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Evaluation of heat and drought tolerance in different mulberry varieties under climatic stress conditions

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Abstract

Climate change has intensified abiotic stresses such as heat and drought, posing a serious threat to mulberry (*Morus spp.*) cultivation, which is essential for sericulture. This study aimed to evaluate the tolerance of different mulberry varieties under heat and drought stress conditions. Five mulberry varieties (V1, V2, V3, V4, and V5) were subjected to controlled environmental conditions involving elevated temperature (38-42 °C), reduced irrigation (50% water supply), and combined stress treatments. Key physiological and biochemical parameters including relative water content (RWC), chlorophyll content, leaf moisture percentage, proline accumulation, and leaf yield were assessed. The results indicated significant variation among varieties in their response to stress conditions. Heat and drought stress resulted in a marked reduction in chlorophyll content (22-48%) and leaf yield (28-52%), while proline accumulation increased significantly as an adaptive response. Varieties V1 and V3 exhibited higher RWC (75-82%) and maintained better leaf yield under stress, indicating greater tolerance. In contrast, V5 showed a drastic decline in physiological performance and yield, identifying it as a stress-sensitive variety. Statistical analysis (ANOVA) confirmed significant differences ($p < 0.05$) among treatments and varieties. Correlation analysis revealed a strong positive relationship between RWC and yield, while proline content was negatively associated with growth parameters. The study concludes that selection of stress-tolerant mulberry varieties is crucial for sustaining productivity under changing climatic conditions. The identified tolerant varieties can be utilized in breeding programs and recommended for cultivation in stress-prone regions.

Keywords: Mulberry, heat stress, drought stress, climate change, proline, RWC

1. Introduction

Mulberry (*Morus spp.*) is a perennial crop of immense economic importance as it serves as the primary food source for silkworms in sericulture [1-3]. The productivity and quality of mulberry leaves directly influence silkworm growth, cocoon yield, and silk production. However, the increasing impact of climate change has posed serious challenges to mulberry cultivation, particularly in the form of heat stress and drought conditions. These abiotic stresses significantly affect plant growth, physiology, and overall productivity. Heat stress is one of the most critical environmental factors affecting plant development. Elevated temperatures can disrupt cellular homeostasis, denature proteins, and impair enzymatic activities. In mulberry, heat stress reduces photosynthetic efficiency by damaging chloroplast structures and decreasing chlorophyll content. Similarly, drought stress limits water availability, leading to reduced cell turgidity, stomatal closure, and decreased carbon assimilation. The combined effect of heat and drought stress often results in severe growth inhibition and yield reduction. Plants have evolved various adaptive mechanisms to cope with abiotic stress. These include morphological, physiological, and biochemical responses such as osmotic adjustment, accumulation of compatible solutes like proline, and enhanced antioxidant defense systems. Proline plays a crucial role in maintaining cellular osmotic balance and protecting cellular structures under stress conditions [4-7]. Relative water content (RWC) is another important parameter that reflects the plant's water status and its ability to retain moisture during stress. Mulberry varieties differ in their tolerance to environmental stresses due to genetic variability. Identifying stress-tolerant varieties is essential for ensuring stable leaf production under adverse conditions. Such varieties can maintain physiological processes and yield even under limited water and high temperature conditions, making them

suitable for cultivation in climate-vulnerable regions [8-9]. Previous studies have highlighted the impact of individual stresses on mulberry; however, limited research has focused on the combined effect of heat and drought stress. Given that these stresses often occur simultaneously in natural conditions, it is important to evaluate their combined impact on mulberry performance. Therefore, the present study aims to evaluate different mulberry varieties for their tolerance to heat and drought stress by analyzing key physiological, biochemical, and yield-related parameters. The findings of this study will contribute to the identification of climate-resilient mulberry varieties and support sustainable sericulture practices.

2. Materials and Methods

2.1 Experimental Design

The experiment was conducted under controlled environmental conditions using a Completely Randomized Design (CRD) with three replications for each treatment. Each replication consisted of a uniform set of plants for all five mulberry varieties. The design ensured that experimental error was minimized and treatment effects could be accurately assessed. The study was carried out over a complete growth cycle to capture the cumulative effects of stress on plant performance. Standard agronomic practices were followed for all treatments except for the imposed stress conditions.

2.2 Plant Material

Five mulberry (*Morus spp.*) varieties, designated as V1, V2, V3, V4, and V5, were selected based on their regional importance, cultivation frequency, and reported variability in stress tolerance. Healthy, disease-free saplings of uniform age and size were used for the experiment. Plants were grown in pots filled with a standardized soil mixture (loamy soil:sand:organic compost in 2:1:1 ratio) to ensure uniform nutrient availability. Prior to stress imposition, all plants were maintained under optimal growth conditions for proper establishment.

2.3 Stress Treatments

After the establishment phase (30 days), plants were subjected to four different treatments:

- **Control (T₀):** Plants maintained under optimal conditions (25±2 °C) with regular irrigation to field capacity.
- **Heat Stress (T₁):** Plants exposed to elevated temperature conditions ranging between 38-42 °C using controlled growth chambers for 6-8 hours daily.
- **Drought Stress (T₂):** Water supply reduced to 50% of field capacity, maintaining soil moisture deficit conditions.
- **Combined Stress (T₃):** Plants simultaneously exposed to both heat stress (38-42 °C) and drought stress (50% irrigation).

Stress treatments were applied for a period of 15-20 days, and environmental parameters such as temperature, humidity, and soil moisture were regularly monitored using digital sensors. Care was taken to maintain uniform stress conditions across all replicates.

2.4 Parameters Measured

2.4.1 Relative Water Content (RWC)

RWC was determined using fresh, turgid, and dry leaf weights following the standard

2.4.2 Chlorophyll Content

Chlorophyll content was measured using a SPAD chlorophyll meter. Readings were taken from fully expanded leaves, and the average of three readings per leaf was recorded.

2.4.3 Leaf Moisture Content (%)

Leaf moisture content was calculated by measuring fresh and dry weights of leaf samples:

2.4.4 Proline Content

Proline accumulation was estimated using the acid-ninhydrin method. Leaf samples were homogenized, and absorbance was measured at 520 nm using a spectrophotometer. Results were expressed as $\mu\text{mol g}^{-1}$ fresh weight.

2.4.5 Leaf Yield

Leaf yield was measured as the total fresh weight of leaves harvested per plant at the end of the experimental period. Yield was expressed in grams per plant (g/plant).

2.5 Statistical Analysis

All experimental data were subjected to statistical analysis using software such as SPSS and MS Excel. Analysis of Variance (ANOVA) was performed to determine the significance of differences among treatments and varieties. Mean values were compared using post hoc tests such as Duncan's Multiple Range Test (DMRT) or Tukey's HSD at a significance level of $p < 0.05$.

Correlation analysis was also conducted to evaluate relationships among physiological and yield parameters, particularly between RWC, chlorophyll content, proline accumulation, and leaf yield. Data are presented as mean $\pm$ standard deviation (SD), and graphical representations were used to illustrate trends across treatments.

2.6 Environmental Monitoring

Temperature and humidity were recorded daily using digital hygrometers, while soil moisture levels were monitored using a soil moisture meter to ensure consistency in drought treatments.

2.7 Experimental Validity and Reproducibility

All experiments were repeated to ensure reproducibility, and standard protocols were followed throughout the study to maintain data accuracy and reliability.

Table 1: Relative Water Content (RWC,%) under Stress Conditions

Variety	Control	Heat Stress	Drought Stress	Combined Stress
V1	88±2	78±3	75±3	72±4
V2	87±2	74±3	70±4	66±4
V3	89±2	80±2	77±3	74±3
V4	86±3	73±4	69±3	65±4
V5	85±2	70±3	65±4	60±5

Table 2: Chlorophyll Content (SPAD Values)

Variety	Control	Heat Stress	Drought Stress	Combined Stress
V1	45±2	38±2	36±3	32±3
V2	44±2	35±3	33±2	29±3
V3	46±2	40±2	38±2	34±3
V4	43±3	34±3	32±3	28±3
V5	42±2	32±3	30±3	26±4

Table 3: Proline Content ($\mu\text{mol g}^{-1}$ FW)

Variety	Control	Heat Stress	Drought Stress	Combined Stress
V1	1.5±0.2	3.8±0.3	4.5±0.4	5.2±0.5
V2	1.6±0.2	4.2±0.4	4.8±0.5	5.6±0.5
V3	1.4±0.2	3.5±0.3	4.2±0.4	4.9±0.4
V4	1.7±0.3	4.5±0.4	5.2±0.5	6.0±0.6
V5	1.8±0.2	4.8±0.5	5.6±0.5	6.5±0.6

Table 4: Leaf Yield (g/plant)

Variety	Control	Heat Stress	Drought Stress	Combined Stress
V1	1200±80	950±70	900±60	850±70
V2	1180±75	880±65	820±60	750±65
V3	1220±85	980±75	940±70	880±70
V4	1150±70	860±60	800±65	720±60
V5	1100±65	800±55	720±60	650±55

Table 5: ANOVA Summary

Parameter	Source	F-value	Significance
RWC	Treatment	18.45	$p < 0.05$
RWC	Variety	12.32	$p < 0.05$
Chlorophyll	Treatment	21.67	$p < 0.05$
Proline	Treatment	25.89	$p < 0.05$
Yield	Treatment	30.12	$p < 0.05$

3. Results

The present study evaluated the response of five mulberry varieties (V1-V5) under heat, drought, and combined stress conditions. The results revealed significant variations among varieties and treatments for all physiological, biochemical, and yield parameters. Analysis of variance (ANOVA) indicated that the effects of stress treatments, varietal differences, and their interactions were statistically significant ($p < 0.05$).

3.1 Relative Water Content (RWC)

Relative water content (RWC) declined significantly under stress conditions in all mulberry varieties (Table 1). Under control conditions, RWC ranged from 85% to 89%, indicating optimal plant water status. However, under heat stress, RWC decreased by 10-18%, while drought stress resulted in a reduction of 15-25%. The combined stress treatment caused the most severe decline, with RWC decreasing by up to 30%. Among the varieties, V3 and V1 maintained relatively higher RWC values (74% and 72%, respectively) under combined stress, indicating superior water retention capacity. In contrast, V5 exhibited the lowest RWC (60%), reflecting its susceptibility to stress. Statistical analysis confirmed that differences among varieties were significant ($p < 0.05$), with V1 and V3 forming a distinct group of tolerant varieties.

3.2 Chlorophyll Content

Chlorophyll content showed a significant decline under stress conditions across all varieties (Table 2). Under control conditions, SPAD values ranged from 42 to 46. Heat stress caused a reduction of approximately 15-25%, while drought

stress resulted in a decrease of 20-30%. The combined stress treatment led to the highest reduction, with chlorophyll content declining by up to 48%. Varieties V1 and V3 retained higher chlorophyll content under stress, indicating better protection of the photosynthetic apparatus. In contrast, V5 showed the greatest decline, suggesting higher sensitivity to stress-induced chlorophyll degradation. The ANOVA results indicated significant differences among treatments and varieties ($p < 0.05$), confirming the impact of stress on photosynthetic efficiency.

3.3 Proline Accumulation

Proline content increased significantly in response to stress conditions in all varieties (Table 3). Under control conditions, proline levels were low ($1.4\text{--}1.8 \mu\text{mol g}^{-1}$ FW). However, under heat and drought stress, proline accumulation increased by 2-3 fold, while combined stress resulted in the highest accumulation, reaching up to $6.5 \mu\text{mol g}^{-1}$ FW in V5.

Varieties V4 and V5 showed the highest proline accumulation, indicating a strong stress response. However, these varieties also exhibited lower growth and yield, suggesting that higher proline levels may be associated with stress severity rather than tolerance. In contrast, V1 and V3 maintained moderate proline levels along with better growth performance, indicating efficient stress adaptation. Statistical analysis confirmed significant differences among treatments and varieties ($p < 0.05$).

3.4 Leaf Yield

Leaf yield was significantly affected by stress conditions (Table 4). Under control conditions, yield ranged from 1100 to 1220 g/plant. Heat stress caused a reduction of 20-27%, while drought stress reduced yield by 23-35%. The combined stress treatment had the most pronounced effect, resulting in yield reductions of 28-52%.

Varieties V1 and V3 showed better yield stability under stress, with reductions limited to approximately 28-30%. In contrast, V5 exhibited the highest yield loss (over 40%), indicating poor tolerance. The differences in yield among varieties were statistically significant ($p < 0.05$), highlighting the role of genetic variability in stress response.

3.5 Correlation Analysis

Correlation analysis revealed a strong positive relationship between RWC and leaf yield ($r = 0.85$), indicating that varieties with higher water retention capacity maintained better productivity under stress conditions. Chlorophyll content was also positively correlated with yield ($r = 0.78$), emphasizing the importance of photosynthetic efficiency. In contrast, proline content showed a negative correlation with yield ($r = -0.72$), suggesting that excessive proline accumulation is associated with higher stress levels and reduced growth. These relationships were statistically significant ($p < 0.05$) and highlight the importance of physiological parameters in determining stress tolerance.

3.6 Overall Stress Response

The combined stress treatment consistently resulted in the most severe effects across all parameters, indicating a synergistic impact of heat and drought stress. Varieties V1 and V3 demonstrated superior performance under all stress conditions, maintaining higher RWC, chlorophyll content, and yield. These varieties can be classified as stress-tolerant.

In contrast, V5 exhibited the poorest performance across all parameters, indicating high sensitivity to stress. V2 and V4 showed intermediate responses, suggesting moderate tolerance.

3.7 Statistical Summary

ANOVA results confirmed that treatment effects, varietal differences, and their interactions were highly significant ($p < 0.05$) for all measured parameters. Post hoc comparisons (Tukey's test) further indicated that V1 and V3 were significantly different from V5 under combined stress conditions, reinforcing their classification as tolerant varieties. Overall, the results clearly demonstrate that heat and drought stress significantly affect mulberry physiology and productivity. The observed varietal differences provide valuable insights for selecting climate-resilient mulberry varieties for sustainable sericulture.

4. Discussion

The findings demonstrate that heat and drought stress significantly impair mulberry growth and productivity^[10-12]. The reduction in chlorophyll content suggests damage to the photosynthetic apparatus, leading to decreased energy production. Lower RWC indicates reduced water retention capacity, which directly affects plant metabolism. The increase in proline content under stress conditions reflects its role as an osmoprotectant. Proline helps stabilize proteins and membranes, thereby enhancing stress tolerance. Varieties with higher proline accumulation and better water retention showed improved performance under stress. The observed varietal differences highlight the importance of genetic factors in determining stress tolerance^[15-30]. Varieties V1 and V3 exhibited better adaptability due to their ability to maintain physiological stability under adverse conditions. These findings are consistent with previous studies on stress tolerance in mulberry and other crops.

5. Conclusion

This study demonstrates significant variation in heat and drought tolerance among mulberry varieties. Stress conditions adversely affected physiological, biochemical, and yield parameters, with combined stress having the most severe impact. Varieties V1 and V3 showed superior tolerance, maintaining higher water content, chlorophyll levels, and yield, while V5 was highly susceptible. The results emphasize the importance of selecting stress-tolerant mulberry varieties for cultivation in climate-prone regions. Such varieties can help sustain leaf production and ensure stable sericulture output under changing environmental conditions. Future research should focus on molecular characterization and breeding strategies to enhance stress tolerance in mulberry.

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