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ARTIKEL JURNAL INTERNASIONAL BEREPOTASI

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2	Penulis	**Fitra Gustiar**, Benyamin Lakitan, Strayker Ali Muda, Rofiqoh Purnama Ria, Indra Advent Simamora
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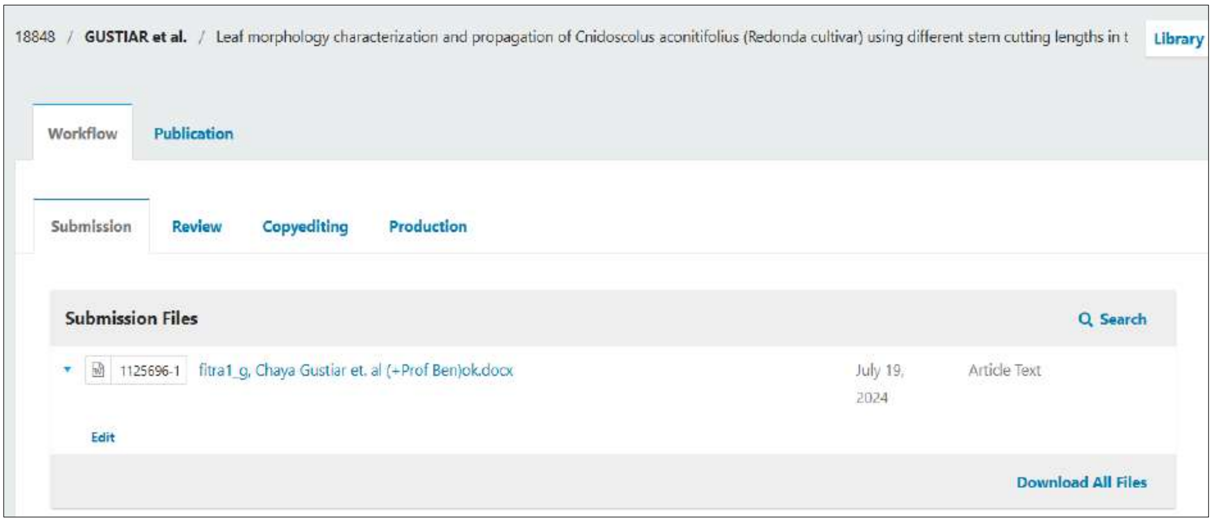
5	Copyediting Discussions	8 September 2024

1. Submission (19 Juli 2024)



2. Proses Review Editor (24 Juli 2024)

7/10/25, 10:54 PM FITRA GUSTIAR, Leaf morphology characterization and propagation of Cnidoscolus aconitifolius (Redonda cultivar) using differen…

Notifications

[biodiv] Editor Decision

2024-07-24 05:50 AM

Fitra Gustiar, Benyamin Lakitan, Strayker Ali Muda, Rofiqoh Purnama Ria, Indra Advent Simamora:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Leaf morphology characterization and propagation of Cnidoscolus aconitifolius (Redonda cultivar) using different stem cutting lengths in the tropical ecosystem". **Complete your revision with a Table of Responses containing your answers to reviewer comments (for multiple comments) and/or enable Track Changes**.

Our decision is: Revisions Required

Reviewer A:

This is a pre-review, kindly revise your paper accordingly based on the comments.

- Please mention all the tables and figures in the text

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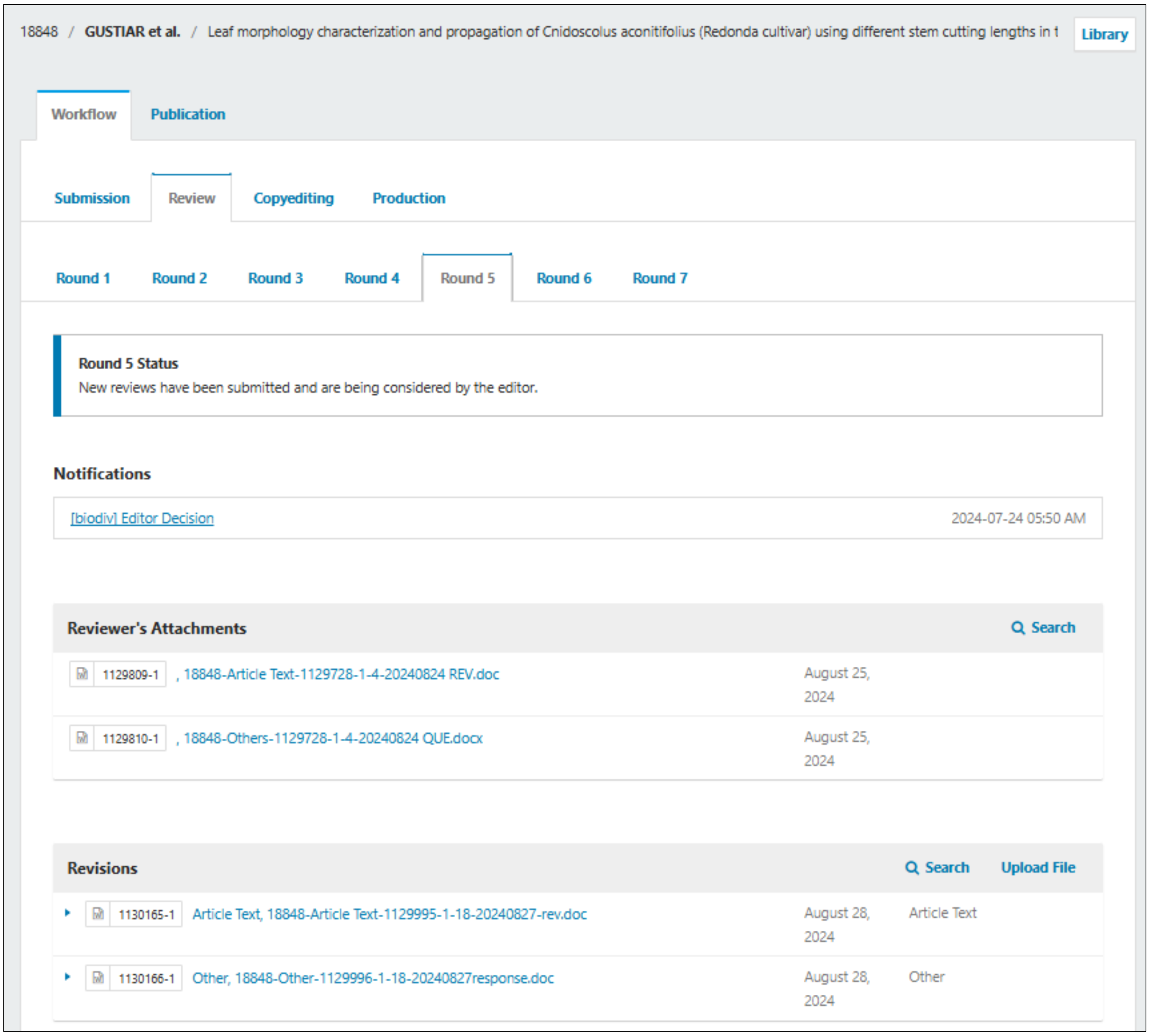

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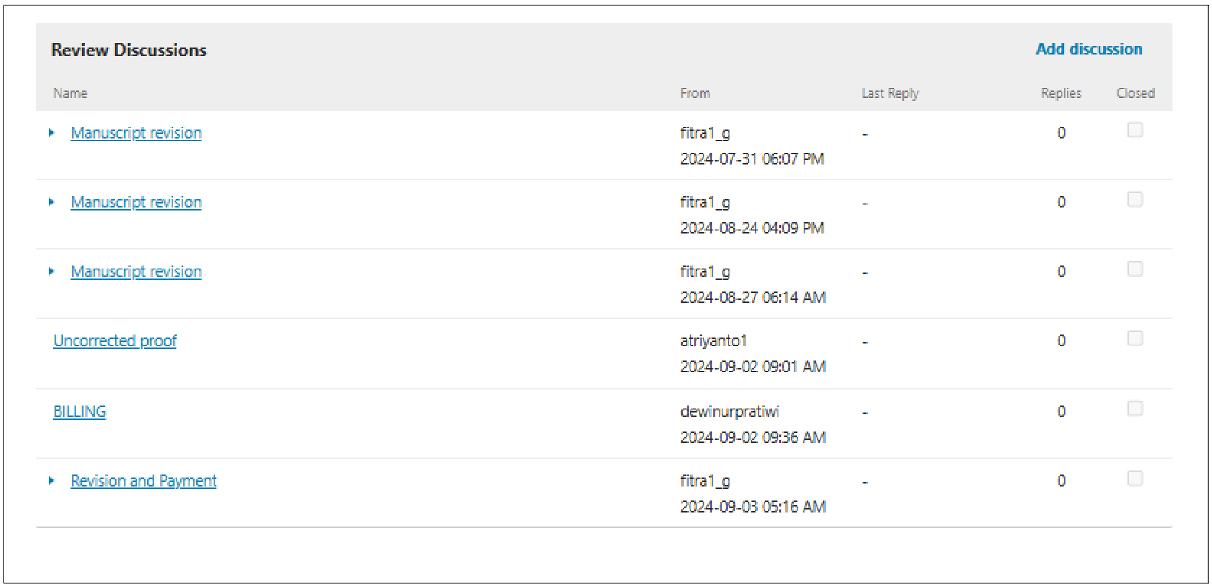

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Manuscript title:

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

Review:

- Generally
    1. Please make the writing of "Redonda" more uniform. The authors should check once again how to write it. Redonda or redonda. Please check the manuscript thoroughly once again. The uniformity includes other cultivar names and even the plant's name.
    2. The authors might also want to consider writing Latin names evenly. For example, in Line 110, the author wrote *Jathropa curcas* L. (Linneaus included). Meanwhile, the rest of the Latin nomenclatures are just genus and species (e.g., Line 123, 124, even citrus is not written in Latin name).
    3. No discussion subsection and conclusion/concluding paragraph?
    4. Noticed that the authors were using reference citation manager. But please recheck the input metadata to make the manuscript more consistent.
- Abstract
    1. The readers could read the aim of the study in the abstract. However, what problem did the authors want to address in this paper? What is the correlation between Chaya originating from Mexico and grown in Asia, its cultivar, and its leaf length? Why length of stem cutting is important to be observed? Is it related to the cultivar type in any way? Please make it clearer.
    2. After reading the whole manuscript, I suggest the authors rephrase Lines 12-14. "study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm". The authors only performed leaf morphological analysis; no observation was completed on other organs.
- Introduction

  Line 26: Somehow, the phrase "as well as different leaf morphology" is like being forced to fit it into one sentence, thus making the sentence itself unclear. I suggest rewriting it into two or three sentences to make it readable, whether there is a correlation between the cultivar and leaf morphology or other aspects that make it different. Although, in my opinion, the authors could remove this part, as they explained further in the following paragraph.

- Material and methods
    1. Line 72-73: Please check how to write the Latin name properly (including the subspecies). I suggest the Authors check the website https://www.ncbi.nlm.nih.gov/taxonomy.
    2. Were the experiments done in a controlled environment or an open area? Please specify the material and methods. External factors (weather, rain period, sun exposure) should always be considered.
- Results and discussion
    1. Line 120, "found" not "founded"

2. Line 117-125: please refer to which figure represents the calculation used for the study.
3. Figure 2, Please check the English vocabulary. Many mistakes. “width” not “widht”; “average” not “avarage”, “length” not “langth”.
4. Figure 2, please include the statistical analysis done for the regression and the name of the software used.
5. Line 132-139: what is WB? The author did not specify in the previous paragraph or figure or material and methods. Why is it necessary to use the calculation in this study?
6. Line 138-139: “This variation of leaf length/width ratio depends on the plant species and growing environment.” This is exactly why the authors should explain the environmental condition during the study in material and method.
7. Line 179-194: in the introduction section, the authors wrote that their study aims to characterize and evaluate leaf morphological organs as genetic diversity for further development both taxonomically. Could the authors elaborate more on how their statistical results could help with the application of taxonomy identification? Based on the authors’ results, what are the differences between Chaya Redonda and other Chaya cultivars? Alternatively, whether the location of cultivation (e.g. Indonesia vs Mexico) affected the leaf length/width ratio.
8. Line 210-212: “The size of the stem cuttings will indicate the amount of nitrogen and carbohydrate content needed for the plant’s initial metabolic process. The longer the size of the stem cutting, the higher the percentage of life.” This phrase needs citation. The authors’ chosen stem lengths (30-25-20 cm) were too narrow to make this conclusion.
9. Figure 7: The authors used different colours for several lines, yet no legends that explain it. Please clarify the differences between those lines in the figure and the figure’s caption.
10. Line 231: What is WAP? Please clarify the abbreviation.
11. Line 239-240: This is a strong statement. Did the authors also measure carbohydrate concentration during the experiments? Suggest rephrasing the sentences and referring to other studies with similar results.
12. Figure 8: please include the information on statistical results in the figure’s caption. What do a, b, ns mean?
13. Figure 8D: Week 6, is the calculation correct? There was no significant difference in canopy area between the three study groups on week 6?
14. Line 246-247: “The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots” >> do the authors mean “the length of cutting had a significant effect on parameters compared to branches and shoots”?

4. Manuskrip accepted (07 September 2024)

7/10/25, 11:01 PM Email Sriwijaya University - [biodiv] Editor Decision

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Team Support Smujo via SMUJO <support@smujo.com> 7 September 2024 pukul 08.07

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FITRA GUSTIAR, BENYAMIN LAKITAN, STRAYKER ALI MUDA, ROFIQOH PURNAMA RIA, INDRA ADVENT SIMAMORA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Leaf morphology characterization and propagation of *Cnidoscolus aconitifolius* (Redonda cultivar) using different stem cutting lengths in the tropical ecosystem".

Our decision is to: Accept Submission

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5. Copyediting Discussions (08 September 2024)

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Kepada: FITRA GUSTIAR <fitragustiar@unsri.ac.id>, BENYAMIN LAKITAN <blakitan60@unsri.ac.id>, STRAYKER ALI MUDA <straykerali@gmail.com>, ROFIQOH PURNAMA RIA <rofiqohpurnamaria@unsri.ac.id>, INDRA ADVENT SIMAMORA <Indraadvent@gmail.com>

FITRA GUSTIAR, BENYAMIN LAKITAN, STRAYKER ALI MUDA, ROFIQOH PURNAMA RIA, INDRA ADVENT SIMAMORA:

The editing of your submission, "Leaf morphology characterization and propagation of Cnidoscolus aconitifolius (Redonda cultivar) using different stem cutting lengths in the tropical ecosystem," is complete. We are now sending it to production.

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- ☑ The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
- ☑ The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
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Leaf morphology characterization and propagation of *Cnidoscolus aconitifolius* (Redonda cultivar) using different stem cutting lengths in the tropical ecosystem

Author(s) name:

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Fitra Gustiar

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

FITRA GUSTIAR[1]♥, BENYAMIN LAKITAN[1,2], STRAYKER ALI MUDA[1],
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Manuscript received: DD MM 2016 (Date of abstract/manuscript submission). Revision accepted: 2016

Abstract. Chaya (*Cnidoscolus aconitifolius*) 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. Therefore, this study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm. 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 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. Cuttings with a length of 30 cm were 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.

Key words: *leaf Area, leaf shape, planting material, redonda*

Abbreviations (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.

Running title: Leaf morphology characterization of Chaya

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) is a perennial vegetable plant originating from southern Mexico and Guatemala, with several cultivars, including redonda, as well as different leaf morphology (Montero-Astúa et al. 2023). Due to the similarity of the agroecosystem with the area of origin, this plant can thrive in Indonesia (Gustiar 2023b. 2023a). 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 leaf can be harvested across the year to fulfill the food needs of the people (Manzanilla & Knerr 2020). Chaya is considered a potential vegetable food ingredient because the leaf has been identified to contain approximately 30% protein based on dry weight (Schwarcz et al. 2022). This protein content is relatively high compared to other plant foods, such as nuts, which comprise only 19.5% - 24.8%. Furthermore, 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). As a nutrient-rich plant, leaf offers many health benefits, including maintaining healthy blood sugar levels, along with providing anti-inflammatory, anti-anemic, anti-microbial, and antioxidant effects (Guevara-Cruz et al. 2021; Guzmán et al. 2023; Manzanilla Valdez et al. 2021). These promote the continuous use of food, fence posts, medicines, and ornamental material, but the 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 & 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. 2023). Leaf organ traits are reported to affect plant development and biomass as they are key to photosynthesis (Hagos Abraha et al., 2024). As the most important organ for photosynthesis, leaves produce a main source of energy not only for plant growth but also for human nutrition and other purposes. The size and shape of the leaves affect the utilization of light energy, thus affecting the development of plants and biomass. In addition, 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 a vegetable. Studies related to the morphology of Redond chaya leaves are still scarce.

Chaya Redonda has been cloned by the people of the Yukatan 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). The ability of stem cuttings to regenerate varies depending on the plant species. Propagation through branch cuttings is influenced by internal and external factors (Zargar & Kumar 2018). The maturity stage and length of planting material have been reported to have an effect on the early growth of stem cuttings of the *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). The same has not been studied in the propagation of the Redonda cultivar chaya, which has different stem characteristics.

Chaya grows quickly with resistance to drought, but it has yet to be cultivated intensively and is only limited to protective planting in land bounded as fences. 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 growth of plants through the propagation of stem cuttings at different lengths.

MATERIALS AND METHODS

Biomaterial and Procedures Study

This study was conducted at Ogan Ilir area (104º46'44" E; 3º01'35" S) in South Sumatra, Indonesia. Chaya plant material used in this study was from the redonda cultivar with the Latin name *Cnidoscolus Aconitifolius ssp. 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 plant can be seen in Figure 1.

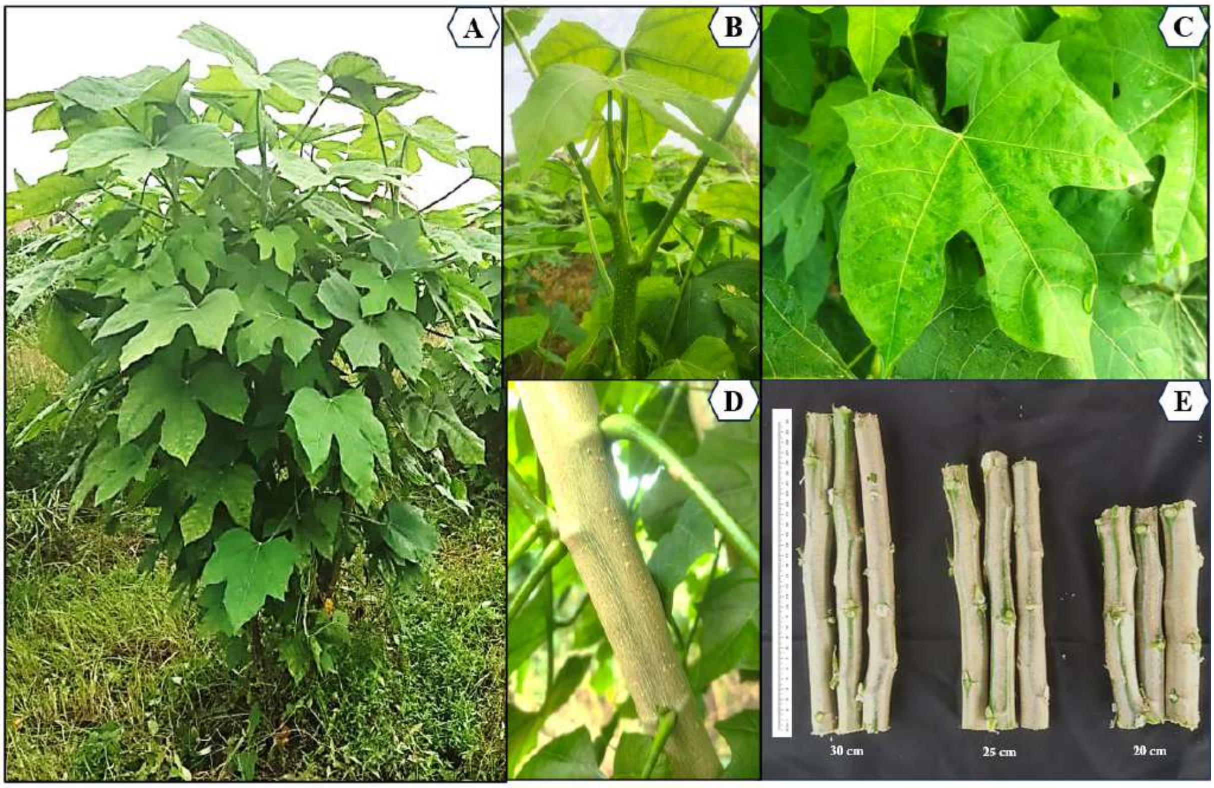

Figure 1. Chaya redonda plant (A), shoot (B), leaf (C), and stem (D), planting material (E)

The planting medium comprised a 2:1 mixture of soil and manure. The polybag used was 15 x 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 containing five plants with four repetitions, leading to a total of 60 plant units.

Leaf Area Estimation

The morphology of the Chaya redonda 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 & Bloom 2014) based on an estimation conducted using power regression models and zero intercept 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 canopy area was measured using the digital camera image method (Easlon & Bloom 2014), and SPAD value was calculated with the Konica Minolta SPAD-502, while dry as well as fresh weight of leaf and stem, total leaf area, and stem diameter were collected at harvest.

Effects of treatments were examined with analysis of variance (ANOVA). Moreover, differences between treatments were determined through least significant difference (LSD) test at $P<5\%$ 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.

RESULTS AND DISCUSSION

Chaya Redonda Leaf Shape

Chaya redonda 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 2. Leaf has a palmate shape with three to five lobes and is petiolated, identical to *Jatropha curcas* L. (R. R. de Oliveira et al. 2016). Length and width are quantitative characters for morphometric analysis used to describe species and assess morphological variations (Chuanromanee et al. 2019; Lestari et al. 2021). This study found that leaf midrib length ratio (L_1/L_2) as well as leaf blade width ratio (W_1/W_2) of chaya redonda remained constant during growth. The results showed leaf shape regularity, which supported the predictor determination, and several related morphological traits were also applied in predicting leaf area (LA), similar to the method used for *Luffa acutangular* plants (Lakitan et al. 2022).

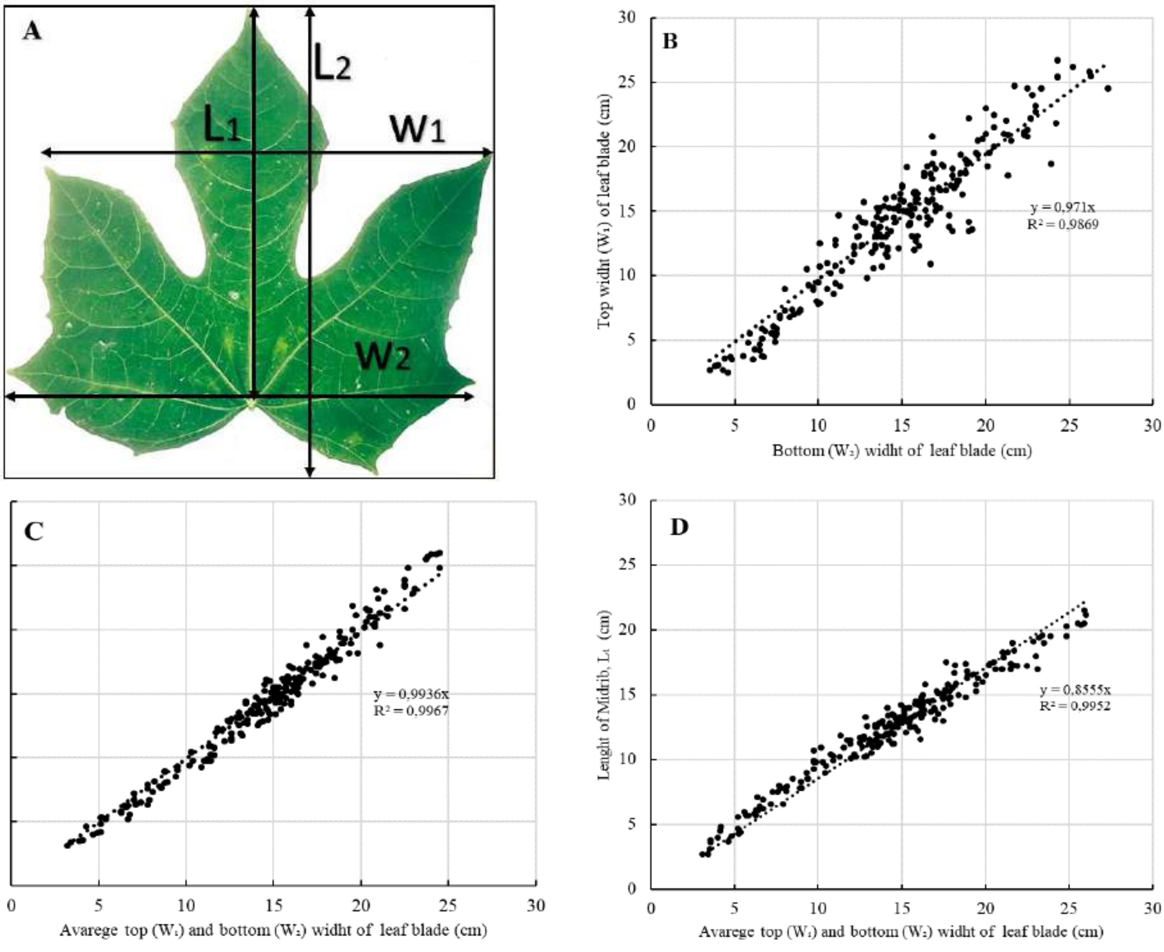

Figure 2. Chaya leaf dimensions (A), regression of between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D)

Leaf shape can be represented through the ratio of length and width, with a value less than 1.0 showing a wide shape, while greater than 1 signifies an elongated shape. Observation results showed length and width ratio values were consistently close to 1.0, proving that the shape had square dimensions with similar length and width. Additionally, the ratio of midrib length and leaf blade width (L_1/WB) or leaf length-width (L_2/WB) did not change during the enlargement process. This fact validated the leaf shape *of Cnidoscolus Aconitifolius ssp.* Breckon's Aconitifolius was constant, as presented in Figure 3.

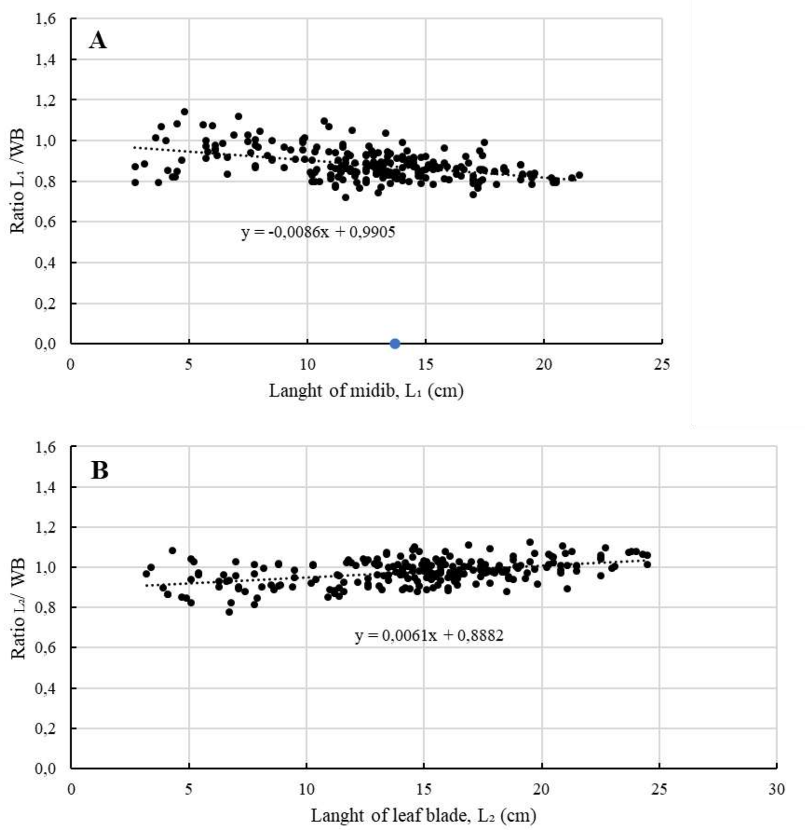

Figure 3. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A), and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda.

Chaya leaf is single, symmetrical, lobed, with a flat surface and constant leaf shape development, while leaf area can be estimated accurately and without damage. 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. 2022).

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 0.909, 0.889, and 0.915 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 4).

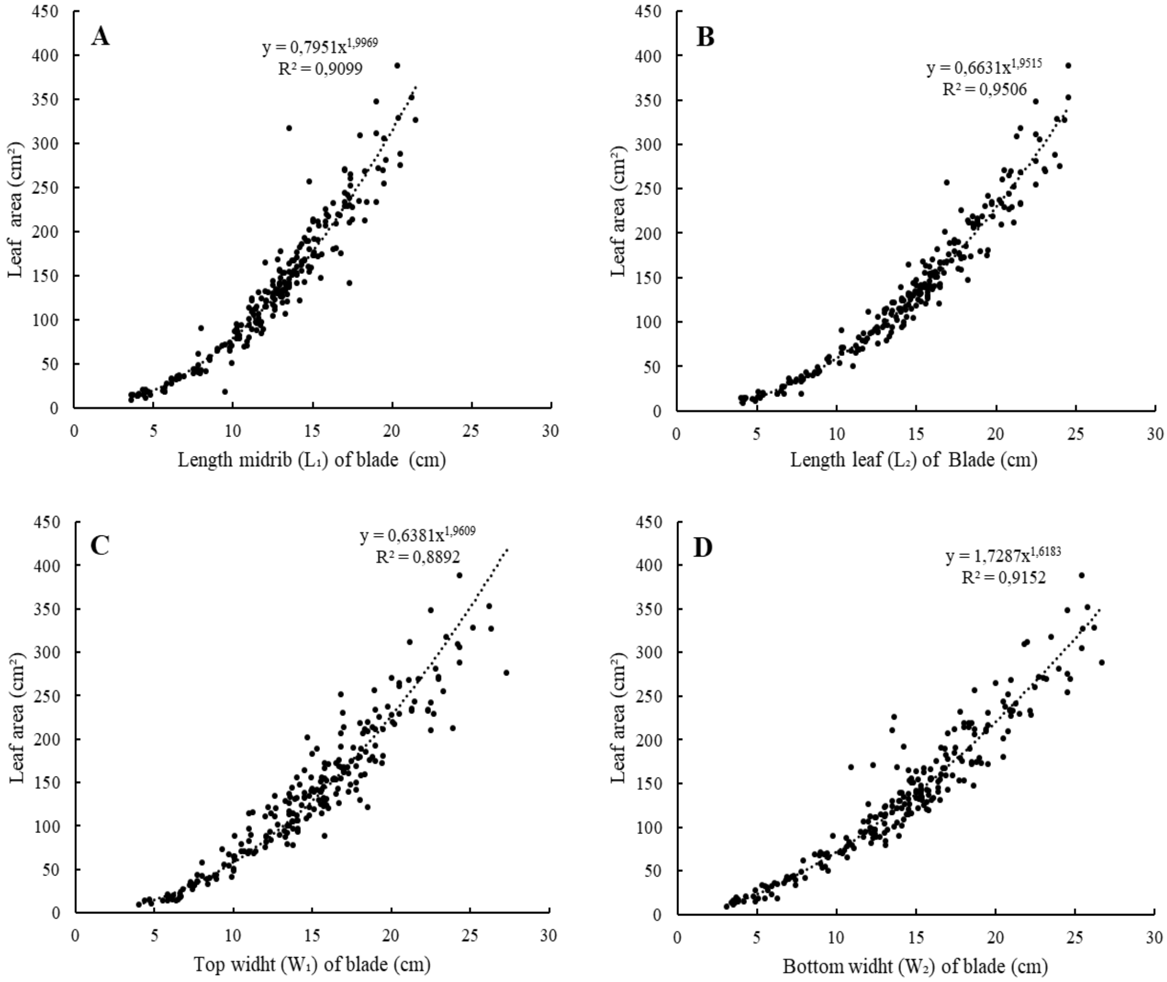

Figure 4. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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 Widht (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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831).

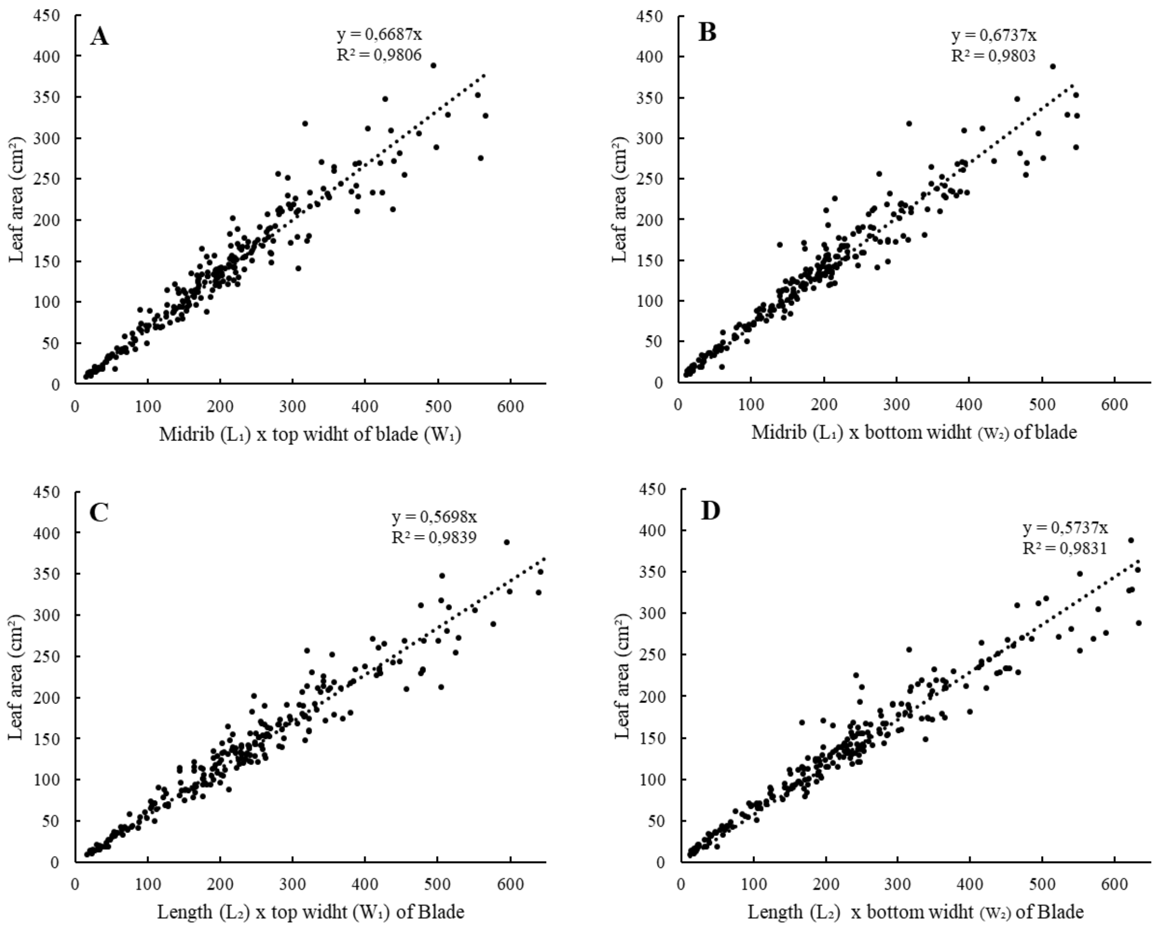

Figure 5. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D).

The resulting model is 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 same time, some leaves have been analyzed for the area through a regression equation method, such as in cassava plants (Lakitan et al. 2022) 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)xW (Figure 5C) was found to be a more accurate predictor in estimating leaf area compared to using length or width separately through linear zero-intercept regression. This statement has also been proven in plants consisting of similar morphology, such as cassava leaf (Lakitan et al. 2022) and Fikuda cultivar (Gustiar et al. 2023).

Early Growth and Yield of Chaya

Chaya redonda propagation can only be carried out vegetatively due to the infertile seeds. 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 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.

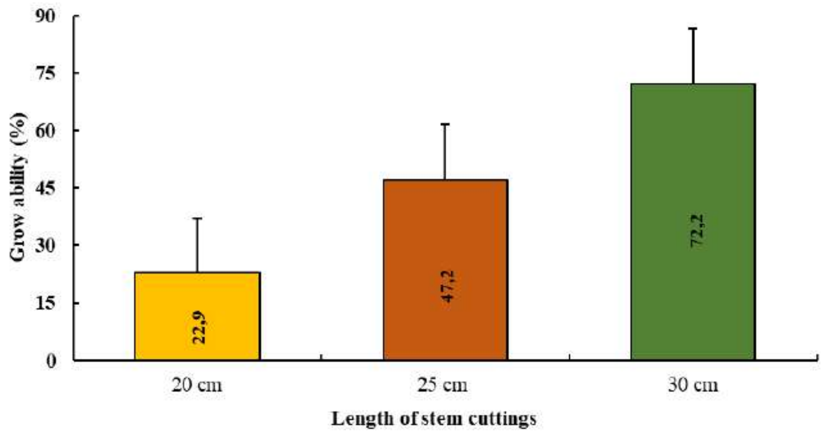

Figure 6. Percentage of growth ability at different stem cutting lengths

After cuttings had developed with vegetative organs, leaf length influenced leaf growth, as shown by the leaf expansion rate. Planting material with stem cuttings of 20 cm in length tended to have smaller leaf sizes than those measuring 25 cm and 30 cm. 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 opening fully. Meanwhile, 20 cm and 25 cm stem cuttings experienced more rapid stagnant growth at 8-9 and 11-12 days, respectively. 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.

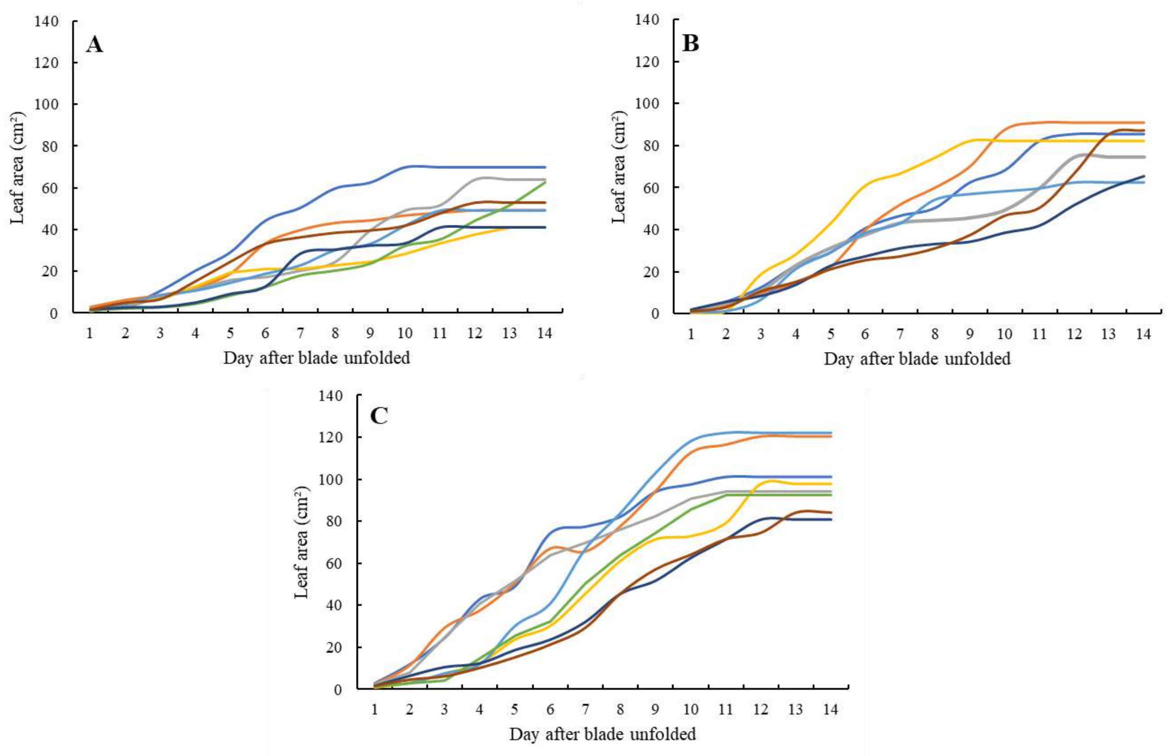

Figure 7. Increasing of chaya redonda leaf area at the onset of plant growth with stem cutting lengths of 20 cm (A), 25 cm (B), and 30 cm (C).

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 8). The large diameter of cuttings did not affect the growth and yield of the vegetative chaya redonda plant, compared to fikuda variety (Gustiar et al. 2023b). This observation corresponded with the result obtained from the analysis of cassava plant cuttings (EJ de Oliveira et al. 2020).

However, cutting length in this study differed significantly from leaf number and canopy area because length affected carbohydrate accumulation in planting material. The accumulation of carbohydrates was previously reported to play an important role before plants actively conduct photosynthetic metabolism (Otiende & 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.

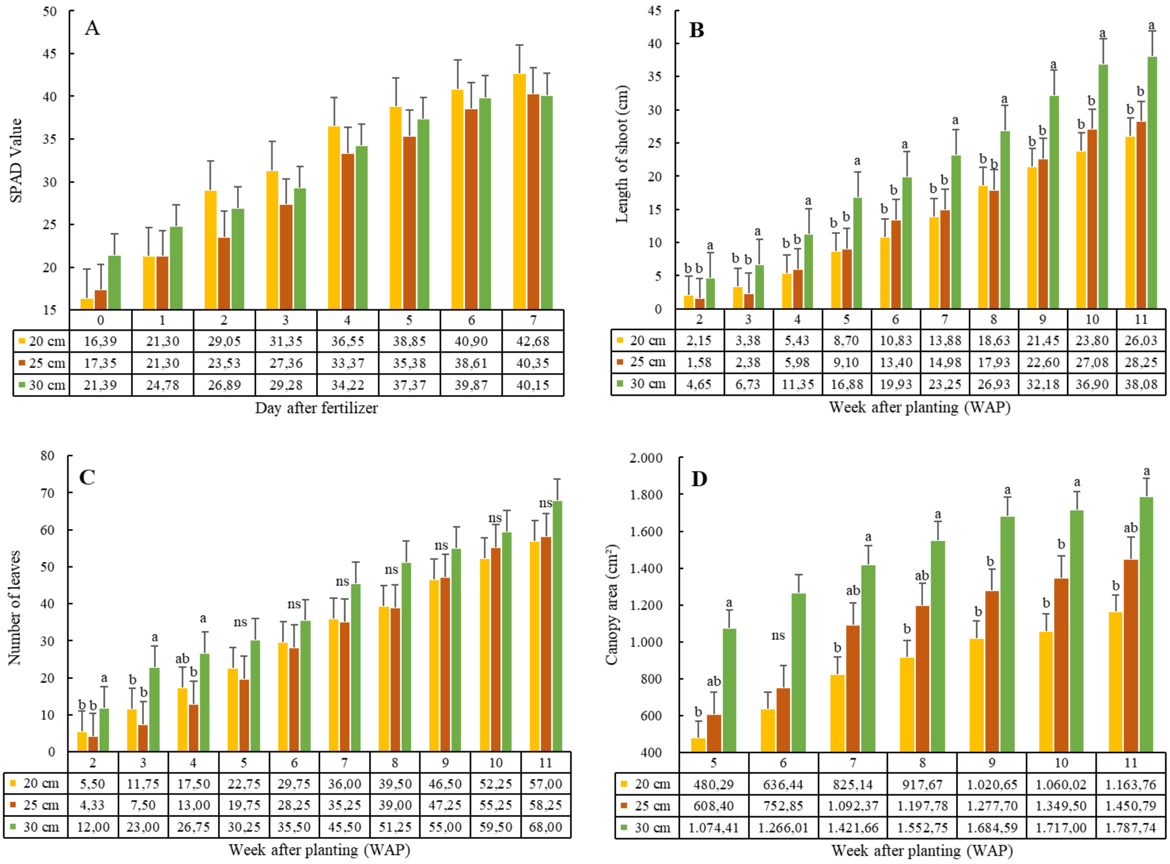

Figure 8. SPAD value (A), shoot length (B), leaf number (C), and canopy area (D) based on differences in stem cutting length

The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots. At the completion of the study at 11 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).

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

	Total leaf area (cm²)	Number of branches	Shoot diameter (mm)	Fresh Weight			Dry Weight		
				Lamina (g)	Petiole (g)	Shoots (g)	Lamina (g)	Petiole (g)	Shoots (g)
20 cm	3134.96 b	5.00 a	17.93 a	48.30 b	31.89 b	128.03 b	10a45 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

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 (Martínez-Vilalta et al. 2016). The carbohydrate accumulation in planting material is stored by the parent plant, which can be mobilized when 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.

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Reviwer A
Round 1

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

Abstract. Chaya (*Cnidoscolus aconitifolius*) 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. Therefore, this study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm. 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 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. Cuttings with a length of 30 cm were 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

Running title: Leaf morphology characterization of Chaya

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) is a perennial vegetable plant originating from southern Mexico and Guatemala, with several cultivars, including redonda, as well as different leaf morphology (Montero-Astúa et al. 2023). Due to the similarity of the agroecosystem with the area of origin, this plant can thrive in Indonesia (Gustiar 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 leaf can be harvested across the year to fulfill the food needs of the people (Manzanilla and Knerr 2020). Chaya is considered a potential vegetable food ingredient because the leaf has been identified to contain approximately 30% protein based on dry weight (Schwarcz et al. 2022). This protein content is relatively high compared to other plant foods, such as nuts, which comprise only 19.5% - 24.8%. Furthermore, 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). As a nutrient-rich plant, leaf offers many health benefits, including maintaining healthy blood sugar levels, along with providing anti-inflammatory, anti-anemic, anti-microbial, and antioxidant effects (Guevara-Cruz et al. 2021; Guzmán et al. 2023; Manzanilla Valdez et al. 2021). These promote the continuous use of food, fence posts, medicines, and ornamental material, but the 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. 2023). Leaf organ traits are reported to affect plant development and biomass as they are key to photosynthesis (Hagos Abraha et al. 2024). As the most important organ for photosynthesis, leaves produce a main source of energy not only for plant growth but also for human nutrition and other purposes. The size and shape of the leaves affect the utilization of light energy, thus affecting the development of plants and biomass. In addition, 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 a vegetable. Studies related to the morphology of Redond chaya leaves are still scarce.

Chaya Redonda has been cloned by the people of the Yukatan 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). The ability of stem cuttings to regenerate varies depending on the plant species. Propagation through branch cuttings is influenced by internal and external factors (Zargar & Kumar 2018). The maturity stage and length of planting material have been reported to have an effect on the early growth of stem cuttings of the *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). The same has not been studied in the propagation of the Redonda cultivar chaya, which has different stem characteristics.

Chaya grows quickly with resistance to drought, but it has yet to be cultivated intensively and is only limited to protective planting in land bounded as fences. 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 growth of plants through the propagation of stem cuttings at different lengths.

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MATERIALS AND METHODS

Biomaterial and procedures study

This study was conducted at Ogan Ilir area (104º46’44” E; 3º01’35” S) in South Sumatra, Indonesia. Chaya plant material used in this study was from the redonda cultivar with the Latin name *Cnidoscolus Aconitifolius ssp. 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 plant can be seen in Figure 1.

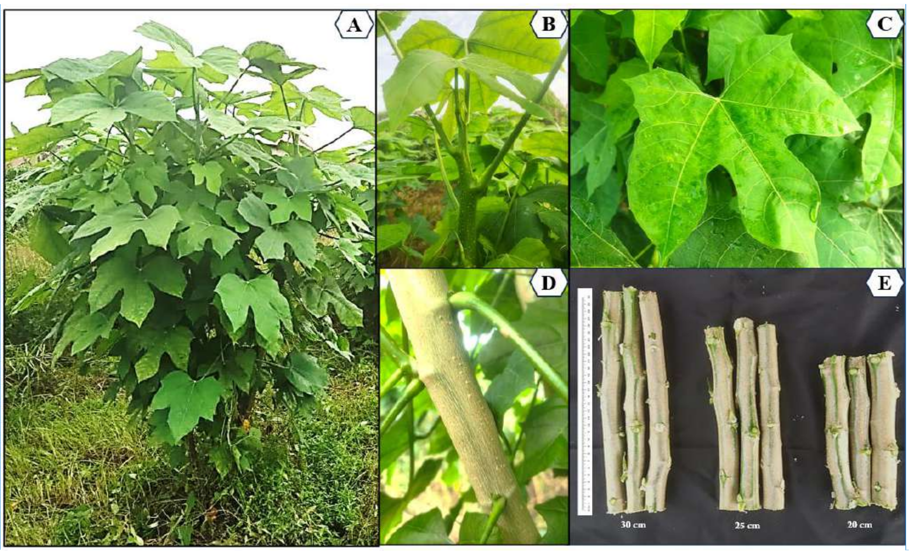

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Figure 1. Chaya redonda plant (A), shoot (B), leaf (C), and stem (D), planting material (E)

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The planting medium comprised a 2:1 mixture of soil and manure. The polybag used was 15 x 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 containing five plants with four repetitions, leading to a total of 60 plant units.

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Leaf area estimation

The morphology of the Chaya redonda 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 intercept 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 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 dry as well as fresh weight of leaf and stem, total leaf area, and stem diameter were collected at harvest.

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Effects of treatments were examined with analysis of variance (ANOVA). Moreover, differences between treatments were determined through 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.

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RESULTS AND DISCUSSION

Chaya redonda leaf shape

Chaya redonda 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 2. Leaf has a palmate shape with three to five lobes and is petiolated, identical to *Jatropha curcas* L. (de Oliveira et al. 2016). The leaf shape of chaya redonda 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 linked to plant water status (Ding et al. 2020). Therefore, it is not surprising that morphological indices have been used to measure different physiological of species or specific conditions in that environment (Yu et al. 2020).

Leaf length and width are quantitative characters for morphometric analysis used to describe species and assess morphological variations (Chuanromanee et al. 2019; Lestari et al. 2021). We divided the quantitative characters of chaya rodenda leaf to four namely midrib length, leaf length, top width, and bottom width. This study founded that leaf midrib length ratio (L_1/L_2) as well as leaf blade width ratio (W_1/W_2) of chaya redonda remained constant during growth. The results showed leaf shape regularity, which supported the predictor determination, and several related morphological traits were also applied in predicting leaf area (LA), similar to the method used for *Luffa acutangular* plants (Lakitan et al. 2022). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies such as *Amorphophallus muelleri* (Nurshanti et al. 2022) and citrus (Muda et al. 2023).

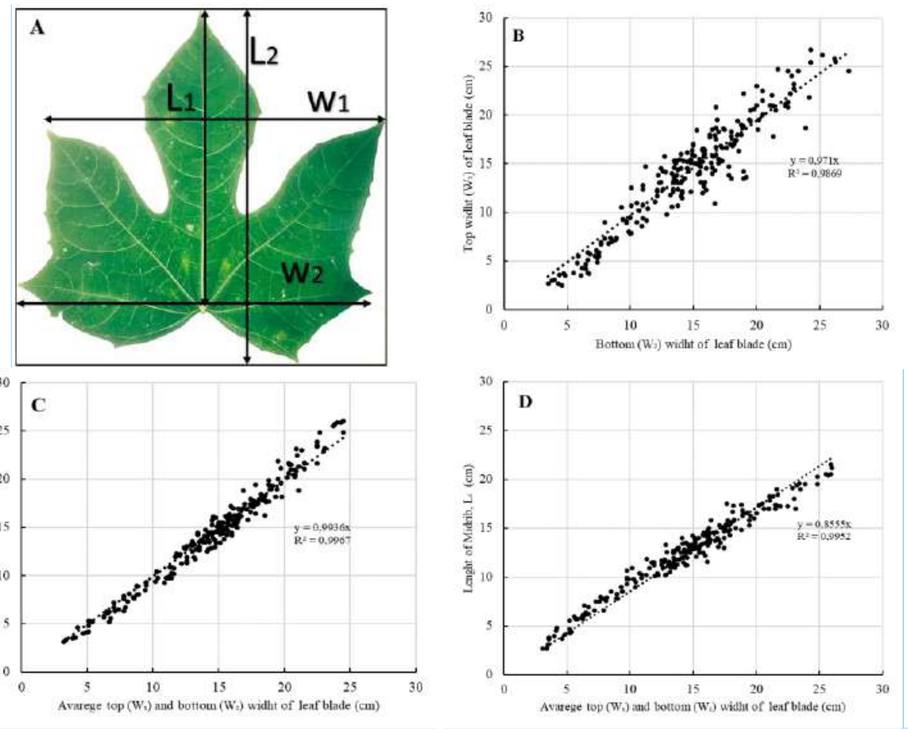

Figure 2. Chaya leaf dimensions (A), regression of between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D)

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Leaf shape can be represented through the ratio of length and width, with a value less than 1.0 showing a wide shape, while greater than 1 signifies an elongated shape. Observation results showed length and width ratio values were consistently close to 1.0, proving that the shape had square dimensions with similar length and width. Additionally, the ratio of midrib length and leaf blade width (L_1/WB) or leaf length-width (L_2/WB) did not change during the enlargement process. This fact validated the leaf shape *of Cnidoscolus Aconitifolius ssp.* Breckon's Aconitifolius was constant, as presented in Figure 3. Several previous studies have reported that each plant has a different leaf length/width ratio. This variation of leaf length/width ratio depends on the plant species and growing environment (Liu et al. 2020; Yu et al. 2020).

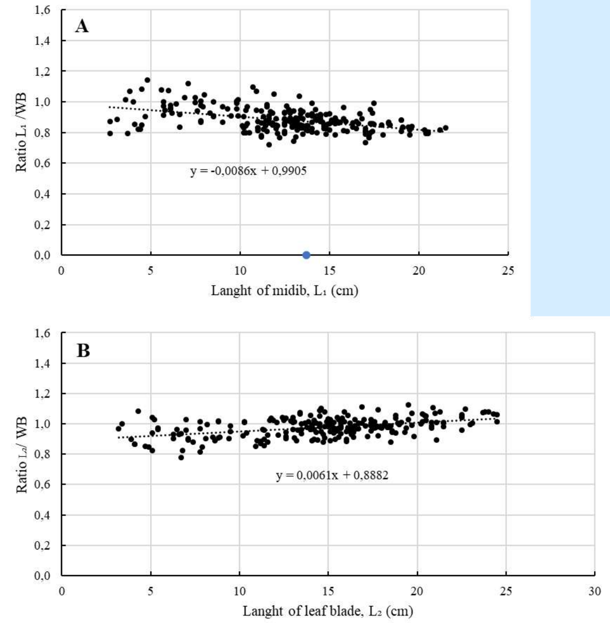

Figure 3. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A), and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda

Chaya leaf is single, symmetrical, lobed, with a flat surface and constant leaf shape development, while leaf area can be estimated accurately and nondestructive. Leaf area is probably one of the most important leaf indices associated with the analysis of plant growth 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. 2022).

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 0.909, 0.889, and 0.915 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 4).

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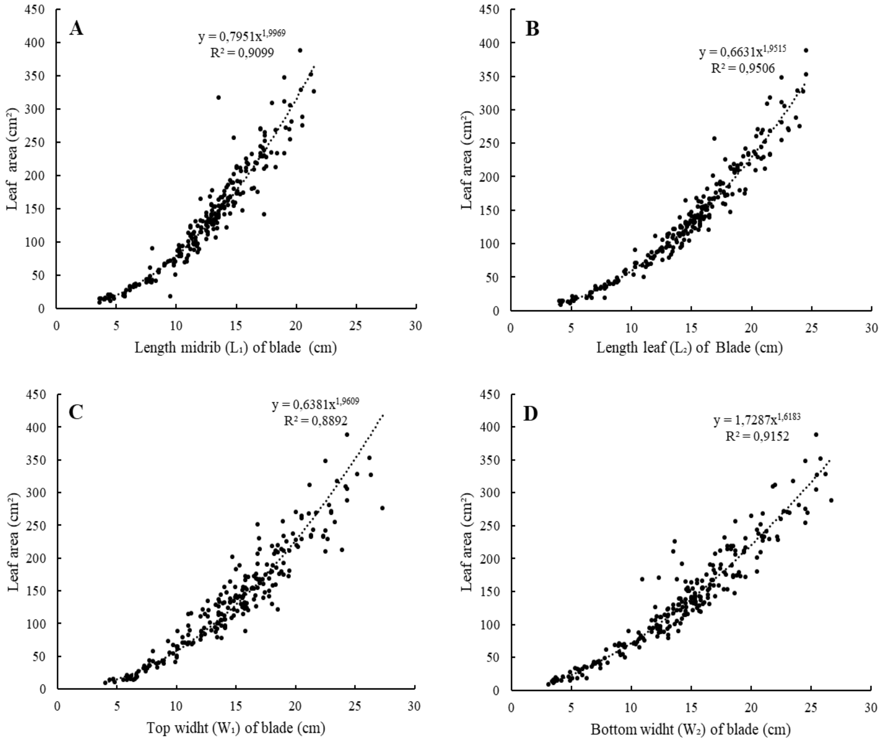

Figure 4. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831), respectively (Figure 5).

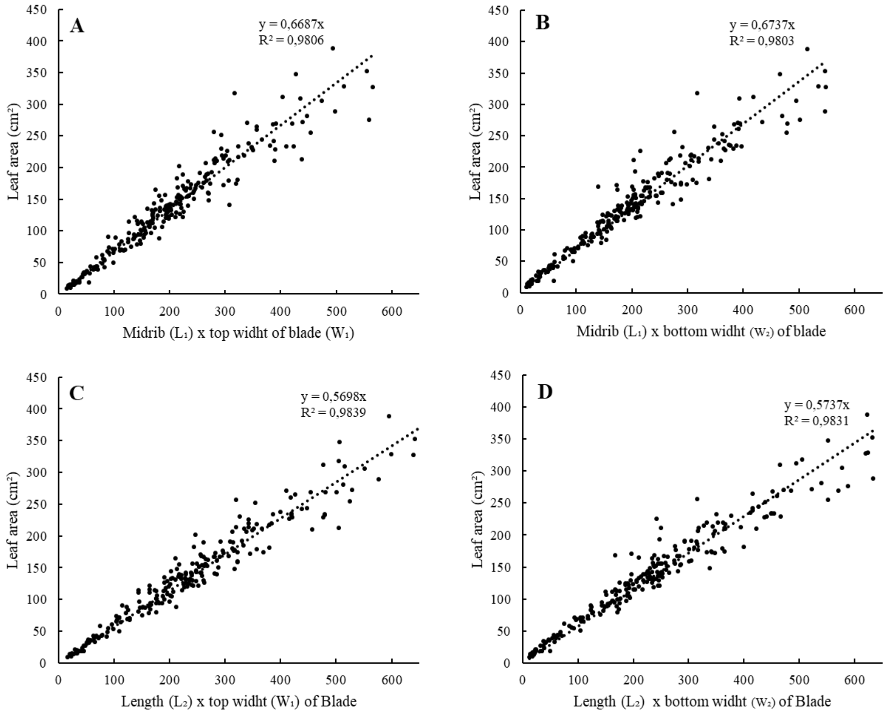

Figure 5. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D)

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, it is necessary 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 same time, some leaves have been analyzed for the area through a regression equation method, such as in cassava plants (Lakitan et al. 2022) 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 x W (Figure 5C) was found to be a more accurate predictor in estimating leaf area compared to using length or width separately through linear zero-intercept regression. This statement has also been proven in plants consisting of similar morphology, such as cassava leaf (Lakitan et al. 2022) and Fikuda cultivar (Gustiar et al. 2023).

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Early growth and yield of Chaya

Chaya redonda propagation can only be carried out vegetatively due to the infertile seeds. 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 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 6).

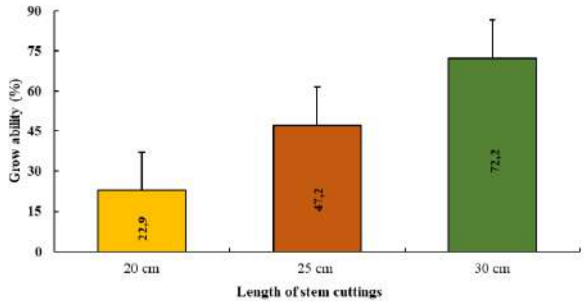

Figure 6. Percentage of growth ability at different stem cutting lengths

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

After cuttings had developed with vegetative organs, leaf length influenced leaf growth, as shown by the leaf expansion rate. 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 gro is very useful in determining the right harvest time for leafy vegetables such as chaya. Monitoring of leaf area by 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. 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 opening fully. Meanwhile, 20 cm and 25 cm stem cuttings experienced more rapid stagnant growth at 8-9 and 11-12 days, respectively (Figure 7). 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.

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If the one you mean is just the growth ability of plant to produce new shoot and root.

Please consider to revise because its fatal to misunderstanding of figure 6 while no information about how the data obtained or analyzed.

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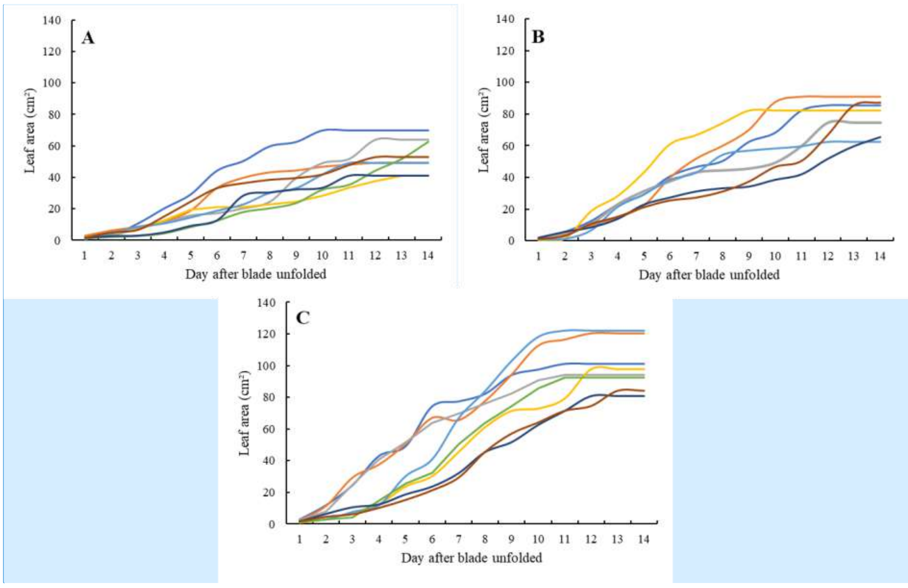

Figure 7. Increasing of chaya redonda leaf area at the onset of plant growth with stem cutting lengths of 20 cm (A), 25 cm (B), and 30 cm (C)

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 8). The large diameter of cuttings did not affect the growth and yield of the vegetative chaya redonda plant, compared to fikuda variety (Gustiar et al. 2023b). This observation corresponded with the result obtained from the analysis of cassava plant cuttings (EJ 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. The accumulation of carbohydrates was previously reported to play an important role before plants actively conduct photosynthetic metabolism (Otiende & 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.

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Suggestion for this data, first combine the 20,25 and 30 as on graphic by averaging each data while you can add the standard deviation to see the data range. I think within one line you can compare each cutting and leaf area expantions.

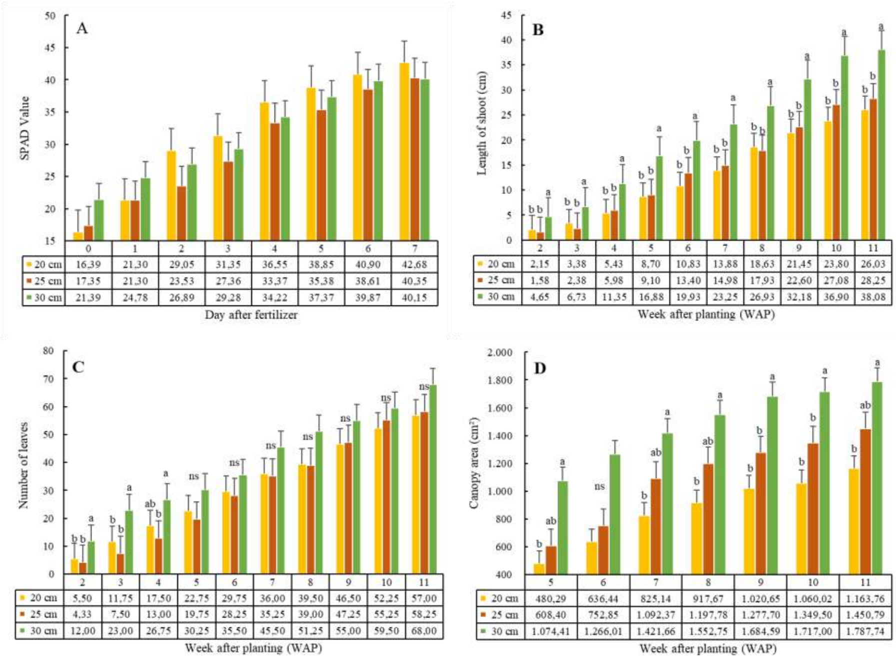

Figure 8. SPAD value (A), shoot length (B), leaf number (C), and canopy area (D) based on differences in stem cutting length

The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots. At the completion of the study at 11 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 shoot (Table 1).

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

	Total leaf area (cm²)	Number of branches	Shoot diameter (mm)	Fresh Weight (g)			Dry Weight		
				Lamina	Petiole	Shoot	Lamina (g)	Petiole (g)	Shoot (g)
20 cm	3134.96 ^{b}	5.00 ^{a}	17.93 ^{a}	48.30 ^{b}	31.89 ^{b}	128.03 ^{b}	10a45 ^{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 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 30 cm that grow more bud structures (buds, stems, and leaves) than shorter. This is in line with the results of research conducted on *Jatropha curcas* (Severino et al. 2011). The cuttings length is one that needs to be considered in plant 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 (Martínez-Vilalta et al. 2016). The carbohydrate accumulation in planting

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material is stored by the parent plant, which can be mobilized when 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.

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ACKNOWLEDGEMENTS

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Reviwer B
Round 1

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

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Abstract. Chaya (*Cnidoscolus aconitifolius*) 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. Therefore, this study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm. 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 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. Cuttings with a length of 30 cm were 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

Running title: Leaf morphology characterization of Chaya

INTRODUCTION

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Chaya (*Cnidoscolus aconitifolius*) is a perennial vegetable plant originating from southern Mexico and Guatemala, with several cultivars, including redonda, as well as different leaf morphology (Montero-Astúa et al. 2023). Due to the similarity of the agroecosystem with the area of origin, this plant can thrive in Indonesia (Gustiar 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 leaf can be harvested across the year to fulfill the food needs of the people (Manzanilla and Knerr 2020). Chaya is considered a potential vegetable food ingredient because the leaf has been identified to contain approximately 30% protein based on dry weight (Schwarcz et al. 2022). This protein content is relatively high compared to other plant foods, such as nuts, which comprise only 19.5% - 24.8%. Furthermore, 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). As a nutrient-rich plant, leaf offers many health benefits, including maintaining healthy blood sugar levels, along with providing anti-inflammatory, anti-anemic, anti-microbial, and antioxidant effects (Guevara-Cruz et al. 2021; Guzmán et al. 2023; Manzanilla Valdez et al. 2021). These promote the continuous use of food, fence posts, medicines, and ornamental material, but the 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. 2023). Leaf organ traits are reported to affect plant development and biomass as they are key to photosynthesis (Hagos

Abraha et al. 2024). As the most important organ for photosynthesis, leaves produce a main source of energy not only for plant growth but also for human nutrition and other purposes. The size and shape of the leaves affect the utilization of light energy, thus affecting the development of plants and biomass. In addition, 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 a vegetable. Studies related to the morphology of Redond chaya leaves are still scarce.

Chaya Redonda has been cloned by the people of the Yukatan 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). The ability of stem cuttings to regenerate varies depending on the plant species. Propagation through branch cuttings is influenced by internal and external factors (Zargar & Kumar 2018). The maturity stage and length of planting material have been reported to have an effect on the early growth of stem cuttings of the *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). The same has not been studied in the propagation of the Redonda cultivar chaya, which has different stem characteristics.

Chaya grows quickly with resistance to drought, but it has yet to be cultivated intensively and is only limited to protective planting in land bounded as fences. 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 growth of plants through the propagation of stem cuttings at different lengths.

MATERIALS AND METHODS

Biomaterial and procedures study

This study was conducted at Ogan Ilir area (104º46’44” E; 3º01’35” S) in South Sumatra, Indonesia. Chaya plant material used in this study was from the redonda cultivar with the Latin name *Cnidoscolus Aconitifolius ssp. 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 plant can be seen in Figure 1.

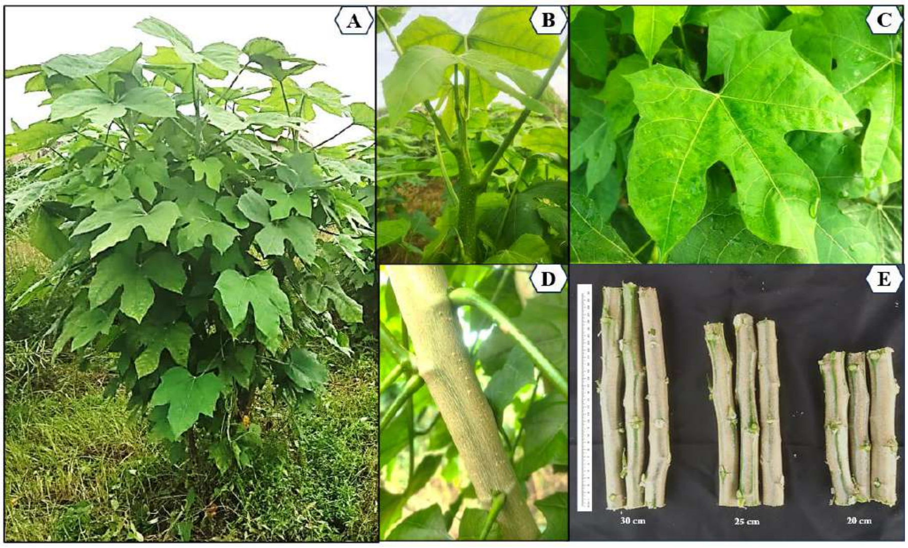

Figure 1. Chaya redonda plant (A), shoot (B), leaf (C), and stem (D), planting material (E)

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The planting medium comprised a 2:1 mixture of soil and manure. The polybag used was 15 x 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 containing five plants with four repetitions, leading to a total of 60 plant units.

Leaf area estimation

The morphology of the Chaya redonda 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 intercept linear regression (Gustiar et al. 2023b).

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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 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 dry as well as fresh weight of leaf and stem, total leaf area, and stem diameter were collected at harvest.

Effects of treatments were examined with analysis of variance (ANOVA). Moreover, differences between treatments were determined through least significant difference (LSD) test at $P<5\%$ 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.

RESULTS AND DISCUSSION

Chaya redonda leaf shape

Chaya redonda 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 2. Leaf has a palmate shape with three to five lobes and is petiolated, identical to *Jatropha curcas* L. (de Oliveira et al. 2016). The leaf shape of chaya redonda 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 linked to plant water status (Ding et al. 2020). Therefore, it is not surprising that morphological indices have been used to measure different physiological of species or specific conditions in that environment (Yu et al. 2020).

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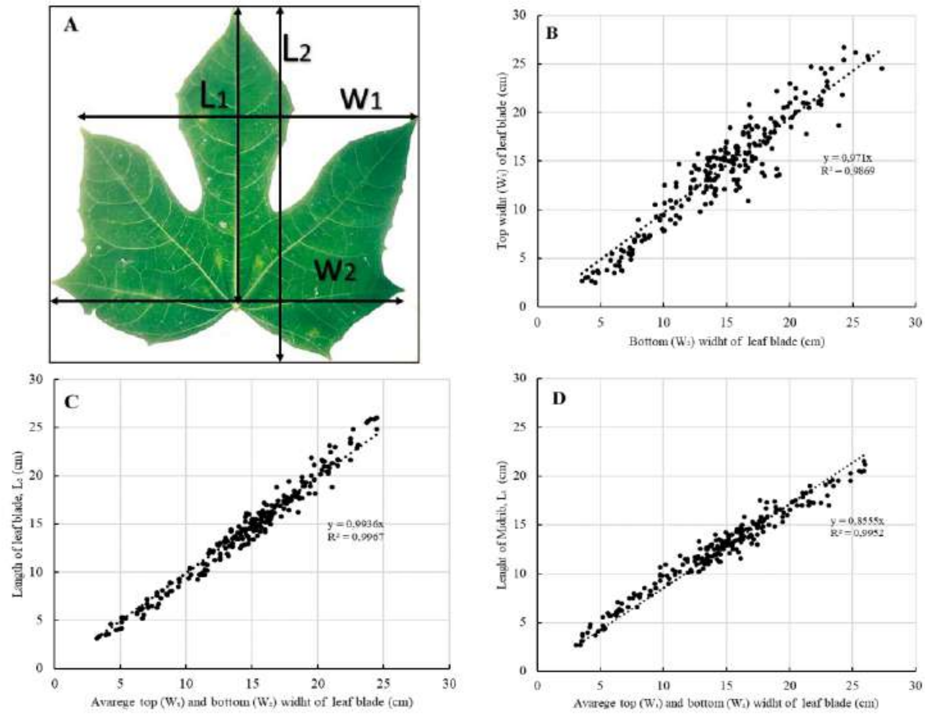
Leaf length and width are quantitative characters for morphometric analysis used to describe species and assess morphological variations (Chuanromanee et al. 2019; Lestari et al. 2021). We divided the quantitative characters of chaya rodenda leaf to four namely midrib length, leaf length, top width, and bottom width. This study founded that leaf midrib length ratio (L_1/L_2) as well as leaf blade width ratio (W_1/W_2) of chaya redonda remained constant during growth. The results showed leaf shape regularity, which supported the predictor determination, and several related morphological traits were also applied in predicting leaf area (LA), similar to the method used for *Luffa acutangular* plants (Lakitan et al. 2022). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies such as *Amorphophallus muelleri* (Nurshanti et al. 2022) and citrus (Muda et al. 2023).



Leaf shape can be represented through the ratio of length and width, with a value less than 1.0 showing a wide shape, while greater than 1 signifies an elongated shape. Observation results showed length and width ratio values were consistently close to 1.0, proving that the shape had square dimensions with similar length and width. Additionally, the ratio of midrib length and leaf blade width (L_1/WB) or leaf length-width (L_2/WB) did not change during the enlargement process. This fact validated the leaf shape *of Cnidoscolus Aconitifolius ssp.* Breckon's Aconitifolius was constant, as presented in Figure 3. Several previous studies have reported that each plant has a different leaf length/width ratio. This variation of leaf length/width ratio depends on the plant species and growing environment (Liu et al. 2020; Yu et al. 2020).

Figure 2. Chaya leaf dimensions (A), regression of between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D)

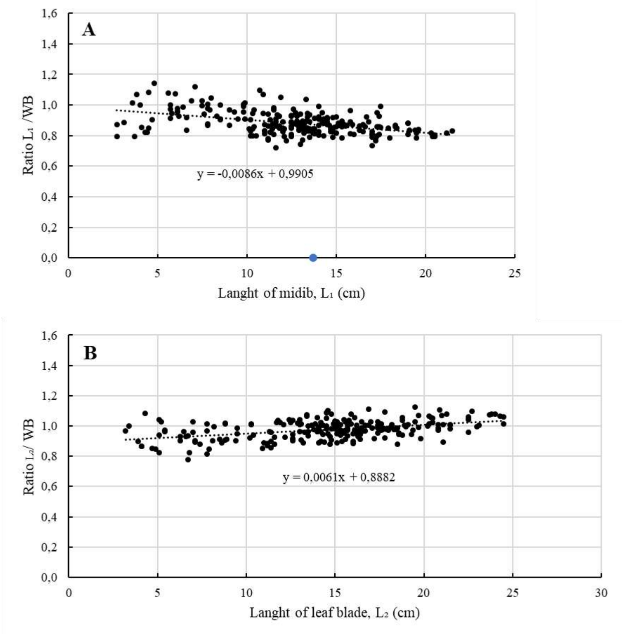


Figure 3. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A), and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda

Chaya leaf is single, symmetrical, lobed, with a flat surface and constant leaf shape development, while leaf area can be estimated accurately and nondestructive. Leaf area is probably one of the most important leaf indices associated with the analysis of plant growth 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. 2022).

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 0.909, 0.889, and 0.915 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 4).

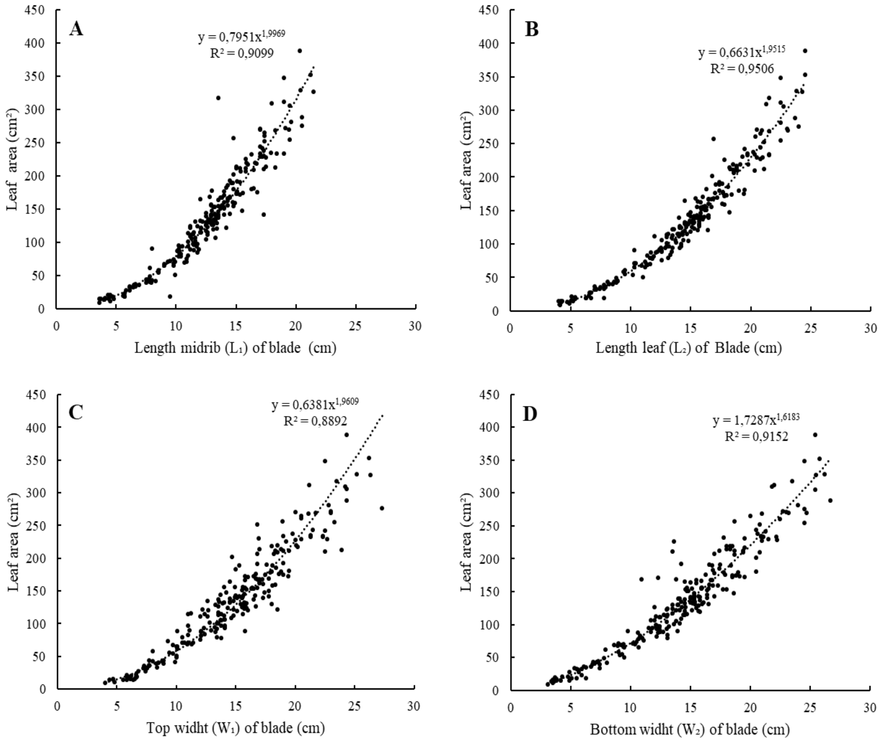


Figure 4. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831), respectively (Figure 5).

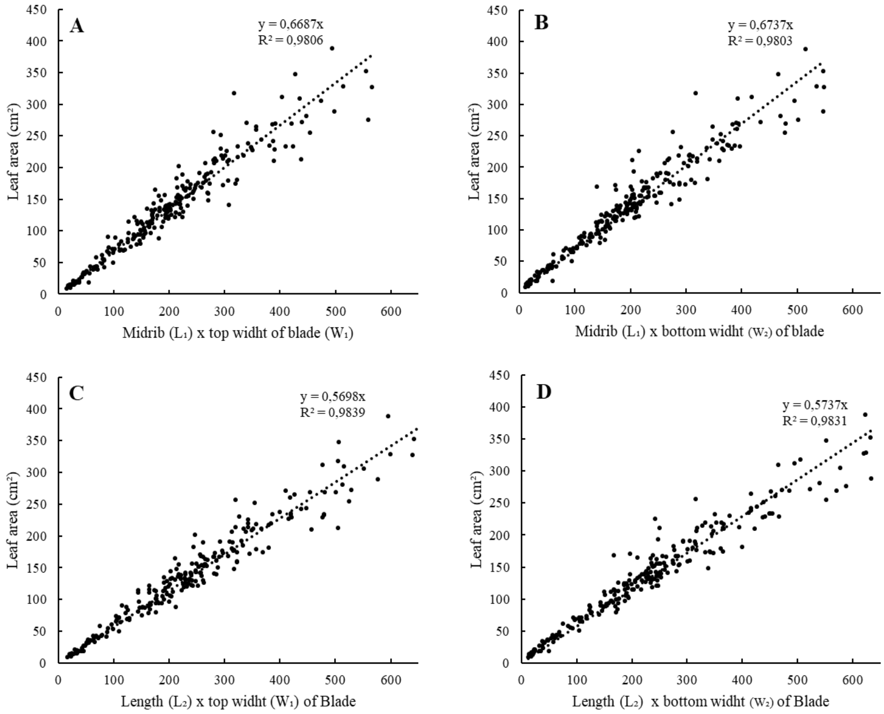


Figure 5. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D)

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, it is necessary 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 same time, some leaves have been analyzed for the area through a regression equation method, such as in cassava plants (Lakitan et al. 2022) 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 x W (Figure 5C) was found to be a more accurate predictor in estimating leaf area compared to using length or width separately through linear zero-intercept regression. This statement has also been proven in plants consisting of similar morphology, such as cassava leaf (Lakitan et al. 2022) and Fikuda cultivar (Gustiar et al. 2023).

Early growth and yield of Chaya

Chaya redonda propagation can only be carried out vegetatively due to the infertile seeds. 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 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 6).

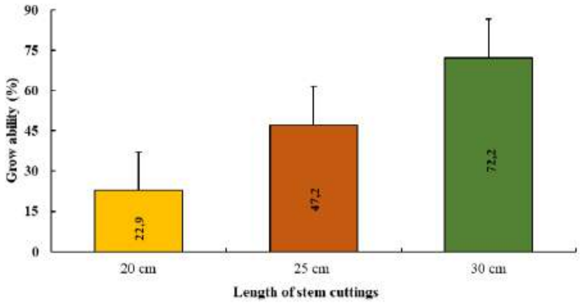


Figure 6. Percentage of growth ability at different stem cutting lengths

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

After cuttings had developed with vegetative organs, leaf length influenced leaf growth, as shown by the leaf expansion rate. 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 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. 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 opening fully. Meanwhile, 20 cm and 25 cm stem cuttings experienced more rapid stagnant growth at 8-9 and 11-12 days, respectively (Figure 7). 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.

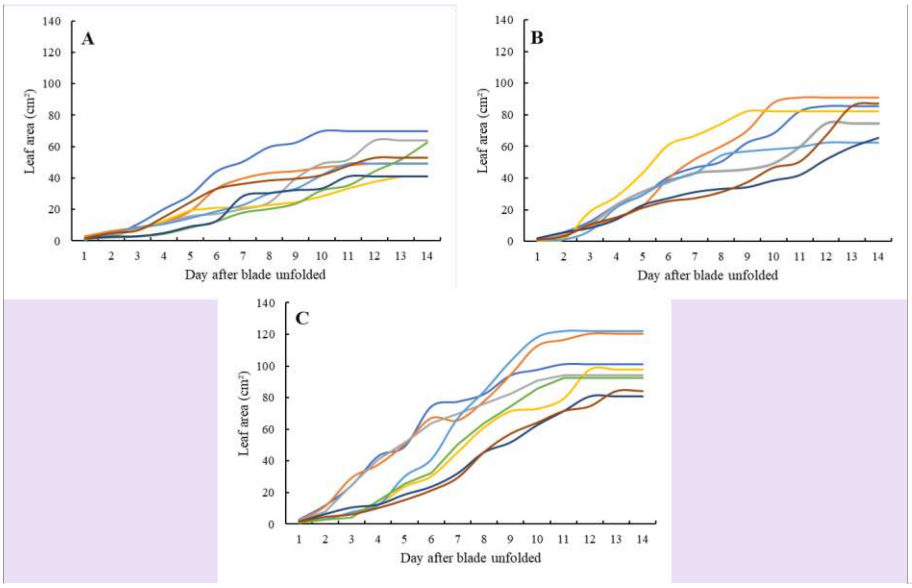


Figure 7. Increasing of chaya redonda leaf area at the onset of plant growth with stem cutting lengths of 20 cm (A), 25 cm (B), and 30 cm (C)

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 8). The large diameter of cuttings did not affect the growth and yield of the vegetative chaya redonda plant, compared to fikuda variety (Gustiar et al. 2023b). This observation corresponded with the result obtained from the analysis of cassava plant cuttings (EJ de Oliveira et al. 2020). However, cutting length in this study differed significantly from leaf number and canopy area because length affected carbohydrate accumulation in planting material. The accumulation of carbohydrates was previously reported to play an important role before plants actively conduct photosynthetic metabolism (Otiende & 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.

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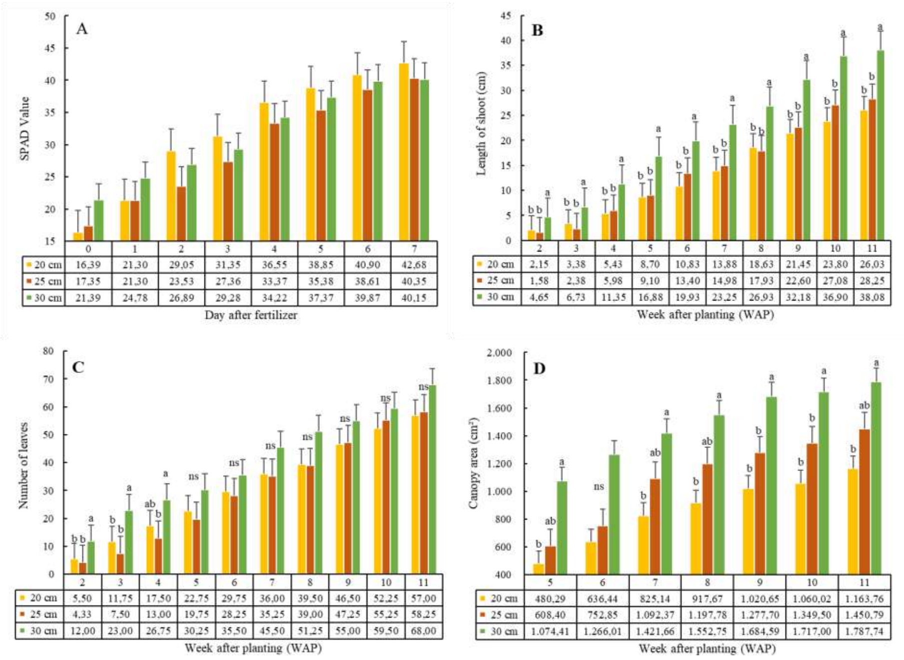

Figure 8. SPAD value (A), shoot length (B), leaf number (C), and canopy area (D) based on differences in stem cutting length

The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots. At the completion of the study at 11 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).

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

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	Total leaf area (cm²)	Number of branches	Shoot diameter (mm)	Fresh Weight			Dry Weight		
				Lamina (g)	Petiole (g)	Shoots (g)	Lamina (g)	Petiole (g)	Shoots (g)
20 cm	3134.96 [b]	5.00 [a]	17.93 [a]	48.30 [b]	31.89 [b]	128.03 [b]	10a45 [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 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 30 cm that grow more bud structures (buds, stems, and leaves) than shorter. This is in line with the results of research conducted on *Jatropha curcas* (Severino et al. 2011). The cuttings length is one that needs to be considered in plant 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 (Martínez-Vilalta et al. 2016). The carbohydrate accumulation in planting material is stored by the parent plant, which can be mobilized when 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.

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Reviwer T
Round 1

Manuscript title:

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

Review:

- Generally
    1. Please make the writing of "Redonda" more uniform. The authors should check once again how to write it. Redonda or redonda. Please check the manuscript thoroughly once again. The uniformity includes other cultivar names and even the plant's name.
    2. The authors might also want to consider writing Latin names evenly. For example, in Line 110, the author wrote *Jathropa curcas* L. (Linneaus included). Meanwhile, the rest of the Latin nomenclatures are just genus and species (e.g., Line 123, 124, even citrus is not written in Latin name).
    3. No discussion subsection and conclusion/concluding paragraph?
    4. Noticed that the authors were using reference citation manager. But please recheck the input metadata to make the manuscript more consistent.
- Abstract
    1. The readers could read the aim of the study in the abstract. However, what problem did the authors want to address in this paper? What is the correlation between Chaya originating from Mexico and grown in Asia, its cultivar, and its leaf length? Why length of stem cutting is important to be observed? Is it related to the cultivar type in any way? Please make it clearer.
    2. After reading the whole manuscript, I suggest the authors rephrase Lines 12-14. "study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm". The authors only performed leaf morphological analysis; no observation was completed on other organs.
- Introduction

    Line 26: Somehow, the phrase "as well as different leaf morphology" is like being forced to fit it into one sentence, thus making the sentence itself unclear. I suggest rewriting it into two or three sentences to make it readable, whether there is a correlation between the cultivar and leaf morphology or other aspects that make it different. Although, in my opinion, the authors could remove this part, as they explained further in the following paragraph.

- Material and methods
    1. Line 72-73: Please check how to write the Latin name properly (including the subspecies). I suggest the Authors check the website https://www.ncbi.nlm.nih.gov/taxonomy.
    2. Were the experiments done in a controlled environment or an open area? Please specify the material and methods. External factors (weather, rain period, sun exposure) should always be considered.
- Results and discussion
    1. Line 120, "found" not "founded"

2. Line 117-125: please refer to which figure represents the calculation used for the study.
3. Figure 2, Please check the English vocabulary. Many mistakes. “width” not “widht”; “average” not “avarage”, “length” not “langth”.
4. Figure 2, please include the statistical analysis done for the regression and the name of the software used.
5. Line 132-139: what is WB? The author did not specify in the previous paragraph or figure or material and methods. Why is it necessary to use the calculation in this study?
6. Line 138-139: “This variation of leaf length/width ratio depends on the plant species and growing environment.” This is exactly why the authors should explain the environmental condition during the study in material and method.
7. Line 179-194: in the introduction section, the authors wrote that their study aims to characterize and evaluate leaf morphological organs as genetic diversity for further development both taxonomically. Could the authors elaborate more on how their statistical results could help with the application of taxonomy identification? Based on the authors’ results, what are the differences between Chaya Redonda and other Chaya cultivars? Alternatively, whether the location of cultivation (e.g. Indonesia vs Mexico) affected the leaf length/width ratio.
8. Line 210-212: “The size of the stem cuttings will indicate the amount of nitrogen and carbohydrate content needed for the plant’s initial metabolic process. The longer the size of the stem cutting, the higher the percentage of life.” This phrase needs citation. The authors’ chosen stem lengths (30-25-20 cm) were too narrow to make this conclusion.
9. Figure 7: The authors used different colours for several lines, yet no legends that explain it. Please clarify the differences between those lines in the figure and the figure’s caption.
10. Line 231: What is WAP? Please clarify the abbreviation.
11. Line 239-240: This is a strong statement. Did the authors also measure carbohydrate concentration during the experiments? Suggest rephrasing the sentences and referring to other studies with similar results.
12. Figure 8: please include the information on statistical results in the figure’s caption. What do a, b, ns mean?
13. Figure 8D: Week 6, is the calculation correct? There was no significant difference in canopy area between the three study groups on week 6?
14. Line 246-247: “The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots” >> do the authors mean “the length of cutting had **a significant effect** on parameters compared to branches and shoots”?

Perbaikan Round 1

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

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Abstract. Chaya (*Cnidoscolus aconitifolius*) 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 cuttings length 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. Cuttings with a length of 30 cm were 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.

Key words: *leaf Area, leaf shape, planting material, Redonda cultivar*

Abbreviations (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.

Running title: Leaf morphology characterization of Chaya

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) is a perennial vegetable plant originating from southern Mexico and Guatemala, with several cultivars, 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 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 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. 2023). 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 a vegetable. Studies related to the morphology of Redond chaya leaves are still scarce.

Chaya Redonda cv. has been cloned by the people of the Yukatan 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 improve plant growth and yields comparing apical cuttings. The ability of stem cuttings to regenerate varies depending on the plant species. Propagation through branch cuttings is influenced by internal and external factors (Zargar & Kumar 2018). The maturity stage and length of planting material have been reported to have an effect on the early growth of stem cuttings of the *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). The same has not been studied in the propagation of the Chaya Redonda cv., which has different stem characteristics.

The research on morphological characteristics and growth performance of Chaya Redonda cv. through stem cuttings has not been widely conducted. Both aspects are very important on 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 growth of plants through the propagation of stem cuttings at different lengths.

MATERIALS AND METHODS

Biomaterial and Procedures Study

This study was conducted at Ogan Ilir area (104º46’44” E; 3º01’35” S) in South Sumatra, 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 *Cnidoscolus 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.

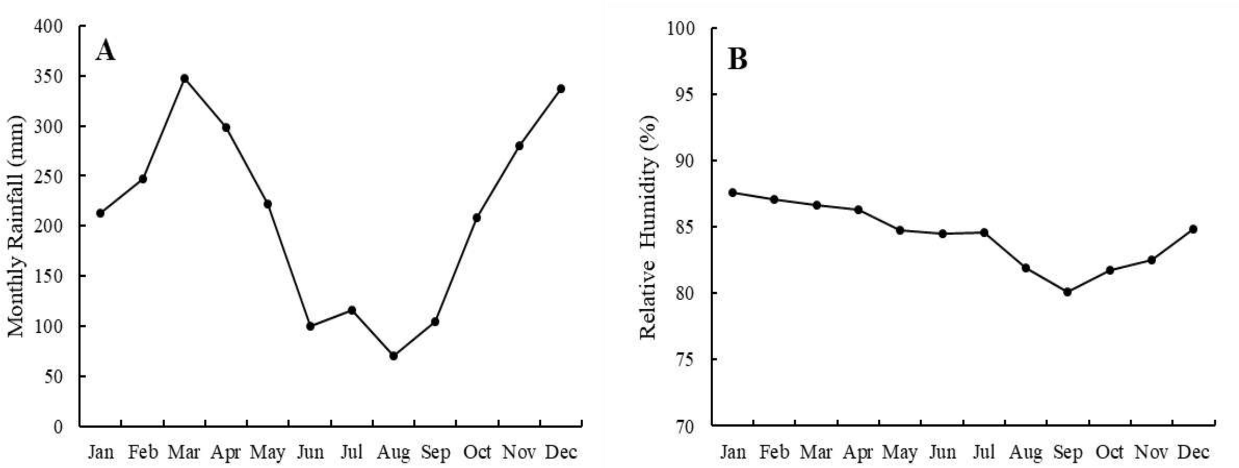


Figure 1. Monthly rainfall (A) and average of relative humidity (B) on research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia).

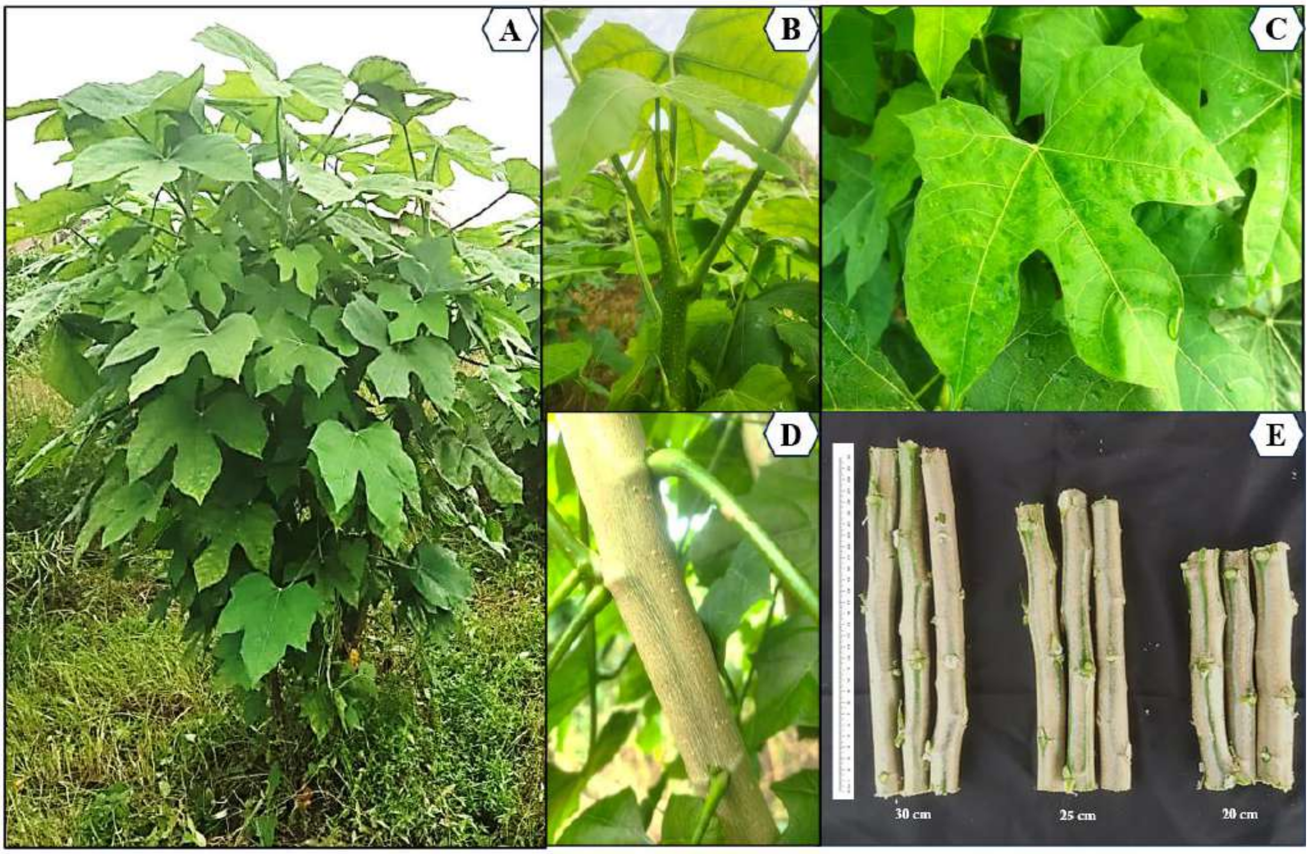


Figure 2. Chaya Redonda cv. (A), young shoot (B), leaf (C), stem (D), and planting material (E)

The planting medium comprised a 2:1 (v/v) mixture of soil and manure. The polybag used was 15 x 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 containing 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 intercept 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 dry as well as fresh weight of leaf and stem, total leaf area, and stem diameter were collected at harvest. To obtain dry weight, each plant organ was dried in an oven at 100°C for 24 hours.

Effects of treatments were examined with analysis of variance (ANOVA). Moreover, differences between treatments were determined through 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.

RESULTS AND DISCUSSION

Chaya Redonda cv. Leaf Shape

Chaya Redonda cv. leaf is founded 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* (de Oliveira et al. 2016). The leaf shape of Chaya Redonda cv. 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 linked to plant water status (Ding et al. 2020). Therefore, it is not surprising that morphological indices have been used to measure different physiological of species or specific conditions in that environment (Yu et al. 2020).

Leaf length and width are quantitative characters for morphometric analysis used to describe species and assess morphological variations (Chuanromanee et al. 2019; Lestari et al. 2021). We divided the quantitative characters of chaya rodenda leaf to four namely midrib length, leaf length, top width, and bottom width (Figure 2A). This study founded that leaf midrib length ratio (L_1/L_2) as well as leaf blade width ratio (W_1/W_2) of Chaya Redonda cv. remained constant during growth. The results showed leaf shape regularity, which supported the predictor determination, and several related morphological traits were also applied in predicting leaf area (LA), similar to the method used for *Luffa acutangula* plants (Lakitan et al. 2022). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies such as *Amorphophallus muelleri* (Nurshanti et al. 2022) and *Citrus sinensis* (Muda et al. 2023).

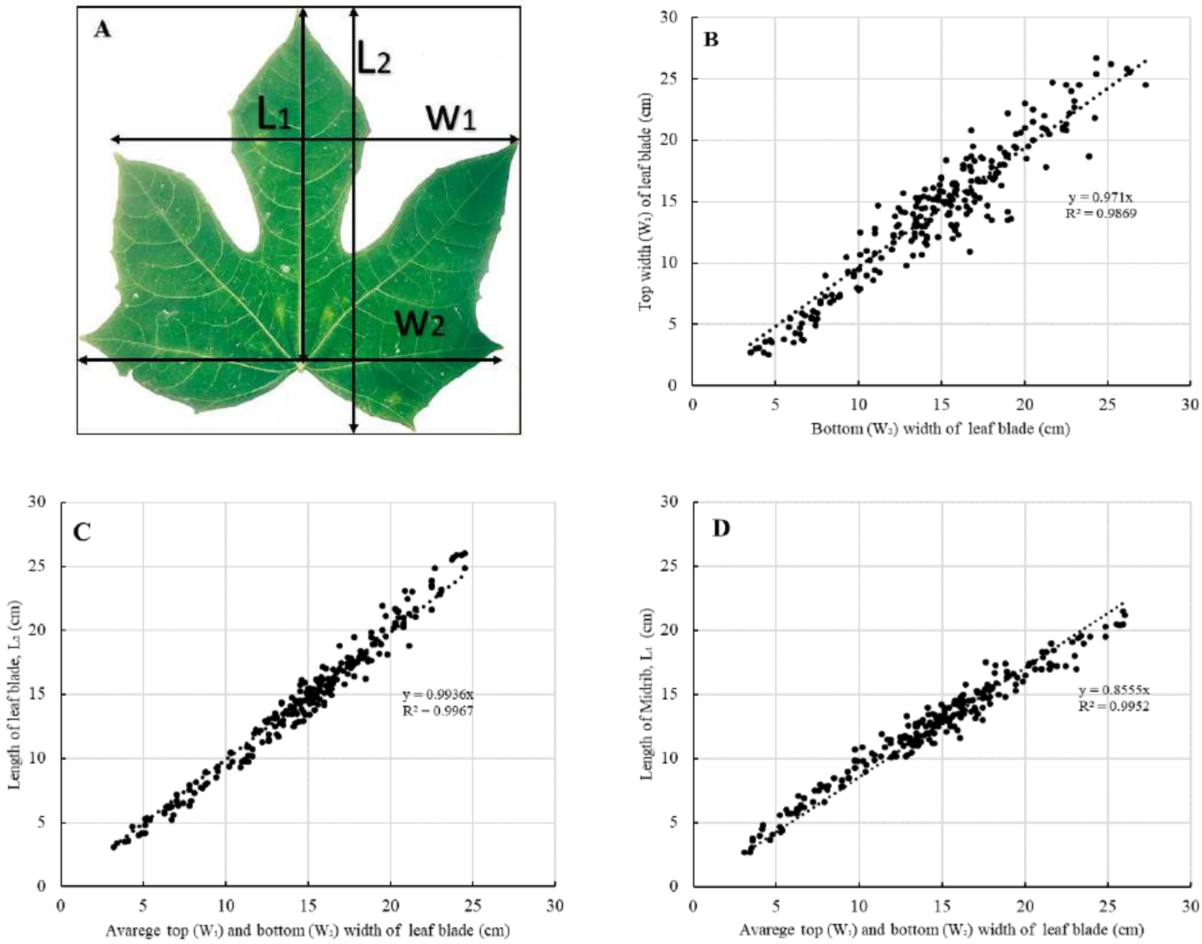


Figure 3. Chaya leaf dimensions (A), linear regression between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D).

Leaf shape can be represented through the ratio of length and width, with a value less than 1.0 showing a wide shape, while greater than 1 signifies an elongated shape. Observation results showed length and width ratio values were consistently close to 1.0, proving that the shape had square dimensions with similar length and width. Additionally, the ratio of midrib length and leaf blade width (L_1/WB) or leaf length-width (L_2/WB) did not change during the enlargement process. This fact validated the leaf shape *of Cnidoscolus Aconitifolius ssp.* Breckon's Aconitifolius was constant, as presented in Figure 4. Several previous studies have reported that each plant has a different leaf length/width ratio. This variation of leaf length/width ratio depends on the plant species and growing environment (Liu et al. 2020; Yu et al. 2020).

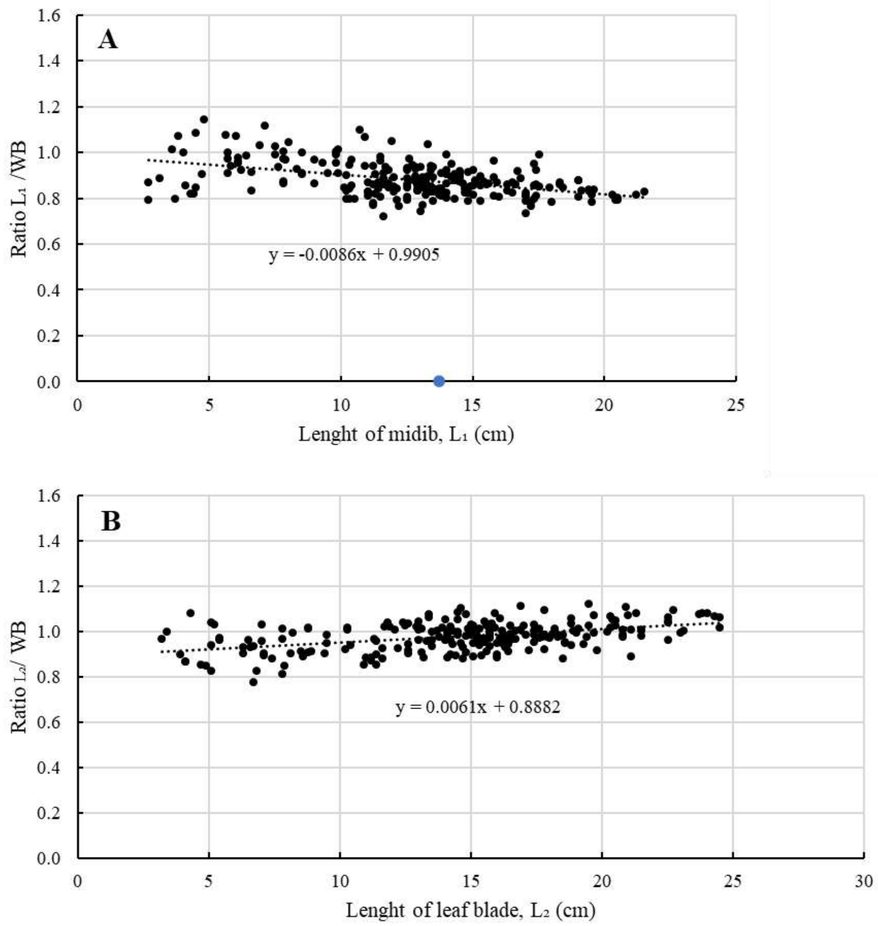


Figure 4. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A), and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda cv. WB means average of top (W_1) and bottom (W_2) width

Chaya leaf is single, symmetrical, lobed, with a flat surface and constant leaf shape development, while leaf area can be estimated accurately and nondestructive. Leaf area is probably one of the most important leaf indices associated with the analysis of plant growth 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. 2022).

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 0.909, 0.889, and 0.915 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 5).

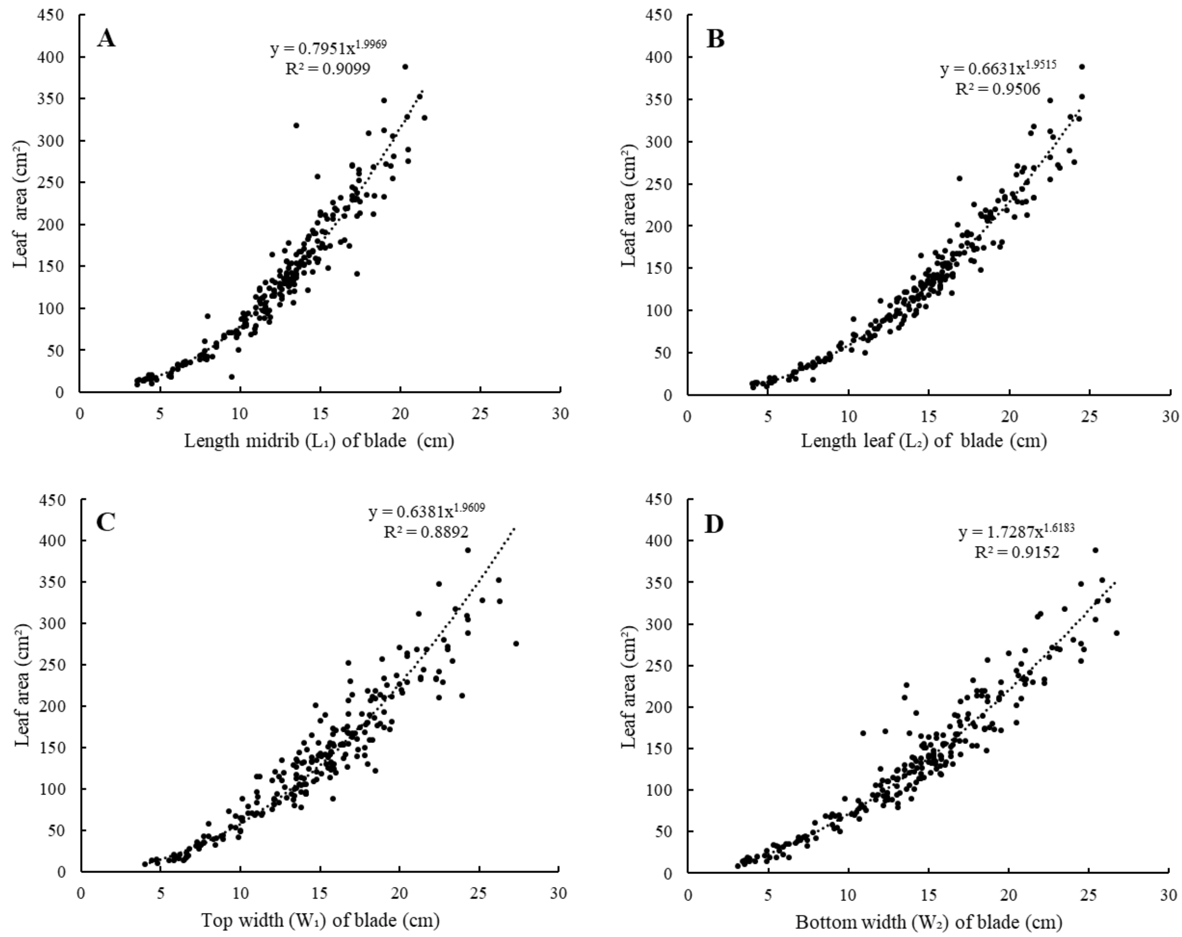


Figure 5. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831), respectively (Figure 6).

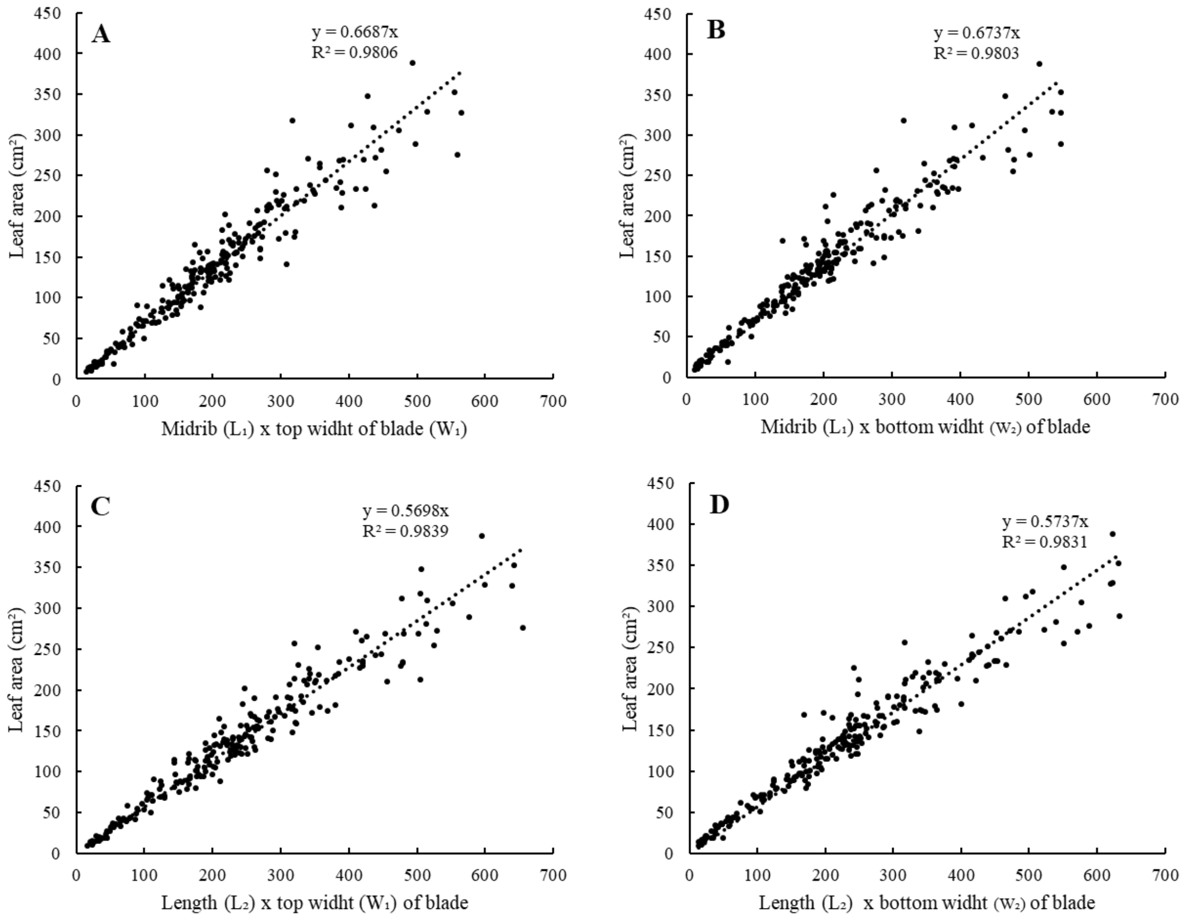


Figure 6. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D).

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, it is necessary 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 same time, some leaves have been analyzed for the area through a regression equation method, such as in cassava plants (Lakitan et al. 2022) 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 x W_1 (Figure 5C) 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 statement has also been proven in plants consisting of similar morphology, such as cassava leaf (Lakitan et al. 2022) and Fikuda cultivar (Gustiar et al. 2023).

Early Growth and Yield of Chaya

Chaya Redonda cv. propagation can only be carried out vegetatively due to the infertile seeds. 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 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).

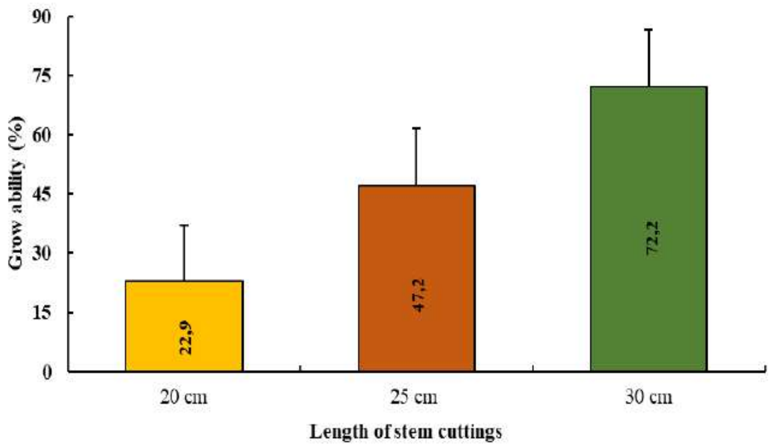


Figure 7. Percentage of growth ability at different stem cutting lengths

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 is influenced by the content of nitrogen, carbohydrates, and auxins as one of the regulators of root growth (Druege et al. 2019; Solikin 2018). The size of stem cuttings indicates the availability of nitrogen and carbohydrate 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 grow is very useful in determining the right harvest time for leafy vegetables such as chaya. Monitoring of leaf area by 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 unfolded. 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.

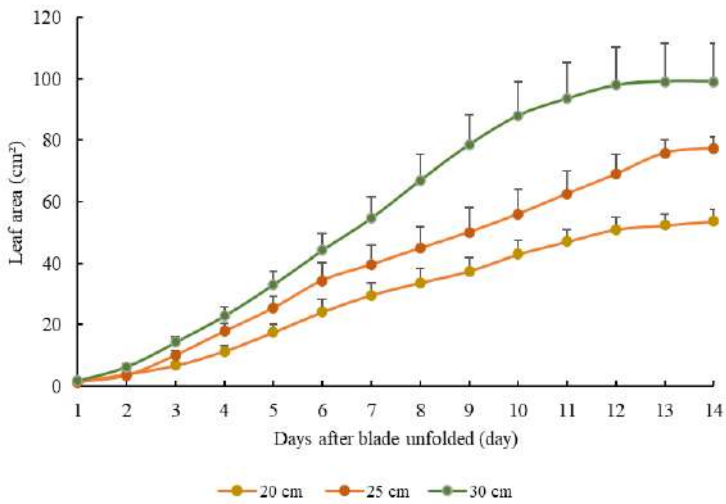
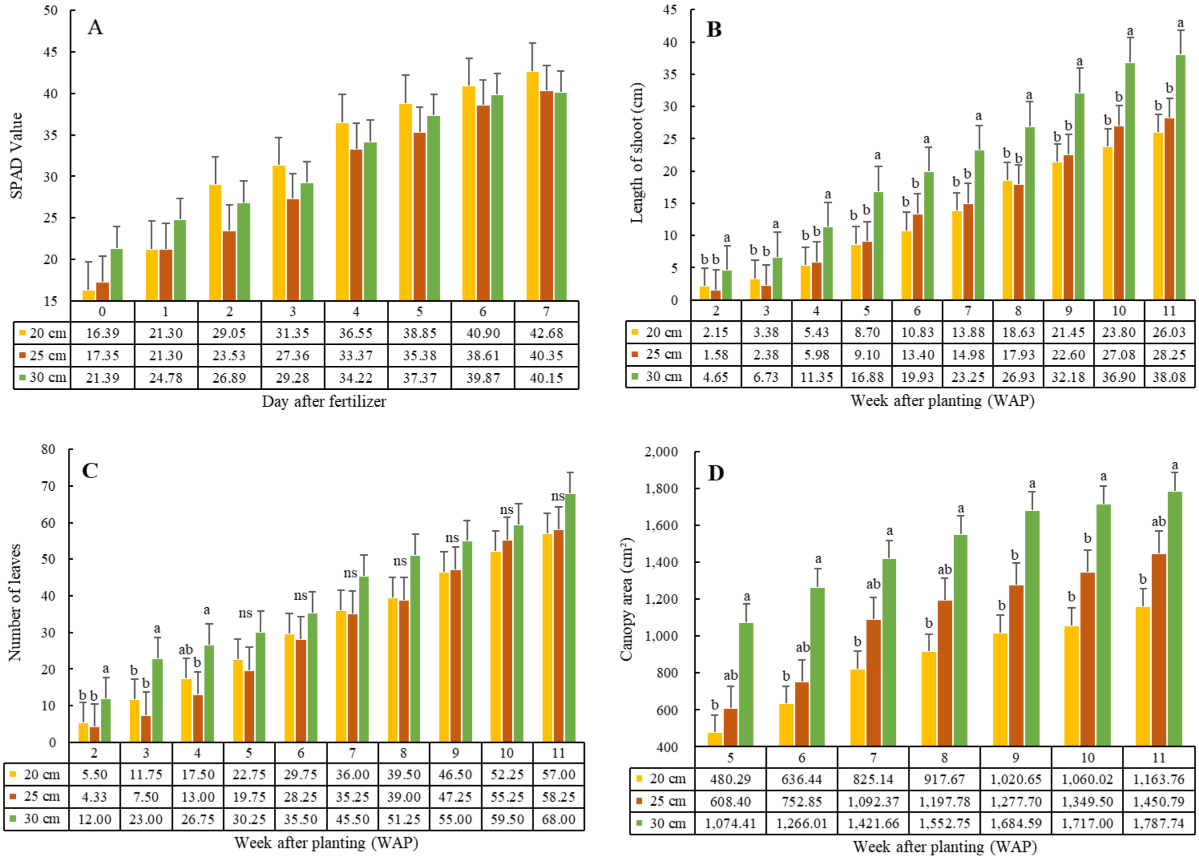


Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm and 30 cm.

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 (EJ de Oliveira et al. 2020). However, cutting length in this study differed significantly from leaf number and canopy area because cuttings 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 & 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.



	0	1	2	3	4	5	6	7
20 cm	16.39	21.30	29.05	31.35	36.55	38.85	40.90	42.68
25 cm	17.35	21.30	23.53	27.36	33.37	35.38	38.61	40.35
30 cm	21.39	24.78	26.89	29.28	34.22	37.37	39.87	40.15

	2	3	4	5	6	7	8	9	10	11
20 cm	2.15	3.38	5.43	8.70	10.83	13.88	18.63	21.45	23.80	26.03
25 cm	1.58	2.38	5.98	9.10	13.40	14.98	17.93	22.60	27.08	28.25
30 cm	4.65	6.73	11.35	16.88	19.93	23.25	26.93	32.18	36.90	38.08

	2	3	4	5	6	7	8	9	10	11
20 cm	5.50	11.75	17.50	22.75	29.75	36.00	39.50	46.50	52.25	57.00
25 cm	4.33	7.50	13.00	19.75	28.25	35.25	39.00	47.25	55.25	58.25
30 cm	12.00	23.00	26.75	30.25	35.50	45.50	51.25	55.00	59.50	68.00

	5	6	7	8	9	10	11
20 cm	480.29	636.44	825.14	917.67	1,020.65	1,060.02	1,163.76
25 cm	608.40	752.85	1,092.37	1,197.78	1,277.70	1,349.50	1,450.79
30 cm	1,074.41	1,266.01	1,421.66	1,552.75	1,684.59	1,717.00	1,787.74

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

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).

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 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 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 (Table 1). This is in line with the results of research conducted on *Jatropha curcas* (Severino et al. 2011). The cuttings length is one that needs to be considered in plant 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 (Martínez-Vilalta et al. 2016). The carbohydrate accumulation in planting material is stored by the parent plant, which can be mobilized when 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.

CONCLUSION

The leaf of Chaya Redonda cv. is a palminerved single leaf characterized by leaf area that strongly correlates with length x 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.

ACKNOWLEDGEMENTS

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Matrik Perbaikan
Round 1

Matrix of responses

Reviewer A

Line/Figure	Reviewer's Comment and Suggestion	Our Response (The revisions have been marked in yellow on the manuscript)
64-65	It does not go well with the next sentences What is the relantionship of the drought and the study aims to characterize and evaluate leaf morphological organs in different stem cuttings?	The manuscript has been revised. "The research on morphological characteristics and growth performance of Chaya Redonda cv. through stem cuttings has not been widely conducted. Both aspects are very important on Chaya Redonda cv. as a leafy vegetable plant propagated through vegetative propagation (In line 56-58)".
78	Can you confirm this picture information? Would you give me a clarification about what is shoot and what is branch?	We confirm that Figure 1B is referred to as a young shoot. The Figure information has been revised (In line 75). Shoot is a developed bud consisting of branches and leaves. Meanwhile, branch is a part of the shoot.
80	Chaya Redonda cv. Is better to indicate the redonda is cultivar or just state the Redonda without chaya in the main text	We agreed. The manuscript has been revised by changing the term Chaya redonda plant to Chaya Redonda cv.
81	By what? Volume?	The ratio of planting media mixture used is by volume. The manuscript has been added (In line 76)
99	Drying method?	The manuscript has been revised. "To obtain dry weight, each plant organ was dried in an oven at 100°C for 24 hours (In line 95-96)".
127	y and x axis title are typo "width" is correct one	The Figure has been revised (In line 123)
140	L1/WB ratio is more make sense Typo again in x axis	The Figure has been revised (In line 133)

187	Need concistency of this part Chaya Fikuda-Chaya Redonda or chaya fikuda – chaya redonda	We agreed to use the term Chaya Fikuda-Chaya Redonda.
191	You can add the information of R^2 result	We agreed. The R^2 information has been added in the manuscript (In line 177-178).
205	How do you calculate this? No information in Materials and method	The information has been added in the manuscript. "The percentage of growth ability was determined through the ratio of the cuttings that produced shoot to the total number of planted cuttings (In line 92-93).
208	This term is a bit off, we usually refer it to plant viability and its not based on ability to develop new roots and shoot but how many plant alive/germinated in one population If the one you mean is just the growth ability of plant to produce new shoot and root. Please consider to revise because its fatal to misunderstanding of figure 6 while no information about how the data obtained or analyzed.	The term has been changed to "growth ability" (In line 188).
208	One plant of chaya is consist of 1 shoot and many branches or many shoots also many branches?	One chaya plant consists of 1 shoot and many branches.
210-211	How do you know the needed of plant? Do you have exact data? Do you mean its the nitrogen and carbohydrate availability?	This statement has been confirmed through previous research that we have cited in the manuscript. "The size of stem cuttings indicates the availability of nitrogen and carbohydrate for the plant's initial metabolic process (Meuriot et al. 2005) (In line 190-192)".
213	Which data? I do not find any leaf expansion rate data Please refere it clearly	The leaf expansion rate is represented by leaf area data. This statement refers to Figure 8. "After cuttings had developed with vegetative organs, leaf length influenced leaf growth, as shown by the leaf expansion rate (Figure 8) (In line 204).
218	Please elaborate, what length?	The term has been elaborated

220	After leaf fully open?	The phrase has been revised
221-222	How thermal dynamic is correalated to leaf expantion that staganat at different days or simply just the growth of leaf?	This statement is intended only for leaf growth in general.
225	We clearly understood about leaf expantion will show an increase therefore this graph is really minimum in information. Is there any important massage that validated by analysis? What is the point of the 7 lines there? Please explain. Suggestion for this data, first combine the 20,25 and 30 as on graphic by averaging each data while you can add the standard deviation to see the data range. I think within one line you can compare each cutting and leaf area expantions.	The 7 lines indicate the number of samples we observed in each treatment. We agree to combine each of 20, 25, and 30 treatments that were averaged and add the standard error.
244-245	Please add the information about SE (n=?) and what analysis you were used, also what the meaning of lowercase letter and ns.	The manuscript has been revised.
255	Please confirm	The data has been confirmed
258	Please refer what is shoot defination in agriculture	Shoot is the part of the plant that is above the surface soil. In this study, the shoot is defined as the developing bud consisting branches and leaves.
259	Data?	The statement has been clarified. "Plants originated from the length stem cuttings 30 cm that grow more bud structures as represented by total leaf area, fresh weight and dry weight of lamina, petiole, and shoots than shorter (Table 1)" (In line 228)
	Please add concluding sentence	The conclusion section has been added (In line 242-244)
	Check the guideliness of references, you missed some point there.	The manuscript has been revised.
		Thank you very much for the suggestions and comments for the improvement our manuscript.

Reviewer B

Line/Figure	Reviewer's Comment and Suggestion	Our Response (The revisions have been marked in yellow on the manuscript)
	Tittle: The margin text according to template	The manuscript has been revised.
	Introduction: 1. Maximum word counts 600 words, 2. Add justification for the advantages of using stem cuttings	1. The Introduction has been revised as the manuscript guidelines. 2. The advantage propagation using stem cutting has been added. "Furthermore, Muda et al. (2022) reported that propagating plants using stem cuttings improve plant growth and yields comparing apical cuttings (In line…)".
80	Stem (D) and planting material (E)	The manuscript has been revised (In line 75)
86	Consistency of writing the word fikuda/picuda in the article	The manuscript has been corrected
111	Consistency of writing the word chaya fikuda/ Chaya Fikuda in the article	The manuscript has been corrected
225	There is no explanation of each line/color in each image.	The Figure has been revised
253	Head of table in the first colomn	The table has been revised (Table 1)
		Thank you very much for the suggestions and comments for the improvement our manuscript.

Reviewer T

Line/Figure	Reviewer's Comment and Suggestion	Our Response (The revisions have been marked in yellow on the manuscript)
	Generally: 1. Please make the writing of "Redonda" more uniform. The authors should check once again how to write it. Redonda or redonda. Please check the manuscript thoroughly once again. The uniformity includes other cultivar names and even the plant's name. 2. The authors might also want to consider writing Latin names evenly. For example, In line 110, the author wrote *Jathropa curcas* L. (Linneaus included). Meanwhile, the rest of the Latin nomenclatures are just genus and species (e.g., Line 123, 124, even citrus is not written in Latin name). 3. No discussion subsection and conclusion/concluding paragraph? 4. Noticed that the authors were using reference citation manager. But please recheck the input metadata to make the manuscript more consistent.	1. The plant name has been uniformed 2. Latin names have been standardized 3. The conclusion section has been added (In line 242-244) 4. The manuscript has been revised.
	Abstract: 1. The readers could read the aim of the study in the abstract. However, what problem did the authors want to address in this paper? What is the correlation between Chaya originating from Mexico and grown in Asia, its cultivar, and its leaf length? Why length of stem cutting is important to be observed? Is it related to the	1. In this manuscript, we would like to address the morphological characteristics of Chaya Rodenda cv. grown in Asia, especially related to the leaves. We believe that the length of cuttings is very important to investigate mainly to improve the effectiveness of planting material used in Chaya Rodenda propagation. 2. We agreed. The aim has been revised. "Therefore, this study aims to assess the leaf morphology of Chaya Redonda cv.

<table>
<tr><td></td><td>cultivar type in any way? Please make it clearer.
2. After reading the whole manuscript, I suggest the authors rephrase Lines 12-14. “study aimed to determine the morphological characteristics of chaya redonda and evaluate the growth during propagation with different stem cutting lengths of 30 cm, 25 cm, and 20 cm”. The authors only performed leaf morphological analysis; no observation was completed on other organs.</td><td>and its growth on different stem cuttings length of 20 cm, 25 cm, and 30 cm, respectively (In line 29-30)”.</td></tr>
<tr><td>26</td><td>Introduction:

Somehow, the phrase “as well as different leaf morphology” is like being forced to fit it into one sentence, thus making the sentence itself unclear. I suggest rewriting it into two or three sentences to make it readable, whether there is a correlation between the cultivar and leaf morphology or other aspects that make it different. Although, in my opinion, the authors could remove this part, as they explained further in the following paragraph.</td><td>This part has been removed</td></tr>
<tr><td>72-73</td><td>Material and methods:
1. Please check how to write the Latin name properly (including the subspecies). I suggest the Authors check the website https://www.ncbi.nlm.nih.gov/taxonomy.

2. Were the experiments done in a controlled environment or an open area? Please specify the material and methods. External factors (weather, rain period, sun exposure) should always be considered.</td><td>1. The Latin name has been revised. “Chaya plant material used in this study was from the Redonda cultivar with the Latin name Cnidoscolus aconitifolius subsp. aconitifolius Breckon, which has been domesticated (In line 75)”.

2. The research was conduct in open area. The monthly rainfall and relative humidity have been added in manuscript (Figure 1).</td></tr>
<tr><td>120</td><td>Results and discussion:

“found” not “founded”</td><td>The phrase has been revised (In line 103)</td></tr>
<tr><td>117-125</td><td>Please refer to which figure represents the calculation used for the study.</td><td>The sentence has been referred by the figure (In line 124-125)</td></tr>
</table>

Figure 2	Please check the English vocabulary. Many mistakes. “width” not “widht”; “average” not “avarage”, “length” not “langth”.	The Figure has been revised.
Figure 2	please include the statistical analysis done for the regression and the name of the software used.	The regression type has been added (In Figure 3). The software has referred in materials and methods.
132-139	what is WB? The author did not specify in the previous paragraph or figure or material and methods. Why is it necessary to use the calculation in this study?	WB means average of top (W_1) and bottom (W_2) width. This calculation is important to demonstrate the consistency of the leaf shape across different sizes.
138-139	“This variation of leaf length/width ratio depends on the plant species and growing environment.” This is exactly why the authors should explain the environmental condition during the study in material and method.	The environmental condition has been referred in Figure 1.
179-194	In the introduction section, the authors wrote that their study aims to characterize and evaluate leaf morphological organs as genetic diversity for further development both taxonomically. Could the authors elaborate more on how their statistical results could help with the application of taxonomy identification? Based on the authors’ results, what are the differences between Chaya Redonda and other Chaya cultivars? Alternatively, whether the location of cultivation (e.g. Indonesia vs Mexico) affected the leaf length/width ratio.	The leaf morphology of Chaya Rodenda and other Chaya cultivars, such as Chaya Fikuda, is different. Chaya Rodenda seems to have 3 main lobes, while Chaya Fikuda has 5 main lobes.
210-212	“The size of the stem cuttings will indicate the amount of nitrogen and carbohydrate content needed for the plant’s initial metabolic process. The longer the size of the stem cutting, the higher the percentage of life.” This phrase needs citation. The authors’ chosen stem lengths (30-25-20 cm) were too narrow to make this conclusion.	This sentence has been added relevant references. “The size of stem cuttings indicates the availability of nitrogen and carbohydrate for the plant’s initial metabolic process (Meuriot et al. 2005) (In line 191-192)”
Figure 7	The authors used different colours for several lines, yet no legends that explain it. Please clarify the differences between those lines in the figure and the figure’s caption.	The Figure has been revised.

231	What is WAP? Please clarify the abbreviation.	WAP stands for weeks after planting. The phrase has been revised (In line 225).
239-240	This is a strong statement. Did the authors also measure carbohydrate concentration during the experiments? Suggest rephrasing the sentences and referring to other studies with similar results.	The sentence has been revised. “However, cutting length in this study differed significantly from leaf number and canopy area because cuttings length affected carbohydrate accumulation in planting material similarly as reported by Meuriot et al. (2005) (In line 191-192).
Figure 8	Please include the information on statistical results in the figure’s caption. What do a, b, ns mean?	The information about figure has been added (in Figure 9).
Figure 8D	Week 6, is the calculation correct? There was no significant difference in canopy area between the three study groups on week 6?	The Figure has been revised
246-247	“The length of cuttings was found to have a significantly affect on leaf parameters, compared to branches and shoots” do the authors mean “the length of cutting had a significant effect on parameters compared to branches and shoots”?	Yes. That's what we mean. The manuscript has been revised. “The length of cuttings had a significant effect on leaf compared to branches and shoots” (In line 224).
		Thank you very much for the suggestions and comments for the improvement our manuscript.

Reviw Round 2

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

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Abstract. Chaya (*Cnidoscolus aconitifolius*) 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: WAP (week after planting), DAP (days after planting), SPAD

Running title: Leaf Morphology Characterization of Chaya

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) 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 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. 2023). 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 Redond 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

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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 *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). 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 at Ogan Ilir area (104º46'44" E; 3º01'35" S) in South Sumatra, 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 *Cnidoscolus 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.

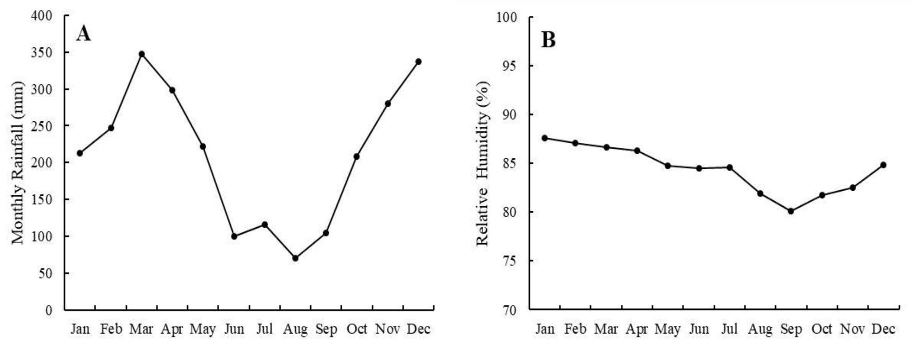

Figure 1. Monthly rainfall (A) and average relative humidity (B) at the research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia).

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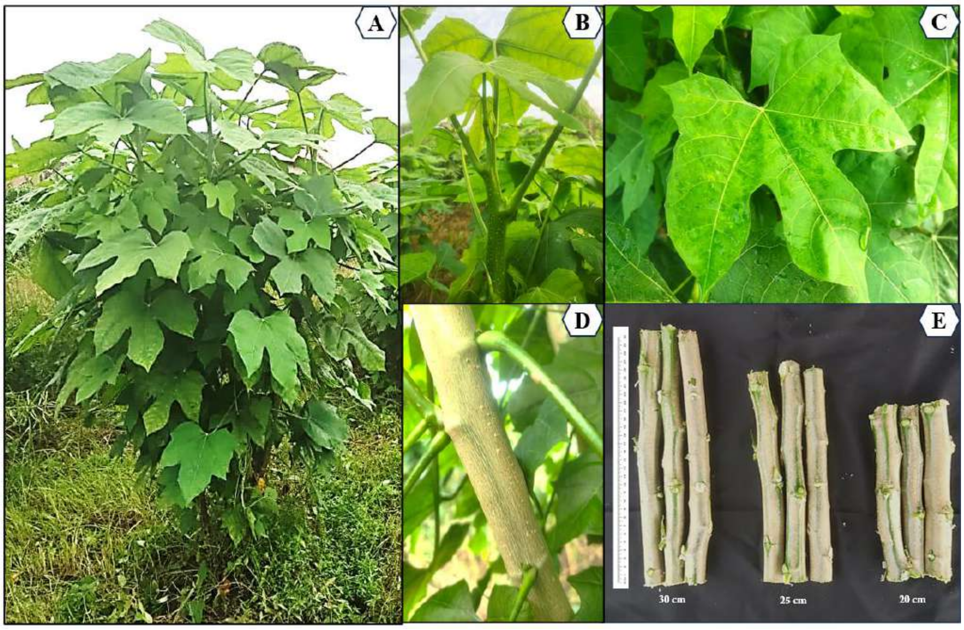

Figure 2. Chaya Redonda cv. (A), young shoot (B), leaf (C), stem (D), and planting material (E)

The planting medium comprised a 2:1 (v/v) mixture of soil and manure. The polybag used was 15 x 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.

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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* (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 leaf into four, namely midrib length, leaf length, top width, and bottom width (Figure 2A). 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. 2022). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies, such as Amorphophallus muelleri (Nurshanti et al. 2022) and *Citrus sinensis* (Muda et al. 2023). The wide applicability of these findings can inspire and intrigue botanists, researchers, and scientists in plant morphology and agriculture.

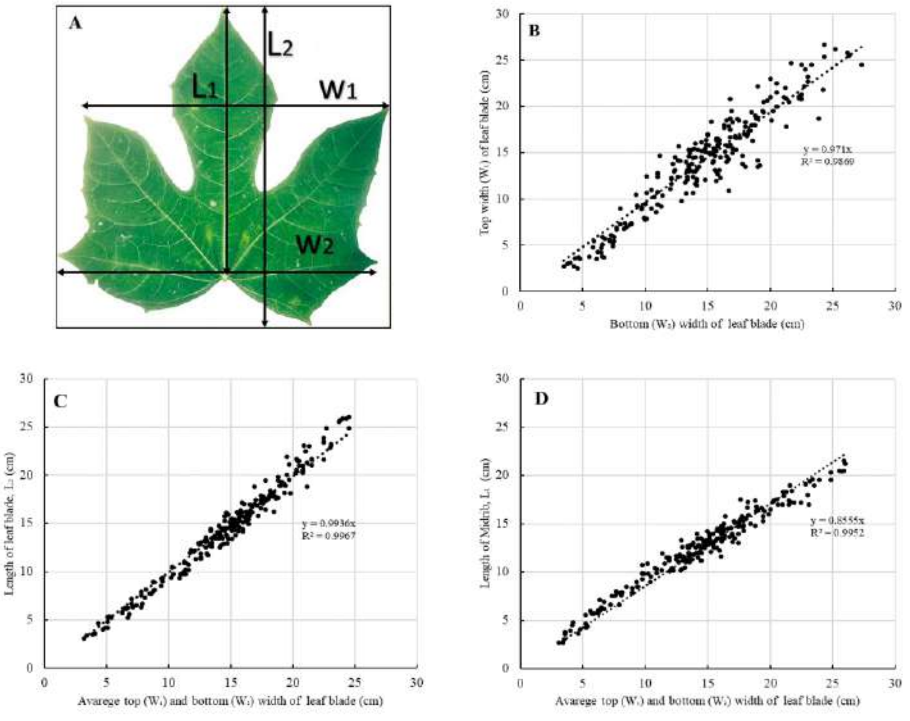

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Figure 3. Chaya leaf dimensions (A), linear regression between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D).

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 *Cnidoscolus 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).

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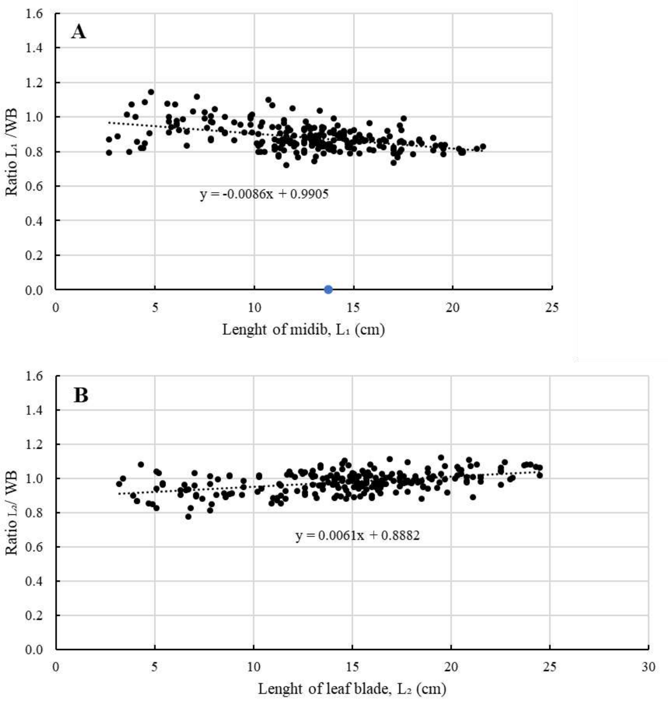

Figure 4. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A) and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda cv. WB means an average of top (W_1) and bottom (W_2) width.

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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. 2022).

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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 0.909, 0.889, and 0.915 obtained for midrib length, as well as upper and lower leaf width, respectively (Figure 5).

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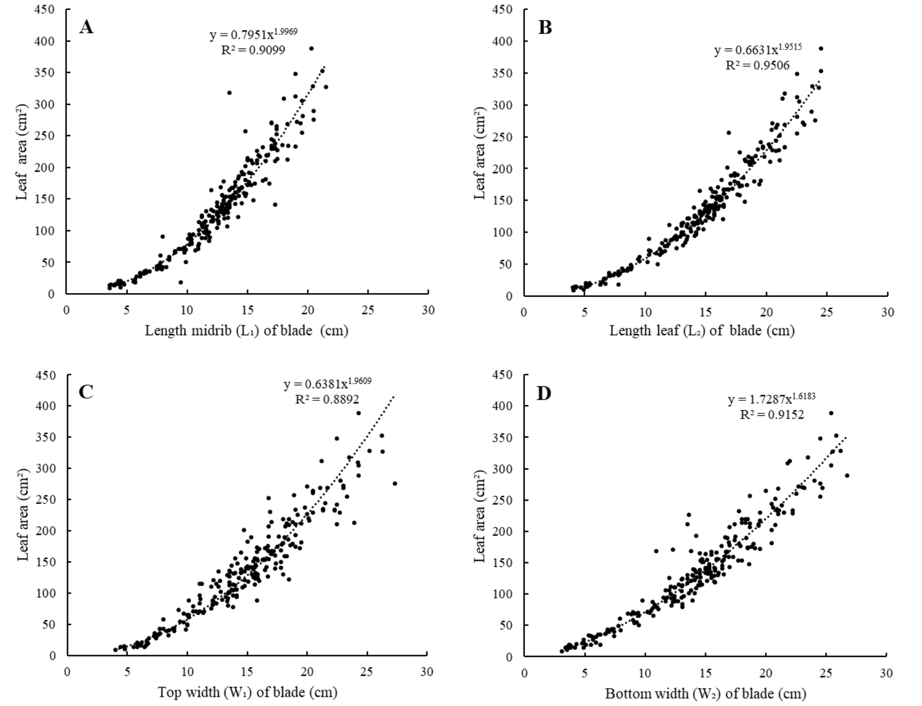

Figure 5. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831), respectively (Figure 6).

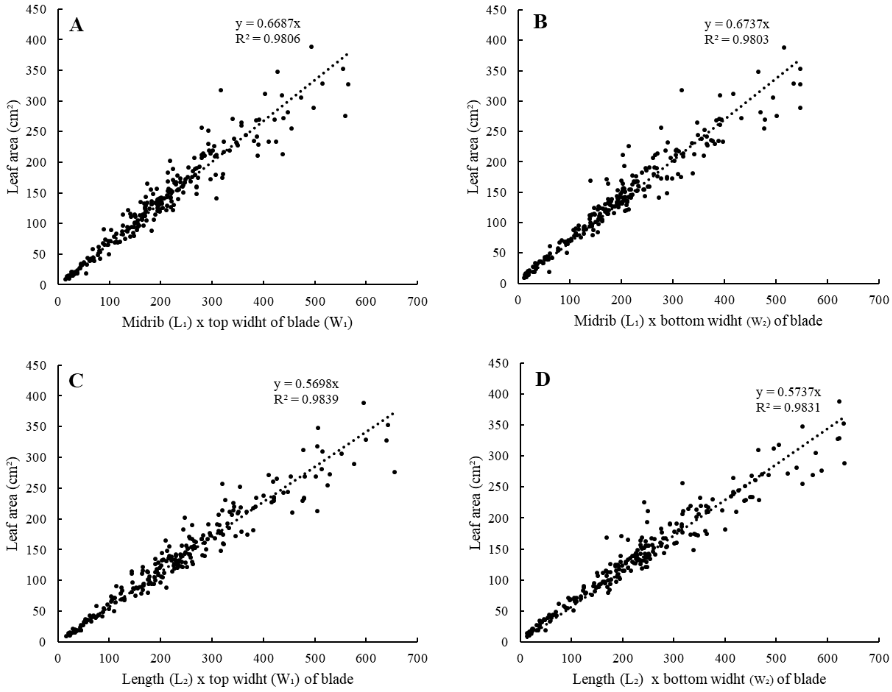

Figure 6. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D).

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. 2022) 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 x W_1 (Figure 5C) 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. 2022) and Fikuda cultivar (Gustiar et al. 2023).

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).

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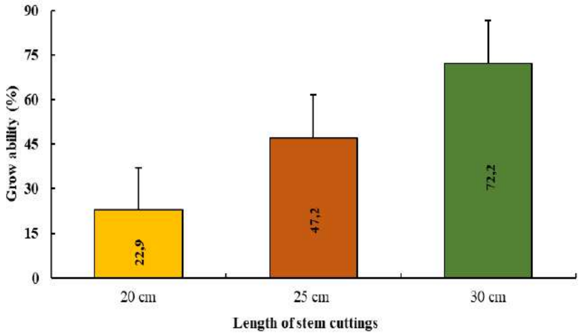

Figure 7. Percentage of growth ability at different stem-cutting lengths

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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 (Druege et al. 2019; Solikin 2018). 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.

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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.

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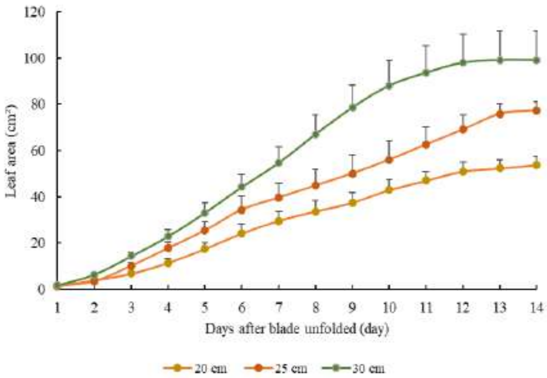

Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm, and 30 cm.

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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

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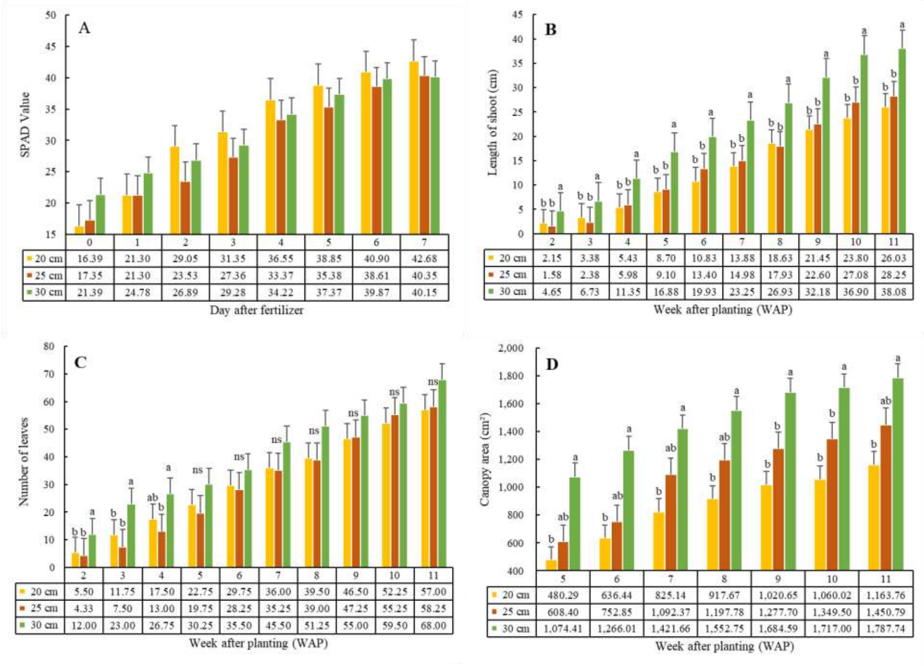
observation corresponded with the result obtained from the analysis of cassava plant cuttings (EJ de Oliveira et al. 2020). However, cutting length in this study differed significantly from leaf number and canopy area because cutting length affected carbohydrate accumulation in planting material, similarly 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.



A	0	1	2	3	4	5	6	7
20 cm	16.39	21.30	29.05	31.35	36.55	38.85	40.90	42.68
25 cm	17.35	21.30	23.53	27.36	33.37	35.38	38.61	40.35
30 cm	21.39	24.78	26.89	29.28	34.22	37.37	39.87	40.15

B	2	3	4	5	6	7	8	9	10	11
20 cm	2.15	3.38	5.43	8.70	10.83	13.88	18.63	21.45	23.80	26.03
25 cm	1.58	2.38	5.98	9.10	13.40	14.98	17.93	22.60	27.08	28.25
30 cm	4.65	6.73	11.35	16.88	19.93	23.25	26.93	32.18	36.90	38.08

C	2	3	4	5	6	7	8	9	10	11
20 cm	5.50	11.75	17.50	22.75	29.75	36.00	39.50	46.50	52.25	57.00
25 cm	4.33	7.50	13.00	19.75	28.25	35.25	39.00	47.25	55.25	58.25
30 cm	12.00	23.00	26.75	30.25	35.50	45.50	51.25	55.00	59.50	68.00

D	5	6	7	8	9	10	11
20 cm	480.29	636.44	825.14	917.67	1,020.65	1,060.02	1,163.76
25 cm	608.40	752.85	1,092.37	1,197.78	1,277.70	1,349.50	1,450.79
30 cm	1,074.41	1,266.01	1,421.66	1,552.75	1,684.59	1,717.00	1,787.74

Figure 9. SPAD value (A), shoot length (B), leaf number (C), and canopy area (D) 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 $P<0.05$

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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).

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

Treatment	Total leaf area (cm²)	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 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 (Martínez-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.

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CONCLUSION

The leaf of Chaya Redonda cv. is a palminerved single leaf characterized by leaf area that strongly correlates with length x 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.

ACKNOWLEDGEMENTS

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Reviw Round 2

Lines	Reviewer Comments/Suggest	Answer
43-46, 50-53, 113-123, 127-135, 233-237	Review the modifications made in this paragraph for accuracy and relevance to the topic.	
152	The manuscript must follow the US English standard; the digits number after the dots were all better stated as the same digits in this manuscript. For example, the coefficient value of 0.9099 (in 4 digits), if expressed in just three digits, should be 0.910 (not 0.909, Figure 5.A).	
243	Please cite the source (s).	
91	SPAD value was not yet being explained.	
222-226	Figure 9.A shows the bar graph with SPAD value and Day After Fertilizer (DAF) parameter, which has not been explained and is different from other measurement methods in WAP; hence, how to compare the DAF (daily) with the WAP (weekly).	
	Plagiarism is under 5% without references, and the shading sentences need more attention. This study was edited with minor corrections on grammar structures and non-influential wording that must be approved. Word deletion, insertion, and paraphrasing were incorporated into the manuscript, maintaining its thought and flow.	
	These statements below do not necessarily require a response that would enrich the analysis and are for counterargument purposes.	
	What would you say to a reader who claims that the study does not sufficiently address the potential effect of environmental factors on the growth of Chaya Redonda cultivar in different locations?	
	Have you considered addressing the possibility that the study's results may not be generalizable due to the limited sample size and replication of treatments?	
	How would you address those who argue that the study does not account for variations in soil type or other local environmental conditions that may impact the growth of Chaya Redonda cultivar?	
	What specific methods were used for assessing the leaf morphology of the Chaya Redonda cultivar?	
	What were the specific findings related to the growth and characteristics of Chaya Redonda cultivar based on different stem cutting lengths?	

	Are there any specific details on the clonal propagation of Chaya Redonda cultivar using stem cuttings that could provide further insights into the study?	
	To ensure that the observed growth in Chaya Redonda cv. due to different stem cutting lengths is not influenced by other environmental factors, you can experiment in a controlled environment such as a greenhouse. This would allow you to regulate factors like soil composition, weather conditions, and pest infestations, reducing their potential impact on the results.	
	To account for the possibility of genetic variations among the stem cuttings, you can use genetically identical plant material for your experiment. This can be achieved through clonal propagation or by using plant material from the same parent plant. By doing so, you can minimize the influence of genetic variability on the observed differences in growth and yield.	
	When addressing concerns about random variability or experimental error, it's important to use proper experimental design and statistical analysis. This includes conducting replicates of the experiment and using appropriate statistical tests to determine the significance of the observed differences. Additionally, conducting a control treatment with standard stem- cutting lengths can help in evaluating the impact of varying stem- cutting lengths. By addressing these concerns, you can ensure the reliability and validity of your experimental results, contributing to a more robust understanding of the relationship between stem cutting lengths and the growth of Chaya Redonda cv.	

Perbaikan
Round 2

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

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Abstract. Chaya (*Cnidoscolus aconitifolius*) 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: WAP (week after planting), WAP (week after planting), SPAD (Index indicating the greenness level of leaves.)

Running title: Leaf Morphology Characterization of Chaya

INTRODUCTION

Chaya (*Cnidoscolus aconitifolius*) 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 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. 2023). 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 *Cnidoscolus aconitifolius* picuda cultivar (Gustiar et al. 2023). 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 at Ogan Ilir area (104º46'44" E; 3º01'35" S) in South Sumatra, 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 *Cnidoscolus 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.

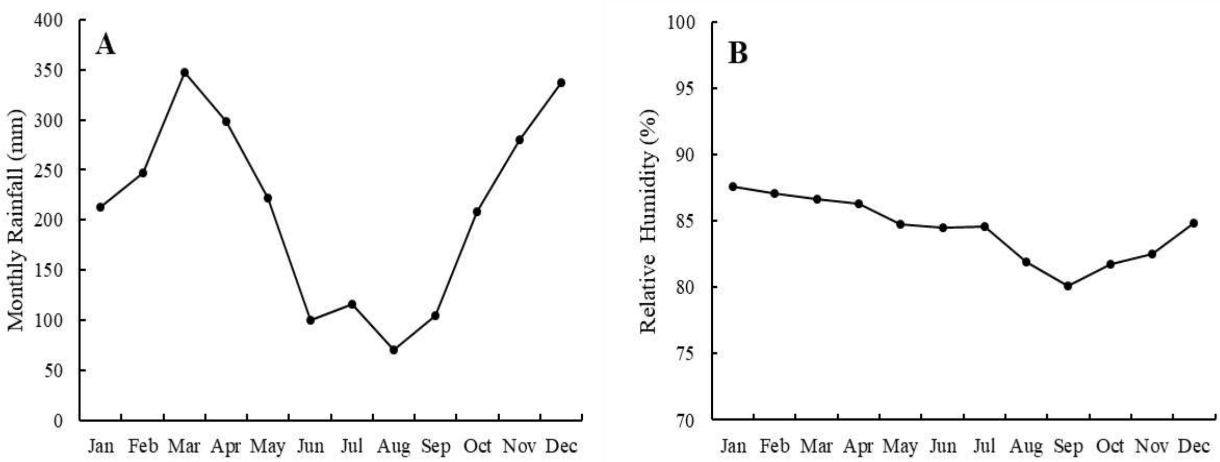

Figure 1. Monthly rainfall (A) and average relative humidity (B) at the research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia).

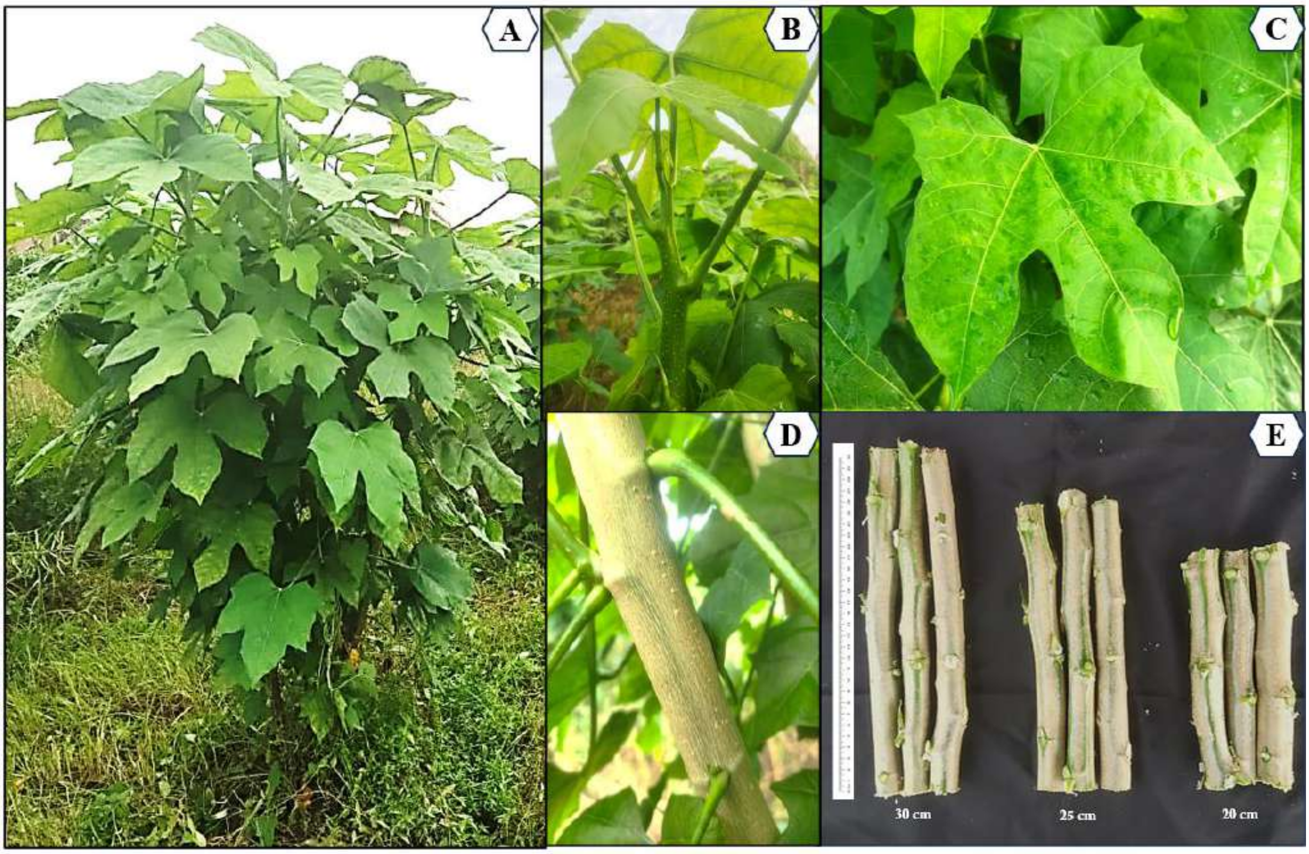

Figure 2. Chaya Redonda cv. (A), young shoot (B), leaf (C), stem (D), and planting material (E)

The planting medium comprised a 2:1 (v/v) mixture of soil and manure. The polybag used was 15 x 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.

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* (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 2A). 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. 2022). The regularities of certain leaf characters have also been utilized to estimate leaves with different morphologies, such as *Amorphophallus muelleri* (Nurshanti et al. 2022) and *Citrus sinensis* (Muda et al. 2023). The wide applicability of these findings can inspire and intrigue botanists, researchers, and scientists in plant morphology and agriculture.

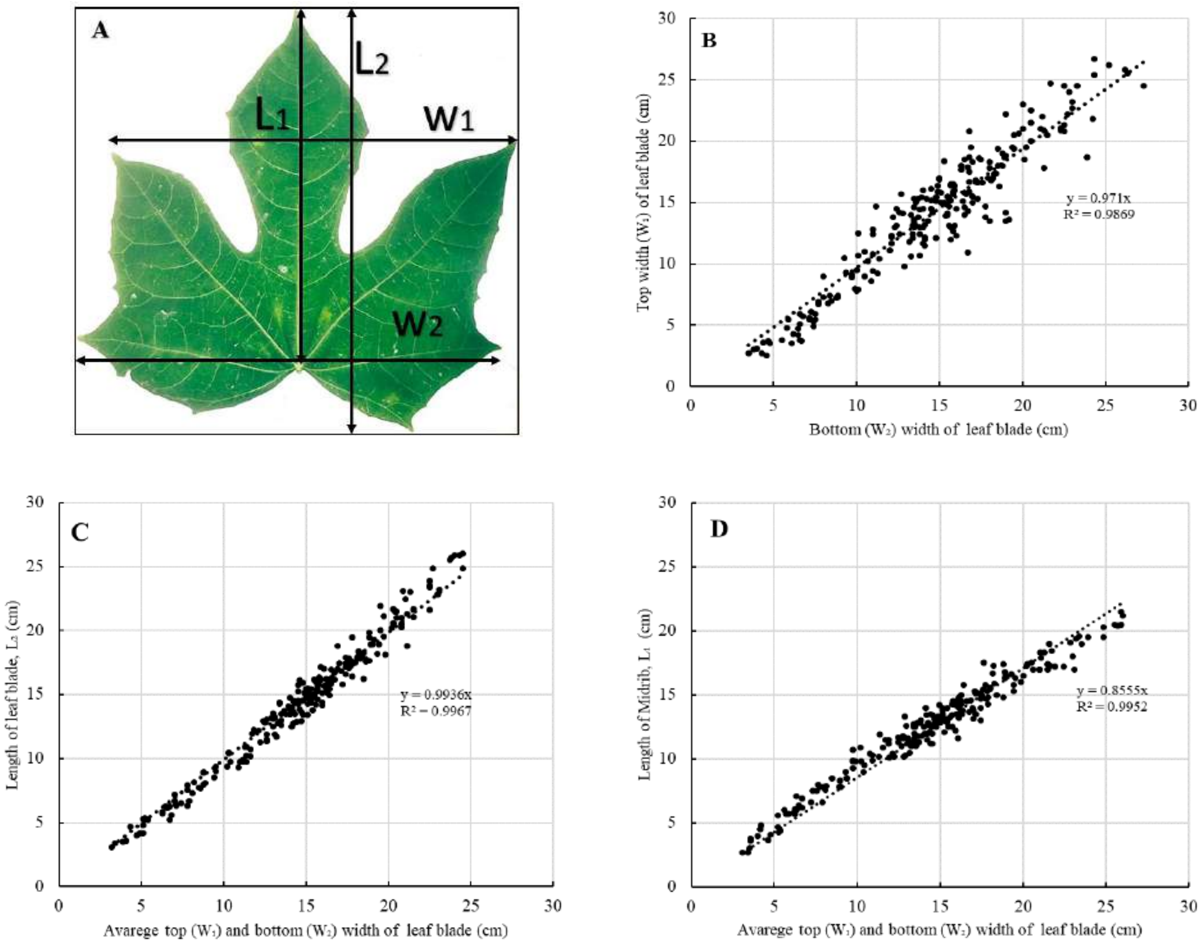

Figure 3. Chaya leaf dimensions (A), linear regression between size leaf traits, top (W_1)-bottom (W_2) width (B), length of leaf blade (L_2)-width (C), and length of midrib (L_1)-width (D).

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 *Cnidoscolus 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).

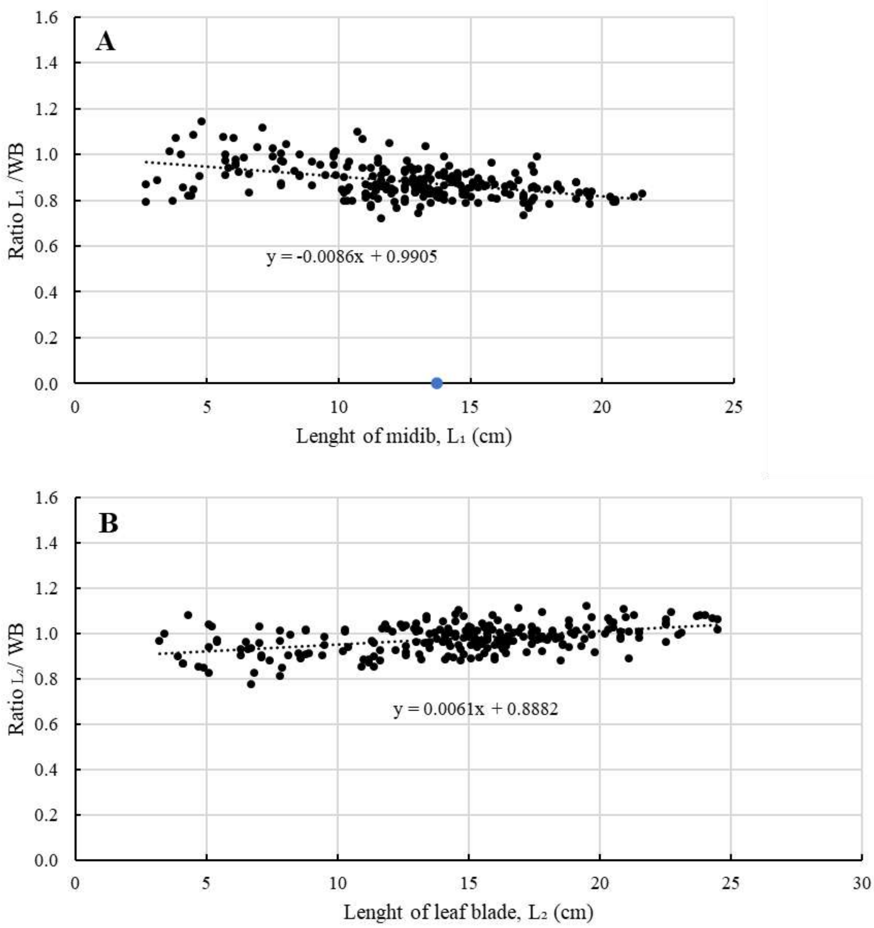

Figure 4. Comparison of midrib length and leaf blade width (L_1/WB) ratio (A) and leaf length-leaf width (L_2/WB) ratio (B) of Chaya Redonda cv. WB means an average of top (W_1) and bottom (W_2) width.

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. 2022).

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).

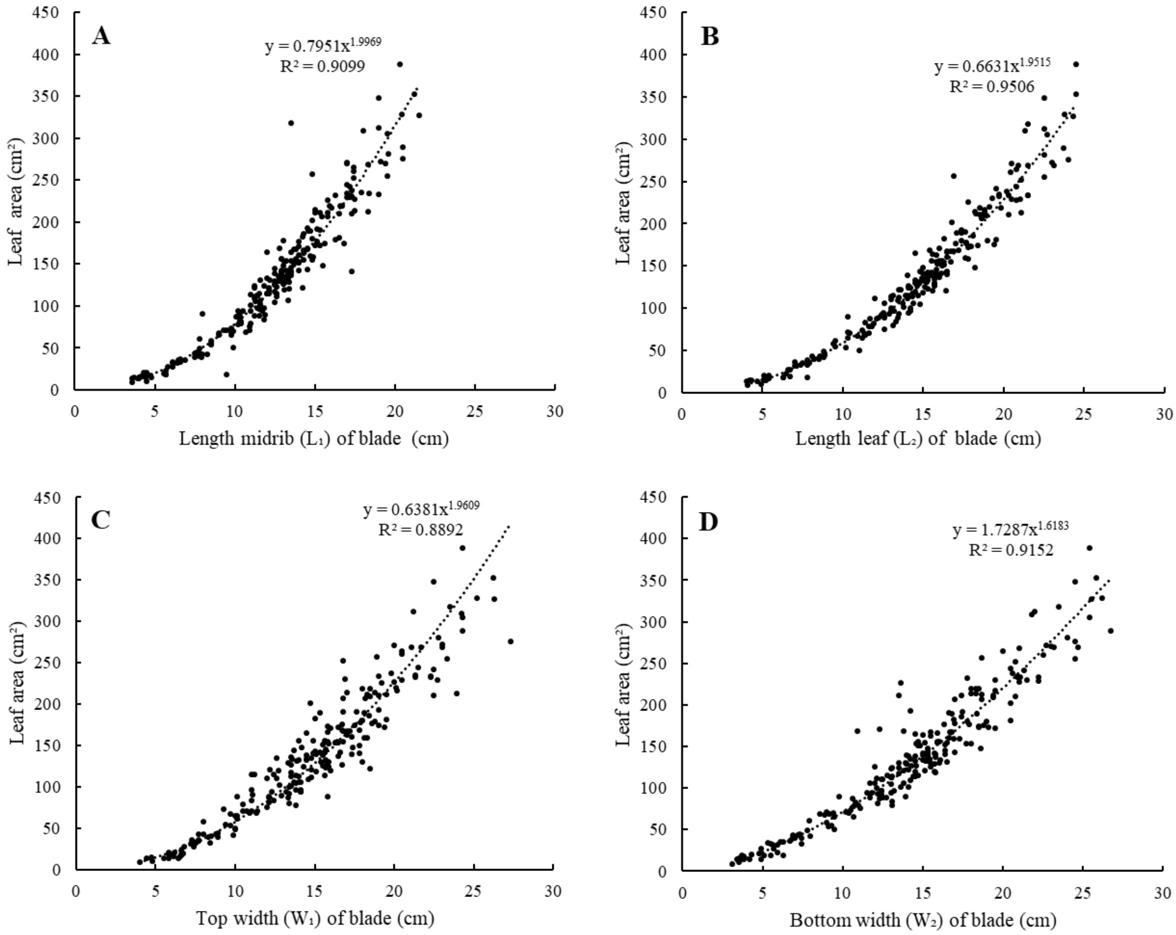

Figure 5. Estimation of leaf area using a single trait with regression power, midrib length (A), blade length (B), top width (C), and bottom width (D)

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) x top width of the blade (R^2= 0.9806), midrib (L_1) x bottom width (W_2) of the blade (R^2 0.9803), and length (L_2) x bottom width of the blade (R^2 = 0.9831), respectively (Figure 6).

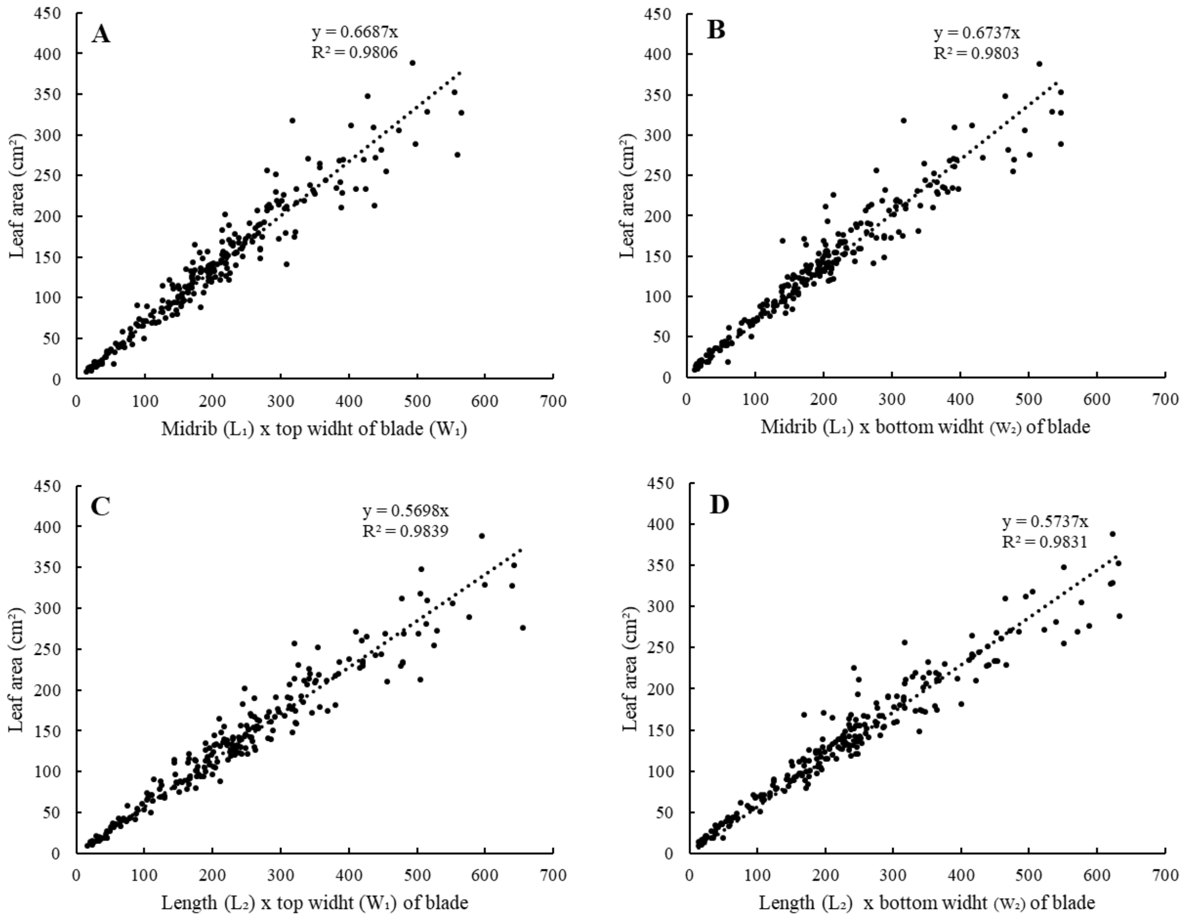

Figure 6. Estimation of leaf area using length (L) and width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using midrib length (A, B) and leaf length (C, D).

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. 2022) 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 x W_1 (Figure 5C) 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. 2022) and Fikuda cultivar (Gustiar et al. 2023).

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).

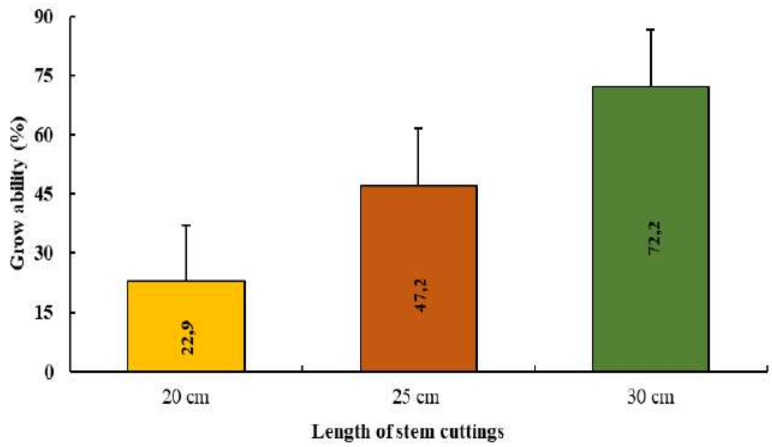

Figure 7. Percentage of growth ability at different stem cutting lengths

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 (Druege et al. 2019; Solikin 2018). 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.

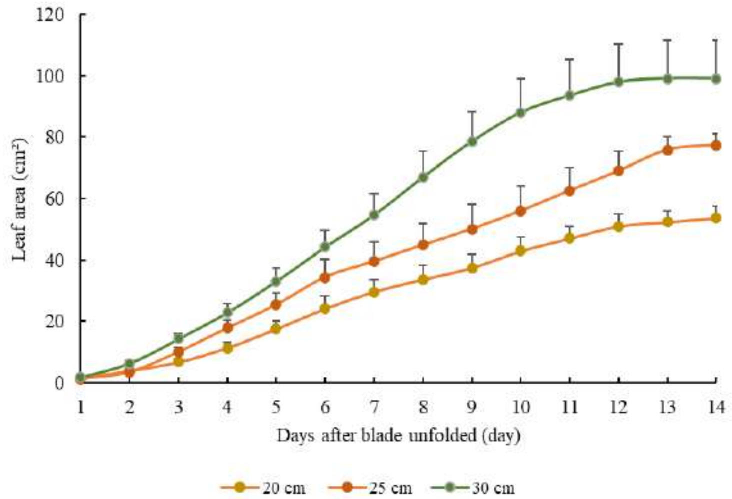

Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm, and 30 cm.

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 (EJ 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.

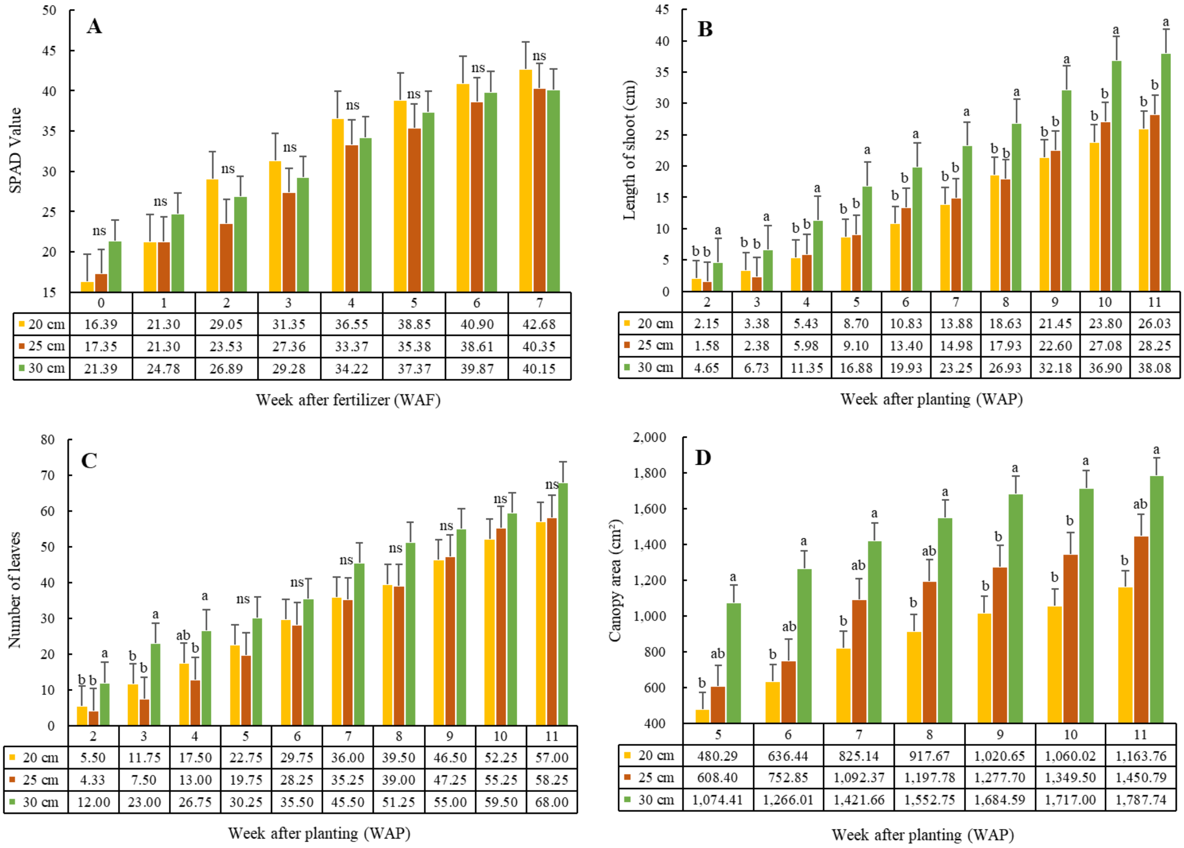

Figure 9. SPAD value (A), shoot length (B), leaf number (C), and canopy area (D) based on differences in stem cutting length. The data is presented as mean ± standard error (n=3). The lowercase letters mean a significant difference through the LSD test at P<0.05. The ns mean non-significant difference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

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).

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 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 (Martínez-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).

CONCLUSION

The leaf of Chaya Redonda cv. is a palminerved single leaf characterized by leaf area that strongly correlates with length x 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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Matrik Perbaikan Round 2

Table of Responses

Lines	Reviewer Comments/Suggest	Answer
43-46, 50-53, 113-123, 127-135, 233-237	Review the modifications made in this paragraph for accuracy and relevance to the topic.	We agreed. The manuscript has been revised.
152	The manuscript must follow the US English standard; the digits number after the dots were all better stated as the same digits in this manuscript. For example, the coefficient value of 0.9099 (in 4 digits), if expressed in just three digits, should be 0.910 (not 0.909, Figure 5.A).	We agreed to express the coefficient value in 4 digits. The manuscript has been revised.
243	Please cite the source (s).	The source has been cited.
91	SPAD value was not yet being explained.	The manuscript has been added.
222-226	Figure 9.A shows the bar graph with SPAD value and Day After Fertilizer (DAF) parameter, which has not been explained and is different from other measurement methods in WAP; hence, how to compare the DAF (daily) with the WAP (weekly).	The measurement of SPAD value was carried out to indicate the plant's response to fertilization. There was a mistake in writing the manuscript. The measurement of SPAD value was carried out on a weekly basis The manuscript has been added (in line 212-213).
	Plagiarism is under 5% without references, and the shading sentences need more attention. This study was edited with minor corrections on grammar structures and non-influential wording that must be approved. Word deletion, insertion, and paraphrasing were incorporated into the manuscript, maintaining its thought and flow.	We agreed. The manuscript has been revised.
	These statements below do not necessarily require a response that would enrich the analysis and are for counterargument purposes.	Thank you very much. We will be preparing these statements below to enrich and respond to each feedback.
	What would you say to a reader who claims that the study does not sufficiently address the potential effect of environmental factors on the growth of Chaya Redonda cultivar in different locations?	We restricted this study to the tropical lowland ecosystem.
	Have you considered addressing the possibility that the study's results may not be generalizable due to the limited sample size and replication of treatments?	The study was conducted during the early vegetative growth stage, hence morphological traits were assumed non-significant difference.
	How would you address those who argue that the study does not account for variations in soil type or other local environmental conditions that may impact the growth of	In the future, it is necessary to conduct research by considering local soil and environmental conditions.

	Chaya Redonda cultivar?	
	What specific methods were used for assessing the leaf morphology of the Chaya Redonda cultivar?	Simple regression model can be used in representing morphology accurately as we have reported in this manuscript.
	What were the specific findings related to the growth and characteristics of Chaya Redonda cultivar based on different stem cutting lengths?	The increasing leaf area in stem cutting of 30 cm in length was better than those of 20 cm and 25 cm. Redonda Chaya cv. performed better through stem cuttings of 30 cm than 20 cm and 25 cm lengths as seen through canopy area, number of leaves, leaf fresh weight and leaf dry weight, and shoots fresh weight and shoots dry weight.
	Are there any specific details on the clonal propagation of Chaya Redonda cultivar using stem cuttings that could provide further insights into the study?	This research provided an understanding of growth analysis using an easy and relevant analysis. This research can be the basis for further research on Redonda Chaya cv.
	To ensure that the observed growth in Chaya Redonda cv. due to different stem cutting lengths is not influenced by other environmental factors, you can experiment in a controlled environment such as a greenhouse. This would allow you to regulate factors like soil composition, weather conditions, and pest infestations, reducing their potential impact on the results.	
	To account for the possibility of genetic variations among the stem cuttings, you can use genetically identical plant material for your experiment. This can be achieved through clonal propagation or by using plant material from the same parent plant. By doing so, you can minimize the influence of genetic variability on the observed differences in growth and yield.	
	When addressing concerns about random variability or experimental error, it's important to use proper experimental design and statistical analysis. This includes conducting replicates of the experiment and using appropriate statistical tests to determine the significance of the observed differences. Additionally, conducting a control treatment with standard stem- cutting lengths can help in evaluating the impact of varying stem- cutting lengths. By addressing these concerns, you can ensure the reliability and validity of your experimental results, contributing to a more robust understanding of the relationship between stem cutting lengths and the growth of Chaya Redonda cv.	
		Thank you very much for the suggestions and comments for the improvement our manuscript.

Perbaikan Proofread

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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: xxxx.* 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 2023a, 2023b). 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 *Cnidoscolus 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 *Cnidoscolus 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.

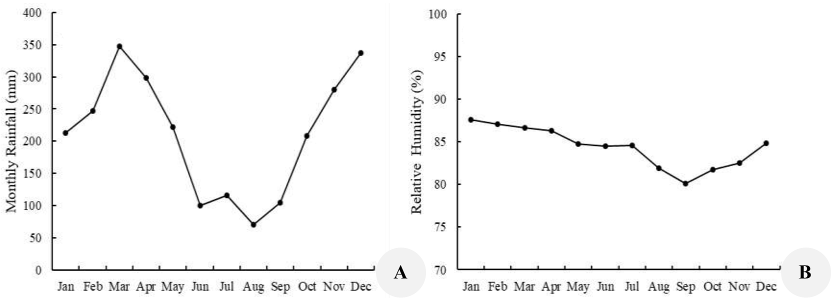

Figure 1. A. Monthly rainfall; and B. Average relative humidity at the research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia)

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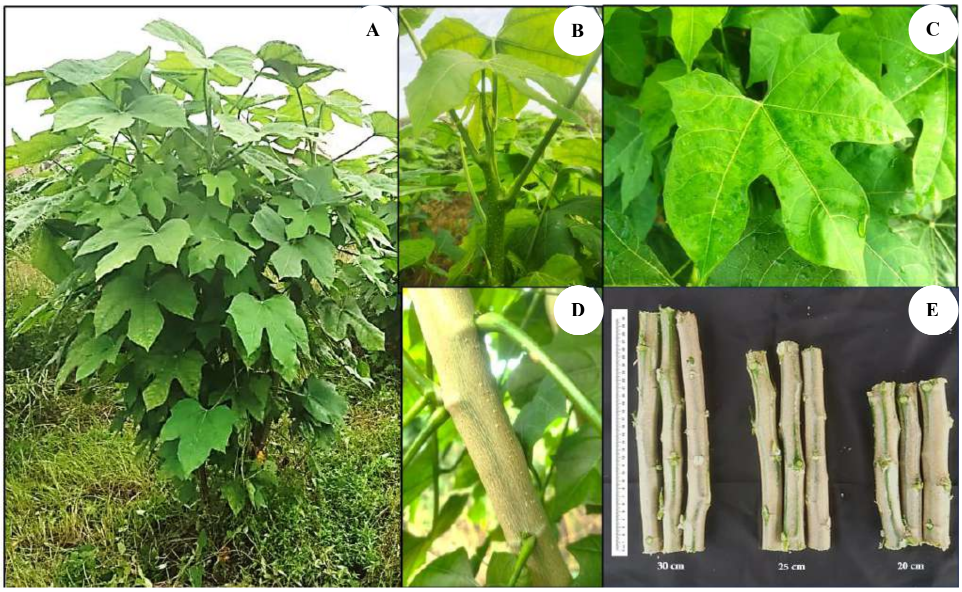

Figure 2. A. Chaya Redonda cv.; B. Young shoot; C. Leaf; D. Stem; and E. Planting material

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. 2022). 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 *Cnidoscolus 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).

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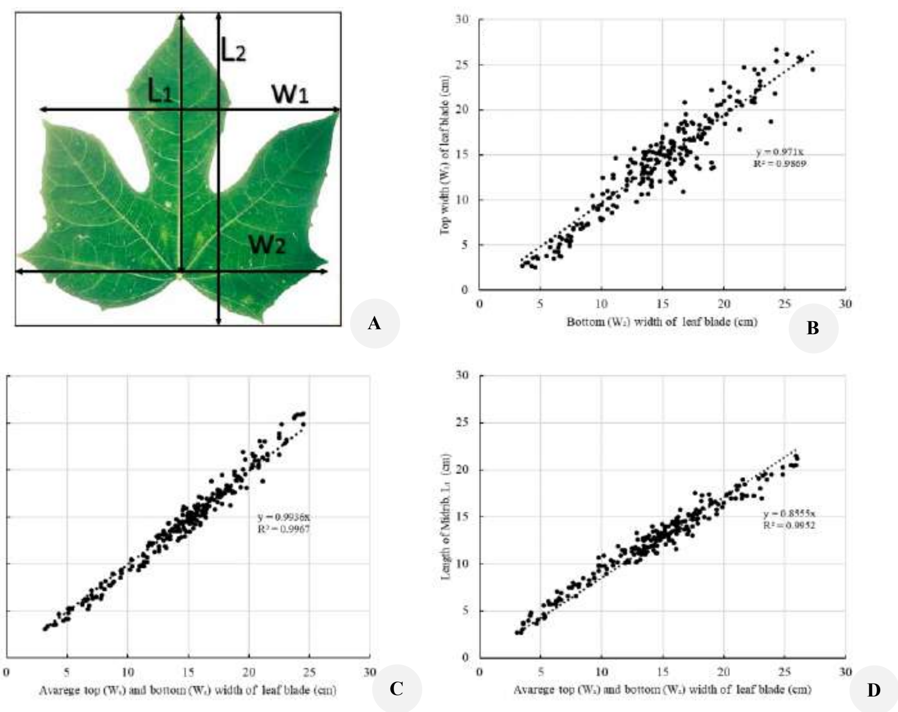

Figure 3. A. Chaya leaf dimensions; B. Linear regression between size leaf traits, top (W_1)-bottom (W_2) width; C. Length of leaf blade (L_2)-width; and D. Length of midrib (L_1)-width

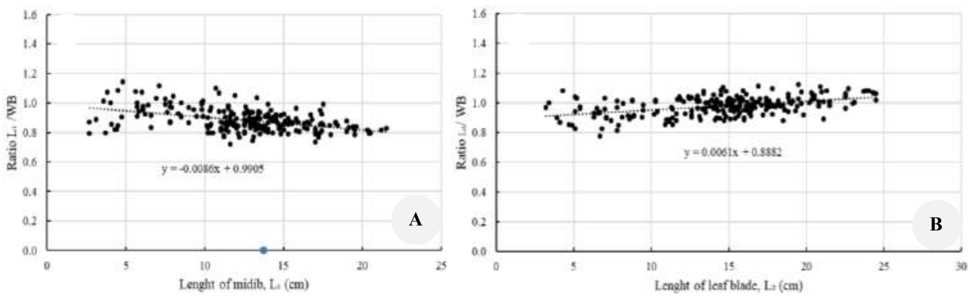

Figure 4. A. Comparison of midrib length and leaf blade width (L_1/WB) ratio; and B. Leaf length-leaf width (L_2/WB) ratio of Chaya Redonda cv. WB means an average of top (W_1) and bottom (W_2) width

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. 2022).

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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. 2022) 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. 2022) and Fikuda cultivar (Gustiar et al. 2023c).

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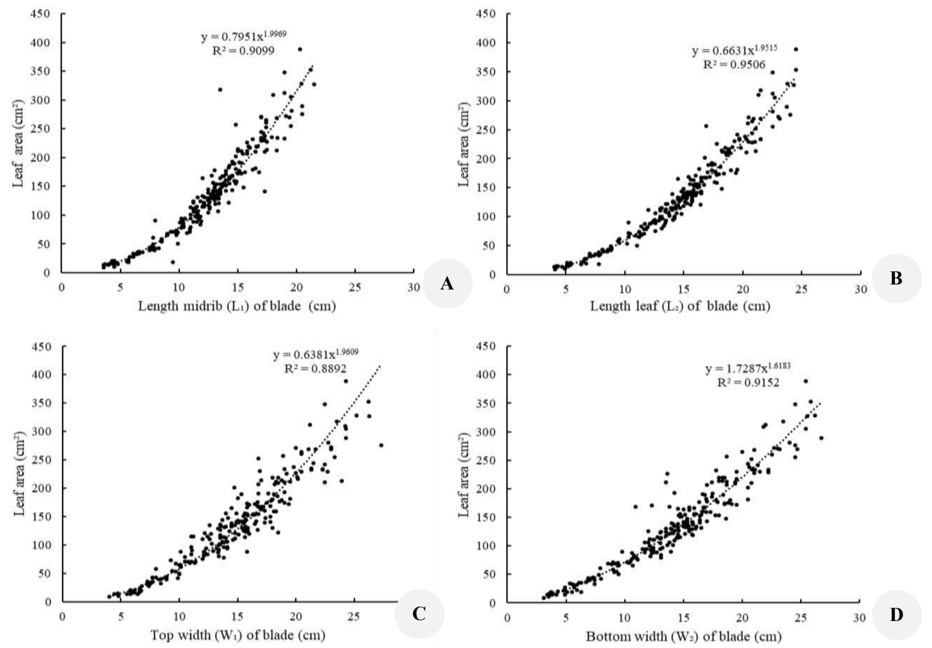

Figure 5. A. Estimation of leaf area using a single trait with regression power, midrib length; B. Blade length; C. Top width, and D. Bottom width

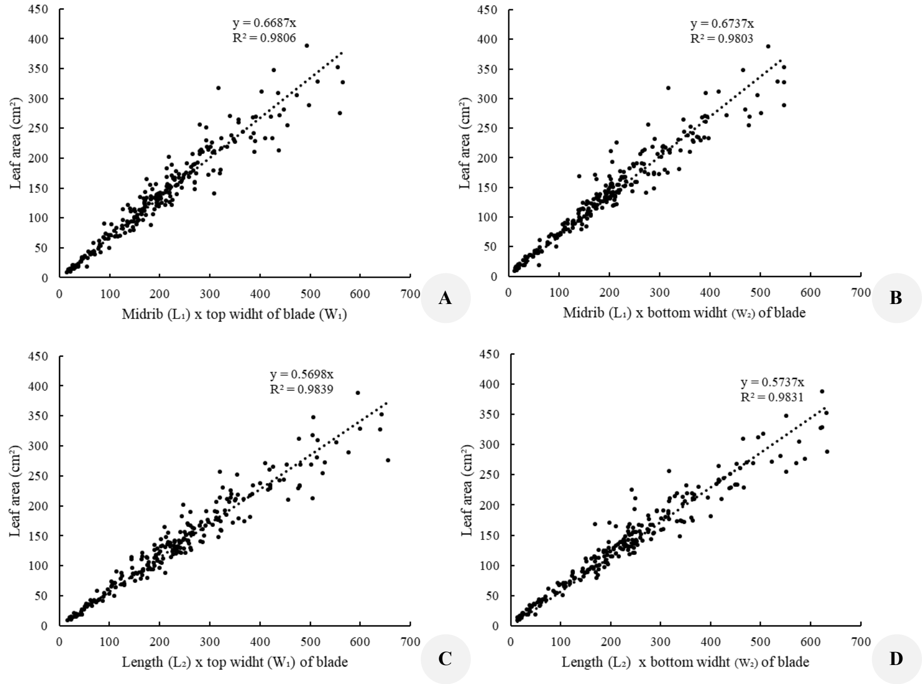

Figure 6. Estimation of leaf area using Length (L) and Width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using A, B. Midrib length; and C, D. Leaf length

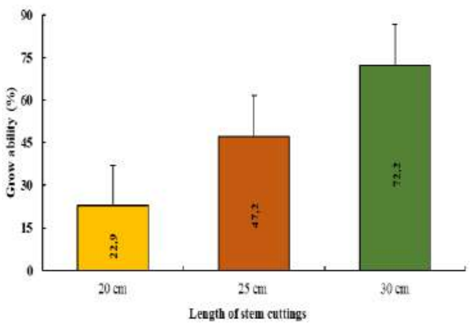

Figure 7. Percentage of growth ability at different stem-cutting lengths

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.

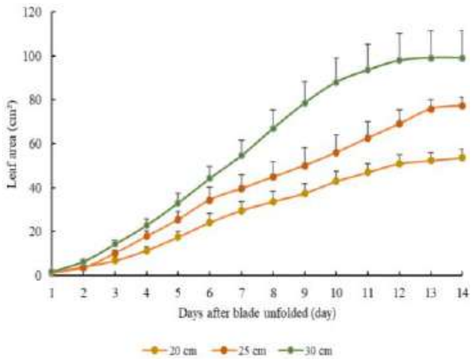

Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm, and 30 cm

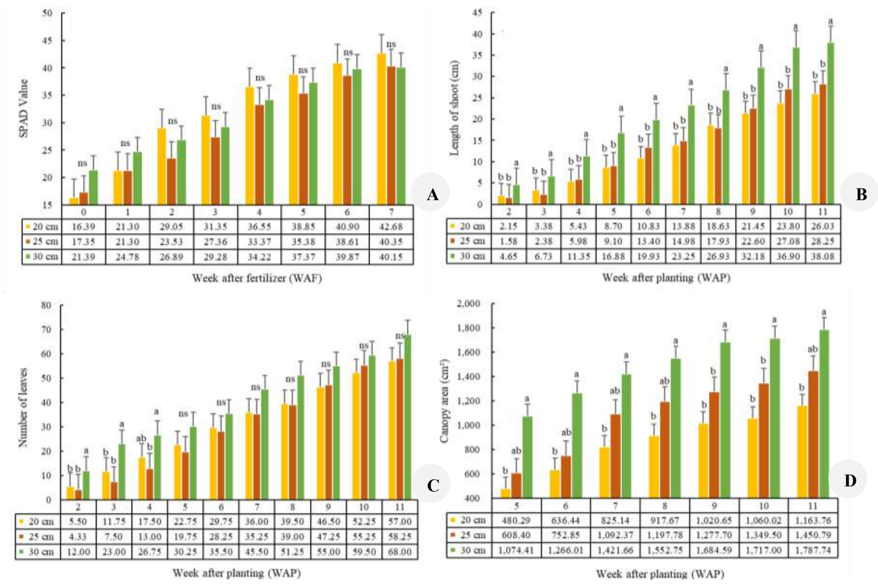

Figure 9. A. SPAD value; B. Shoot length; C. Leaf number; and D. Canopy area based on differences in stem cutting length. The data is presented as mean ± standard error (n = 3). The lowercase letters mean a significant difference through the LSD test at P<0.05. The ns mean non-significant difference 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 (Martínez-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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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: xxxx.* 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 2023a, 2023b). 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 *Cnidoscolus 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 *Cnidoscolus 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.

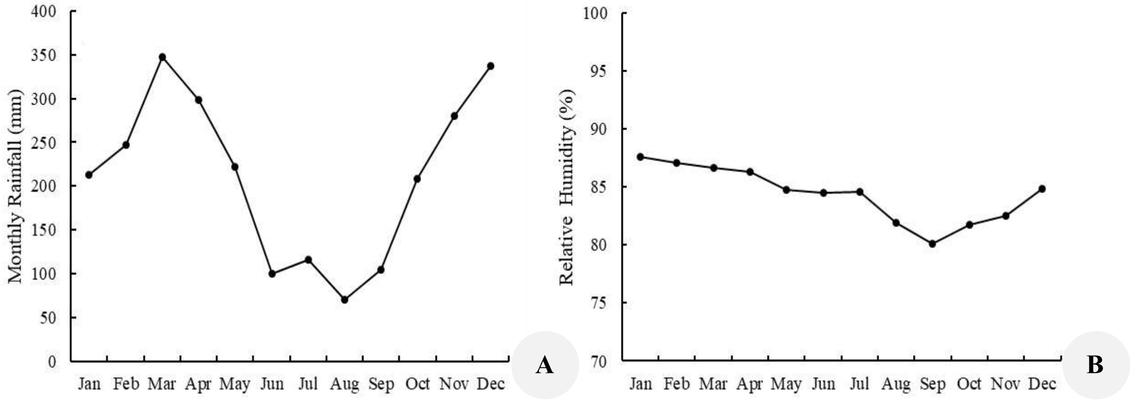

Figure 1. A. Monthly rainfall; and B. Average relative humidity at the research location during 2019-2023 (Source: Meteorological, Climatological, and Geophysical Agency of Indonesia)

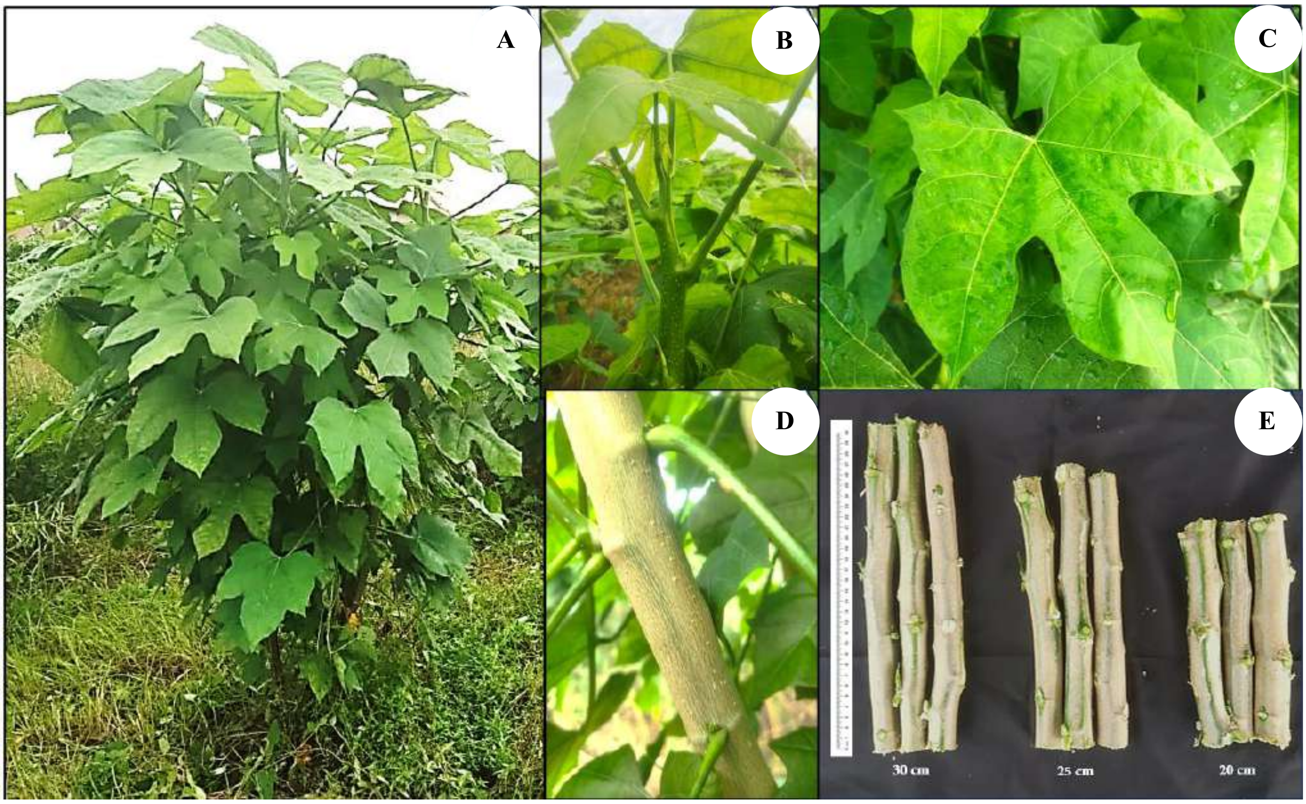

Figure 2. A. Chaya Redonda cv.; B. Young shoot; C. Leaf; D. Stem; and E. Planting material

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 *Cnidoscolus 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).

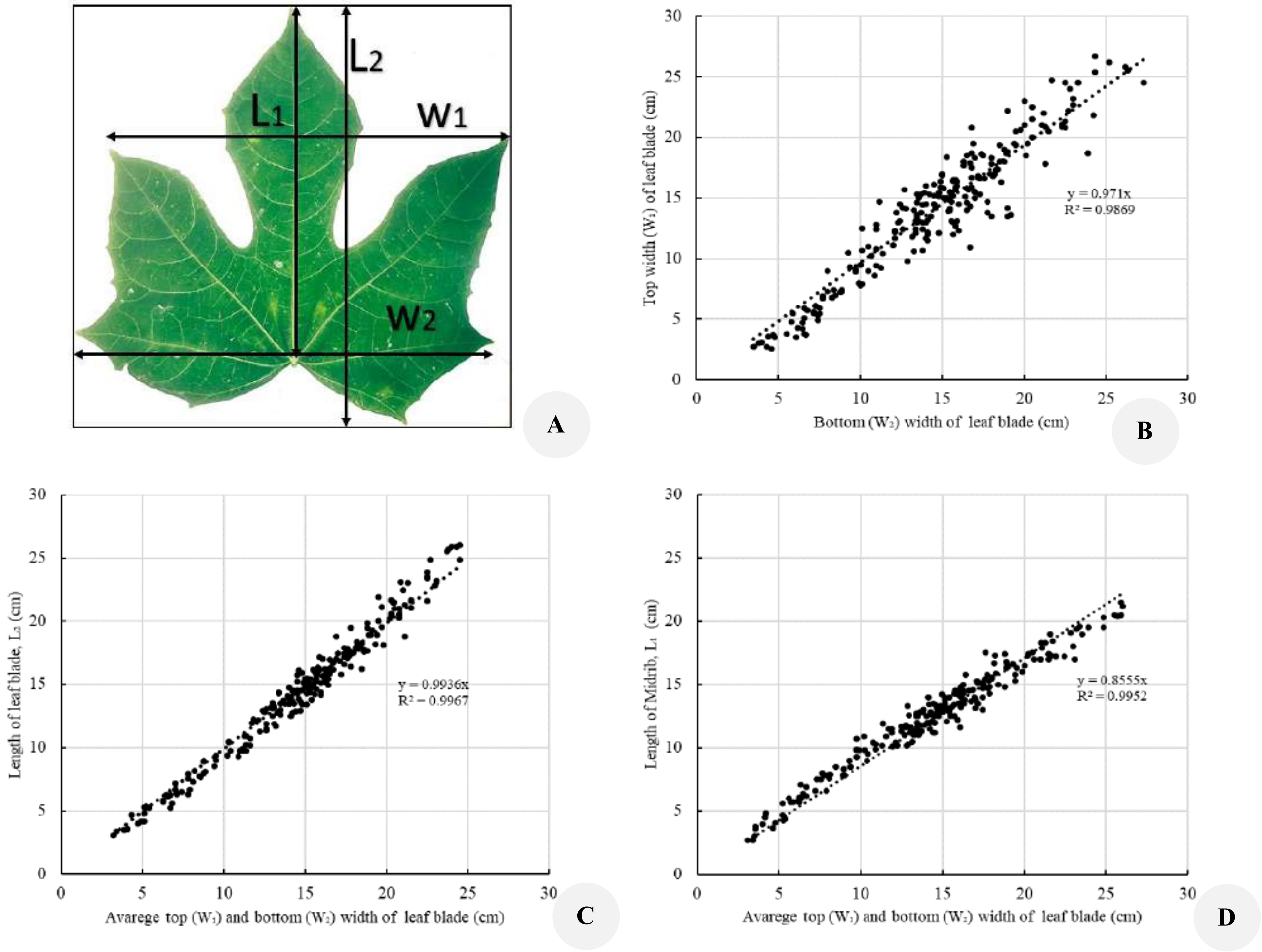

Figure 3. A. Chaya leaf dimensions; B. Linear regression between size leaf traits, top (W_1)-bottom (W_2) width; C. Length of leaf blade (L_2)-width; and D. Length of midrib (L_1)-width

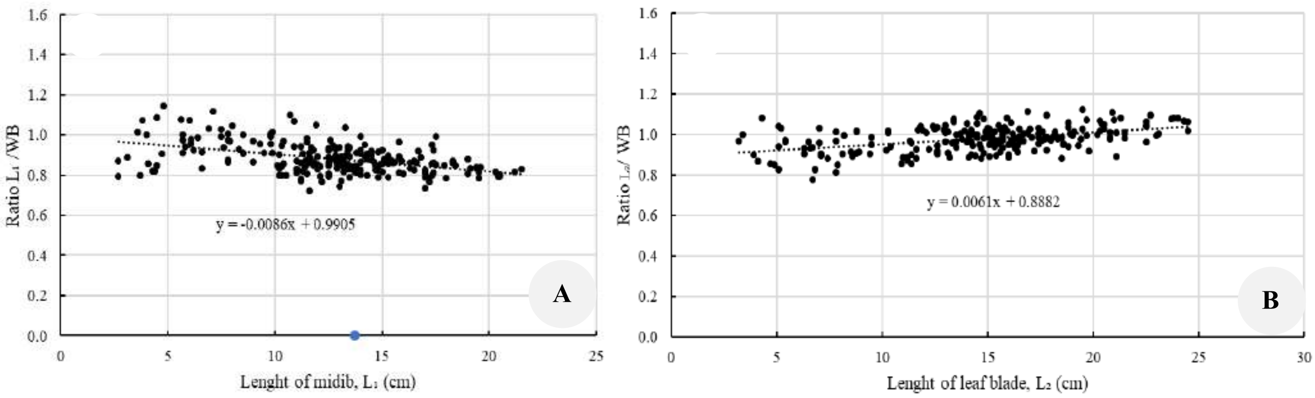

Figure 4. A. Comparison of midrib length and leaf blade width (L_1/WB) ratio; and B. Leaf length-leaf width (L_2/WB) ratio of Chaya Redonda cv. WB means an average of top (W_1) and bottom (W_2) width

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).

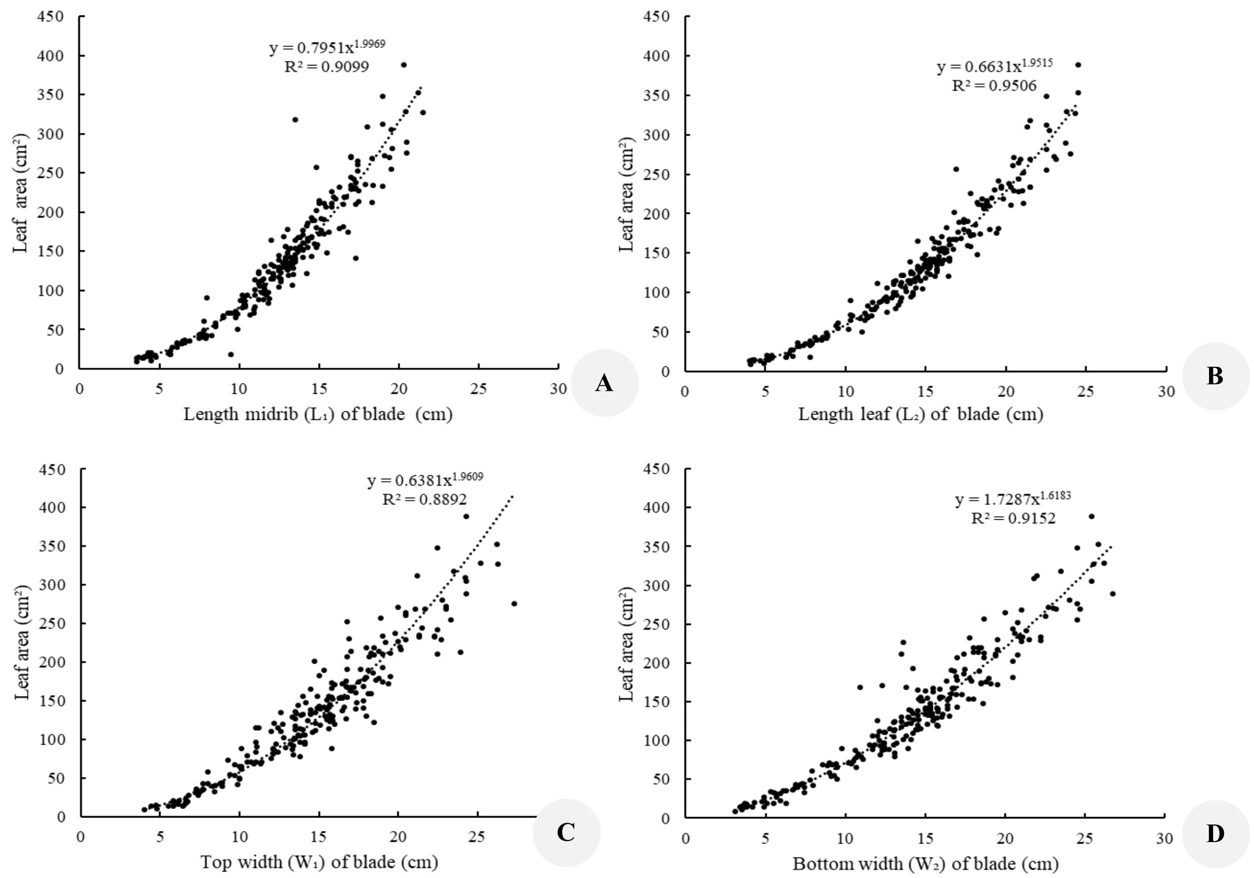

Figure 5. A. Estimation of leaf area using a single trait with regression power, midrib length; B. Blade length; C. Top width, and D. Bottom width

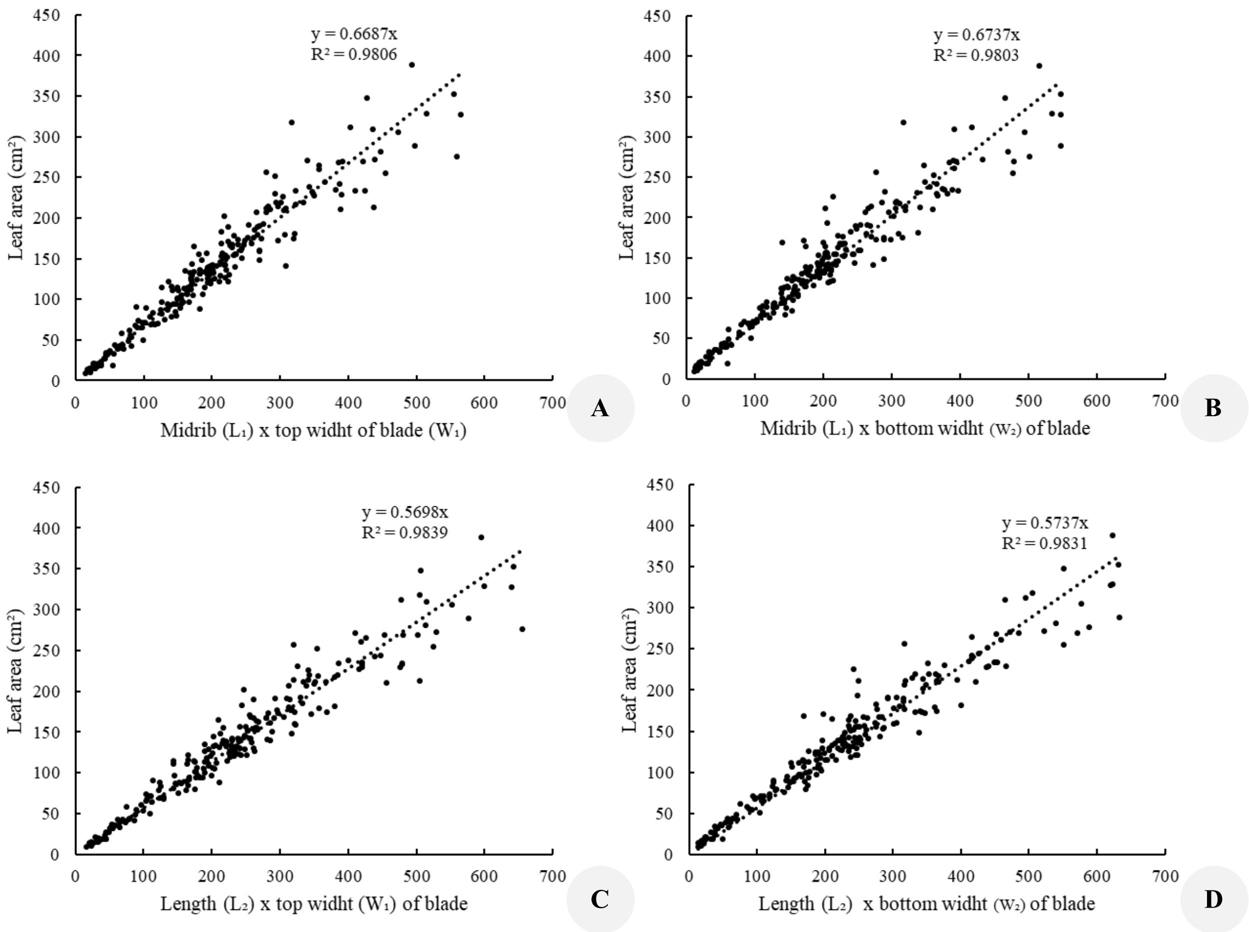

Figure 6. Estimation of leaf area using Length (L) and Width (W) of the lobes with the linear zero-intercept quadratic model. Estimation using A, B. Midrib length; and C, D. Leaf length

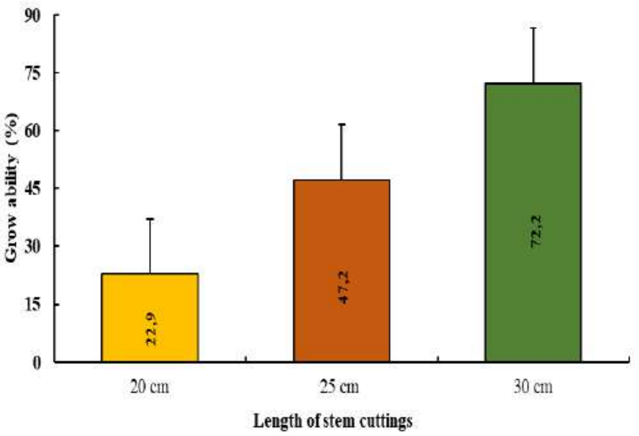

Figure 7. Percentage of growth ability at different stem-cutting lengths

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.

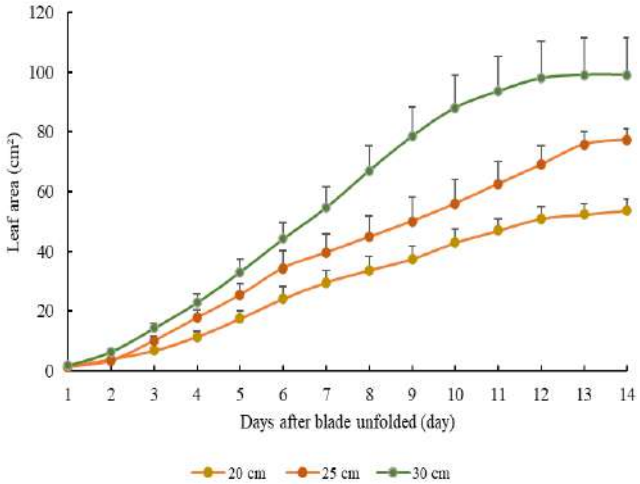

Figure 8. Increasing of Chaya Redonda cv. leaf area at the onset of plant growth with stem cutting lengths of 20 cm, 25 cm, and 30 cm

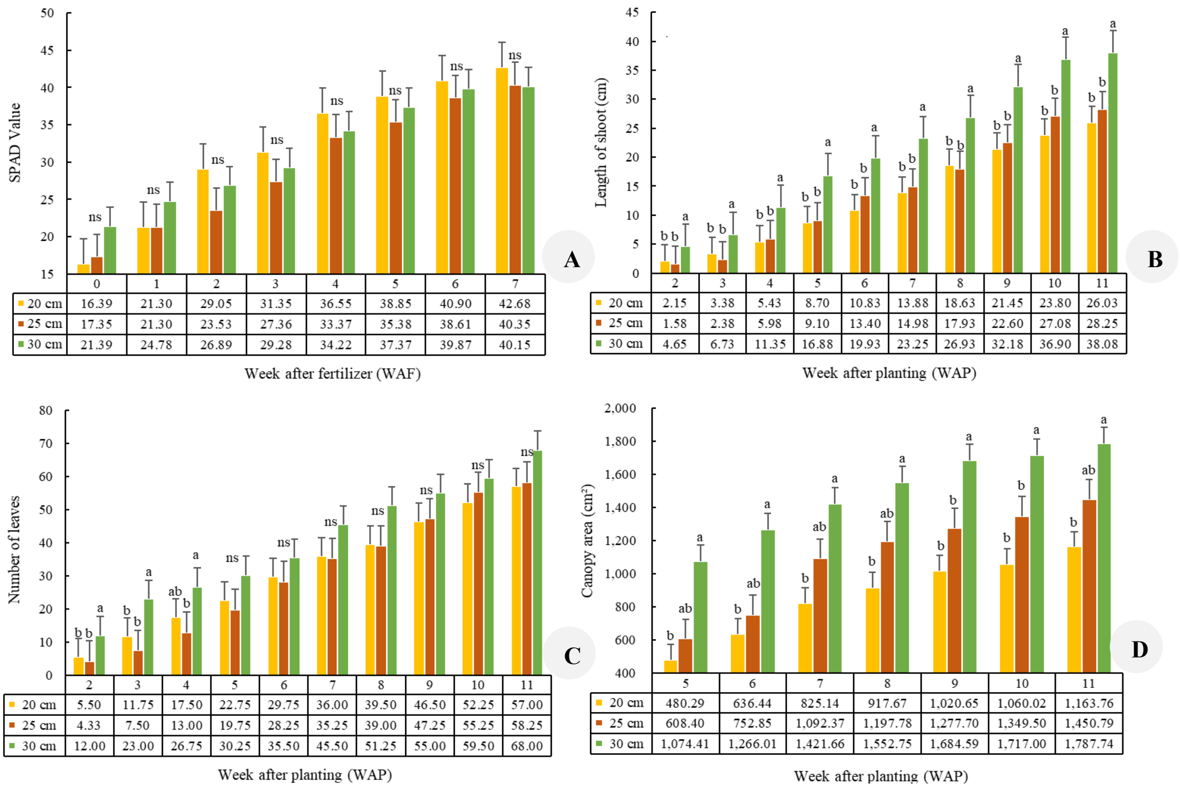

Figure 9. A. SPAD value; B. Shoot length; C. Leaf number; and D. Canopy area based on differences in stem cutting length. The data is presented as mean ± standard error (n = 3). The lowercase letters mean a significant difference through the LSD test at P<0.05. The ns mean non-significant difference 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 (cm²)	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 (Martínez-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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