Growth and Morphological Responses of Chaya (*Cnidoscolus aconitifolius*) under Different Drought Stress Durations

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Abstract. Chaya (*Cnidoscolus aconitifolius*) is a perennial leafy vegetable known for its high nutritional value and tolerance to marginal environmental conditions, including drought stress. However, prolonged water deficit may significantly affect its growth and productivity. This study aimed to evaluate the morphological and growth responses of chaya under different durations of drought stress. The experiment was conducted under greenhouse conditions using a randomized block design with five drought treatments: control (well-watered), and drought periods of 4, 8, 12, and 16 days. Growth parameters, morphological traits, and biomass accumulation were measured and analyzed using ANOVA followed by LSD test at 5% significance level. The results showed that increasing drought duration significantly affected soil moisture, leaf temperature, and plant growth parameters. Mild drought stress (4 days) still supported optimal growth, while prolonged drought (12–16 days) led to a significant reduction in leaf area, biomass, and overall plant performance. Conversely, root length increased under severe drought conditions, indicating an adaptive response to water deficit. Despite its tolerance, chaya exhibited optimal growth under adequate water availability or mild drought conditions.In conclusion, chaya demonstrates adaptive morphological strategies under drought stress; however, prolonged water deficit negatively impacts its growth and biomass production. These findings highlight the importance of proper water management to optimize chaya cultivation under changing climatic conditions.

1 Introduction

Chaya (*Cnidoscolus aconitifolius* (Mill.) I.M. Johnst.) is a perennial leafy vegetable with considerable potential to be developed as a nutritious food source. The plant is characterized by semi-woody stems and palmately lobed leaves resembling those of papaya; consequently, it is commonly known in Indonesia as "Japanese papaya." Although chaya has not yet been widely cultivated, it is promising as a leafy vegetable because it can be harvested repeatedly and contains valuable nutrients. Chaya leaves have been reported to contain protein, fat, carbohydrates, calcium, phosphorus, iron, vitamins, and various bioactive compounds, such as flavonoids, tannins, saponins, and alkaloids, which may support their utilization as a functional food source [1], [2]. Therefore, the development of chaya cultivation requires adequate agronomic information, particularly regarding the ability of the plant to adapt to unfavorable environmental conditions.

One of the major constraints in vegetable crop cultivation is limited water availability. Water plays an essential role in maintaining cell turgor, supporting cell division and expansion, transporting nutrients, and sustaining photosynthesis and biomass formation. When water availability in the growing medium decreases, plant growth may be impaired due to the reduced ability of roots to absorb water and nutrients. Water deficit can also induce stomatal closure as a mechanism to reduce water loss through transpiration. However, this response simultaneously restricts carbon dioxide entry into the leaves, thereby reducing photosynthetic activity and plant growth [3], [4].

Drought stress can cause substantial changes in plant morphology and growth. Reduced water availability is generally associated with decreases in plant height, leaf number, leaf size, canopy area, and biomass accumulation. In leafy vegetables such as chaya, these effects are particularly important because the leaves represent the primary economically valuable harvested organ. A reduction in leaf area and leaf biomass may therefore directly decrease crop yield. On the other hand, plants may exhibit adaptive mechanisms in response to water deficit, such as increased root growth to expand the soil volume explored for water uptake or the maintenance of certain traits that support survival during periods of drought. Accordingly, the evaluation of growth and morphological characteristics can be used to assess the tolerance of chaya to drought stress.

Chaya is known for its ability to grow under relatively marginal environmental conditions and is considered potentially tolerant to limited water availability. Nevertheless, the ability of a plant to survive does not necessarily indicate that it can maintain optimal growth and leaf production. Previous studies on chaya have primarily examined plant responses to irrigation amounts or volumes and their interactions

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with other cultivation inputs [5]. Information regarding the growth and morphological responses of chaya to different durations of drought stress remains limited. This aspect is important because the longer a plant experiences water deficit, the greater the likelihood of reductions in vegetative growth, morphological changes, and biomass loss.

Evaluating chaya responses to different drought durations is therefore necessary to determine the limits of its adaptive capacity under water-deficit conditions. Such information is important for supporting more appropriate water management in chaya cultivation, particularly in areas with limited water availability or irregular rainfall distribution. Therefore, this study aimed to evaluate the growth and morphological responses of chaya (*Cnidoscolus aconitifolius*) under different durations of drought stress. The results of this study are expected to provide information on the drought tolerance of chaya and to support the development of suitable cultivation strategies under water-limited conditions.

2 Materials and methods

2.1 Experimental Site and Period

The experiment was conducted from June to September 2025 in the greenhouse of the Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Ogan Ilir, South Sumatra, Indonesia. The experimental site is located at approximately 3°13′ S and 104°39′ E. The greenhouse conditions were used to minimize the influence of rainfall and to allow controlled application of drought stress treatments.

2.2 Experimental Design and Drought Stress Application

Chaya (*Cnidoscolus aconitifolius* (Mill.) I.M. Johnst.) plants were grown individually in 35 cm × 40 cm polybags containing a growing medium consisting of topsoil classified as an Inceptisol and goat manure mixed at a ratio of 3:1 (v/v). The components were thoroughly homogenized before filling the polybags. Prior to drought stress application, the plants were maintained under well-watered conditions during the establishment period.

The experiment was arranged in a randomized block design (RBD) with five drought stress treatments and three replications. Each experimental unit consisted of four plants, resulting in a total of 60 plants. The treatments consisted of a well-watered control (C1), water withholding for 4 days (C2), 8 days (C3), 12 days (C4), and 16 days (C5). The control plants were supplied with adequate water throughout the treatment period, whereas irrigation was withheld from drought-stressed plants according to the designated treatment duration. These treatments were applied to evaluate changes in the growth and morphological responses of chaya under progressively prolonged water-deficit conditions.

2.3 Plant Measurements

Plant responses to different drought stress durations were evaluated through microenvironmental, physiological, growth, morphological, and biomass measurements. Soil moisture, soil temperature, leaf temperature, and leaf greenness were measured every two days during the 16-day treatment period. Soil moisture was measured using a soil moisture meter (Lutron PMS-714), while soil temperature was determined using a digital soil thermometer. Leaf temperature was measured using a thermal imaging camera (FLIR E4 Thermal Imager) by capturing thermal images of fully expanded leaves under ambient conditions. SPAD value (SPAD-502 Plus) as a nondestructive indicator of relative chlorophyll content. Soil moisture was recorded to indicate water availability in the growing medium, whereas soil temperature and leaf temperature were measured to characterize changes in the growing environment and plant thermal response under drought conditions

Vegetative growth measurements were also conducted every two days from the beginning of drought treatment until 16 days after treatment application. The measured variables included plant height, number of leaves, number of shoots, shoot diameter, leaf length, and leaf width. Plant height was measured from the base of the plant to the highest growing point. The number of leaves and shoots was counted manually, while shoot diameter was measured on the main shoot. Leaf length and leaf width were measured on representative fully expanded leaves.

At the end of the treatment period, morphological measurements were conducted, including canopy area, total leaf area, edible leaf area, root length, and root diameter. Canopy area was measured to describe overall aboveground plant development, while total leaf area and edible leaf area were determined to evaluate leaf development and the harvestable portion of the plant. After harvest, the roots were carefully removed from the growing medium and cleaned. Root length was measured from the stem base to the longest root tip, and root diameter was recorded to assess root morphological responses to drought stress.

Biomass measurements were performed at final harvest. Plants were separated into edible leaves, non-edible leaves, edible petioles, non-edible petioles, edible stems, main stems, and roots. Fresh weight was recorded immediately after separation of each plant component. Subsequently, all plant parts were dried to a constant weight, and their dry weights were recorded to determine biomass accumulation and biomass partitioning under different drought stress durations.

2.4 Statistical Analysis

Data obtained from the observations were subjected to analysis of variance (ANOVA) based on the randomized block design. When the treatment effect was significant, mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% significance level. Statistical analyses were conducted using RStudio software.

3 Results and discussion

3.1 Soil Moisture, Soil Temperature, and Leaf Thermal Response

Drought duration markedly affected soil moisture and leaf thermal response in chaya plants (Figure 1). Soil temperature showed a relatively similar fluctuation pattern among treatments, decreasing until approximately day 8–10, increasing toward day 14, and declining again on day 16 (Figure 1A). Although the differences among treatments were not highly distinct, plants subjected to longer water withholding tended to show slightly higher soil temperatures. This condition may be associated with the lower water content of the growing medium, as dry soil has a reduced capacity to buffer temperature changes compared with moist soil.

A clearer treatment effect was observed in soil moisture (Figure 1B). The well-watered control (C1) maintained relatively higher soil moisture throughout the observation period, whereas drought treatments showed a progressive decline in soil water availability according to the duration of water withholding. The most rapid decline was observed in C5, followed by C4, indicating that prolonged water withholding successfully created severe water-deficit conditions. This decline in soil moisture represents the primary driver of plant stress under drought, as limited water availability directly reduces root water uptake and disrupts plant water balance.

Leaf temperature increased as soil moisture decreased (Figure 1C). Plants exposed to longer drought durations, particularly C4 and C5, tended to have higher leaf temperatures than the control, especially around days 12–14. This response indicates reduced transpirational cooling under water-deficit conditions. Under drought stress, stomatal closure is one of the earliest responses to reduce water loss; however, it also restricts transpiration and increases leaf surface temperature. Recent studies have confirmed that drought-induced stomatal closure reduces evaporative cooling and can increase canopy or leaf temperature, making thermal traits useful indicators of plant water stress [6].

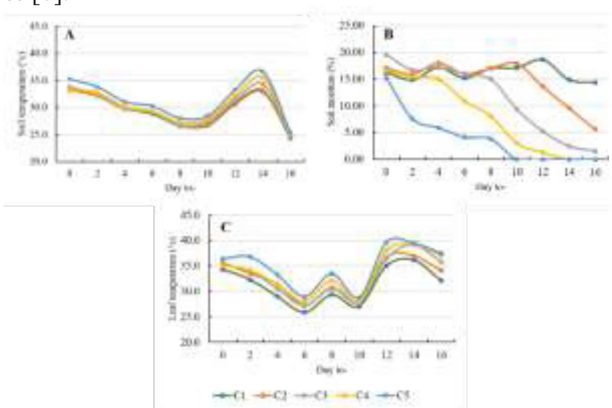

Fig. 1. Changes in soil temperature (A), soil moisture (B), and leaf temperature (C) of chaya under different drought stress durations.

Thermal images further supported this pattern (Figure 2). Well-watered plants showed lower leaf surface temperatures, approximately 32.3 °C, whereas drought-stressed plants showed higher temperatures, reaching approximately 38.4–39.0 °C under longer drought durations. The stronger yellow to white color intensity in drought-stressed leaves indicates hotter leaf surfaces, reflecting reduced transpiration and impaired leaf water status. Thermal imaging has been widely recognized as a rapid, nondestructive method for detecting plant stress because plant surface temperature closely reflects the balance between water uptake, stomatal conductance, and transpiration [7], [8].

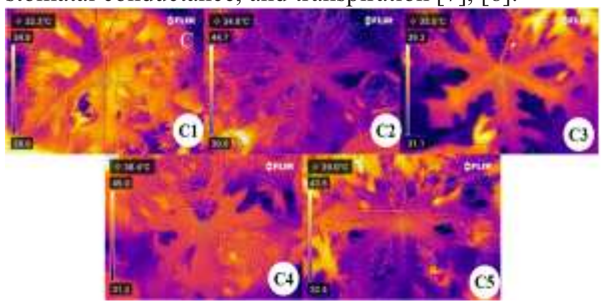

Fig. 2. Thermal images of chaya leaves under different drought stress durations. Prolonged drought increased leaf surface temperature, indicating reduced transpirational cooling and greater plant water stress.

These results indicate that chaya responded to prolonged drought through clear changes in soil water status and leaf thermal regulation. Although chaya is considered a promising leafy vegetable for marginal environments, the increase in leaf temperature under 12–16 days of drought suggests that prolonged water deficit can impose substantial physiological stress. A recent study on chaya under tropical conditions also reported that water limitation reduced growth and yield performance, indicating that adequate water management remains important for maintaining productivity despite the adaptive capacity of this species [5].

3.2 Vegetative Growth Responses of Chaya to Different Drought Stress Durations

Drought stress duration affected the dynamics of vegetative growth in chaya, particularly plant height, leaf formation, and shoot development (Figure 3). SPAD values showed a fluctuating pattern among treatments during the observation period (Figure 3A), but did not show a consistent decline with increasing drought duration. This indicates that relative leaf chlorophyll content was still maintained during short-term drought exposure, even though the plants had already experienced limited water availability. This response suggests that, in chaya, morphological growth changes may occur earlier than visible reductions in leaf greenness. Similar responses have been reported in several crops under drought stress, where the inhibition of leaf expansion and shoot growth often occurs before substantial chlorophyll degradation becomes evident [9], [10].

Plant height increased in all treatments during the observation period; however, the well-watered control showed a higher growth rate than the drought-stressed plants (Figure 3B). The control treatment produced the tallest plants at the end of the observation period, whereas plants exposed to the longest drought duration tended to show the lowest height. The reduced growth rate under drought stress indicates that limited water availability restricted stem elongation. Physiologically, water deficit reduces cell turgor pressure, thereby limiting cell division and expansion. Consequently, stem elongation and canopy development are inhibited. Recent studies have also emphasized that drought stress reduces plant height, leaf size, and biomass accumulation by disrupting plant water status, stomatal conductance, and photosynthetic activity [6], [11]

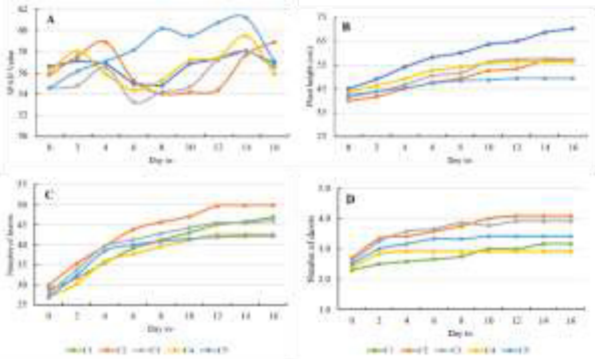

Fig. 3. Changes in SPAD value (A), plant height (B), number of leaves (C), and number of shoots (D) of chaya under different drought stress durations.

The number of leaves increased in all treatments; however, the increase was generally greater under well-watered and mild drought conditions than under longer drought durations (Figure 3C). The 4-day drought treatment showed a relatively high number of leaves at the end of the observation period, whereas plants subjected to 12 and 16 days of drought showed more restricted leaf formation. Reduced leaf production under water deficit may be associated with limited canopy expansion and a decline in the formation of new organs as part of a water-conservation strategy. Under drought conditions, plants commonly reduce the transpiring surface area by producing fewer or smaller leaves, thereby minimizing water loss. However, this strategy also reduces the photosynthetic area and may limit yield potential, particularly in leafy vegetables such as chaya.

Shoot number also increased during the observation period, but prolonged drought tended to restrict the development of new shoots (Figure 3D). The 4- and 8-day drought treatments still maintained relatively good shoot development, whereas the 12- and 16-day drought treatments resulted in fewer shoots. This indicates that chaya can maintain vegetative growth under mild to moderate drought stress, but prolonged water deficit begins to suppress shoot initiation and development. In chaya, shoot development is an important trait because it is directly related to the formation of new leaves and the potential for repeated harvesting.

chaya was able to maintain vegetative growth during drought exposure, but its growth rate declined as drought duration increased. Short to moderate drought durations, particularly 4–8 days, still allowed relatively favorable vegetative performance, whereas 12–16 days of drought increasingly suppressed plant height, leaf formation, and shoot development. These findings support the report of Gustiar *et al* [5], who showed that chaya has adaptive capacity under limited water conditions, although water limitation still reduces growth and yield performance under tropical cultivation. Therefore, drought tolerance in chaya should be interpreted as the ability to survive and maintain partial growth, rather than the ability to sustain optimal productivity under prolonged water deficit.

3.3 Leaf Area and Root Morphological Responses to Drought Stress

Drought duration significantly altered leaf area development and root morphology in chaya (Table 1; Figure 4). Total leaf area declined progressively as drought duration increased, from 1,321.84 cm² in the well-watered control (C1) to 886.04 cm² under 16 days of drought stress (C5). A similar pattern was observed for edible leaf area, which decreased from 21.45 cm² in C1 to 11.38 cm² in C5. These results indicate that prolonged water deficit restricted leaf expansion and reduced the harvestable leaf portion of chaya. This response is agronomically important because chaya is cultivated primarily as a leafy vegetable, and reductions in leaf area directly affect potential yield.

Table 1. Total leaf area, edible leaf area, root length, and root diameter of chaya under different drought stress durations.

Treatment	TLA (cm^2)	ELA (cm^2)	RL (cm)	RD (mm)
C_1	1.321.84 a	21.45 a	37.00 b	2.57 a
C_2	1.166.58 ab	16.38 ab	40.17 ab	1.97 ab
C_3	1.119.67 ab	18.03 ab	43.33 ab	2.32 a
C_4	1.017.25 ab	15.22 ab	47.00 ab	2.17 ab
C_5	886.04 b	11.38 b	50.67 a	1.64 b
$LSD_{0.05}$	385.59	6.76	1.54	2.31

Note : Means followed by the same letter in each column are not significantly different according to the LSD test at $p \leq 0.05$. Total Leaves area (TLA), Edibale Leaves area (ELA), Root langth (RL), Root Diameter (RD)

The reduction in leaf area under drought stress is a common morphological adjustment that enables plants to reduce transpirational water loss. However, this adaptive response also decreases light interception and photosynthetic surface area, thereby limiting biomass accumulation and productivity. Recent reviews have emphasized that drought stress commonly reduces leaf size, leaf length, shoot dry matter, and overall crop growth rate due to reduced turgor pressure, stomatal limitation, and impaired photosynthesis [9], [10]. Similar drought-induced reductions in leaf functional traits and biomass allocation have also been reported in other plant species, where severe drought substantially reduced total biomass and leaf biomass while increasing allocation toward roots [12], [13].

In contrast to leaf area, root length increased with increasing drought duration. The shortest root length was recorded in the control treatment (37.00 cm), whereas the longest root length was observed under 16 days of drought stress (50.67 cm). The visual observation in Figure 4B also supports this pattern, showing more elongated root systems in drought-stressed plants, particularly under the longer drought durations. This suggests that chaya responded to declining water availability by promoting root elongation, likely as an adaptive mechanism to explore a greater volume of growing medium for water uptake. Increased root growth under drought is widely recognized as an important drought-avoidance strategy, allowing plants to improve access to deeper or residual soil water [9], [12].

Fig. 4. Morphological performance of chaya plants under different drought stress durations: aboveground plant appearance (A) and root morphology (B).

However, root diameter decreased under prolonged drought, from 2.57 mm in the control to 1.64 mm in C5. This indicates that although drought stimulated root elongation, it limited radial root development. Such a response reflects a shift in growth strategy from thicker root formation toward longer and finer roots, which may increase soil exploration efficiency under water-limited conditions. Nevertheless, reduced root diameter under severe drought may also indicate restricted assimilate supply to roots as shoot photosynthesis and leaf area decline. Therefore, the root response observed in this study represents an adaptive but potentially costly adjustment under prolonged water deficit.

Figure 4A further confirms the negative effect of prolonged drought on aboveground growth. Plants under C1 showed more vigorous canopy development, whereas plants under C4 and C5 were visibly smaller, with reduced leaf development and canopy volume. These visual differences are consistent with the quantitative reduction in total and edible leaf area shown in Table 1. A recent study on chaya also reported that water limitation reduced growth and yield performance under tropical conditions, indicating that although chaya has adaptive capacity under drought, adequate water availability remains essential for maintaining leaf productivity [5].

3.4 Fresh and Dry Biomass Responses of Chaya under Different Drought Stress Durations

Drought stress duration affected both fresh and dry biomass accumulation in chaya (Tables 2). Fresh weight of edible leaves (FWEL) declined from 10.45 g in the well-watered control (C1) to 6.07 g under 16 days of drought stress (C5), indicating a reduction of approximately 41.9%. A similar decreasing trend was observed for fresh weight of edible stems (FWES), which declined from 8.97 g in C1 to 5.12 g in C5, and fresh weight of non-edible stems (FWNES), which decreased from 56.75 g to 25.80 g. In contrast, fresh weight of non-edible leaves (FWNEL) and roots (FWR) did not differ significantly among treatments, although both showed lower values under prolonged drought. These results indicate that prolonged water deficit primarily reduced the harvestable and structural aboveground biomass of chaya.

Table 2. Fresh weight of chaya plant organs under different drought stress durations.

Treatment	FWEL (gr)	FWNEL (gr)	FWES (gr)	FWNES (gr)	FWR (gr)
C_1	10.45 a	646.40 a	8.97 a	56.75 a	9.65 a
C_2	9.08 ab	560.19 a	6.35 b	37.95 ab	6.95 a
C_3	9.67 ab	607.25 a	8.88 a	41.63 ab	9.22 a
C_4	8.08 ab	505.35 a	6.57 ab	38.47 ab	9.20 a
C_5	6.07 b	437.50 a	5.12 b	25.80 b	6.25 a
$LSD_{0.05}$	4.26	365.70	2.40	19.32	3.95

Note: Means followed by the same letter in each column are not significantly different according to the LSD test at $p \leq 0.05$. FWEL = fresh weight of edible leaves; FWNEL = fresh weight of non-edible leaves; FWES = fresh weight of edible stems; FWNES = fresh weight of non-edible stems; FWR = fresh weight of roots.

The reduction in fresh biomass under drought stress is closely associated with decreased tissue water content and restricted vegetative growth. Fresh biomass reflects both plant structural growth and water status; therefore, its decline under prolonged drought suggests reduced water uptake, lower cell turgor, and limited organ expansion. This response is consistent with recent evidence that drought stress reduces leaf size, shoot dry matter, and crop growth rate through impaired plant water relations, stomatal closure, and reduced photosynthetic activity [9], [10]. For a leafy vegetable such as chaya, the decline in edible leaf fresh weight is particularly important because it directly reflects reduced marketable yield.

Dry biomass data further confirmed the negative effect of prolonged drought on assimilate accumulation (Table 3). Dry weight of non-edible leaves (DWNEL) decreased from 4.92 g in C1 to 2.47 g in C5, while dry weight of non-edible stems (DWNES) decreased from 5.62 g to 2.87 g. Dry weight of edible stems (DWES) was also lower under drought, especially in C2, C4, and C5. However, dry weight of edible leaves (DWEL) was not significantly different among treatments, suggesting that chaya may partly maintain dry matter allocation to harvestable leaves during short-term drought exposure. Interestingly, root dry weight (DWR) was highest in C3 and lowest in C5, indicating that moderate drought may stimulate root biomass allocation, whereas prolonged drought restricts overall biomass accumulation.

Table 3. Dry weight of chaya plant organs under different drought stress durations.

Treatment	DWEL (gr)	DWNEL (gr)	DWES (gr)	DWNES (gr)	DWR (gr)
C_1	1.95 a	4.92 a	0.75 a	5.62 a	1.22 ab
C_2	1.75 a	3.65 ac	0.47 b	3.82 ab	1.10 ab
C_3	2.03 a	4.25 ab	0.78 a	4.25 ab	1.38 a
C_4	1.33 a	3.33 bc	0.58 b	3.98 ab	1.27 ab
C_5	1.30 a	2.47 c	0.48 b	2.87 b	0.97 b
$LSD_{0.05}$	0.86	1.52	0.14	2.42	0.39

Note: Means followed by the same letter in each column are not significantly different according to the LSD test at $p \leq 0.05$. DWEL = Dry weight of edible leaves; DWNEL = Dry weight of non-edible leaves; DWES = Dry weight of edible stems; DWNES = Dry weight of non-edible stems; DWR = Dry weight of roots.

The contrasting response between moderate and severe drought suggests that chaya can temporarily adjust its biomass partitioning under limited water availability. Under moderate drought, plants may allocate more assimilates to roots to improve water acquisition, while reducing shoot expansion to limit water loss. However, under severe or prolonged drought, reduced photosynthetic area and impaired carbon assimilation eventually limit biomass production in both shoot and root organs. A global synthesis also showed that drought commonly alters biomass allocation by reducing aboveground growth while increasing or maintaining root investment, depending on species and drought intensity [14]. A recent chaya study similarly reported that water limitation reduced growth and yield performance under tropical conditions, confirming that adequate water supply remains important despite the adaptive capacity of this species [5].

4 Conclusion

Drought stress duration significantly affected the growth and morphological performance of chaya. Prolonged drought reduced soil moisture, increased leaf temperature, suppressed leaf area development, and decreased fresh and dry biomass. Chaya exhibited an adaptive response through root elongation, particularly under moderate drought, but this response did not fully compensate for the reduction in shoot growth and edible leaf production. The plant tolerated short drought periods of 4–8 days, whereas 12–16 days of water withholding caused substantial growth inhibition. Therefore, chaya can be considered moderately drought-tolerant, but proper water management is required to maintain optimum productivity.

Acknowledgements

The authors would like to express their sincere gratitude to the Faculty of Agriculture, Universitas Sriwijaya, for providing the facilities and support during the implementation of this study. The authors also thank all individuals who assisted in plant maintenance, data collection, and laboratory activities throughout the experiment. The publication of this article was funded by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology and managed under the EQUITY Program (Contract Numbers 0011/UN9/SK.BUK.HT/2025 and 0042/UN9.R/SK.SU/2026).

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