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Climate Change and Its Influence on Anatomical and Physiological Features of Pomegranate (Punica Granatum L.) Leaves

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ABSTRACT: Climate change presents a significant barrier to agricultural sustainability, particularly in arid and semiarid zones. Pomegranate (*Punica granatum* L.) is a crop of major nutritional and economic importance in Najaf, Iraq, where prolonged heat waves and scarce rainfall increasingly threaten orchard viability. This study investigated anatomical and physiological adjustments in leaves of the local Hamadhy cultivar and the introduced 'Wonderful' cultivar across two growing seasons. Measured traits included epidermal thickness, mesophyll structure, stomatal density, chlorophyll levels, proline accumulation, and cuticular wax deposition. Findings showed reductions in leaf area and chlorophyll content under summer stress, accompanied by thicker epidermis layers, higher proline concentration, and greater wax accumulation. 'Wonderful' maintained larger leaf area and higher pigment levels, whereas Hamadhy accumulated more proline, reflecting differing adaptive strategies. These outcomes emphasize the need for cultivar selection and climatesmart practices to sustain pomegranate production under intensifying climate stress .

KEYWORDS: Climate change, Pomegranate, Leaf anatomy, Physiology, Iraq, Adaptation

1. INTRODUCTION

Agricultural systems worldwide are increasingly challenged by climate variability, including rising global temperatures, erratic rainfall, and more frequent extreme events. These effects are particularly severe in the Middle East and North Africa (MENA), where limited water resources and fragile soils exacerbate vulnerability [1,2,3].

Pomegranate (*Punica granatum* L.), an ancient fruit crop, is well adapted to semiarid regions and has become vital for both food security and income generation in Iraq. Nevertheless, orchards in Najaf experience extreme summer heat often exceeding 45°C and declining rainfall, which jeopardize productivity and fruit quality [4].

Leaves are highly sensitive to environmental fluctuations. Their structural and physiological traits including epidermal thickening, mesophyll adjustments, stomatal responses, pigment stability, and osmolyte accumulation serve as indicators of stress adaptation [5,6]. While international studies have reported such responses in Iran, Morocco, and India, relatively little is known about how Iraqi cultivars respond compared with introduced varieties such as 'Wonderful.'

This study addresses that gap by examining leaf anatomical and physiological responses of the local Hamadhy cultivar and the introduced 'Wonderful' under Najaf's climate. The objective was to evaluate adaptation strategies, highlight cultivar specific resilience, and propose practices for climate smart pomegranate cultivation in Iraq

2. MATERIALS AND METHODS

2.1 Study Area

The fieldwork was carried out in Najaf Governorate (32°00′N, 44°20′E), central Iraq. The climate is classified as extremely hot desert with summer temperatures up to 46°C, annual rainfall of 100–150 mm concentrated in winter, and relative humidity dropping to 15% in summer [2]. Soils are sandyloam, moderately saline, and poor in organic matter.

2.2 Plant Material

Two cultivars were selected:

Hamadhy (local): A traditional Najafi cultivar noted for its acidity and tolerance to drought.

Wonderful (introduced): A globally cultivated cultivar recognized for productivity and adaptability [1].

2.3 Sampling Design

Sampling was conducted in 2023 and 2024 at three stages:

Spring growth (April),

Peak summer stress (July–August),

Autumn recovery (October).

Five trees per cultivar were selected, and 20 fully expanded leaves were collected per tree.

2.4 Anatomical Analysis

Leaf cross sections (15–20 µm) were prepared using standard histological methods, stained with safranin and fast green, and observed under light microscopy. Traits measured included epidermis thickness, mesophyll dimensions, stomatal density, and total leaf area, using ImageJ software [7].

2.5 Physiological Analysis

Chlorophyll content: Determined spectrophotometrically (Arnon's method).

Proline: Measured using the ninhydrin assay [8].

Cuticular wax: Extracted with chloroform and expressed as mg cm^{-2} leaf area [4, 5].

2.6 Environmental Monitoring

Meteorological data (temperature, humidity, rainfall) were obtained from Najaf Weather Station. Soil moisture was measured using TDR probes.

2.7 Statistical Analysis

Data were analyzed using two way ANOVA ($p < 0.05$). Means were separated using Tukey's HSD test. Correlation analysis and PCA were performed to identify trait interactions [6].

3 RESULTS

3.1 Climatic Conditions

Summer maxima reached 46.8°C in 2022 and 45.9°C in 2023, with rainfall restricted to winter months. Relative humidity dropped to 14–15% during summer, confirming severe stress conditions [7,8].

Table1. Climatic conditions in Najaf during 2023–2024 (temperature, rainfall, humidity) .

Year	Max Temp (°C)	Rainfall	Min RH (%)	Stress Condition
2023	46.8	Winter only	14-15	Severe
2024	45.9	Winter only	14-15	Severe

3.2 Anatomical Adjustments

The studied pomegranate cultivars exhibited distinct anatomical responses under water stress conditions table 1 and 2. Epidermal thickness showed a noticeable increase, with values rising by approximately 20% in Hamadhy and 15% in Wonderful , reflecting an adaptive reinforcement of the protective leaf layer. In contrast, the palisade mesophyll thickness decreased significantly, by about 28% in Hamadhy compared to 18% in Wonderful . Similarly, spongy mesophyll tissues exhibited reductions of 35% and 22% in Hamadhy and Wonderful , respectively, indicating a decline in internal leaf porosity and potential photosynthetic capacity .

Leaf area was markedly reduced, declining by 45% in Hamadhy and 30% in Wonderful , suggesting a strong morphological adaptation to minimize transpiration losses under drought stress. Stomatal density displayed a slight increase in Hamadhy , while it remained nearly unchanged in Wonderful , highlighting cultivar-specific variations in gas exchange regulation mechanisms Fig 1 and 2 this agree with [9, 10] . .

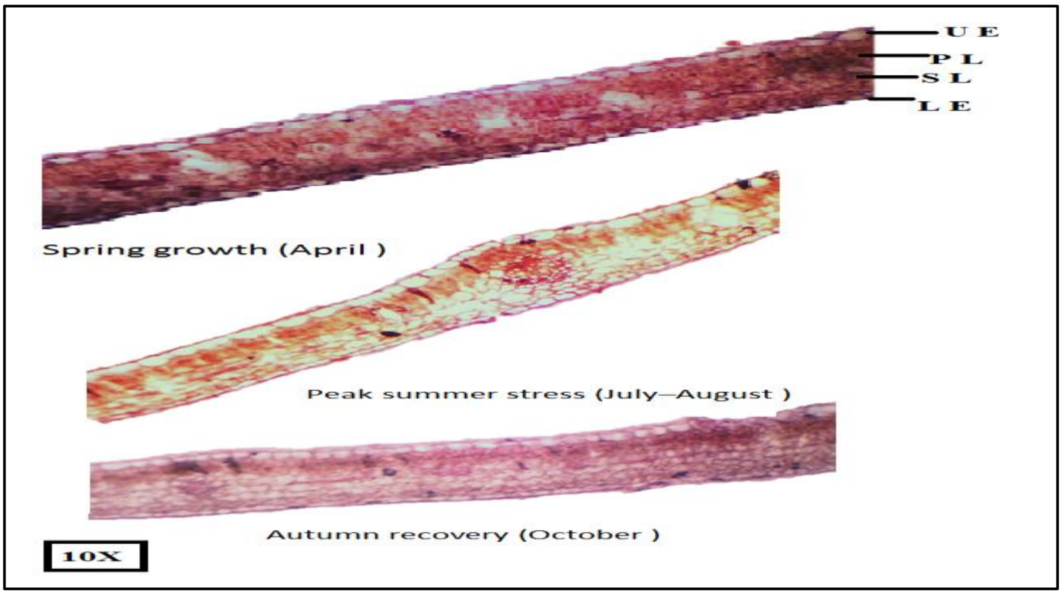

Figure 1. Microscopic cross section of Hamadhy leaf showing reduced mesophyll under stress. Were UE= Upper Epidermis, P L= Placid Layer, SL= Spongy Layer and LE= Lower Epidermis

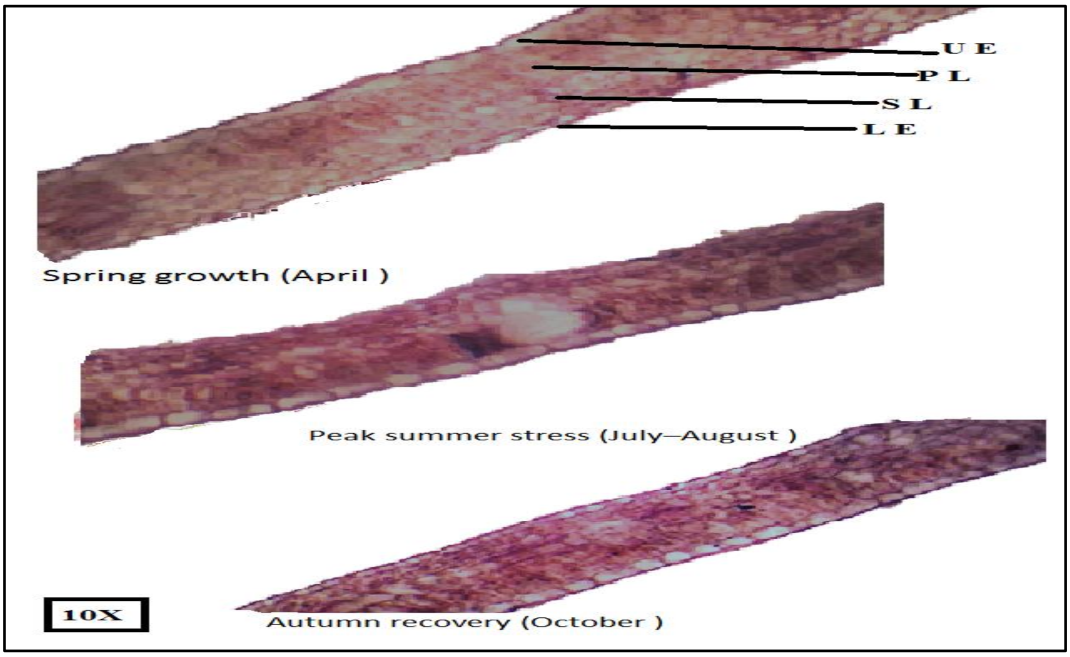

Figure 2. Microscopic cross section of Wonderful leaf showing thicker epidermis and moderate mesophyll reduction. Were UE= Upper Epidermis, P L= Placid Layer, SL= Spongy Layer and LE= Lower Epidermis.

Table 2. Leaf anatomical traits of Hamadhy and Wonderful cultivars under summer stress conditions.

Trait	Hamadhy (Control)	Hamadhy (Stress)	Change (%)	Wonderful (Control)	Wonderful (Stress)	Change (%)
Epidermis thickness (μm)	23.4 ± 1.2	28.1 ± 1.4	+20	22.8 ± 1.0	26.2 ± 1.1	+15
Palisade mesophyll (μm)	76.5 ± 2.3	55.1 ± 2.0	−28	78.2 ± 2.1	64.2 ± 1.8	−18
Spongy mesophyll (μm)	89.7 ± 2.5	58.3 ± 2.1	−35	91.2 ± 2.2	71.1 ± 2.0	−22
Leaf area (cm^2)	8.4 ± 0.4	4.6 ± 0.3	−45	8.9 ± 0.5	6.2 ± 0.4	−30
Stomatal density (mm^2)	112 ± 4.5	118 ± 5.0	+5	115 ± 4.2	116 ± 4.4	≈0

3.3 Physiological Responses

Marked physiological variations were observed between the two cultivars in response to water stress. Chlorophyll pigments exhibited substantial declines, with chlorophyll a decreasing by 40% in Hamadhy compared to 25% in Wonderful , while chlorophyll b showed reductions of 35% and 20%, respectively. These reductions indicate a cultivar-dependent impairment of the photosynthetic apparatus. In contrast, osmoprotectant accumulation was strongly stimulated under stress. Proline levels increased by 220% in Hamadhy and 150% in Wonderful , reflecting enhanced biochemical adjustments to maintain cellular osmotic balance. Additionally, cuticular wax deposition increased significantly, with Hamadhy showing a 45% rise and Wonderful a 60% rise. This suggests that Wonderful adopts a more pronounced cuticular barrier strategy to reduce transpirational water loss under drought conditions Table 3 this agree with [11, 12].

Table 3. Physiological traits of Hamadhy and Wonderful cultivars under climate stress.

Trait	Hamadhy (Control)	Hamadhy (Stress)	Change (%)	Wonderful (Control)	Wonderful (Stress)	Change (%)
Chlorophyll a (mg g^{-1} FW)	1.21 ± 0.05	0.73 ± 0.04	−40	1.28 ± 0.06	0.96 ± 0.05	−25
Chlorophyll b (mg g^{-1} FW)	0.87 ± 0.04	0.57 ± 0.03	−35	0.91 ± 0.05	0.73 ± 0.04	−20
Proline (μmol g^{-1} FW)	1.85 ± 0.09	5.92 ± 0.21	+220	1.76 ± 0.08	4.40 ± 0.19	+150
Cuticular wax (mg cm^{-2})	2.1 ± 0.12	3.05 ± 0.16	+45	2.3 ± 0.11	3.68 ± 0.18	+60

3.4 Recovery Phase

Partial recovery of pigments and leaf area was observed in autumn. However, epidermis thickening and wax accumulation persisted, suggesting stable acclimation traits Fig 1 and 2.

3.5 Comparative Performance

Figure 3. Comparative physiological responses of Hamadhy and Wonderful cultivars under water stress. Both cultivars exhibited a marked decline in chlorophyll pigments, with reductions more pronounced in Hamadhy (40% in chlorophyll a and 35% in chlorophyll b) than in Wonderful (25% and 20%, respectively). Conversely, osmoprotective mechanisms were more evident in Hamadhy , as reflected by a 220% increase in proline compared to 150% in Wonderful . In contrast, Wonderful showed greater investment in structural adaptation, with a 60% rise in cuticular wax deposition compared to 45% in Hamadhy . These findings highlight that Wonderful maintains pigment stability and cuticular protection, while Hamadhy relies primarily on metabolic adjustments through proline accumulation this agree with [13, 14] .

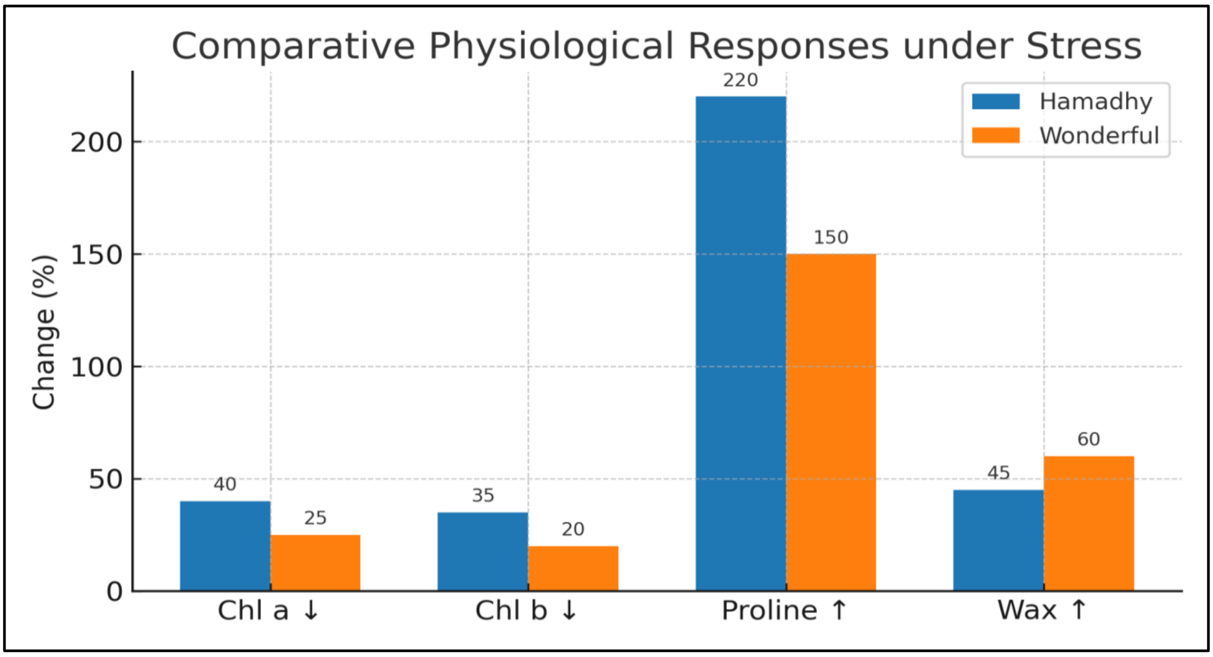

Figure 3. Comparative physiological responses (chlorophyll, proline, wax) in Hamadhy vs Wonderful.

4 DISCUSSION

Najaf's climate imposed combined heat and drought stress [15], leading to both structural and biochemical leaf modifications [16]. Epidermal thickening and enhanced wax deposition in both cultivars demonstrate a defense strategy to minimize transpiration [17,18]. However, reductions in mesophyll thickness and leaf area confirm reduced photosynthetic efficiency, particularly in Hamadhy. The elevated proline in Hamadhy reflects osmotic adjustment, a common drought tolerance mechanism [20, 19]. Yet this reactive strategy may compromise longterm productivity by diverting resources from photosynthesis. By contrast, Wonderful maintained chlorophyll stability and invested more in structural reinforcement, supporting sustained photosynthetic activity.
These findings agree with earlier work highlighting cultivarspecific resilience in pomegranate and other fruit trees [22, 21]. For Najaf, 'Wonderful' shows greater suitability under projected climate stress, though local cultivars like Hamadhy represent valuable genetic resources for breeding [24,23]. Integrated management practices mulching, regulated irrigation, and shading remain essential for orchard resilience [26, 25].

5 CONCLUSIONS

Climate change significantly influences pomegranate physiology and anatomy in Najaf. While both cultivars adapted through epidermal reinforcement and wax deposition, Hamadhy relied heavily on osmolyte accumulation, and Wonderful sustained higher pigment levels and leaf area.
Recommendations:
1Prioritize resilient cultivars such as Wonderful for new orchards.
2Use local cultivars as breeding material for combined resilience traits.
3Integrate climatesmart practices (mulching, shading, efficient irrigation) into orchard management.
4Strengthen climate monitoring and adaptive planning for longterm sustainability.

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