Mesophyll thickness and sclerophylly among Calotropis procera morphotypes reveal water-saving adaptation to environments (Postprint)

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Abstract

Calotropis procera (Aiton) Dryand (Apocynaceae) is a native species in tropical and subtropical Africa and Asia. However, due to its fast growing and drought-tolerant, it has become an invasive species when it was introduced into Central and South America, as well as the Caribbean Islands. Currently, C. procera displays a wide distribution in the world. Invasiveness is important, in particular, because many invasive species exert a high reproductive pressure on the invaded communities or are highly productive in their new distributed areas. It has been suggested that a very deep root system and a high capacity to reduce stomatal conductance during water shortage could allow this species to maintain the water status required for a normal function. However, the true mechanism behind the successful distribution of C. procera across wet and dry environments is still unknown. C. procera leaves were collected from 12 natural populations in Brazil, Colombia and Mexico, ranging from wet to dry environments during 2014–2015. Many traits of morphology and anatomy from these distinct morphotypes were evaluated. We found that C. procera leaves had a considerable capacity to adjust their morphological, anatomical and physiological traits to different environments. The magnitude of acclimation responses, i.e., plasticity, had been hypothesized to reflect the specialized adaptation of plant species to a particular environment. However, allometric models for leaf area (LA) estimation cannot be grouped as a single model. Leaves are narrower and thicker with low amounts of air spaces inside the leaf parenchyma in wet

environments, while they are broader and thinner with a small number of palisade cell layers in dry environments. Based on these, we argue that broader and thinner leaves of C. procera dissipate incident energy at the expense of a higher rate of transpiration to survive in environments in which water is the most limiting factor and to compete in favorable wet environments.

Full Text

Preamble

Mesophyll thickness and sclerophylly among Calotropis procera morphotypes reveal water-saved adaptation to environments

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Abstract

Calotropis procera (Aiton) Dryand (Apocynaceae) is native to tropical and subtropical Africa and Asia. However, due to its rapid growth and drought tolerance, it has become invasive in Central and South America as well as the Caribbean Islands, now displaying a wide global distribution. Invasiveness is particularly significant because many invasive species exert high reproductive pressure on invaded communities or achieve high productivity in their new ranges. It has been suggested that a very deep root system and high capacity to reduce stomatal conductance during water shortage enable this species to maintain water status for normal function. Yet the true mechanism behind C. procera's successful distribution across wet and dry environments remains unknown. We collected C. procera leaves from 12 natural populations in Brazil, Colombia, and Mexico spanning wet to dry environments during 2014–2015 and evaluated numerous morphological and anatomical traits from these distinct morphotypes. We found that C. procera leaves possess considerable capacity to adjust their morphological, anatomical, and physiological traits to different environments. While the magnitude of these acclimation responses (i.e., plasticity) has been hypothesized to reflect specialized adaptation to particular environments, allometric models for leaf area (LA) estimation could not be grouped into a single model. Leaves in wet environments are narrower and thicker with low amounts of air spaces within leaf parenchyma, whereas those in dry environments are broader and thinner with fewer palisade cell layers. Based on these findings, we argue that the broader, thinner leaves of C. procera dissipate incident energy at the expense of higher transpiration rates to survive in water-limited environments and compete effectively in favorable wet environments.

Keywords: invasive plant; energy budget; leaf anatomy; morphological trait; specific leaf area

1 Introduction

Invasive plants are non-native species that affect the biodiversity of regions they colonize (Colautti et al., 2004). Over the past two decades, local invasions by non-native species have become a major focus of ecological research, primarily due to their extensive ecosystem impacts and the novel ecological strategies they employ when colonizing new environments at both small geographical scales and macroscales (Alberio and Comparatore, 2014; Pagnucco et al., 2015; Dawson et al., 2017). Invasiveness is particularly important because many invasive species exert high reproductive pressure on invaded communities (Simberloff et al., 2012) or achieve high productivity in their new ranges (Blossey and Nötzold, 1995). Calotropis procera (Aiton) Dryand (Apocynaceae), known as milkweed, exemplifies such an invasive species (Muriira et al., 2018). Native to tropical and subtropical Africa and Asia, it has been introduced into Central and South America and the Caribbean Islands, now displaying a wide distribution (Singh, 1995) from dry to wet environments, even growing at elevations up to 2000 m a.s.l. (Hassan et al., 2015). C. procera can quickly establish itself as a weed along degraded roadsides, lagoon edges, and overgrazed native pastures, with morphological variability existing among different provenances (populations) (Peguero-Pina et al., 2014).

C. procera is an evergreen shrub capable of maintaining high net photosynthetic rates even during the dry season (Rivas et al., 2017), a key feature contributing to its competitive success under diverse environmental conditions. Ecophysiological adaptations such as a very deep, stout taproot reaching depths of 1.7-3.0 m in Indian sandy desert soils may aid survival in extreme drought (Sharma, 1968). Additionally, low stomatal conductance during water shortage has been identified as an important mechanism for maintaining relatively high water content and water potential, described as intrinsic water use efficiency (WUEi) (Rivas et al., 2017), forming this species into a classical desiccation-postponement morphotype (Antunes et al., 2017).

Characterizing plant form and function at large scales is the first step toward improving the predictive capability of vegetation models (Wang et al., 2013; Díaz et al., 2016). Leaves are highly sensitive to environmental conditions (Fahn, 1990; Wright et al., 2004; Evert, 2013). The internal structure of leaves, such as the arrangement of palisade and spongy parenchyma, affects gas diffusion and partially determines photosynthetic and non-photosynthetic tissues (Peguero-Pina et al., 2016a). Specific leaf area (SLA, the ratio of leaf area to leaf dry weight) serves as an indicator of morphological and anatomical traits (Villar et al., 2013). Generally, low SLA results from thicker leaves caused by large volumes of mesophyll and air spaces, as well as high leaf density due to high volumetric fractions of mesophyll and low fractions of air spaces and epidermis (Villar et al., 2013). Decreases in SLA have been postulated to result from denser leaf laminae associated with lignification, thickened cell walls, cuticle waxes, trichomes, and other non-photosynthetic structures—collectively termed sclerophylly (Villar et al., 2013). Conversely, low SLA is often considered an indicator of high photosynthetic potential (Reich et al., 1992). Poorter et al. (2006) found that low SLA relates to long leaf lifespan and conservative growth rate, with higher construction costs for leaf area. Additionally, thicker leaves display greater light absorbance (Falcioni et al., 2017), partly due to chloroplasts stacked in small areas, while thinner leaves spread chloroplasts throughout larger areas—both common physiological strategies in response to light intensity (Evans and Poorter, 2001; Poorter et al., 2009). Large leaves have smaller convection coefficients and higher resistance to heat transfer through leaf boundary layers than small leaves, and leaf size may change to optimize leaf temperature (Leigh et al., 2016). Larger leaves may be intrinsically vulnerable to drought-induced embolism due to lower vein length and larger xylem conduit diameters (Peguero-Pina et al., 2014; Gil-Pelegrín et al., 2017). These findings could be extrapolated to leaves developing in arid (e.g., Mediterranean) environments (Peguero-Pina et al., 2014; Peguero-Pina et al., 2016b). Sclerophylly may be an important mechanism for adapting to extremely hot, drought-prone environments with high light intensities. However, the mechanisms facilitating C. procera’ s successful distribution across wet and arid environments remain unknown.

This study aimed to determine the morphological traits of C. procera leaves that enable successful distribution in wet or dry environments and facilitate its invasive success. Additionally, we evaluated which leaf anatomical and morphological traits relate to drought tolerance. We hypothesized that C. procera possesses various anatomical and morphological traits that vary according to provenance.

2 Materials and Methods

2.1 Plant materials

C. procera leaves were collected from 12 natural populations along a macro-environmental gradient (rainforest, arid, and semi-arid regions) in Mexico, Colombia, and Brazil during 2014–2015. Approximately 10 populations were from Brazil, one from the Caribbean Atlantic region in Colombia, and one from a mountain rainforest in southern Mexico (Fig. 1 [Figure 1: see original paper]). Geoclimatic details are described in Table 1 . Climatic datasets from the 12 populations over the past 15 years were downloaded from https://pt.climate-data.org. The aridity index was estimated using the Martonne aridity index (MAI; MAI = P/(T + 10), where P is annual precipitation (mm) and T is monthly mean temperature (°C)). Based on MAI and climatic characteristics (Sant' anna-Neto et al., 2015), we classified Brazilian populations as: (i) Atlantic Rainforest (MAI $>$ 35), (ii) Semi-arid region (25 $\leq$ MAI $\leq$ 35), and (iii) Arid region (MAI $<$ 25). The population collected in Montería, Colombia is referred to as Caribbean, and the population collected in Oaxaca, Mexico is referred to as Mountain Rainforest (Fig. 1).

2.2 Leaf morphology and specific leaf area (SLA) measurements

A total of 5,937 fully expanded leaves were harvested (approximately 500 leaves per population) in the field at the end of the growing season during 2014–2015.
Leaves were scanned at 1200\$$\times 1200 dpiresolution(HPPSC1410, PaloAlto, CA, USA)andanalyzedusingImage$
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we oven-dried 50 randomly selected leaf discs (12 mm diameter) from each population for 72 h at 70°C and weighed them (Witkowski and Lamont, 1991).

2.3 Allometric model for leaf area estimation

Allometric models were developed following Antunes et al. (2008) and Pompelli et al. (2012) to estimate leaf area (LA) using L, W, or L×W. Estimated leaf area served as the dependent variable and observed leaf area as the independent variable (n = 500). After initial runs, preliminary models were evaluated for bias. The best unbiased models were validated using an independent dataset (n = 200) from the same morphotype. The validated, unbiased power models from each morphotype were then tested for equality using the procedure described in Graybill (2000). First, we tested whether all equations were equal ($\alpha < 0.05$; F test). If not, populations were excluded and others were pooled into a single model. This procedure was repeated for all possible combinations until $\alpha > 0.05$ (F test).

2.4 Leaf anatomy and leaf symmetry measurements

Leaf anatomy was compared among four populations highly divergent in leaf morphology (i.e., SLA and L/W ratio), mean annual precipitation, and MAI:

(i) Atlantic Rainforest of Pernambuco State (Köppen classification: equatorial climate)—Paulista-PE (#3), (ii) arid region of Pernambuco State (Köppen classification: desert climate)—Afrânio-PE (#10), (iii) Caribbean region with climate strongly influenced by North Pacific ocean currents (Köppen classification: semi-humid tropical climate)—Montería-CO (Colombia) (#11), and (iv) mountain rainforest with annual precipitation of ~2,170 mm in Mexico (Köppen classification: semi-humid tropical climate)—Oaxaca-MX (#12) (Table 1). All sampled tissues were fixed in formalin-acetic-alcohol (FAA; 10% formaldehyde, 5% acetic acid, 50% ethanol), dehydrated (ethanol series), and embedded in methacrylate. Paradermic sections (4 m) and cross-sections (7 m) were stained with toluidine blue. Anatomical data were quantified using Image Pro® Plus software, measuring: (i) total leaf thickness, (ii) adaxial and abaxial epidermal thicknesses, (iii) palisade and spongy parenchyma thicknesses, (iv) adaxial palisade number, and (v) palisade and spongy air spaces as a percentage of total mesophyll cross-sectional area. Except for palisade and spongy air spaces, all measurements were conducted randomly 10 times per micrograph using 20 light micrographs per morphotype. To illustrate morphotype differences, we traced drawings from representative photomicrographs.

For leaf symmetry measurements, 50 fully expanded leaves per morphotype were measured for (i) leaf width and (ii) absolute interrib distance using the method of Møller and Eriksson (1994). Measured data were tested using the Shapiro-Wilk test; non-normal distributions were normalized via Box-Cox transformation (Souza et al., 2005).

2.5 Plasticity index

Plasticity index ranged from 0.0 (no plasticity) to 1.0 (maximal plasticity), calculated as the difference between minimum and maximum values divided by the maximum average value for each morphological and anatomical trait (Valladares et al., 2002).

2.6 Pearson's correlation coefficient and principal component analysis (PCA)

Pearson' s correlation coefficient examined relationships among variables, with similarity between data groups calculated using adjusted r coefficients via the Corrplot Package in R (Wei and Simko, 2016). To test whether alteration patterns in specific trait sets (morphological and anatomical) were consistent across populations, we used data from all 12 C. procera populations. Magnitude issues were avoided by standardizing all variables (Z-score) before PCA to control measurement differences and compare variables with different units (Gotelli and Ellison, 2012). Z standardization satisfied basic requirements for hypothesis testing, including data normality and variance homogeneity (Gotelli and Ellison, 2012). One-way ANOVAs complemented by Tukey' s test compared the full range of traits (multivariate attribute vector) among populations using the most variable axis scores (PC1 and PC2) (Gotelli and Ellison, 2012). This procedure was performed for both morphological and anatomical traits. Finally, all PCA layers were combined on a standard scale in a single graph using ggplot2 and Vegan packages in R (Husson et al., 2017).

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3 Results

3.1 Allometric model for leaf area estimation

Allometric models predicted leaf area from linear dimensions (L, W, or L×W). The best unbiased equation was a power model: $Y = \beta_0 \times (L\times W)\hat{}\beta_1 \times e$, where β_0 and β_1 are coefficients, L is leaf length, W is leaf width, and e is random error representing approximation discrepancy (Fig. S1). The equation was validated; estimated parameters (β' s) for model calibration were not statistically different ($P > 0.05$) from validating equations using independent leaf sets from the same morphotype. Most calibrating equations for single morphotypes were not statistically similar ($P > 0.05$) by model identity test (Table 2). Thus, we grouped morphotypes as: G1: #12; G2: #10; G3: #11; G4: #8; G5: #1; G6: #7; G7: #6 and #9; and G8: #2, #3, #4, and #5 (Table S1) based on successive exclusion from pooled data.

3.2 Leaf morphological trait

Leaf morphological traits differed significantly between semi-arid and arid populations versus other provenances (Table 3). The Paulista-PE population (#3) exhibited greater leaf width, length, and area than other populations (Table 3). Morphological traits showed highly significant differences among four C. procera populations from arid region, Atlantic Rainforest, Caribbean, and Mountain Rainforest (Table 3; Fig. 2 [Figure 2: see original paper]). The L/W ratio indicated that Oaxaca-MX leaves (#12) were broader (1.28 ± 0.14) than Afrânio-PE leaves (#10; 2.19 ± 0.44) (Table 3; Fig. 2). SLA values in Afrânio-PE leaves were 47% higher than in Oaxaca-MX leaves. Consequently, relationships between L/W ratio and SLA, and between L/W ratio and mean annual precipitation, showed negative correlations, while SLA and mean annual precipitation were positively correlated (Fig. 3 [Figure 3: see original paper]).

3.3 Leaf anatomical trait

C. procera leaves had uniseriate epidermis formed by cells with curved anticlinal walls and covered by a thick cuticle along their entire length (Fig. 4 [Figure 4: see original paper]). All morphotypes were amphistomatic, with stomata arranged in a plane above the epidermis (data not shown). Except for the Afrânio-PE population (#10), C. procera presented dorsiventral mesophyll parenchyma consisting of three to four layers of palisade parenchyma with slightly elongated, juxtaposed, thin, compactly arranged cells. However, Afrânio-PE leaves displayed isobilateral mesophyll (Fig. 4). In all populations, spongy parenchyma consisted of loosely arranged cells of various shapes with numerous intercellular spaces (Fig. 5 [Figure 5: see original paper]).

In-depth analysis among four populations (#3, #10, #11, #12) revealed that Afrânio-PE leaves had leaf, adaxial epidermis, abaxial epidermis, and palisade and spongy parenchyma thicknesses 23%, 26%, 13%, and 26% greater, respectively, than Montería leaves (Table 4). Despite these differences, Afrânio-PE leaves had 3.7 ± 0.1 adaxial palisade layers versus 3.3 ± 0.1 in Montería-CO leaves—a 13% increase. Palisade and spongy parenchyma thicknesses in Afrânio-PE leaves were 214% and 40% greater, respectively, than in Montería-CO leaves (Table 4).

3.4 Leaf symmetry trait and plasticity index

C. procera leaves demonstrated fluctuating asymmetry (data not shown). The plasticity index indicated that while foliar plasticity was quite high for some populations and lower for others, it did not differ significantly among assessed populations—i.e., plasticity index was not provenance-dependent. However, morphological traits (0.77 ± 0.09) showed higher plasticity than anatomical traits (0.34 ± 0.04) (Table S2).

3.5 Pearson' s correlation coefficient and PCA

Mean annual precipitation correlated strongly and negatively with L/W ratio ($r = -0.41$), total leaf thickness ($r = -0.31$), adaxial epidermis thickness ($r = -0.35$), abaxial epidermis thickness ($r = -0.54$), and palisade thickness ($r = -0.32$), but positively with elevation ($r = 0.26$), SLA ($r = 0.55$), L ($r = 0.28$), W ($r = 0.33$), LA ($r = 0.29$), and air spaces in spongy mesophyll ($r = 0.28$; Fig. 6 [Figure 6: see original paper]). No correlation was observed between mean annual precipitation and leaf symmetry. Across all populations, elevation effects on anatomical characteristics were marginal compared to precipitation effects, with no significant correlation ($P = 0.985$).

Significant patterns in morpho-anatomical traits were observed across provenances (Atlantic Rainforest, Semi-arid region, Arid region, Caribbean, and Mountain Rainforest). For morphological traits (L, W, LA, L/W ratio), the first two PCA axes (cumulative proportion = 98.11%) indicated partial separation of natural morphological variation. Leaves from Rainforest and Caribbean environments showed greater expression and dominance of these traits, as indicated by relatively larger leaves (Fig. 6). Although group overlaps occurred, ANOVA confirmed consistent differences among populations ($P < 0.001$) except between Atlantic Rainforest and Caribbean, which systematically presented more similar leaf morphological traits. Anatomical traits also showed clear separation among provenances, with two axes explaining 66.00% of variance. Air spaces in palisade and spongy parenchyma (upper left-hand portion of PC1 axis) showed expressive values for more humid environments (Rainforests and Caribbean). The opposite axis end was dominated by palisade thickness, total leaf thickness, and spongy thickness traits, representing specialization for semi-arid and arid populations. Anatomical traits differed significantly among provenances ($P < 0.001$; Fig. 6), except between Arid region and Atlantic Rainforest. Coordinated changes in the two-dimensional axis indicated that increased palisade mesophyll air spaces related to decreased adaxial epidermis thickness. Climatic variations were significantly associated with morpho-anatomical changes ($P < 0.001$), and SLA also showed precipitation-related changes.

4 Discussion

This study demonstrated that C. procera plants from wet-to-dry environments have developed a wide range of morphological traits, resulting in unique models (for each morphotype or small population) for leaf area estimation based on linear leaf dimensions, along with anatomical and physiological adaptations. The major differences in L/W ratio and SLA among C. procera populations may be the most important traits for survival in dry environments. Parkhurst and Loucks (1972) stated that "natural selection leads to organisms having a combination of form and function optimal (i.e., phenotypic plasticity) for growth and reproduction in the environments in which they live." Thus, through natural selection, population members may be expected to approach optimum performance. In this case, C. procera presented distinct models, diverging from Coffea spp. (Antunes et al., 2008) or Jatropha curcas trees growing in wet or dry environments (Pompelli et al., 2012).

One might ask: why are leaves narrower in dry environments and broader in wet ones? Maximum leaf size undergoes considerable acclimation to water stress, whereas leaf shape is largely controlled by heredity (Arterburn et al., 2010). Plant ecologists have emphasized extensive relationships between native plant leaf traits and climate (Wright et al., 2004; Dainese et al., 2015; Díaz et al., 2016). A common strategy in arid and semi-arid plants is developing thicker leaves with low SLA (Bacelar et al., 2007; Evert, 2013). However, coordination between foliar traits in multivariate environments appears more complex and at least partially climate-dependent (Wright et al., 2004).

Rivas et al. (2017) found that C. procera leaf temperatures in wet environments were lower than air temperature (-1.33°C), whereas under drought they were 12.61°C higher than air temperature. Under drought, C. procera reduces stomatal conductance and increases leaf temperature (Antunes et al., 2017; Rivas et al., 2017). High leaf plasticity contributes to radiation interception and radiative/sensible heat loss, increasing intrinsic water use efficiency and partially mitigating extreme water vapor gradients between mesophyll and surrounding environment, similar to Quercus coccifera under Mediterranean conditions (Peguero-Pina et al., 2016b).

Multi-layered parenchyma has been identified in various plant families (Fahn, 1990; Evert, 2013), but variation in parenchyma thickness and layers along natural precipitation gradients is first reported here. We argue that more palisade layers or isobilateral mesophyll may protect photosynthetic tissue (Chazdon and Kaufmann, 1993). Michaletz et al. (2015) found that reducing transpiration by altering leaf morphology decreases hydraulic conductance loss via xylem embolism under drought. Larger leaves have smaller convection coefficients and greater boundary layer resistance than smaller leaves, with leaf size changing to optimize temperature (Leigh et al., 2016). Narrower, smaller, thicker leaves in drier environments exhibit greater radiative and sensible heat loss with lower latent (transpiration) heat loss (Peguero-Pina et al., 2014). Thicker leaves retain more water and become warmer, mainly due to high near-infrared radiation absorption by water, which dissipates energy via non-transpiring systems. They open stomata less frequently and close them earlier in the day (Rivas et al., 2017) to save water, displaying a drought-postponement morphotype (Antunes et al., 2017). Thus, low SLA is an important trait for adjusting leaf energy budget, significantly increasing radiative heat loss in dry environments. Sclerophylly (low SLA) is common in semi-arid and arid species and may function to save water (Monk, 1966). C. procera presents a morphotype with thinner leaves in wet environments and thicker leaves in dry environments. Thinner leaves dissipate incident energy at the expense of higher transpiration (latent heat loss). Thinner leaves with larger areas may lead to high whole-plant transpiration rates, but this is neutral for survival because water is plentiful in their original environment. When combined with higher stomatal conductance, increased CO_2 influx, larger leaf area, and greater net photosynthesis, thinner leaves become a positive trait. The thinner leaves of C. procera allow survival in both dry and wet environments, consistent with Lindorf et al. (1991) and Witkowski and Lamont (1991), who reported that C. procera leaves are more succulent in arid environments with thicker cuticles and smaller leaf areas.

Thinner leaves are often short-lived (e.g., Montería-CO (#11) or Oaxaca-MX (#12)), possibly associated with lower leaf area construction costs (Villar et al., 2013). Lower area-based construction costs were verified for invasive Lythrum salicaria compared to five co-occurring native herbaceous plants and shrubs (Nagel and Griffin, 2001). The authors suggested that producing larger photosynthetic surface area at lower energy expense—resulting from lower requirements or higher efficiency—helps plants outcompete neighboring natives in low-stress, low-disturbance, high-competition wet environments. Research shows that increased mesophyll thickness or more compact mesophyll improves CO_2 diffusion through intercellular spaces from sub-stomatal cavity to mesophyll cell surfaces (Tezara et al., 2011). We imply that plants in more xerophytic environments might have lower stomatal conductance to mitigate atmospheric water loss without large photosynthetic capacity losses, thus increasing WUEi as reported by Rivas et al. (2017).

Leaf area changes systematically with leaf dimensions. The Afrânio-PE morphotype appeared better drought-adapted with smaller leaves. Selecting thinner, larger leaves with lower L/W ratio associates with maximum light capture at the expense of abundant water use (White and Montes-R, 2005). Thicker palisade parenchyma, more adaxial palisade layers, and less air space may importantly influence photosynthetic capacity. Thicker leaves with lower SLA present greater living photosynthetic cell volume per unit leaf area (Villar et al., 2013). In arid environments, leaves often experience very high direct irradiance and diffuse light fluency, so thicker leaves absorb more light than thinner ones (Falcioni et al., 2017). Upper palisade parenchyma cells efficiently absorb blue and red light (Terashima et al., 2009; Falcioni et al., 2017; Smith et al., 2017), while sunlight' s abundant yellow and green light (Terashima et al., 2009) penetrates deeper, enabling light reactions in middle spongy parenchyma. Isobilateral palisade parenchyma symmetry in Afrânio-PE may be another adaptive trait for absorbing soil-reflective and diffuse light, efficiently driving photosynthesis on both leaf surfaces while reducing transpiration pressure from larger leaf areas.

5 Conclusions

C. procera leaves demonstrate considerable capacity to adjust morphological, anatomical, and physiological traits to different environments, with plasticity values ranging from 0.115 to 0.999. Morphological traits are more plastic than anatomical traits (0.77 ± 0.09 vs. 0.34 ± 0.04), indicating leaf morphology is profoundly affected by environmental conditions. For example, increased leaf thickness, lower L/W ratio, and isobilateral mesophyll in the Afrânio-PE morphotype strongly contributed to dry environment adaptation. At least three-quarters of morpho-anatomical traits varied across environments, consistent with C. procera' s eco-physiological mechanism changes and efficiency. We demonstrated that morphological traits are good predictors of plant form and function at macroscale.

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Appendix

Fig. S1 Deviation of estimated leaf area from observed leaf area of *Calotropis procera* from different population groups. Group information is listed in Table S1. Leaf area was estimated using four models where β_0 and β_1 are coefficients, L is length, W is width, and e is random error representing approximation discrepancy. Vertical bars denote mean values and spreads denote 95% confidence intervals of the difference. Asterisks denote significant difference ($P < 0.05$, Student' s t test) from observed values. See Figure 1 and Table 1 for population details.

Table S1 Group, coefficient (β_0), standard error (SE), coefficient of determination (R^2), degrees of freedom (df), sum of squares (SS), calculated F, P-value, and leaf area estimation model as a function of linear dimensions (L, length; W, width) for *Calotropis procera*

Group	β_0	β_1	R^2	F	P	Model
All (G1)/#12	0.71924	1.01867	557,318	<0.0001	Y = 0.71924 × (L×W)^1.01867	
(G2)/#10	1.22999	0.91999	325,645	<0.0001	Y = 1.22999 × (L×W)^0.91999	
(G3)/#11	0.75486	1.00023	114,472	<0.0001	Y = 0.75486 × (L×W)^1.00023	
(G4)/#8	0.61262	1.04114	10,010	<0.0001	Y = 0.61262 × (L×W)^1.04114	
(G5)/#1	0.66429	1.03074	256,617	<0.0001	Y = 0.66429 × (L×W)^1.03074	

Group	β_0	β_1	R^2	F	P	Model
(G6)/#7	0.64241	1.03857	488,336	<0.0001	Y = 0.64241 × (L×W)^1.03857	
(G7)/#6, #9	0.69267	1.03196	206,263	<0.0001	Y = 0.69267 × (L×W)^1.03196	
(G8)/#2, #3, #4, #5	0.68226	1.02959	16,503	<0.0001	Y = 0.68226 × (L×W)^1.02959	
	0.63834	1.04269	57,371	<0.0001	Y = 0.63834 × (L×W)^1.04269	

Note: #01, Aimorés-MG; #02, Jaboatão Guararapes-PE; #03, Paulista-PE; #04, Pombos-PE; #05, Santa Terezinha-PB; #06, Alagoinha-PE; #07, Jaramataia-AL; #08, Serra Talhada-PE; #09, Parnamirim-PE; #10, Afrânio-PE; #11, Montería-CO; #12, Oaxaca-MX.

Table S2 Plasticity index of leaf morphological and anatomical traits of *Calotropis procera* from four populations

Morphological trait	Afrânio-PE (#10)	Paulista-PE (#3)	Oaxaca-MX (#12)	Montería-CO (#11)	Mean±SD
Specific leaf area	0.79$±0.10|0.68±0.06|0.22±0.04|0.97±0.02|0.77±0.09||*Leaflength(L)*|0.79±0.05|0.97±0.02|0.9				

Anatomical trait	Mean±SD
Total leaf thickness	0.34$±0.06 ∗ ||*Abaxialepidermisthickness*|0.30±0.05∗ ||*Adaxialepidermisthickness*|0.30±0.03∗ ||*Palisademesophyllthickness*|0.35±0.05∗ ||*Spongymesophyllthickness*|0.36±0.03||*Palisadenumbe*

Note: * denotes statistically significant differences among anatomical traits across populations at $P < 0.01$. Morphological trait means did not differ significantly at $P < 0.05$.