W_2) width; C. Length of leaf blade (L_2)-width and D. Length of midrib (L_1)-width

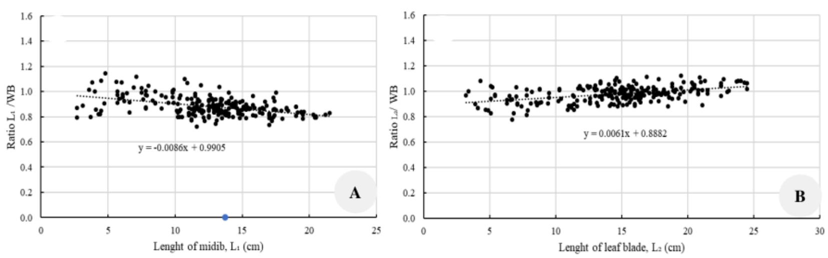

Figure 4. A. Comparison of midrib length and leaf blade width (L_1/WB) ratio and B. Leaf length-leaf width (L_2/WB) ratio of Chaya Redonda cv. WB means an average of top (W_1) and bottom (W_2) width

Chaya leaf is single, symmetrical, lobed, with a flat surface and constant leaf shape development, while leaf area can be estimated accurately and non-destructive. Leaf area is probably one of the most important leaf indices associated with plant growth analysis and development in different environments because it can provide a direct relationship to photosynthetic capacity and because it is a useful substitute measure of other functional traits such as specific leaf area (Shi et al. 2020). Morphological characteristics in the form of length and width can serve as an accurate predictor of leaf area (Hernández-Fernandéz et al. 2021). Several models are used to estimate leaf area, including power regression, 2nd order polynomial, or zero intercept linear regression (Lakitan et al. 2022b).

Leaf area estimation

Chaya leaf area estimation models can be developed through linear regression with zero-intercept, while the unique shape and properties allow predictors to be categorized into single and combined forms. Several single predictors often used to estimate the area are midrib length, leaf length, as well as upper and lower leaf width. The study results showed that length was the most reliable in estimating the area, as proven by the 0.951 coefficient of determination (R^2) for leaf length. This coefficient value was higher than 0.9099, 0.8892, and 0.9152 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 5).

Different levels of reliability were obtained from leaf area estimation performed using a combination of two characteristics as predictors. The calculation results showed that Length (L_2) and Top Width (W_1) had the highest reliability level according to R^2 of 0.9839. This was confirmed through comparison with several other combinations of leaf characteristics, including midrib (L_1) × top width of the blade (R^2 = 0.9806), midrib (L_1) × bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) × bottom width of the blade (R^2 = 0.9831), respectively (Figure 6).

The leaf area is one of the most important leaf parameters related to plant growth and development due to its direct relationship with photosynthetic activity. Most of the methods used to measure leaf area are destructive by requiring the removal of leaves from branches. As a result, it is not possible to note the expansion of the development of the same lamina area during growth (Yu et al. 2020). Continuous measurement is necessary for many studies that focus on the process of plant growth. Therefore, the resulting model can be further used in predicting leaf area non-destructively, rapidly, and accurately, hence, the growth analysis can be calculated realistically due to using the same leaf. Most area estimation models are based on regression equations using morphological traits as predictors. At the same time, some leaves have been analyzed for the area through a regression equation method, such as in cassava plants (Lakitan et al. 2022b) and Chaya Fikuda cultivar (Gustiar et al. 2023b). Development of the area estimation model adapted to leaf morphology, as many types and combinations of characteristics have been used in the area estimation models. However, the types of characteristics that are often consistent and accurate are directly related to the dimensions, namely length and width. In this study, the combination $L_2 \times W_1$ (Figure 5.C) was found to be a more accurate predictor in estimating leaf area compared to using length or width separately through linear zero-intercept regression (R^2 = 0.9839). This is significant because it provides a more accurate and efficient method for estimating leaf area, which has also been proven in plants that have similar morphology, such as cassava leaf (Lakitan et al. 2022b) and Fikuda cultivar (Gustiar et al. 2023c).

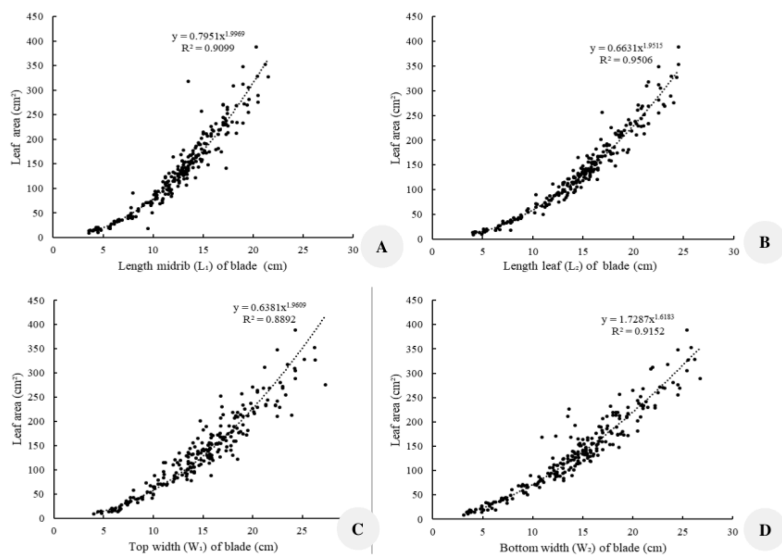

Figure 5. A. Estimation of leaf area using a single trait with regression power, midrib length; B. Blade length; C. Top width and D. Bottom width

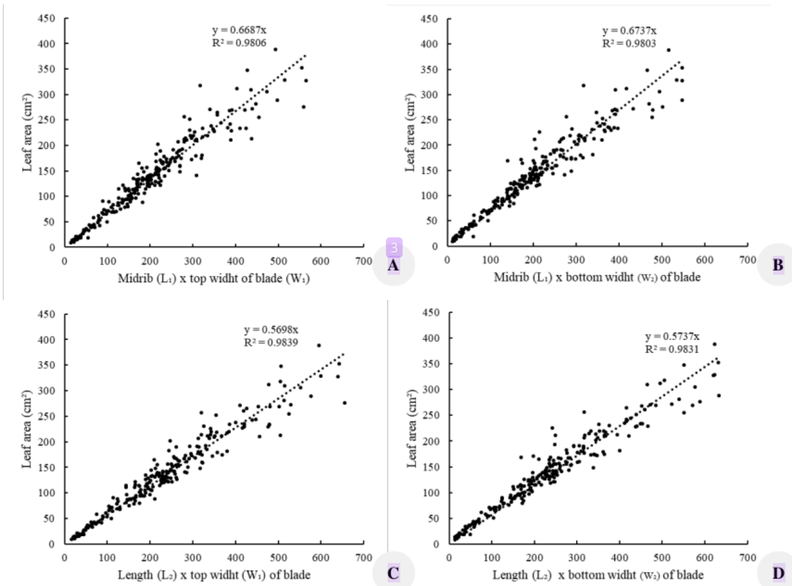

Figure 6. Estimation of leaf area using Length (L) and Width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using A, B. Midrib length and C, D. Leaf length

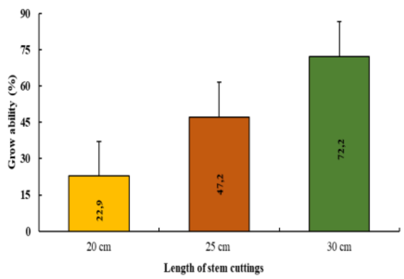

Figure 7. Percentage of growth ability at different stem-cutting lengths

Early growth and yield of chaya

Due to the infertile seeds, Chaya Redonda cv. propagation can only be carried out vegetatively. Hence, stem cuttings with different lengths were selected in this study to reproduce the plants. Observations showed that planting material length greatly affected the survival of cuttings, where a length of 30 cm increased the potential of living cuttings by approximately 72.2%. Meanwhile, survival has been confirmed to be at lower rates of 22.9 % and 47.2% in lengths of 20 cm and 25 cm, respectively (Figure 7).

The growth ability of stem cuttings is determined by their ability to develop new roots and shoots. Meanwhile, the ability to grow roots and shoots was influenced by the content of nitrogen, carbohydrates, and auxins as one of the regulators of root growth (Solikin 2018; Druege et al. 2019). The size of stem cuttings indicates the availability of nitrogen and carbohydrates for the plant's initial metabolic process (Meuriot et al. 2005). The longer the size of the stem cutting, the higher the percentage of growth ability.

After cuttings had developed with vegetative organs, leaf length influenced leaf growth, as shown by the leaf expansion rate (Figure 7). The surface area of the leaves is measured daily for 14 consecutive days from the time the leaves begin to open until they reach their full size. Information on when the leaves stop growing is very useful in determining the right harvest time for leafy vegetables such as chaya. Monitoring of leaf area by a non-destructive method based on length and width has been shown in Figure 4 and Figure 5. Planting material with stem cuttings of 20 cm in length tended to have smaller leaf sizes than those measuring 25 cm and 30 cm. The leaf length affected the period of reaching the maximum area, as chaya propagated through stem cuttings of 30 cm produced leaf that continued growing until 13-14 days after fully unfolding. Meanwhile, 20 cm and 25 cm stem cuttings experienced more rapid stagnant growth at 8-9 and 11-12 days, respectively (Figure 8). The thermal dynamics were strongly affected by the two-dimensional size and shape of the leaf blade and thickness (Anfodillo et al. 2012). Additionally, leaf growth fluctuated, and the area increased depending on shape and position, with shape enlarging persistently during active cell division.

Fertilization was able to increase SPAD values in all stem-cutting length treatments. However, the SPAD values in each stem cutting length were not significantly different. In line with Du et al. (2022) stated that the SPAD value is an effective indicator to determine the plant's response to fertilization. Moreover, shoot length generally continued to extend as cutting age increased, starting from the onset of growth until 11 WAP. Chaya propagated by stem cuttings of 30 cm showed longer shoot growth compared to planting material with other cutting lengths. The dominant growth of 30 cm stem cuttings could also be observed through the variables of leaf number and canopy area (Figure 9). The large diameter of cuttings did not affect the growth and yield of the vegetative Chaya Redonda cv., compared to Fikuda variety (Gustiar et al. 2023b). This observation corresponded with the result obtained from the analysis of cassava plant cuttings (de Oliveira et al. 2020). However, cutting length in this study differed significantly from leaf number and canopy area because cutting length affected carbohydrate accumulation in planting material, similarly as reported by Meuriot et al. (2005). The accumulation of carbohydrates was previously reported to play an important role before plants actively conduct photosynthetic metabolism (Otiende and Maimba 2020). Therefore, cuttings with higher carbohydrate accumulation had better growth performance, as those measuring 30 cm grew optimally in terms of shoot length, leaf number, and canopy area.

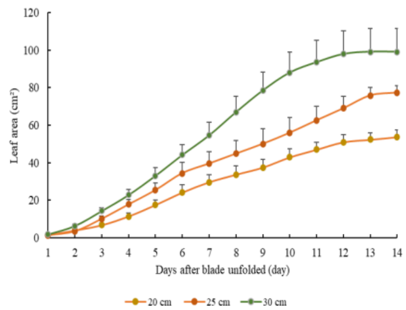

Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm, and 30 cm

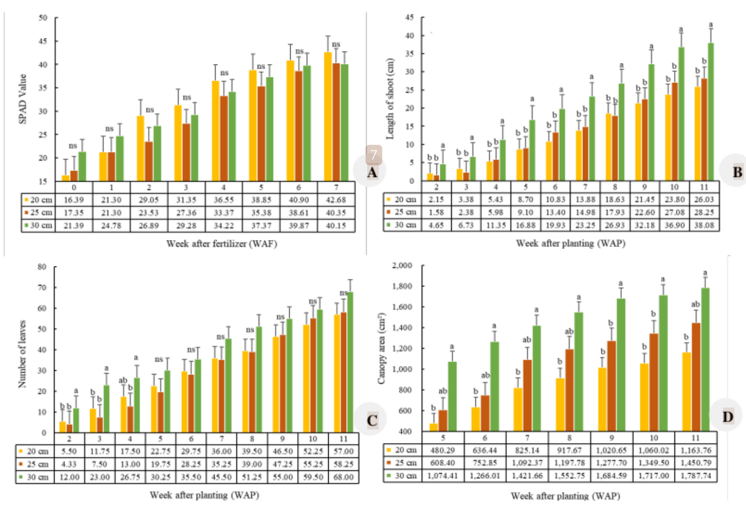

Figure 9. A. SPAD value; B. Shoot length; C. Leaf number and D. Canopy area based on differences in stem cutting length. The data is presented as mean ± standard error (n = 3). The lowercase letters mean a significant difference through the LSD test at P<0.05. The ns mean non-significant difference through LSD test at P<0.05

Table 1. Effect of planting material length on the growth and weight of chaya biomass

Treatment	Total leaf area (cm^2)	Number of branches	Branch diameter (mm)	Fresh weight (g)			Dry weight (g)		
				Lamina	Petiole	Shoots	Lamina	Petiole	Shoots
20 cm	3134.96 [b]	5.00 [a]	17.93 [a]	48.30 [b]	31.89 [b]	128.03 [b]	10.45 [b]	4.05 [a]	15.11 [b]
25 cm	3448.74 [ab]	5.25 [a]	17.68 [a]	56.76 [ab]	36.84 [ab]	158.99 [ab]	13.02 [ab]	4.01 [a]	17.53 [ab]
30 cm	4418.38 [a]	5.50 [a]	15.74 [a]	71.22 [a]	49.42 [a]	202.48 [a]	16.96 [a]	5.86 [a]	24.49 [a]
$LSD_{0.05}$	1037.87	1.65	3.70	16.88	14.39	52.35	4.93	2.63	8.06

The length of cuttings had a significant effect on leaf compared to branches and shoots. At the completion of the study at 11 Weeks After Planting (WAP), 30 cm stem cuttings had a greater canopy area representing the total leaf area, compared to cuttings measuring 25 cm and 20 cm. This phenomenon is consistent with the growth of plant organs, including the fresh and dry weight of lamina, petioles, and shoots (Table 1).

The length of the stem cuttings affects the growth and development of the chaya plant. Long stem cuttings encourage more shoot and root growth which will further accelerate leaf growth. Plants originated from the length stem cuttings of 30 cm that grow more bud structures as represented by total leaf area, fresh weight, and dry weight of lamina, petiole, and branch than the shorter ones (Table 1). This is in line with the results of research conducted on *Jatropha curcas* (Severino et al. 2011); the cutting length needs to be considered in plants cultivated using stem cuttings. Different cutting lengths show variations in carbohydrate content, which is often converted by the plant to stimulate cell development, thereby supporting the growth of organs such as shoots, stems, and roots (Martinez-Vilalta et al. 2016). The carbohydrate accumulation in planting material is stored by the parent plant, which can be mobilized when it is needed for metabolism (El Omari 2022). In this case, stem cuttings need carbohydrates for further growth due to being produced through photosynthetic metabolism. Optimal photosynthesis promotes good growth, followed by an increase in dry weight, reflecting plant nutritional status because dry weight depends on cell activity, size, and quality (Sales et al. 2021).

In conclusion, the leaf of Chaya Redonda cv. is a palminerved single leaf characterized by leaf area that strongly correlates with length × top width of leaf blade. The recommendation for Chaya Redonda cv. propagation is through stem cuttings measuring 30 cm in length to have optimum leaf growth.

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