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Thermal Adaptation Strategies in Waterleaf (*Talinum triangulare* Jacq.): Balancing Biomass Production and Nutritional Quality Under Climate Change

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ABSTRACT

This study investigated the morphophysiological and biochemical responses of waterleaf (*Talinum triangulare* Jacq.) to different temperature regimes (18°C, 22°C, 25°C, and 35°C) to assess its potential as a climate-resilient crop. Through controlled-environment experiments, we demonstrated that temperature significantly ($p < 0.05$) influenced all measured morphophysiological and biochemical parameters. Plants grown at 35°C showed maximal biomass production (57.80 g fresh weight; 2.4-fold higher than at 25°C) but exhibited oxidative stress (8.7 nmol/g MDA) and altered metabolite profiles. The 25°C regime optimized the balance between yield and nutritional quality, maintaining high photosynthetic efficiency (Fv/Fm = 0.80) while accumulating beneficial phytochemicals (15.2 mg GAE/g phenolics). Cooler temperatures (18°C) severely limited growth (4.55 g fresh weight) despite some metabolic advantages of high accumulation of antioxidant compounds. Principal component analysis distinguished treatment responses along two primary axes: PC1 (68.83% variance) separated 35°C-grown plants, characterized by vigorous growth (2.4-fold greater biomass than 25°C), from 18°C plants showing metabolic suppression ($p < 0.05$). PC2 (22.97% variance) revealed a secondary gradient of photosynthetic efficiency (Fv/Fm = 0.81 at 35°C vs. 0.74 at 18°C) and oxidative stress (MDA levels 2.7 vs. 2.4 nmol/g FW), with 25°C plants exhibiting optimal balance between these parameters. These findings establish that waterleaf employs distinct adaptation strategies across temperature regimes, with 25°C representing the optimal compromise between productivity and nutritional value. The study provides critical data for cultivating waterleaf as a sustainable crop under climate change, recommending temperature-specific management strategies tailored to either biomass production (35°C) or nutrient-dense leaf production (25°C).

1 | Introduction

Climate change poses a significant threat to global food and nutritional security, affecting food availability, accessibility, utilization, and stability. As the world population continues to grow and the demand for nutritious food increases, there is an urgent need to explore and develop underutilized food crops that can withstand changing environmental conditions [1–3].

Waterleaf (*Talinum triangulare* (Jacq.) Wild.), also known as Ceylon spinach or Surinam purslane, is an indigenous

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tropical leafy vegetable cultivated across Africa, Asia, and Central and South America [4]. This perennial herbaceous plant, belonging to the Talinaceae family, typically grows to a height of 1–3 m and is characterized by succulent stems, spirally arranged leaves, and small pink self-pollinating flowers [5]. Nutritionally, waterleaf is a rich source of essential minerals including calcium, magnesium, potassium, iron, copper, and zinc, as well as vitamins A, C, and E [5, 6]. It also contains squalene, which may contribute to its potential anticancer properties, particularly against colon and breast cancer [7] as well as anti-inflammatory properties [8]. Additionally, waterleaf has been associated with cardiovascular health benefits and weight management due to its high fiber content [9]. In traditional medicine, waterleaf has been utilized to treat various ailments including measles, asthma, scabies, anemia, and hypertension [8]. Its potential health benefits, coupled with its nutritional profile, make waterleaf an attractive food and nutrition security crop for cultivation and consumption. As waterleaf gains attention in regions like Nigeria, Benin, Ghana, Kenya, and other developing countries, there is a pressing need to establish optimal growing conditions for optimum yield.

The impact of rising global temperatures on crop production is a matter of serious concern. Many staple crops including leafy vegetables such as lettuce, amaranths, and spinach are showing decreased yields and nutritional quality as temperatures increase, threatening food security for millions of people worldwide [10, 11]. In this context, understanding the response of alternative crops like waterleaf to various temperature regimes becomes critically important.

Waterleaf represents a promising crop that could play a crucial role in enhancing food security and dietary diversity in the face of climate change. Focusing on underutilized crops like waterleaf aligns with global efforts to increase agricultural biodiversity, which is key to creating resilient food systems [2, 3, 12, 13]. Understanding how waterleaf's nutritional qualities are affected by temperature changes is vital for assessing its potential contribution to nutritional security under future climate scenarios. There is a significant lack of comprehensive research on the temperature responses of many underutilized crops, including waterleaf [14, 15]. This knowledge gap emphasizes the urgent need for rigorous scientific research to understand the agronomic and nutritional properties of waterleaf, thereby potentially facilitating its integration into sustainable agricultural systems and enhancing food security in regions where it is endemic [1, 3].

Our study aims to provide a comprehensive assessment of waterleaf's performance under different temperature regimes. By examining both morphophysiological and biochemical responses, we seek to offer valuable insights that can inform agricultural practices, future breeding programs, and policy decisions aimed at enhancing food and nutrition security in the face of climate change and population growth [1]. This research represents a critical step toward harnessing the potential of underutilized crops to address one of the most pressing challenges of our time: ensuring global food security in a changing climate. This knowledge will be fundamental in promoting the adoption and sustainable production of this nutritionally valuable crop.

2 | Materials and Methods

2.1 | Study Area, Plant Materials, and Growth Conditions

The experiment was carried out in the Department of Plant, Food, and Environmental Sciences at Dalhousie University, Truro, Canada, between January and August 2024. Waterleaf seeds were initially sown in 32-cell pack trays. After 2 weeks, the seedlings were transferred to 2.84 L pots containing a soilless growth medium (Pro-Mix® BX). A balanced NPK fertilizer (20-20-20 Miracle Gro®), sourced from Canadian Tire in Truro, NS, was applied weekly at the recommended concentration of 6 mL/L. Fertilization continued until the final harvest, conducted 4 weeks after transplanting (WAT). Throughout the growth period, plants were watered consistently to maintain soil moisture at field capacity.

2.2 | Experimental Treatments and Design

The study investigated the effects of different temperature regimes on waterleaf growth using CONVIRON controlled growth chambers (GC) (Controlled Environments Limited, Manitoba, Canada). Plants were exposed to daytime temperatures of 35°C, 25°C, 22°C, and 18°C under a 12-h photoperiod with a light intensity of 150 μmol·m^{-2}·s^{-1}. The experiment followed a completely randomized design with six replicates per treatment. Each replicate consisted of six plants randomly assigned to different temperature conditions. To minimize potential bias from environmental variations within the growth chambers, a pseudo-replication approach was implemented. This involved weekly rearrangement of the pots, a technique previously described by Schank and Koehnle [16] and later utilized in studies by Abbey and Rao [17], Nutsukpo et al. [18], and Ofori [19]. The entire experiment was conducted twice to ensure reproducibility.

2.3 | Plant Growth, Fresh and Dry Weight

Plant growth parameters were assessed 4 WAT. Plant height was determined by measuring from the base to the apex of the tallest leaf using a standard ruler. Leaf length was recorded from the petiole junction with the stem to the leaf tip. The stem diameter at the base of each plant was measured using a digital Vernier caliper (Mastercraft, ON, Canada). The total number of leaves and branches per plant was counted and documented for each treatment. Leaf area was quantified using an LI-3100 leaf area meter (LI-COR Inc., Lincoln, Nebraska, USA).

2.4 | Leaf Greenness and Photosynthetic Activities

Leaf chlorophyll content was quantified using a SPAD-502 chlorophyll meter (Spectrum Technologies Inc., Aurora, IL, USA), with measurements taken on the third and fourth fully expanded leaves of each plant. Photosynthetic functionality was assessed through chlorophyll fluorescence analysis conducted with a portable chlorophyll fluorometer (Opti-Sciences, Inc., Hudson, NY, USA). Prior to fluorescence measurements, the same leaves (excluding midrib sections) were dark-adapted for 25 min using specialized leaf clips. Fluorescence parameters were calculated as follows: variable fluorescence (Fv) was determined by subtracting minimum fluorescence (Fo) from maximum fluorescence (Fm) (Fv = Fm − Fo). The maximum

photochemical efficiency of photosystem II (PSII) was expressed as the Fv/Fm ratio, while the potential activity of PSII reaction centers was represented by the Fv/Fo ratio.

Photosynthesis parameters were examined using an LCi-SD portable photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, UK). The middle portion of the third and fourth leaves (avoiding midrib areas) was positioned in the measurement chamber for a 3-min period to record multiple photosynthetic parameters. These included net photosynthetic rate (A), stomatal conductance (Gs), transpiration rate (E), and intercellular CO_2 concentration (Ci).

2.5 | Biochemical Analysis

At the experimental endpoint (4 weeks post-transplanting), mature, healthy leaves from each treatment group were collected and flash-frozen in liquid nitrogen to preserve biochemical integrity. The frozen leaf tissue was subsequently pulverized to a homogeneous powder using a prechilled mortar and pestle, with care taken to prevent thawing during processing. The resulting powder was aliquoted into sterile microcentrifuge tubes and maintained at −80°C until required for downstream biochemical assays.

2.6 | Chlorophylls and Carotenoid Assay

Photosynthetic pigment concentrations were determined following established spectrophotometric procedures by Linchtenthaler [20]. Fresh leaf tissue (0.2 g) was mechanically homogenized in 2 mL of ice-cold 80% acetone solution. The resulting slurry was centrifuged at 15000 × g for 15 min at 4°C to obtain a clarified pigment extract. Absorbance measurements of the supernatant were conducted at three specific wavelengths: 663.2 nm (chlorophyll a), 646.8 nm (chlorophyll b), and 470 nm (carotenoids). Spectrophotometric readings were referenced against an 80% acetone blank for baseline correction. Final concentrations were calculated and normalized to fresh weight ($\mu g \cdot g^{-1}$ FW). All extractions and measurements were performed under low-light conditions to prevent pigment degradation.

2.7 | Total Phenolics Content Determination

The total phenolic content in leaf samples was quantified using an optimized Folin-Ciocalteu method by Ainsworth and Gillespie [21]. Leaf tissue (0.2 g fresh weight) was homogenized in 1.5 mL of chilled 95% methanol and extracted in complete darkness at ambient temperature for 48 h. Following extraction, the homogenate was clarified by centrifugation at 13000 × g for 5 min. For colorimetric analysis, 100 μL of the methanolic extract was reacted with 200 μL of 10% (v/v) Folin-Ciocalteu reagent for 5 min with constant agitation. The reaction was then developed by adding 800 μL of 700 mM sodium carbonate solution. After thorough mixing, the samples were protected from light and maintained at room temperature for a 2-h incubation period to allow full color development. Absorbance measurements were performed at 765 nm using a spectrophotometer, with appropriate reagent blanks for baseline correction. Total phenolic content was determined by comparison with a standard curve prepared from gallic acid solutions (0–500 μg/mL) and expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

2.8 | Total Flavonoids Content Analysis

Total flavonoid content was determined using an adapted colorimetric method by Chang et al. [22]. Fresh leaf tissue (0.2 g) was homogenized in 1.5 mL of ice-cold 95% methanol, then centrifuged at 15000 × g for 10 min at 4°C to obtain a clear supernatant. For the assay, 500 μL of supernatant was combined with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was thoroughly mixed and allowed to stand at room temperature for 30 min to develop color. Absorbance was measured at 415 nm using a spectrophotometer, with a blank sample (prepared without aluminum chloride) serving as the reference. Total flavonoid content was quantified against a quercetin standard curve and expressed as percentage flavonoid content relative to fresh weight.

2.9 | Total Soluble Sugar Determination

Total soluble sugars were quantified using the phenol-sulfuric acid method by Dubois et al. [23]. Leaf samples (0.2 g) were extracted in 10 mL of 90% ethanol, followed by vortex mixing for 3 min and incubation at 60°C for 60 min. The extract volume was adjusted to 5 mL with 90% ethanol and centrifuged at 12000 × g for 3 min. For color development, 1 mL of supernatant was combined with 1 mL of 5% phenol in a glass test tube. After thorough mixing, 5 mL of concentrated sulfuric acid was added rapidly, followed by immediate vortexing for 20 s. The reaction mixture was kept in darkness for 15 min to allow color development. Following cooling to room temperature, absorbance was measured at 490 nm using a blank containing deionized water, phenol, and sulfuric acid as reference. Sugar concentrations were determined from a glucose standard curve (0–100 μg/mL) and expressed as glucose equivalents per gram fresh weight (μg/g FW).

2.10 | Total Ascorbate Determination

Total ascorbate content was determined using an adapted protocol from Ma et al. [24]. Fresh leaf samples (0.2 g) were homogenized in 1.5 mL of ice-cold 5% trichloroacetic acid (TCA). The homogenate was vortexed for 2 min and centrifuged at 12000 × g for 10 min at 4°C to obtain a clear supernatant. For analysis, 100 μL of supernatant was mixed with 400 μL of phosphate buffer (150 mM KH_2PO_4, pH 7.4, containing 5 mM EDTA), followed by the addition of 100 μL of 10 mM dithiothreitol (DTT) and vortexing for 30 s. Color development was achieved by adding 400 μL each of 10% TCA, 44% phosphoric acid, and 4% α, α'-dipyridyl in 70% ethanol, plus 200 μL of 3% ferric chloride. The mixture was incubated at 40°C for 60 min to develop color. Absorbance was measured at 525 nm, with concentrations calculated using an L-ascorbic acid standard curve (0–100 μM) and expressed as μmol/g fresh weight.

2.11 | Total Protein Content Quantification

Protein extraction was performed by homogenizing 0.2 g of lyophilized plant material in 3 mL of chilled extraction buffer (50 mM potassium phosphate, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone and 0.1 mM EDTA. The homogenate was centrifuged at 15000 × g for 20 min at 4°C to pellet cellular debris. The resulting supernatant, containing soluble proteins, was carefully transferred to prechilled microcentrifuge tubes

TABLE 1 | Morphological characteristics of waterleaf grown under different temperature regimes (18°C, 22°C, 25°C, and 35°C).

Temperatures	Plant height (cm)	Leaf length (cm)	Leaf number	Leaf area (cm^2)	Branch number	Stem girth (mm)	Fresh weight (g)	Dry weight (g)
35°C	31.68a	14.84ab	114.5a	33.93b	12.75a	7.89a	57.80a	3.60a
25°C	25.03b	20.71a	24.25b	49.54a	5.00b	6.17b	23.20b	1.06b
22°C	23.00b	12.63ab	23.25b	45.20a	4.25b	5.17b	17.03c	0.82c
18°C	13.48c	8.74b	10.00c	21.98c	0.00c	3.57c	4.55d	0.20d
p value	0.001	0.007	0.001	0.001	0.001	0.001	0.001	0.001

Note: Values represent means ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$, Tukey's HSD test) in a column.

maintained on ice. Protein quantification was conducted using the Bradford assay [25]. Briefly, 100 μL of the crude extract was mixed with 5 mL of Bradford reagent and allowed to incubate for 5 min at room temperature. Absorbance readings were taken at 595 nm using a spectrophotometer. A standard curve was prepared using bovine serum albumin (BSA) solutions ranging from 200 to 900 $\mu g \cdot mL^{-1}$ to calculate protein concentrations in the samples.

2.12 | Statistical Analysis

The experimental data were analyzed through one-way analysis of variance (ANOVA) implemented in Minitab statistical software (version 21; Minitab Inc., State College, PA, USA). Post hoc comparisons of treatment means were performed using Tukey's honestly significant difference (HSD) test at a 95% confidence level ($\alpha = 0.05$). To examine relationships between variables, we conducted bivariate correlation analysis. Additionally, multivariate patterns in the dataset were explored using two-dimensional principal component analysis (PCA), performed with XLSTAT statistical software (version 2022.3; Addinsoft, New York, USA). Graphical representations of the results were generated using GraphPad Prism (GraphPad Software, San Diego, CA).

3 | Results

The study revealed significant temperature-dependent variations in the morphophysiological and biochemical characteristics of waterleaf (*Talinum triangulare*). The morphological analysis revealed striking temperature-dependent growth patterns in waterleaf (Table 1). Plants grown at 35°C exhibited the highest plant height, representing a 1.3-fold increase compared to those at 25°C and a 2.4-fold increase relative to plants at 18°C (Figure 1). Leaf production showed a particularly strong temperature response, with plants at 35°C producing approximately 4.7-fold more leaves than those at 25°C and 11.5-fold more than those at 18°C. Leaf length followed a different pattern, with maximum leaf length at 25°C exceeding 35°C values by 1.4-fold and 18°C by 2.4-fold. Interestingly, plants grown at 25°C and 22°C produced broader leaves, with areas of 49.54 cm^2 and 45.20 cm^2, respectively. In contrast, plants grown at 35°C and 18°C had smaller leaves, with areas of 33.93 cm^2 and 21.98 cm^2, respectively (Figure 1).

Stem development exhibited trends consistent with overall growth, with stem girth at 35°C being 1.3-fold, 1.5-fold, and 2.2-fold greater than at 25°C, 22°C, and 18°C, respectively. Branching was most pronounced at 35°C, representing a 2.6-fold and 3-fold increase over 25°C and 22°C, respectively, and complete absence of branching at 18°C (Figure 1). Biomass accumulation showed similar temperature sensitivity, with fresh weight at 35°C being 2.5-fold higher than at 25°C, 3.4-fold higher than at 22°C, and 12.7-fold greater than at 18°C. The dry weight ratio followed this pattern exactly, confirming consistent biomass partitioning across temperatures.

Physiological measurements demonstrated temperature-dependent responses in photosynthetic efficiency. The SPAD values, indicating chlorophyll content, were highest at 35°C, showing 1.3-fold and 1.6-fold increase compared to 25°C and 18°C, respectively (Table 2). However, photosynthetic efficiency showed a different pattern, with the maximum quantum efficiency of PSII (Fv/Fm) remaining relatively stable across higher temperatures, thus, 0.81 at 35°C vs 0.80 at 25°C but declining significantly at 18°C. Stomatal conductance (gs) peaked at 25°C, representing a 3.4-fold increase over the 35°C treatment. This pattern was also shown in transpiration rates (E), with maximum values at 25°C being 2.6-fold higher than at 35°C. On the other hand, the net photosynthetic rate (A) showed temperature resilience, maintaining similar levels at 35°C and 25°C, but decreasing significantly at 22°C and 18°C. Intercellular CO_2 concentration (Ci) was lowest at 35°C, representing a 1.4-fold reduction compared to 25°C.

The biochemical analysis revealed complex temperature-dependent patterns across all measured metabolites. Photosynthetic pigments exhibited differential responses to temperature regimes (Figure 2). Chlorophyll *a* concentration reached its highest level at 25°C, showing a 23% reduction at 35°C and a 49% decrease at 18°C. Chlorophyll b demonstrated similar sensitivity, with 25°C values exceeding those at 35°C by 12.8% and at 18°C by about 47%. Carotenoid content exhibited similar pattern, maintaining optimal levels at 25°C with 12.5% and 60% reductions at 35°C and 18°C, respectively.

The carbohydrate metabolism analysis revealed contrasting temperature responses. Total soluble sugars showed an inverse relationship with temperature, with maximum accumulation at 18°C that declined by 22.4% at 25°C and 44.6% at both 22°C and 35°C. Protein content displayed an inverse temperature dependence, reaching maximum levels at 18°C and declining progressively at both higher and lower temperatures—showing 22%, 27%, and 33% reductions at 22°C, 25°C, and 35°C, respectively. Maximum ascorbate content occurred at 18°C, with temperature-dependent reductions of 14%, 20%, and 26.5% at 22°

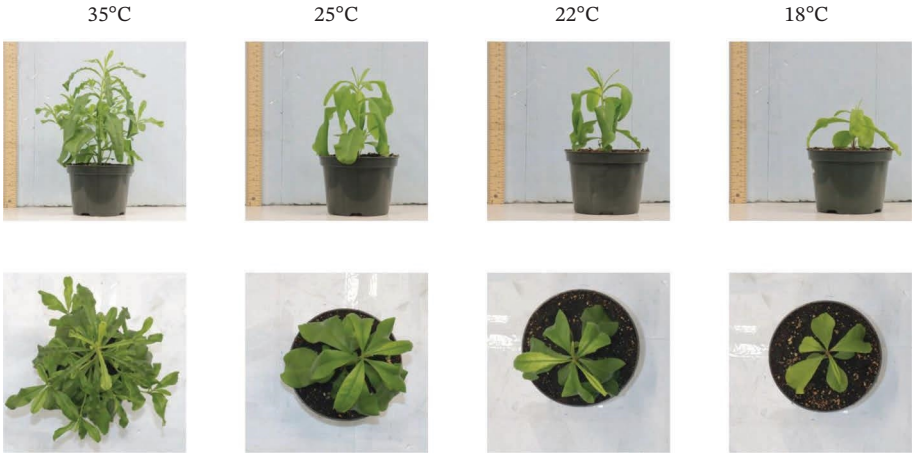


FIGURE 1 | Phenotypic variation in waterleaf plants grown at 35°C, 25°C, 22°C, and 18°C. The top panel displays a frontal (side) view, while the lower panel shows an aerial (top-down) view of potted plants at 4 weeks after transplanting.

C, 25°C, and 35°C, respectively. Malondialdehyde (MDA) accumulation, an indicator of oxidative stress, showed temperature-dependent increases, peaking at 35°C. In contrast, plants cultivated at 18°C, 22°C, and 25°C showed similar MDA levels, all approximately 22% lower than the maximum observed at 35°C (Figure 3).

The antioxidant system components displayed temperature-specific regulation (Figure 4). Total phenolic content peaked at 35°C, declining by approximately 25.5% at 22°C and by 32% at both 22°C and 18°C. Flavonoid accumulation followed an identical trend, reaching its maximum at 35°C and showing progressive decreases of 16% at 25°C, 39% at 22°C, and 57% at 18°C relative to the peak value.

The 18°C treatment resulted in significant metabolic suppression, particularly affecting photosynthetic efficiency and growth parameters. Meanwhile, the 22°C condition displayed intermediate characteristics, bridging the responses observed at 18°C and 25°C. These multivariate patterns confirm that waterleaf undergoes coordinated physiological and biochemical adjustments across different temperature regimes, with 25°C representing the most favorable condition for balanced growth and metabolic homeostasis.

3.1 | PCA of Waterleaf Responses to Temperature Variation

PCA effectively discriminated the temperature treatments based on their integrated morphophysiological and biochemical profiles (Figure 5).

PCA employed in this study offers a powerful multivariate perspective on the integrated responses of waterleaf to temperature stress, revealing not only treatment segregation but also fundamental connections between morphophysiological and biochemical variables. The first two principal components collectively explained 91.80% of the total variance, with PC1 accounting for 68.83% and PC2 contributing 22.97% of the variation. The clear separation along PC1 (68.83% of variance) shows a primary trade-off between growth-related parameters and stress-induced metabolic responses. Variables such as plant height, leaf number, fresh weight, and stem girth loaded strongly on the positive axis, closely associated with the 35°C treatment. Conversely, the negative PC1 axis was characterized by elevated levels of soluble sugars, proteins, and ascorbate metabolites that accumulated significantly under cooler conditions (18°C). PC2 (22.97% of variance) further refined this interpretation by highlighting a gradient related to photosynthetic performance and oxidative stress. Photosynthetic parameters such as Fv/Fm and stomatal conductance (gs) loaded positively, aligning with the 25°C treatment, which exhibited optimal photosynthetic efficiency. In contrast, MDA, a marker of oxidative damage and phenolic compounds loaded negatively, correlated strongly with the 35°C treatment.

4 | Discussion

The impact of rising global temperatures on crop production is a matter of serious concern. Many staple crops including leafy vegetables such as lettuce, amaranths, and spinach are showing decreased yields and nutritional quality as temperatures

TABLE 2 | Physiological response of waterleaf plants exposed to varying temperature conditions (18°C, 22°C, 25°C, and 35°C).

Temperatures	SPAD value	Fv/Fm	Fv/Fo	A (μmol·m^{-2}·s^{-1})	E (mol·m^{-2}·s^{-1})	Ci (μmol·mol^{-1})	gs (mol·m^{-2}·s^{-1})
35°C	35.21a	0.81a	4.32a	8.50a	2.24b	277.63b	0.11b
25°C	26.54b	0.80b	3.90b	8.38a	5.79a	394.63a	0.37a
22°C	25.48b	0.79b	3.71b	5.03a	2.87b	367.00a	0.14b
18°C	21.69c	0.74c	2.81c	6.64a	3.91ab	399.38a	0.22ab
p value	0.001	0.001	0.001	0.242	0.004	0.001	0.003

Note: Values represent mean ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$, Tukey's HSD test) in a column. Fv/Fm, Maximum quantum yield of PSII; Fv/Fo, electron transport rate; A, net photosynthetic rate; Ci, stomatal conductance; E, transpiration rate; SPAD value, chlorophyll content index.

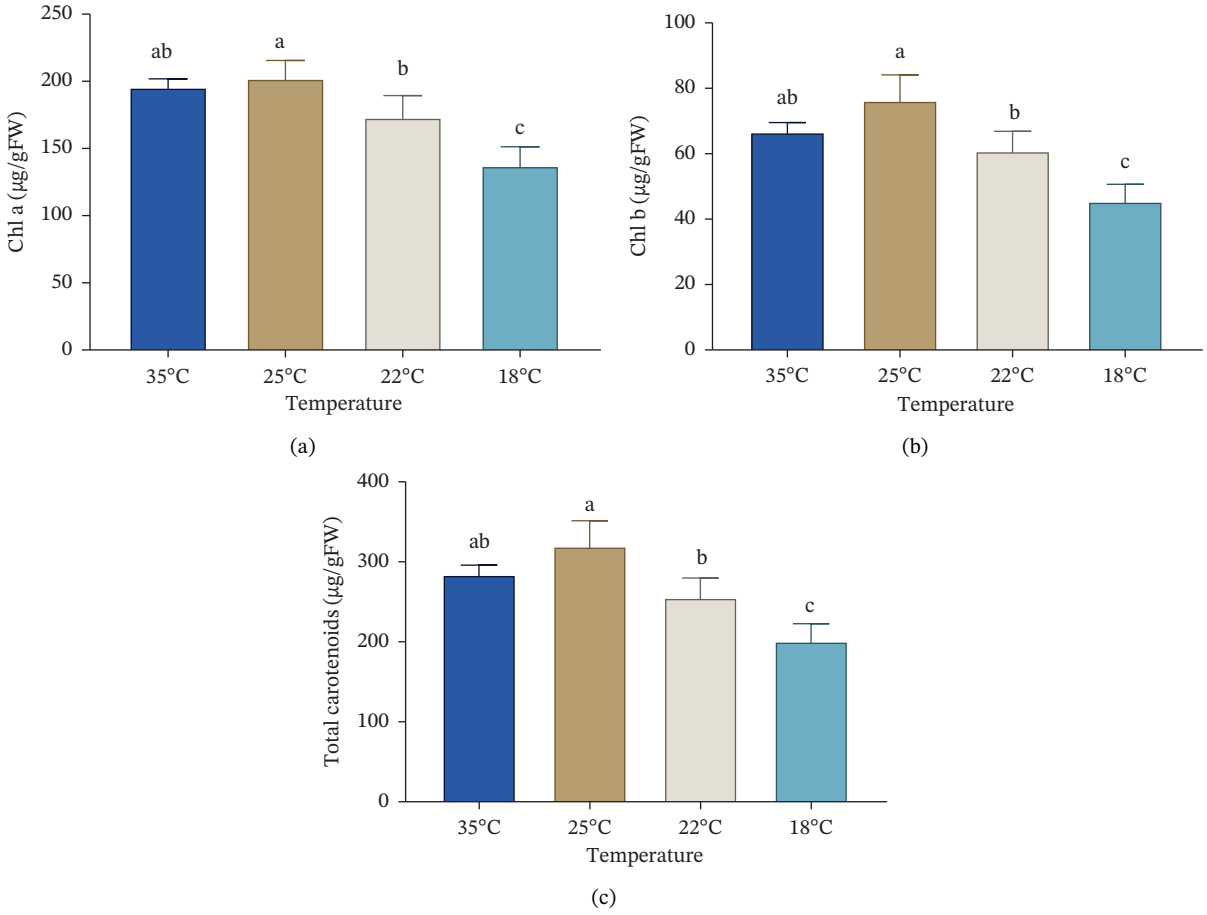


FIGURE 2 | Photosynthetic pigment composition: (a) chlorophyll a, (b) chlorophyll b, and (c) total carotenoid concentrations in waterleaf leaves across temperature treatments. Bars show mean ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$, Tukey's HSD test).

increase, threatening food security for millions of people worldwide [10, 11]. In this context, understanding the response of alternative crops like waterleaf to various temperature regimes becomes critically important.

The present study provides a comprehensive evaluation of the morphophysiological and biochemical responses of waterleaf (*Talinum triangulare*) grown under different temperature regimes, offering critical insights into its adaptability and potential as a climate-resilient crop. The results demonstrate a clear temperature-dependent variation in growth, physiological performance, and biochemical composition, highlighting a trade-off between biomass production and phytochemical accumulation [26, 27]. Thus, waterleaf exhibited remarkable phenotypic plasticity in response to temperature variation, demonstrating distinct adaptation strategies across different thermal regimes. This fundamental trade-off between biomass production and metabolic regulation revealed by our findings provides important implications for cultivating this underutilized crop under changing climatic conditions.

The morphological responses demonstrate that while 35°C promotes maximum biomass production, 25°C may represent a better compromise between growth quantity and quality, as evidenced by larger individual leaves despite lower total leaf numbers. The superior vegetative growth observed at 35°C, evidenced by 2.4-fold greater plant height and 11.5-fold higher leaf production compared to 18°C, suggests waterleaf possesses inherent thermotolerance mechanisms. This aligns with its tropical origins [5] and exceeds the thermal tolerance reported for conventional leafy vegetables like spinach [11]. However, the reduced leaf area at 35°C (33.93 cm^2) vs. (49.54 cm^2) at 25°C indicates potential heat-induced limitations in cellular functions [28, 29], a phenomenon documented in other heat-stressed crops such as wheat [30], potato [31], and lettuce [32].

Our physiological data revealed a complex temperature adaptation strategy. While chlorophyll content peaked at 35°C, the maintenance of stable Fv/Fm ratios up to 25°C suggests photosystem II remains functional across a broad thermal range [33, 34]. The optimal stomatal conductance at 25°C coupled with sustained photosynthetic rates at 35°C, demonstrates waterleaf's ability to balance carbon fixation with water conservation under heat stress [35], a critical trait for climate-resilient crops. Under extreme temperatures, waterleaf may maintain water balance and cellular homeostasis which is a unique trait also observed in alfalfa [36]. The 3.4-fold higher stomatal conductance at 25°C compared to 35°C may reflect strategic water conservation under extreme heat, consistent with observations in other drought-adapted species [37–39].

The biochemical profiles demonstrate temperature-mediated metabolic reprogramming with nutritional implications. The peak phenolic and flavonoid content at 35°C contrasts with

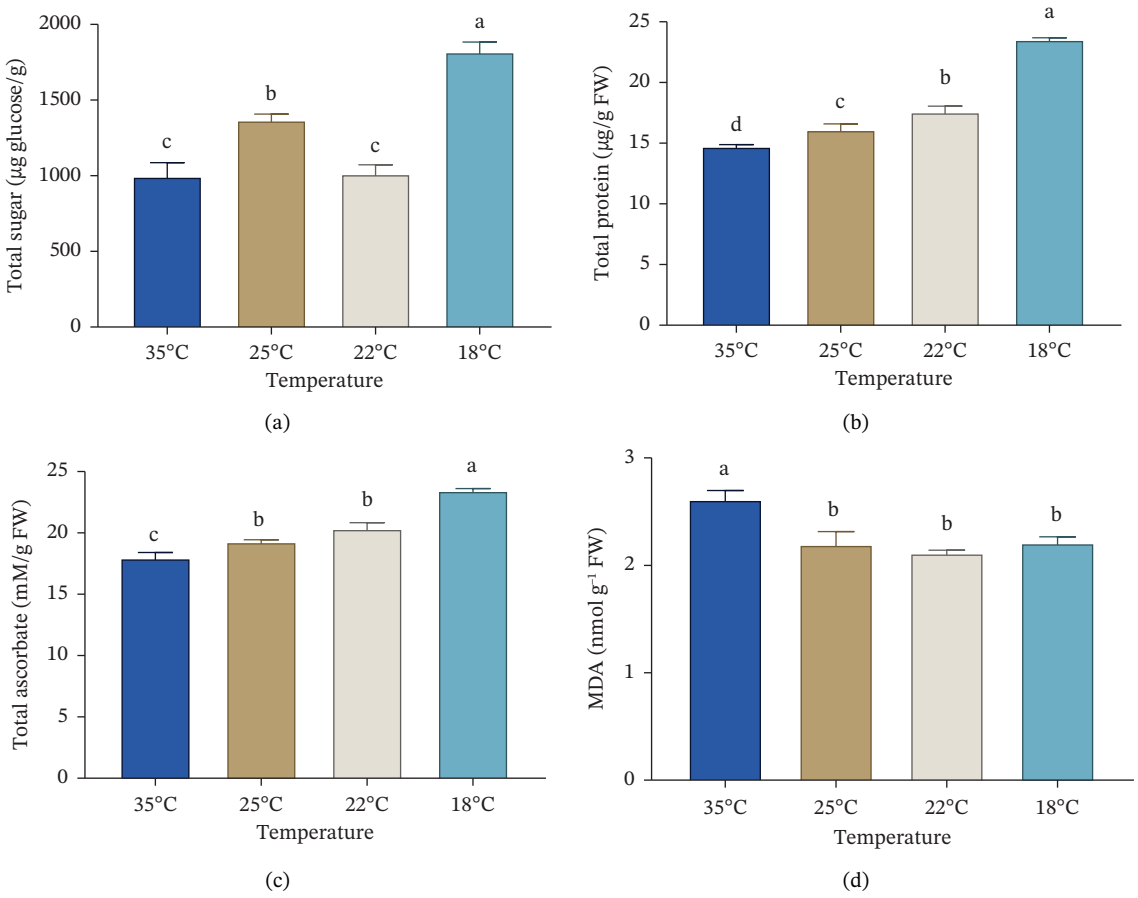


FIGURE 3 | Primary metabolic responses: (a) soluble sugars, (b) protein content, (c) ascorbate levels, and (d) malondialdehyde (MDA) accumulation in waterleaf plants grown at four temperatures. Bars show mean ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$, Tukey's HSD test).

typical stress-response patterns, suggesting waterleaf may employ alternative antioxidant strategies under heat stress. This is supported by the concurrent 26.5% reduction in ascorbate at 35°C and elevated MDA levels, indicating potential oxidative stress despite high phenolic accumulation [40, 41]. The inverse temperature relationship shown by soluble sugars (maximum at 18°C) versus proteins (maximum at 18°C) reveals fundamental shifts in carbon partitioning [42, 43], possibly

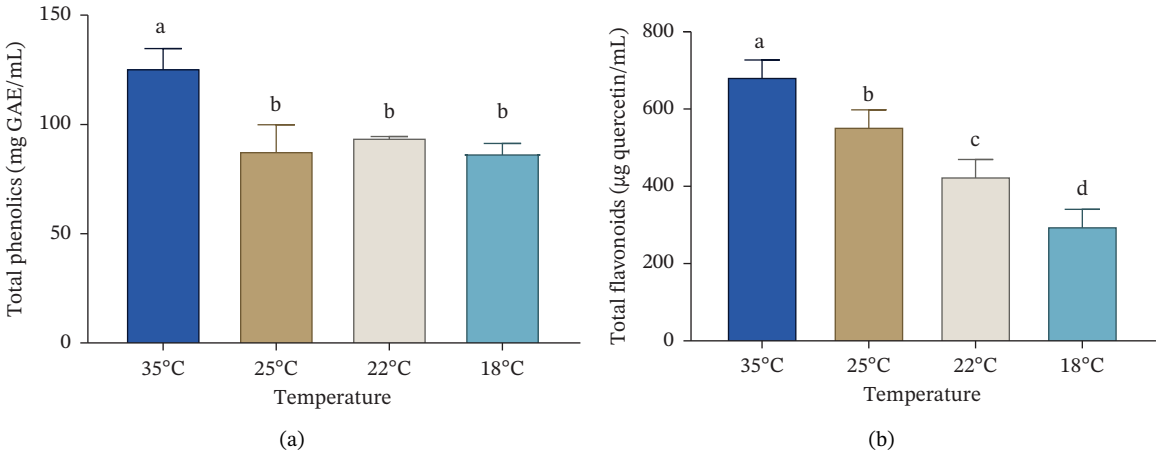


FIGURE 4 | Secondary metabolite accumulation in waterleaf leaves: (a) total phenolics and (b) flavonoid content under different temperature conditions. Bars show mean ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$, Tukey's HSD test).

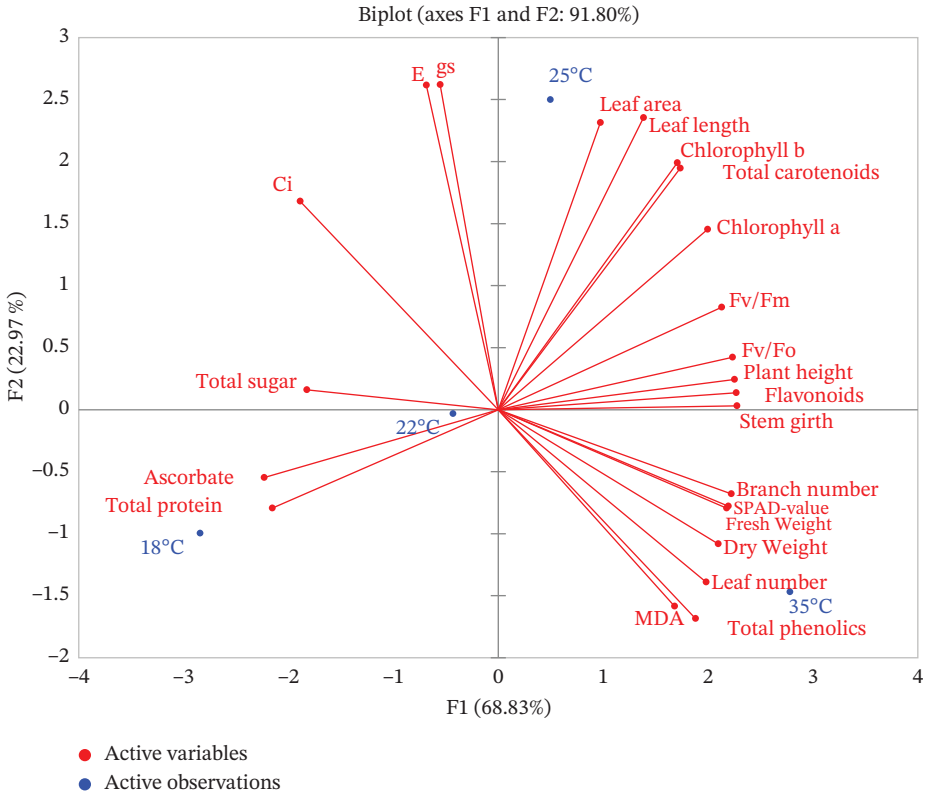


FIGURE 5 | Principal component analysis of waterleaf (*Talinum triangulare*) responses to different temperature regimes. (a) Score plot showing separation of treatments along PC1 (68.83% variance) and PC2 (22.97% variance). Ellipses represent 95% confidence intervals for each temperature group ($n = 6$). (b) Loading plot displaying variable contributions, where vector length and direction indicate influence on component axes. Key: Red = morphophysiological and biochemical parameters; Blue = temperature treatments (18°C, 22°C, 25°C and 35°C). Physiological parameters abbreviated as Fv/Fm (PSII efficiency), Fv/Fo (Electron transport rate), A (Net photosynthetic rate), Ci (Stomatal conductance), E (Transpiration rate), SPAD value (Chlorophyll content index), and gs (stomatal conductance); biochemical as MDA (malondialdehyde).

reflecting altered sink–source relationships under different thermal regimes [44].

PCA revealed a clear trade-off between growth and stress mitigation. High temperature (35°C) promoted biomass accumulation but was associated with oxidative stress markers like MDA, indicating a significant physiological cost. Conversely, cooler conditions (18°C) favored the accumulation of protective osmolytes and antioxidants, shifting the plant's strategy toward defense. Critically, the PCA results provide integrative evidence that 25°C represents the optimal compromise, sustaining growth while maintaining metabolic homeostasis. The clear separation of treatments along PC1 (62.3% variance), primarily loaded by growth parameters versus antioxidant compounds, shows the physiological cost of thermal adaptation. These patterns align with recent findings in other crops, supporting the concept that nutritional quality and yield are often inversely related under abiotic stress [45–47]. This demonstrates that waterleaf employs distinct, temperature-dependent adaptive strategies to balance growth with metabolic protection.

These results carry significant implications for multiple agricultural applications. For cultivation practices in warming climates, they suggest strategic temperature management could optimize either biomass or nutritional quality depending on production goals. Breeding programs targeting climate resilience may benefit from selecting genotypes that maintain the favorable traits observed at 25°C under warmer conditions. Furthermore, the temperature-dependent variation in bioactive compounds supports potential nutritional interventions [48, 49] using waterleaf as a functional food with customizable phytochemical profiles.

5 | Conclusion

This study demonstrates that waterleaf exhibits distinct temperature-dependent strategies for growth and metabolic regulation. While cultivation at 35°C maximizes biomass production, it triggers oxidative stress and alters metabolite profiles, potentially compromising leaf quality. In contrast, 25°C emerges as the optimal temperature, providing a critical balance between yield and nutritional value by maintaining robust photosynthetic performance while accumulating beneficial phytochemicals. The 18°C regime severely restricts productivity despite some metabolic advantages, whereas 22°C induces transitional responses between cold-limited and optimal growth conditions.

These findings establish waterleaf as a promising climate-resilient crop, with its phenotypic plasticity offering adaptive potential for tropical agriculture under warming scenarios. However, they also underscore the need for strategic temperature management—targeting 35°C for maximal biomass or 25°C for

quality-focused production—depending on whether the crop is destined for fresh markets or nutraceutical applications.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data presented in this study are available upon request from the corresponding author.

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