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CHLOROPLAST PIGMENTS VARIABILITY IN COTTON (*GOSSYPIUM HIRSUTUM* L.) UNDER DIVERSE WATER REGIMES

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SUMMARY

This study aimed to evaluate the physiological performance of 13 promising advanced lines and the cultivar Namangan-77 of upland cotton (*Gossypium hirsutum* L.) under different water supply conditions. Genotypes enunciated significant differences for plastid pigments under optimal and water-stressed conditions. The results revealed that a marked decrease in pigment content expressed impaired photosynthesis, weakened leaf energetic status, and increased metabolic disruptions in drought-sensitive genotypes, whereas pigment retention reflected robust adaptive mechanisms in drought-tolerant cotton genotypes. These physiological mechanisms confirmed that pigments served as reliable biomarkers for assessing drought-tolerant cotton genotypes. The results exhibited water deficit conditions significantly affect key processes in cotton plant leaves, from water balance to the stability of the photosynthetic apparatus. Under optimal conditions, all physiological processes proceeded efficiently, while under modeled drought, it activated complex adaptive mechanisms to preserve more water and pigments, reduce transpiration, and stabilize the leaf structure.

Keywords: Upland cotton (*G. hirsutum* L.), water regimes, drought stress conditions, physiological traits, chlorophyll, carotenoids, morphological traits, agronomic variables

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Key findings: The plastid pigments' analysis showed drought-tolerant cotton (*G. hirsutum* L.) genotypes (T-217, T-4, and Xi Zhong 52) retained higher pigment levels under water stress, while drought-sensitive genotypes (Namangan-77, T-2, and Xi Zhong 78) exhibited a significant decrease in pigment content. The results highlighted the effectiveness of using a comprehensive assessment of physiological, morphological, and agronomic traits to select drought-tolerant cotton genotypes.

INTRODUCTION

Drought is one of the major abiotic stress factors that considerably affect cotton productivity worldwide. On seed cotton yield, the water stress impact depends on the duration and intensity of drought and the plant's developmental stages. Water deficit conditions disrupt various physiological and biochemical processes in cotton plants, leading to a sharp decline in growth, development, and productivity. Water plays a decisive role in a plant's life at all stages, from seed germination to fruit maturation (Chakma *et al*., 2021).

Drought stress adversely affects both the quantity of seed cotton yield and the development of fiber quality traits in cotton. Therefore, under scarce water conditions, it is vital to develop drought-tolerant cultivars that can grow efficiently to produce high-quality fibers, which earnestly require an in-depth study of their different morphological, physiological, and molecular adaptation mechanisms in plants. Drought stress reduces photosynthetic activity and eventually alters plants through both stomatal and non-stomatal limitations (Pilon *et al*., 2018).

Under water-deficit conditions, stomatal closure leads to reduced gas exchange, transpiration, and nutrient uptake (Zhang *et al*., 2021). However, in severe drought, the reduction in photosynthesis is mainly because of non-stomatal factors, whereas under mild drought conditions, the stomatal limitations play a dominant role. In cotton cultivars, a negative correlation has been evident between the guard cell length and stomatal density on both leaf surfaces, revealing an architectural synchronization between the stomatal number and guard cell morphology in response to water availability (Dubey *et al*., 2023).

The physiological mechanisms by which protective solutes prevent damage to cellular components in stress conditions have not yet attained full elucidation. However, studies have shown that under adverse conditions, such as water deficit, high temperature, and elevated salinity, the concentration of these compounds increases in plant tissues where they exert protective effects (Chen *et al*., 2018).

Drought modifies and reduces boll retention and leaf area in cotton plants. Increasing the cultivation of drought-tolerant cotton cultivars, improving crop field practices, and developing innovative technologies proved crucial to ensure sustainable cotton production under harsh environmental stress conditions. These advanced agro-technological approaches can further enhance crop tolerance to extreme stress conditions, such as drought, salinity, and high temperature, thereby improving both yield and quality (Ju *et al*., 2023).

Upland cotton (*G. hirsutum* L.), being the most important fiber crop, supplies raw material to the global textile industry. However, its productivity has been unstable over the years due to climate change–induced biotic stresses, such as insects, diseases, and weeds, as well as abiotic stresses, including drought, salinity, heat, and cold (Ahmed *et al*., 2024). Drought stress is an unavoidable factor that disrupts crop production by altering various morphological, physiological, biochemical, and molecular functions in crop plants.

Breeding for drought tolerance urgently requires comprehensive knowledge of the molecular factors controlling stress-responsive pathways. Generally, crop plants respond to drought stress conditions through four main mechanisms, including avoidance, escape, tolerance, and recovery. Although applying traditional plant breeding tools has been successful to improve drought tolerance in cotton, the complex nature of drought tolerance has limited the effectiveness of these conventional approaches (Rasheed *et a*l., 2023). Therefore, the presented study aimed to evaluate the physiological performance of promising advanced lines and the cultivar Namangan-77 of upland cotton (*G. hirsutum* L.) under optimal and water-stressed conditions.

MATERIALS AND METHODS

Experimental site and genetic material

The concerned research on cotton (*G. hirsutum* L.) crops commenced during 2024–2025 at the Institute of Genetics and Plant Experimental Biology of the Academy Sciences of Uzbekistan, Tashkent, Uzbekistan (with an altitude of 398 masl). The climate exhibited sudden fluctuations, with the highest temperature during summer months (June, July, and August) and a sharp air temperature drop in winter months (December and January). Sunny days prevailed for 175–185 days and non-cold days for 200–210 days. The rainfall was notable in the fall, winter, and spring, with dry air in the summer. According to the granulometric composition, the experimental field soil was low in humus, typically gray, and moderately sandy. The terrain was slightly sloping, not saline, and naturally damaged by whitish (verticillus) silt. The measured soil bulk density was 1.32–1.33 g/cm^3, with a limited field moisture capacity (LFMC) of 22%. Groundwaters go deep (8 m and more) (Matniyazova *et al.,* 2025).

The study comprised the evaluation and response of 13 advanced lines and the cultivar Namangan-77 of upland cotton (*G. hirsutum* L.) based on physiological and yield-related traits under water non-stress and stressed conditions. Cottonseeds, sown in the field after 20 days in April, used a randomized design. Each variety reached planting in three rows, with 25 seeds sown per row. In optimum and controlled water regimes, the cotton genotypes received irrigation four times (scheme 1:2:1) during the vegetative and flowering stages, using 4800–5000 m^3 of water per hectare. However, in stress conditions, the genotypes received only two irrigations (scheme 1:1:0), and the total water volume used for irrigation was 2800–3000 m^3/ha. Under the water-stressed environment, the cotton genotypes received irrigation once each during the seedling and flowering stages to develop artificial water scarcity (modeled drought). In cotton genotypes, the identification of essential physiological indicators of water metabolism proceeded with the following methods. The determined levels of cotton lines' adaptation to water deficit conditions employed the method according to Eberhart and Russell (1966).

Extraction and determination of leaf pigments

For pigment determination, leaf samples randomly collected from three replicate cotton plants continued from the third to the fourth leaves counted from the growth point. Each 50-mg leaf, as placed in a test tube, reached homogenization in 5 ml of 95% ethyl alcohol solution (Lichtenthaler and Wellburn, 1983). The homogenate underwent centrifugation at the speed of 5000 for 12 min. The amounts of chlorophyll a and b and carotenoid determination used an Agilent Cary 60 UV-Vis spectrophotometer at 664, 649, and 470 nm in the resulting extract. Based on these indicators, measuring amounts of chlorophyll a, b, and carotenoids ensued in the cotton leaves using the following equations (Nayek *et al*., 2014):

$$\text{Ch-}a = (13.36 \times \text{A664}) - (5.19 \times \text{A649})$$

$$\text{Ch-}b = (27.43 \times \text{A649}) - (8.12 \times \text{A664})$$

$$\text{C x+c} = ([1000 \times \text{A470}] - [2.13 \times \text{Ch-}a] - [97.63 \times \text{Ch-}b])/209$$

Statistical analysis

The obtained data analysis ran through Microsoft Excel 2016 by using the analysis of variance (ANOVA) in the STAT VIEW software at a significance level of $P < 0.05$.

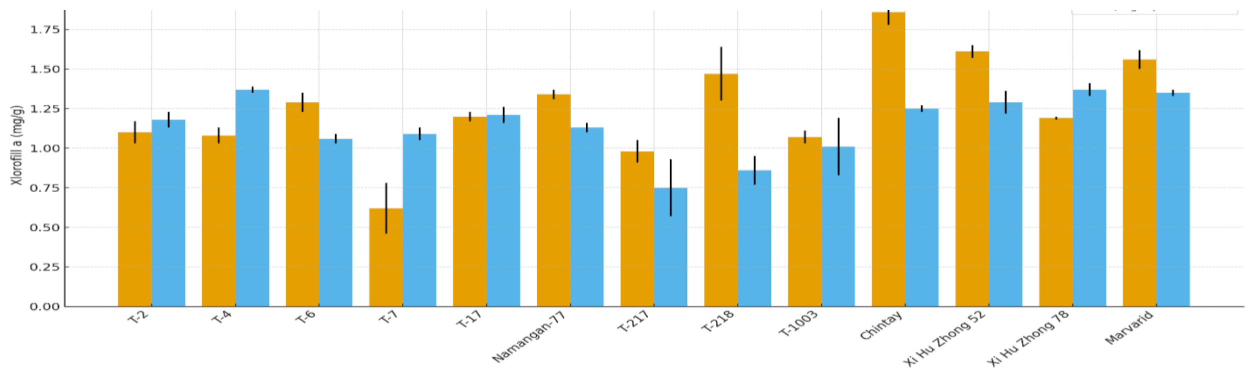

Figure 1. Chlorophyll a content in cotton genotype leaves under optimal and modeled drought conditions (Mean ± SE).

RESULTS AND DISCUSSION

Chlorophyll, being the primary pigment playing a key role in the photosynthetic process of plant leaves and its content, is a widely used indicator for assessing the physiological status of crop plants, stress tolerance, and photosynthetic efficiency. However, the drought stress condition can accelerate pigment degradation, slow down pigment biosynthesis, and disrupt photochemical systems in plant leaves. Therefore, analyzing genotypes with their varied responses to water deficit conditions provides an important scientific basis in plant breeding programs (Matniyazova *et al.,* 2023). This study analyzed the chlorophyll-a content of 13 cotton genotypes grown under optimal and simulated drought conditions. The results served as an essential source for elucidating genotype-specific mechanisms of pigment response to stress. According to results, pigment content appeared to be significantly dependent on the level of available water, and genotype responses considerably varied under optimal and drought stress conditions (Ashurova *et al*., 2025).

Overall, under optimal conditions, most cotton genotypes exhibited higher levels of chlorophyll a, revealing that the photosynthetic apparatus of leaves was well developed, with physiological activities carried out normally (Figure 1). Under drought stress conditions, however, two distinct response patterns were evident in the genotypes—some genotypes maintained or even increased their chlorophyll content, whereas others showed a sharp decline. Genotypes T-2, T-4, and Xi Lu Zhong-78 displayed the most adaptation to drought conditions, maintaining and increasing chlorophyll a content even when in stress conditions. The results suggested these genotypes possess well-developed mechanisms, limiting chlorophyll degradation in leaf tissues, such as enhanced antioxidant activity, protection of photosystems, and accumulation of osmoprotectants.

In contrast, genotypes T-6, T-217, T-218, Namangan-77, and Chintay-2 exhibited a significant reduction in chlorophyll a content under drought stress conditions. Such decreases indicated damage to the photosynthetic apparatus and increased activity of degradative enzymes, i.e., chlorophyllase and peroxidase. These genotypes therefore displayed considerable sensitivity to water deficit conditions. Under optimal conditions, genotypes with higher pigment content, such as Chintay, Xi Lu Zhong-52, and Marvarid, showed the highest photosynthetic potential. However, the sharp reduction in pigment content observed in the genotype Chintay-2 within stress conditions confirmed its sensitivity to drought stress.

The results further showed both environmental (A) and genotypic (G) factors significantly affected chlorophyll a content, indicating that water deficit conditions robustly influenced the pigment synthesis and degradation processes. However, the

Table 1. Analysis of variance of backgrounds, genotypes, and A × G interactions for factors affecting chlorophyll a content in cotton genotype leaves under diverse water regimes.

Factor	SS	d.f.	MS	F	p-value
Background (A)	2184	1	2184	16.9	0.004
Genotype (G)	4342	12	361	2.8	0.027
A × G	1711	12	142	1.1	0.38
Error	3350	26	129	—	—

environment–by-genotype interactions were nonsignificant, revealing that drought determined an overall trend of pigment change across the genotypes, while variations mainly depended on the cotton genotype. In general, the cotton genotypes exhibited pronounced differences in their physiological responses to water deficit conditions. The preservation of chlorophyll a pigment signified a direct association with genotype adaptability to drought conditions. In this regard, genotypes T-2, T-4, and Xi Lu Zhong-78 were distinctly highly tolerant (Figure 1). In contrast, genotypes, viz., T-6, T-217, and T-218, demonstrated characteristics of rapid pigment degradation, obtaining a drought-sensitive classification.

The multifactorial analysis of variance revealed pigment content had a notable association with water regimes, genotypes, and their interactions (Table 1). The level of water supply has a significant ($P < 0.004$) effect on chlorophyll a content. The results confirmed that under drought conditions, chlorophyll a levels decrease in plant leaves due to enhanced degradation of photosynthetic pigments. The cotton genotypes indicated significant ($P < 0.027$) differences for chlorophyll a content. An explanation for this can be evident in the genetic variations in the photosynthetic apparatus, pigment biosynthesis capacity, and chloroplast stability among genotypes. However, some cotton genotypes were capable of maintaining pigment levels, while others showed higher sensitivity to water deficit conditions.

The environment-by-genotype interactions were noticeably nonsignificant for chlorophyll a content. This indicates the drought stress influences chlorophyll a content in the same general direction across all genotypes. Although the degree of variations differed among the cotton genotypes, these differences were nonsignificant as an interaction effect. Thus, the direction of the water stress response was the same, while each genotype exhibited a distinct level of responsiveness. Overall, the results demonstrated that in cotton genotypes, chlorophyll a content was robustly dependent on both water regimes and genotypes' genetic makeup. The water deficit conditions substantially affected the reduction of this pigment, and therefore, the observed genotypic differences reflect varying levels of adaptive capacity. The findings confirmed that chlorophyll a could effectively serve as an important physiological indicator for identifying drought-tolerant cotton genotypes.

In this study, investigating the chlorophyll b content also occurred among the cotton genotype leaves under optimal and drought stress conditions. The water regimes had differential effects on chlorophyll b content, and the genotypes responded to stress conditions with individual physiological reactions. In general, under optimal conditions, all cotton genotypes exhibited relatively stable pigment levels, whereas under drought stress conditions, the pigment retention varied among genotypes. The results highlighted the considerable differences in the sensitivity of chloroplasts to stress among cotton genotypes. The path coefficient analysis found that leaf chlorophyll content could remarkably be one of the indicators in the improvement of seed cotton yield in cotton under drought stress conditions (Karademir *et al.,* 2009).

Study results revealed chlorophyll b content did not change sharply in cotton genotypes T-2, T-4, T-17, Xi Lu Zhong-78, and Marvarid under drought conditions (Figure 2). In some cases, the genotype Xi Lu Zhong-78 showed a slight increase in pigment content.

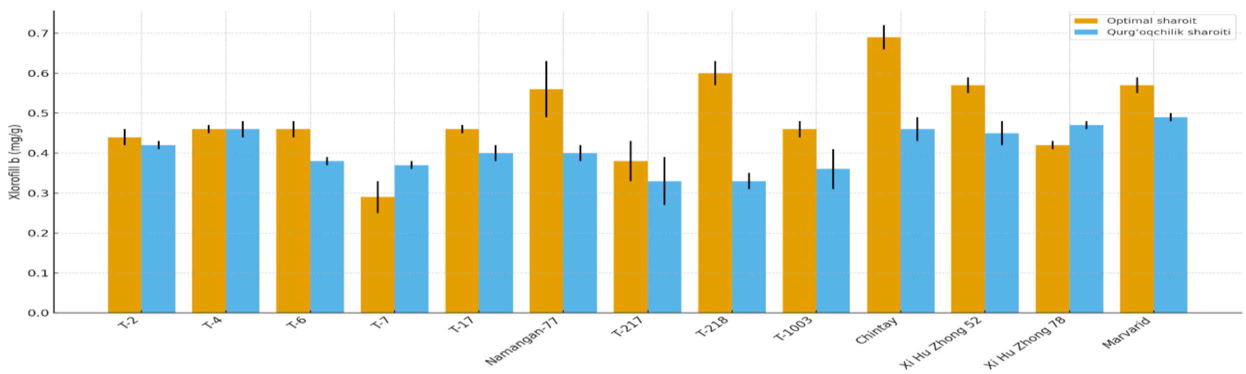

Figure 2. Chlorophyll b content in cotton genotype leaves under optimal and modeled drought conditions (Mean ± SE).

Table 2. Analysis of variance of backgrounds, genotypes, and A × G interactions for factors affecting chlorophyll b content in cotton genotype leaves under diverse water regimes.

Factor	SS	d.f.	MS	F	p-value
Background (A)	0.114	1	0.114	9.42	0.006
Genotype (G)	0.298	12	0.025	2.09	0.042
A × G	0.167	12	0.014	1.18	0.31
Error	0.316	26	0.012	—	—

The genotypes possess a relatively stable photosynthetic apparatus in their leaves, demonstrating the ability to preserve pigments within stress conditions. Therefore, such genotypes can be considerably drought-tolerant due to chlorophyll b. In contrast, genotypes T-6, T-7, Namangan-77, T-217, T-218, and Chintay-2 exhibited a significant decrease in chlorophyll b content under drought stress conditions. In particular, the greater pigment loss resulted in genotypes T-218, Namangan-77, and Chintay-2. This indicates intensified oxidative stress in leaves of these genotypes and higher sensitivity of chloroplast membranes and photosystem II structures. Thus, these genotypes can receive a drought-sensitive classification based on chlorophyll b levels (Figure 2).

The cotton genotypes revealed obvious differences in their degree of adaptation to drought stress conditions based on chlorophyll b content. Preservation of the pigment enhances a genotype's drought tolerance, whereas a pronounced decrease indicates sensitivity to stress conditions. The results confirmed that chlorophyll b could serve as an important physiological marker for identifying drought-tolerant genotypes in cotton breeding programs. According to the multifactorial analysis of variance to evaluate chlorophyll b content in cotton genotypes under different water supply conditions, it clearly confirmed that pigment variation was substantially dependent on water regimes, genotypes, and certain agro-biological characteristics.

Environmental conditions had a meaningful effect on chlorophyll b content, particularly the level of water supply ($P < 0.006$). Under drought conditions, the reduction in this pigment can refer to the chloroplast damage, accelerated chlorophyll degradation, and limited regeneration of accessory photosynthetic pigments. The pronounced sensitivity of chlorophyll b to water deficit has an apparent biological basis, which has an association with its crucial role in the light-harvesting complex of photosystem II (Table 2). The genotypes exhibited the presence of significant ($P < 0.042$) differences among cotton genotypes for chlorophyll b content. This suggested genotype genetically differed in the stability of the photosynthetic

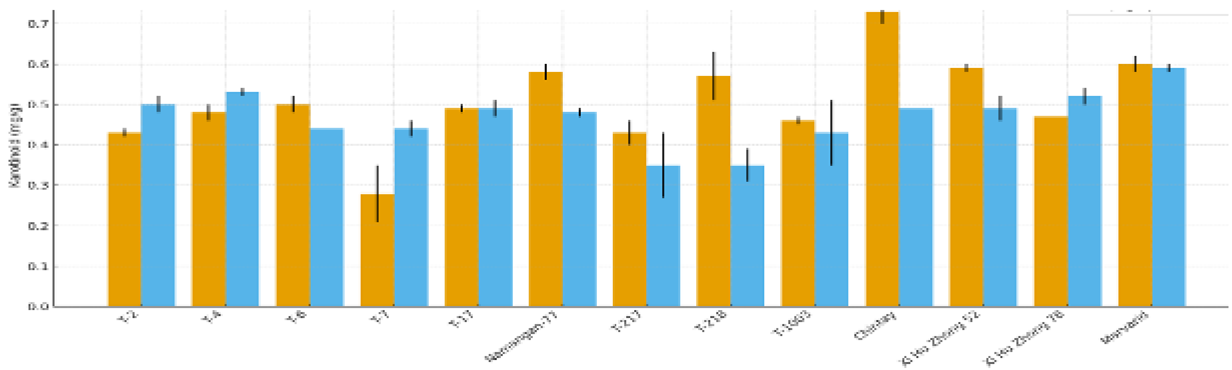

Figure 3. Carotenoid content in cotton genotype leaves under optimal and modeled drought conditions (Mean ± SE).

apparatus, pigment biosynthesis capacity, membrane integrity, and antioxidant system activity. However, some cotton genotypes were capable of maintaining pigment levels, while others expressed greater sensitivity to water deficit conditions.

The environment-by-genotype interactions appeared to be nonsignificant for chlorophyll b content. The results revealed that drought stress affected the chlorophyll b content in the same direction across all genotypes. Although the magnitude of the effect varied among the cotton genotypes, these differences were nonsignificant at the interaction level. In other words, drought's impact on the pigment was general, and the individual genotypic responses followed a similar trend. The analysis of variance enunciated that chlorophyll b content signifies a notable association with both water regime and genotype-specific characteristics. Meanwhile, water deficit led to a decrease in pigment content, and the differences observed among cotton genotypes reflect variations in chloroplast stress tolerance, photoprotective mechanisms, and pigment retention capacity. The outcomes confirmed that chlorophyll *b* could benefit as a reliable physiological marker in the selection of drought-tolerant cotton cultivars. Past studies reported that chlorophyll content indicated direct positive effects on the seed cotton yield (Reddy and Kumari, 2004).

In this study, the carotenoid pigments in the cotton genotype leaves sustained comparative evaluation under optimal and drought stress conditions. The results exhibited that carotenoid content was robustly dependent on variations in water availability, and cotton genotypes displayed varied response patterns. Overall, under optimal conditions, cotton genotypes maintained relatively stable carotenoid levels, indicating a normal functioning of the photosynthetic apparatus. Under drought conditions, however, some genotypes preserved carotenoid content to a considerable extent and even showed a slight increase, whereas other genotypes exhibited an evident decline. The results reflect variations in the development of antioxidant defense systems and photoprotective mechanisms among the cotton genotypes.

For carotenoid content, genotypes T-2, T-4, T-17, Xi Hu Zhong-78, and Marvarid displayed small variations and a slight increase under drought conditions (Figure 3). The results suggested these genotypes have a high capacity to reduce oxidative stress in leaf tissues, neutralize free radicals, and protect the chloroplasts. Therefore, these genotypes could entail classification as relatively stable in stress based on carotenoid pigments. In contrast, cotton genotypes T-6, T-7, Namangan-77, T-217, T-218, and Chintay showed a significant reduction in carotenoid content under drought stress conditions. Such a sharp decrease led to intensified oxidative stress, insufficient regeneration of carotenoids, and damage to chloroplast structure. The greater losses manifested in genotypes T-218, Namangan-77, and Chintay, which confirmed

Table 3. Analysis of variance of backgrounds, genotypes, and A × G interactions for factors affecting carotenoid content in cotton genotype leaves under diverse water regimes.

Factor	SS	d.f.	MS	F	p-value
Background (A)	0.241	1	0.241	12.5	0.004
Genotype (G)	0.466	12	0.039	2.03	0.049
A × G	0.228	12	0.019	0.98	0.48
Error	0.501	26	0.019	—	—

their highest sensitivity to drought stress conditions.

The maintenance and reduction of carotenoid pigments under drought stress conditions determines the level of stress adaptation of cotton genotypes. Preservation of pigments enunciates the protection of the photosynthetic apparatus, whereas a sharp decline reflects rapid susceptibility to stress conditions. The results confirmed that carotenoid pigments could be applicable as vital physiological indicators for identifying drought-tolerant genotypes in cotton breeding programs. The multifactorial analysis of variance evaluated carotenoid content in cotton leaves under different water supply conditions, which obviously confirmed this pigment was significantly dependent on water stress, genotypes' genetic makeup, and their physiological responses.

The environments revealed that water availability had a significant ($P < 0.004$) effect on carotenoid content in cotton genotypes (Table 3). Under drought stress conditions, the decrease in some genotypes provided the carotenoids' maintenance being associated with their antioxidant properties, ability to scavenge free radicals, and activity of photoprotective systems. The pronounced response of carotenoids to water deficit conditions can refer to the genotypes' crucial role in protecting the photosynthetic apparatus. The genotypes demonstrated significant ($P < 0.049$) differences in carotenoid content. The findings indicated that carotenoid synthesis and degradation were more stable in some genotypes, whereas other cotton genotypes were more sensitive to drought stress conditions. These differences in carotenoid levels implied a close association with the strength of the antioxidant system and stress-protection mechanisms of cotton genotypes.

The interactions between environments and genotypes were nonsignificant. The results enunciated that the overall effect of drought on carotenoid content followed the same trend across the genotypes. Although the degree of change varied among the genotypes, these differences were nonsignificant as interaction effects. The drought stress tended to reduce carotenoid content in all genotypes; however, each genotype exhibited a unique individual physiological response.

Overall, results exhibited that carotenoid pigments respond significantly to water stress, with their levels influenced by both the genetic makeup of genotypes and environmental factors. The water deficit conditions caused a general reduction in carotenoids, and differences among cotton genotypes reflected the efficiency of their antioxidant defense systems, pigment retention capacity, and the stress tolerance of their photosynthetic apparatus. Moreover, study findings suggested that carotenoid pigments could serve as an influential physiological biomarker for selecting drought-tolerant cotton cultivars.

The variation in the levels of major photosynthetic pigments—chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in cotton genotypes under different water availability conditions—fully reflected the plants' physiological responses to drought stress conditions. The analysis of variance confirmed these variations were significant ($P < 0.05$), revealing that pigment systems were reliable indicators for assessing drought tolerance in cotton genotypes.

Specifically, the effect of water availability was significant for all the pigment types, i.e., chlorophyll a ($P < 0.004$), chlorophyll b ($P < 0.006$), total chlorophyll ($P < 0.002$), and carotenoids ($P < 0.004$). Under water deficit conditions, pigment degradation accelerated and chloroplast membrane stability declined, impairing the activities of photosystems I and II. These observations align with past studies of Li *et al*. (2025), who reported a parallel decline in chlorophyll b and hydraulic conductivity, while Shani *et al*. (2025a, 2025b) highlighted the carotenoids as key biomarkers of stress tolerance.

Significant ($P < 0.05$) differences were evident among the cotton genotypes for all pigments, reflecting genetic variations in photosynthetic apparatus structure, pigment synthesis, membrane stability, and antioxidant defense mechanisms. According to results, cotton genotypes T-2, T-4, T-17, Xi Hu Zhong 78, and Marvarid maintained their pigment levels relatively well during stress conditions, indicating the highest drought tolerance. These genotypes prevented sharp declines in chlorophyll a and b, sustained total chlorophyll levels, and enhanced antioxidant protection through carotenoids.

In contrast, cotton genotypes T-6, T-7, Namangan-77, T-217, T-218, and Chintay exhibited significant reduction in pigment content, confirming sensitivity to drought stress conditions. Particularly, genotypes T-218 and Namangan-77 showed the most pronounced decline, suggesting oxidative stress at the cellular level increased, with chloroplast structures and photochemical systems severely affected.

CONCLUSIONS

The significant reduction in pigment levels suggested impaired photosynthesis, weakened energetic status of plant leaves, and increased metabolic disruptions in drought-sensitive cotton genotypes. However, the retention of pigments reflected robust adaptive mechanisms in drought-tolerant genotypes. The results exhibited that water deficit conditions remarkably affect key processes in cotton leaves, including water balance, to the stability of the photosynthetic apparatus. Under optimal conditions, physiological processes proceeded actively, while under modeled drought, it activated complex adaptive mechanisms in plants to preserve pigments and stabilize leaf structure in cotton plants.

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