Enhanced Diurnal Crassulacean Acid Metabolism (CAM) Activity in *Kalanchoe pinnata* (Lam.) Pers. under Drought Stress: Physiological Plasticity and Climate Adaptation Potential in Ghana

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Abstract

Kalanchoe pinnata, commonly known in Ghana as Africa Never Die, is a succulent species with significant ethomedicinal and ecological value. This study evaluated its CAM photosynthetic plasticity under drought stress using leaf tissue pH as a proxy for nocturnal acid accumulation. Ten replicate plants per treatment were grown in pots and subjected to well-watered (control) or drought-stress conditions. Experimental tracking was initiated at the University of Energy and Natural Resources (UENR) campus vegetation structures. Leaf samples were collected at 04:00 h and 16:00 h. Tissue pH was significantly lower at night than during the day in both treatments (paired t-tests, $p < 0.001$). Drought-stressed plants showed significantly stronger nocturnal acidification (3.38 ± 0.03 vs 4.17 ± 0.05 in controls; $t(18) = 14.01$, $p < 0.001$) and a larger diurnal pH amplitude ($\Delta pH = 2.97 \pm 0.07$ vs. 1.90 ± 0.05); $t(18) = -12.77$, $p < 0.001$). These results demonstrate enhanced CAM activity under water deficit, highlighting the species' adaptive capacity in the face of climate variability in tropical West

Africa. The findings support the potential use of *K. pinnata* in restoration and climate-resilient agroforestry systems.
Keywords: Crassulacean Acid Metabolism, drought stress, leaf acidity, *Kalanchoe pinnata*, Ghana, climate resilience, succulent physiology

1. Introduction

Crassulacean Acid Metabolism (CAM) is a specialized photosynthetic pathway that enables plants to minimize water loss by opening stomata at night for CO{2} uptake. *Kalanchoe pinnata*(Crassulaceae), a widely distributed succulent, exhibits constitutive CAM with reported facultative plasticity under environmental stress. In Ghana, the plant is culturally significant and locally called Africa Never Die. or Resurrection Plant due to its ability to propagate vegetatively from detached leaf margins and tolerate extreme environmental conditions.

Climate change is increasing the frequency and intensity of seasonal drought events within the dry semi-deciduous forest zones of the Bono and Ashanti Regions. Understanding how naturalized succulents respond physiologically to water limitations is critical for developing local ecological conservation strategies and sustainable land management models. This study quantified the diurnal fluctuations of leaf tissue pH—a highly reliable, cost-effective indirect indicator of CAM activity—in a local population of *K. pinnata* subjected to controlled drought stress to evaluate its inherent physiological plasticity.

2. Materials and Methods

2.1 Study Site and Sourcing

Experimental plant stocks were sourced from established wild populations in Nagoode, located within the Atwima Mponua District of the Ashanti Region, Ghana (approx. 6°45′N, 2°10′W). The source area experiences a bimodal rainfall regime with an annual mean range of 1,400–1,600 mm and mean ambient temperatures between 24 °C and 33 °C.

2.2 Experimental Design and Environment

Uniform, healthy juvenile clones of *K. pinnata* were transplanted into individual 10-L experimental pots containing a uniform, steam-sterilized 2:1:1 mixture of local topsoil, river sand, and organic manure. The experiment was set up within a protected vegetation house structure at the University of Energy and Natural Resources (UENR), Sunyani, Ghana.

Following a strict four-week acclimatization phase under a standard watering regime to ensure uniform root development, twenty identical pot units were randomized into two distinct treatment groups ($n = 10$) plants per group):

- **Control Treatment:** Plants were maintained under optimal water conditions, brought back to full field capacity every alternating morning.
- **Drought Treatment:** Irrigation was completely withheld for 14 consecutive days until visible macromorphological onset of

moderate stress was induced (characterized by standard soft-leaf turgidity loss without entering permanent, irreversible cellular wilting).

2.3 Leaf Tissue pH Measurement

Fully expanded, mature leaves from the third upper nodal tier of each replicate plant were sampled systematically on a single experimental day at two distinct temporal peaks: 04:00 h (nocturnal acid accumulation peak) and 16:00 h (diurnal acid depletion peak). Approximately 1.5 g of fresh leaf tissue was excised per sample, rinsed with distilled water, blotted dry, and thoroughly homogenized using a mortar and pestle in 15 mL of chilled distilled water (pH 7.0).

The pH value of the resulting tissue homogenate was determined immediately using a benchtop digital pH meter with an analytical resolution of 0.01, pre-calibrated against standard technical buffer solutions (pH 4.01) and (7.00).

2.4 Data Analysis

Raw data parameters were evaluated for normal distribution characteristics using the Shapiro-Wilk test and variance homogeneity using Levene's test. Within-treatment variations across the day-to-night timeline were evaluated using paired-samples t-tests. Variations across the two separate treatment structures at specific collection times were analyzed using independent-samples t-tests.

The individual diurnal CAM acid amplitude (ΔpH value) was calculated mathematically for each individual plant replicate as follows:

$\Delta pH = \Delta pH16:00h - \Delta pH04:00h$

Statistical computations were executed utilizing IBM SPSS Statistics Version 29. Main effects were determined based on a significance threshold of $(p < 0.05)$. Descriptive parameters are stated as Mean $\pm$ Standard Error (SE)

3. Results

Leaf tissue pH profiles displayed distinct diurnal oscillations across both groups, validating the functional execution of CAM carbon pathways (Table 1). Nighttime tissue pH levels dropped significantly lower than corresponding daytime values in both experimental groups (Paired t-tests: Control ($t(9) = 36.30$, $p < 0.001$; Drought $t(9) = 45.67$, $p < 0.001$.

Exposing *K. pinnata* to prolonged drought stress resulted in enhanced nighttime acidification, dropping significantly to 3.38 ± 0.03 relative to the control group value of 4.17 ± 0.05 (Independent t-test: $t(18) = 14.01$, $p < 0.001$). Conversely, daytime pH was significantly higher in drought-stressed tissues (6.35 ± 0.04) than in well-watered tissues (6.08 ± 0.05; $t(18) = -4.31$, $p < 0.001$). Consequently, the net diurnal CAM amplitude (ΔpH}) widened drastically under water-deficit conditions (2.97 ± 0.07) compared to controls (1.90 ± 0.05), yielding a highly significant treatment effect ($t(18) = -12.77$, $p < 0.001$).

Table 1. Effect of drought stress on tissue pH and diurnal amplitude (ΔpH)in *Kalanchoe pinnata* leaves.

Treatment Group	Night pH (04:00 h)	Day pH (16:00 h)	Diurnal Amplitude (ΔpH)
Control (n=10)	4.17 ± 0.05	6.08 ± 0.05	1.90 ± 0.05
Drought (n=10)	3.38 ± 0.03	6.35 ± 0.04	2.97 ± 0.07

Values represent Mean ± Standard Error (SE).

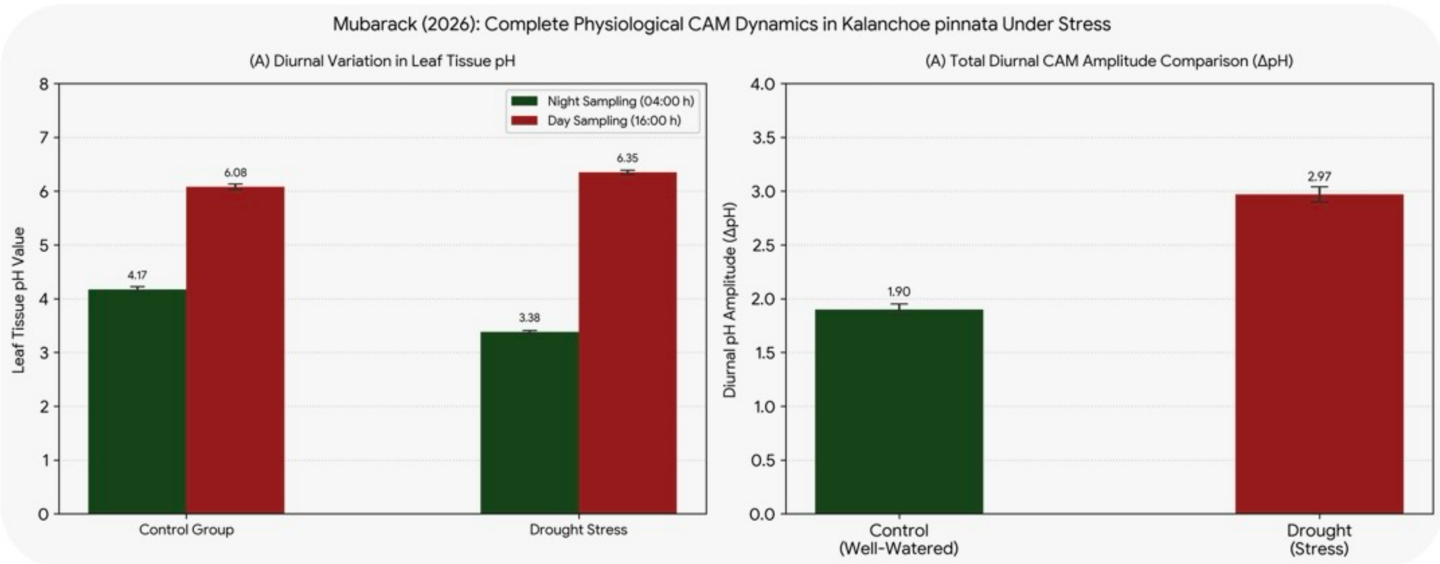

Figure 1. Complete physiological CAM dynamics in *Kalanchoe pinnata* under stress. Panel (A) shows the precise diurnal variation in leaf tissue pH between 04:00 h and 16:00 h. Panel (B) shows the total calculated diurnal pH amplitude comparison (ΔpH) between well-watered control and drought stress groups. Error bars denote

±SE (n=10).

4. Discussion

This investigation delivers empirical evidence regarding the metabolic flexibility of a Ghanaian population of *Kalanchoe pinnata* under water stress. The substantial drop in nighttime pH alongside the expanded ΔpH configuration under drought confirms an accelerated accumulation of vacuolar malic acid during dark hours. This physiological adjustment allows the plant to seal its stomatal apertures during hot daytime periods, avoiding rapid transpiration while using internally stored CO_2 for photosynthesis.
This metabolic plasticity provides a clear explanation for the plant's local name ("Africa Never Die"). It explains how the species survives and capitalizes on highly degraded, dry, or rocky terrain across the Bono and Ashanti regions where native C_3 or C_4 plants face mortality. Given current climate projections indicating extended dry seasons across Ghana, *K. pinnata* presents practical characteristics for incorporation into urban green-roofing frameworks, soil stabilization interventions, and climate-smart landscaping systems.
A noted limitation of this research includes its focus on short-term drought observations and its reliance on tissue pH values as an indirect proxy for absolute acid synthesis. Future research lines should focus on tracking long-term seasonal shifts, measuring direct titratable acidity values, monitoring precise gas-exchange dynamics, and analyzing chemical transformations using high-performance

liquid chromatography.

5. Conclusion

Drought stress significantly upregulates CAM activity in *Kalanchoe pinnata*, as evidenced by a substantial increase in diurnal pH fluctuations and nocturnal acidification. This inherent physiological flexibility highlights the species' potential for ecological deployment in climate-resilient restoration projects across seasonally dry tropical agro-ecological zones in West Africa.

Acknowledgements

The author expresses sincere appreciation to the University of Energy and Natural Resources for providing laboratory infrastructure and academic support. Special thanks are extended to the technical staff within the Department of Biological Sciences for guidance throughout the laboratory processing phase.

Funding

This research project received no external funding from public, private, commercial, or non-profit grant bodies.

Conflicts of Interest

The author declares no conflicts of interest regarding this manuscript.

Data Availability Statement

The datasets generated and analyzed during this study are fully available from the corresponding author upon reasonable request.

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