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Determining Optimal Measurement Time Points for SPAD and Canopy Temperature in Drought Tolerant Cotton (*Gossypium hirsutum* L.) Breeding

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Abstract

This study investigates optimal physiological parameter measurement times in *Gossypium hirsutum* L. to assess the effects of drought stress. Observations of SPAD (Leaf Chlorophyll Content) and canopy temperature depression (CMD) were made at different growth stages, including Early Growth, Flowering, Bolling, and Boll Opening stages. These measurements were linked to yield and fiber quality parameters across the cotton grown stages.Data was analyzed using Principal Component Analysis (PCA) to uncover complex trait variations and genetic diversity under water-limited conditions. Specifically, PCA revealed significant variations in key traits such as yield and fiber quality under water stress conditions.Canopy temperature measurements highlighted the reliability of assessments made during specific stages, particularly the Bolling stage, for evaluating trait relationships.Leaf chlorophyll content (SPAD) analysis emphasized the significant correlation of SPAD4 with fiber elongation (FE) and lint percentage (LP).Overall, the results demonstrate the critical importance of timely SPAD and CMD measurements, especially during the Bolling stage (SPAD3 and CHD3), for understanding cotton traits under drought stress.These findings underscore the importance of strategic physiological parameter assessments in cotton breeding under challenging environmental conditions.

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1. Introduction

Drought stress presents a significant challenge to cotton (*Gossypium hirsutum* L.) cultivation, impacting various physiological parameters crucial for plant growth and productivity. Understanding the physiological responses of cotton plants to drought stress is essential for developing strategies to enhance drought tolerance and mitigate yield losses. In this context, two key physiological parameters, Two important physiological parameters used to assess drought stress in cotton are the Spad chlorophyll meter readings and canopy temperature depression (Guendouz et al., 2021).

The Spad chlorophyll meter is a non-destructive tool widely used to measure the relative chlorophyll content in plant leaves, serving as an indicator of photosynthetic activity and overall plant health. Under drought conditions, cotton plants often exhibit a decrease in chlorophyll content due to reduced photosynthetic activity and premature leaf senescence. By measuring Spad readings at various growth stages, growers can monitor the impact of drought stress on cotton's physiological status and make informed decisions regarding irrigation management and other agronomic practices (Ata-Ul-Karim et al., 2016). Canopy temperature depression (CTD) refers to the temperature difference between the air under the cotton canopy and the ambient air temperature. Drought stress typically leads to higher canopy temperatures as a result of reduced transpiration rates and increased stomatal closure in response to water scarcity. Elevated canopy temperatures can exacerbate drought stress effects, negatively impacting cotton growth and development. Monitoring CTD provides valuable insights into the extent of water stress experienced by cotton plants, allowing growers to adjust irrigation schedules and implement supplemental watering strategies to alleviate stress and minimize yield losses (Singh et al., 2014; Cakalogullari and Tatar, 2020; Özkul et al., 2022).

In addition to Spad readings and CTD, other physiological parameters such as leaf water potential, stomatal conductance, and relative water content are also commonly used to assess drought stress in cotton. Integrating these physiological indicators with agronomic observations and remote sensing technologies enables comprehensive monitoring of crop water status and facilitates timely interventions to mitigate the adverse effects of drought stress on cotton production. In conclusion, drought stress poses a significant threat to cotton cultivation, impacting various physiological processes essential for plant growth and productivity. By utilizing physiological parameters such as Spad chlorophyll meter readings and canopy temperature depression, growers can accurately assess drought stress levels in cotton fields and implement targeted management strategies to enhance crop resilience and sustainability in water-limited environments.

The timing of physiological observations under drought stress in plants is a critical aspect that requires careful consideration. According to Arabaci et al. (2017), some different perdiod of harvest has an effect on quality characteristics of plants. Research by Suzuki et al. (2014) highlighted the complexity of correlations between growth and physiological parameters under combined drought and salt stress, suggesting a nuanced relationship that may vary under different stress conditions. Similarly, Chen et al. (2016) emphasized the importance of physiological changes like leaf water potential, chlorophyll content, and Fv/Fm as reliable indicators for selecting drought-adaptive genotypes in maize seedlings. Physiological studies, such as those conducted by Hayano-Kanashiro et al. (2009) and (Ahmed et al., 2022), have demonstrated the accumulation of osmolytes like sugars and amino acids under drought stress, indicating plant responses to dehydration. However, the optimal timing for assessing these physiological responses remains a subject of debate due to the dynamic nature of plant stress responses. Integrating genetic and molecular approaches, as shown in studies by Abtahi et al. (2019) and Zhang et al. (2012), can provide valuable insights into the genetic basis of physiological responses to drought stress. By mapping physiological traits associated with drought tolerance, researchers can identify selectable features that contribute to plant resilience under stress conditions. In conclusion, the confusion regarding when to take physiological observations under drought stress for a insufficient to understand the timing and relevance of physiological assessments. Further research, as demonstrated by various studies (Barik et al., 2020; Lanceras et al., 2004; Oğuz, 2021; Tang et al., 2023; Balkan et al., 2023), is essential to elucidate the optimal timing for evaluating physiological responses to drought stress, ultimately enhancing our ability to develop drought-resilient crop varieties.

It is critical to obtain physiological parameters at the right time to evaluate the effects of drought stress. This study aims to determine the most appropriate physiological parameter measurement time in *Gossypium hirsutum* L. For this purpose, Early Growth Stage Flowering Stage Boll Setting Stage: Boll Opening Stage, spad and canopy temperature depression (CMD) observations were taken at different stages of the plant's life cycle. In this study, SPAD and CMD measurements taken at different growth stages were compared with the yield and fiber quality parameters of the plant. Observations made during seed germination and seedling periods have been linked to root development and initial growth of the plant. Data taken during the flowering period focused on assessing effects on the plant's pollen production and fertilization ability. Observations made during fruit formation and harvest periods aimed to determine the effects of the plant on seed maturation and harvest yield.

2. Metarials and Metods

The study material obtained breeding in 2008 by using line tester method with five cotton varieties which are most cultivated and high yielding in Turkey. In this paper, we evaluate selected ten homozygous lines which tolerant deficit stress in a randomized block design with 4 replicates in deficit water conditions (F9 generation). The study was conducted at the Faculty of Agriculture, Adnan Menderes University, in Turkey.

Understanding the impact of drought stress during these important growth stages is crucial for developing effective strategies to increase cotton resilience to water deficit conditions and improve overall crop performance. The following important yield and fiber parameters were used to determine the purpose of physiological parameters for developing these strategies.

Observations were recorded on five randomly selected each line were used for observing the following traits Observations, boll weight (g), lint percentage, and seed cotton yield (kg da^{-1}). Lint percentage was calculated by using formula suggested by Ghule et al. (2013).

Lint percentage (%) = (Weight of lint / Weight of seed cotton) x 100

Three samples were collected from each line for fiber quality analysis after the lint cleaner. The fiber quality traits were measured using HVI instruments (fiber length, elongation, micronaire, strength).

The four most important periods during which cotton plants are significantly affected by drought stress can be identified based on the following: SPAD and Conopy Heat Stress traits were taken in these four plant grown stages.

- ✓ Early Growth Stage: Khan et al. (2018) highlighted the structural, physiological, and molecular damage induced by drought stress in cotton plants. This early growth stage is crucial for establishing a strong foundation for plant development, making it vulnerable to the adverse effects of drought stress.
- ✓ Flowering Stage: Temple et al. (1988) discussed the interaction between drought stress and ozone effects on cotton yield. Drought stress during the flowering stage can have a substantial impact on cotton sensitivity to environmental stressors, potentially leading to yield reductions.
- ✓ Boll Setting Stage: Chen et al. (2022) investigated the role of the GhBEE3-Like gene in cotton drought tolerance. The boll setting

stage is critical for cotton yield determination, and drought stress during this period can significantly affect boll development and ultimately impact crop productivity.

✓ Boll Opening Stage: Guo et al. (2022) evaluated cotton drought resistance using hyperspectral imaging and artificial intelligence techniques during the boll opening stage. This period is crucial for fiber maturation and quality, and drought stress at this stage can lead to reduced fiber yield and quality.

Leaf Chlorophyll Content (SPAD): It was measured with “Konica Minolta SPAD-502 Plus” portable chlorophyll meter in the fully-developed flag leaves and determined as “SPAD value” (Pask et al., 2012). It was taken three averages of five leaves per plot, and they were done from 11:00h to 14:00h.

Canopy Temperature (CT-°C): It was measured with a portable infrared thermometer (Extech Mini IR Thermometer Modell 42500) as o C (Reynolds et al., 2001). It was taken as two measurements per plot during the day between (11:00h to 14:00h). Canopy temperature depression (CTD) is the difference between ambient air temperature and canopy temperature in degree centigrade.

2.1. Irrigation method

The experiment included 50 % irrigation regime. Well-watered (control treatment) was designed to provide 100 % replenishment of soil water depletion. A surface drip irrigation system was used for irrigation. A 16 mm diameter polyethylene pipe with in-line pressure compensating drippers at 0.33 m intervals was placed on one side of each cotton row. The different irrigation treatments were started on 18 June and continued according to regional practice until late August when 10% of bolls on a plant were fully open. The average amount of water applied was 400 mm for 50% (deficit water). Soil water levels were monitored by gravimetric method in the control plots (James, 1988).

2.2. Statistical analysis

Principal component analysis (PCA) was employed to extract the maximum variance from the data set, with each component reducing a large number of variables to a smaller number of components. Furthermore, correlation heat maps and PCA were applied to SPAD and CHD measurements taken at four different times. In these analyses, cotton selection parameters in drought, yield, and fibre quality parameters were calculated according to SPAD and CHD components. The principal component analysis (PCA) was conducted using the JMP Pro16 software (SAS Institute, Cary, NC, USA). The correlation heatmap was analysed with the help of the R package program.

3. Results

3.1. Principal component analysis (PCA)

Principal component analysis (PCA) is a powerful statistical approach to analyze and simplify complex and extensive datasets. In this study, PCA was employed to investigate the variation patterns in cotton genotypes and assess their genetic diversity in relation to the studied traits.

For the genotypes grown under water deficit condition (50 % water availability), the total variation was divided into 15 Principal Components (PCs) (Table 1). Notably, the first five PCs exhibited eigenvalues greater than 1, indicating their significance in explaining the variation among the trait (Figure 1). The cumulative contribution of the first seven PCs accounted for 80.836 % of the total variability in yield and fiber quality traits, reflecting their strong association with these traits under deficit conditions.

In the analysis of cotton genotypes under drought stress (DS) conditions, notable trait correlations and variability patterns were observed, shedding light on the genetic diversity of the studied traits. The boll number (BN) trait displayed a strong negative correlation with FS (fiber strenght), FU (Fiber Lenght) and FUI (Fiber uniformity). The length of vectors originating from the biplot's center depicted the correlation among traits. Traits, CHD3 was associated with long vectors, indicating higher variation among these traits. On the other hand, CHD1 exhibited the least variability, as evidenced by its shorter vector length. This information is illustrated in Figure 1, providing a visual representation of the correlations and variability among traits under DS conditions.

When we examined the relationship between canopy temperature depression (CTD) values and yield and fiber parameters; a positive correlation was found with CHD1 (Early Growth Stage) and FS, FU, FUI. This means that if these three traits are the traits we target, canopy temperature taken at Early Growth Stage can be used to determine these traits. There was a strong correlation between CHD2, CHD3 and CH4 periods and seed cotton yield. It is concluded that if a yield-oriented study is carried out, it is appropriate to take observations during these periods. In addition, since CHD3 has the longest vector length, it is concluded that the most reliable result will be the canopy temperature taken in the boll setting period.

Table 1. PCA obtained from SPAD, CHD, yield and fiber parameters deficit under deficit irrigation condition

Number	Eigen value	Percent	Cum percent
1	3.4892	23.261	23.261
2	2.0473	13.648	36.909
3	1.9120	12.747	49.656
4	1.3981	9.321	58.977
5	1.3261	8.841	67.818
6	1.0945	7.297	75.115
7	0.8582	5.722	80.836
8	0.6885	4.590	85.426
9	0.6678	4.452	89.878
10	0.4902	3.268	93.146
11	0.3820	2.547	95.693
12	0.2408	1.605	97.298
13	0.1902	1.268	98.566
14	0.1425	0.950	99.515
15	0.0727	0.485	100.000

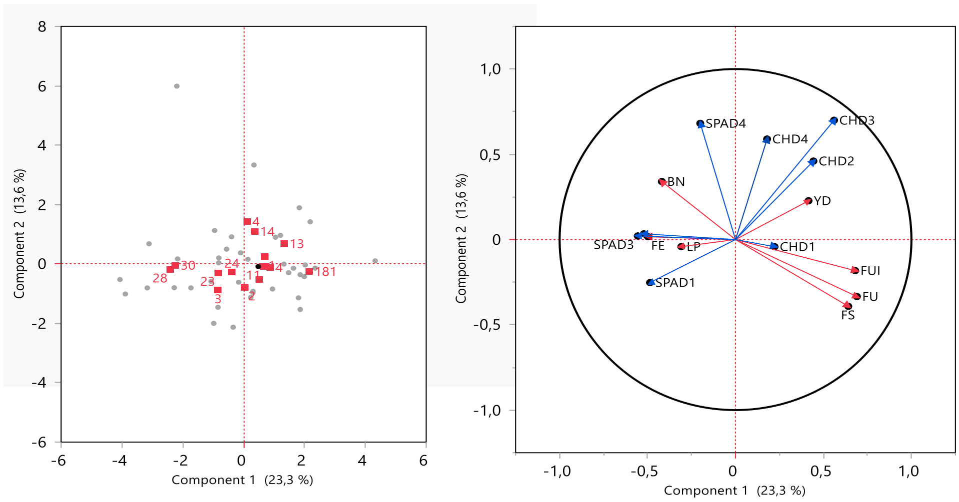

Figure 1. Summary of bar chart displaying eigenvalue and variation percentage contribution by all principal components (PCs), a biplot between PC1 and PC2 displaying the distribution of SPAD, CHD and yield and fiber parameters under deficit irrigation

*BN: Boll number. LP: Lint percentage. FU: Fiber length. FS: Fiber strength. FF:Fiber fineness. FUI: fiber uniformity index. FE: Fiber elongation. YD: Seed cotton yield. SPAD1 and CHD1: Early Growth Stage; SPAD2 and CHD2: Flowering Stage; SPAD3 and CHD3: Boll Setting Stage; SPAD4 and CHD4: Boll Opening Stage

When we examined the relationship between Leaf Chlorophyll Content (SPAD) and yield and fiber parameters; SPAD4 had the longest vector length. According to the results of the analysis. no positive correlation was found between SPAD and fiber properties except FE (fiber elongation). The highest positive correlation was observed in LP (lint percantage) trait.

The purpose of this analysis is to answer the question whether the measurements for these two traits should be made more than once or whether the two traits can be measured in the same period. Evaluating the SPAD and CHD subjected to drought stress condition (50 % water availability) observed in 4 different periods. the total variation was distributed among 8 PCs. the first three PCs displayed eigenvalues greater than 1, indicating their relevance in explaining the observed variation (Figure 2). The cumulative contribution of the first four PCs accounted for 76.901 % of the total variance in yield and fiber quality traits under drought-stress conditions (Table 2).

When these 2 physiological parameters are analysed for each period of observation; it is seen that the CHD1 parameter is the shortest vector. And there is no positive correlation with the measurements taken. CHD2, CHD3 and CHD4 are in positive correlation with each other. If only the CHD parameter is to be used. it is determined that the measurements are suitable for this observation. especially at the Boll Setting Stage. Flowering Stage and Boll Opening Stage. When the SSAP parameter was analysed. the highest effect was found in SPAD3, SPAD1, SPAD2 and SPAD3 showed positive correlation with each other (Figure 2).

As a result of this analysis. SPAD3 and CHD3 were determined as the longest proxy. And it is concluded that the most appropriate observation time for these two features is Boll Setting Stage.

Table 2. PCA obtained from SPAD1, SPAD2, SPAD3, SPAD4 and CHD1, CHD2, CHD3, CHD4 under deficit irrigation condition

Number	Eigenvalue	Percent	Cum Percent
1	2.3851	29.814	29.814
2	1.6590	20.738	50.552
3	1.2016	15.020	65.572
4	0.9064	11.330	76.901
5	0.8519	10.649	87.550
6	0.4702	5.877	93.427
7	0.3971	4.963	98.390
8	0.1288	1.610	100.000

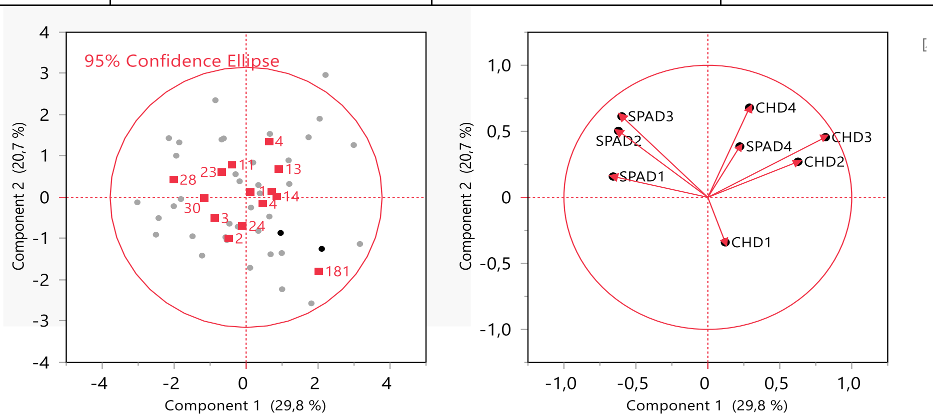

Figure 2. Summary of bar chart displaying eigenvalue and variation percentage contribution by all principal components (PCs), a biplot between PC1 and PC2 displaying the distribution of SPAD1, SPAD2, SPAD3, SPAD4 and CHD1, CHD2, CHD3, CHD4 under deficit irrigation

*SPAD1 and CHD1: Early Growth Stage; SPAD2 and CHD2: Flowering Stage; SPAD3 and CHD3: Boll Setting Stage; SPAD4 and CHD4: Boll Opening Stage:.

3.2. Correlation analysis

Correlation coefficients between yield. yield components. SPAD and CHD of cotton under water deficit conditions are presented in Figure 3. This analysis was carried out to measure whether the correlations are important to use for selection traits changed under deficit irrigation conditions.

As shown in Figure 3. LP was negatively and significantly correlated with FU, BN was positively and significantly correlated with SPAD1 and SPAD2 and SPAD4 while fiber strenght (FS) was negatively correlated with BN but FS positively and significantly correlated with YD. On the other hand. FU was negatively and significantly correlated with SPAD4. FE and LP while FU positively and significantly correlated FS. Also FS was negatively and significantly correlated with SPAD4 and positively and significantly correlated with FU, FUI and YD.

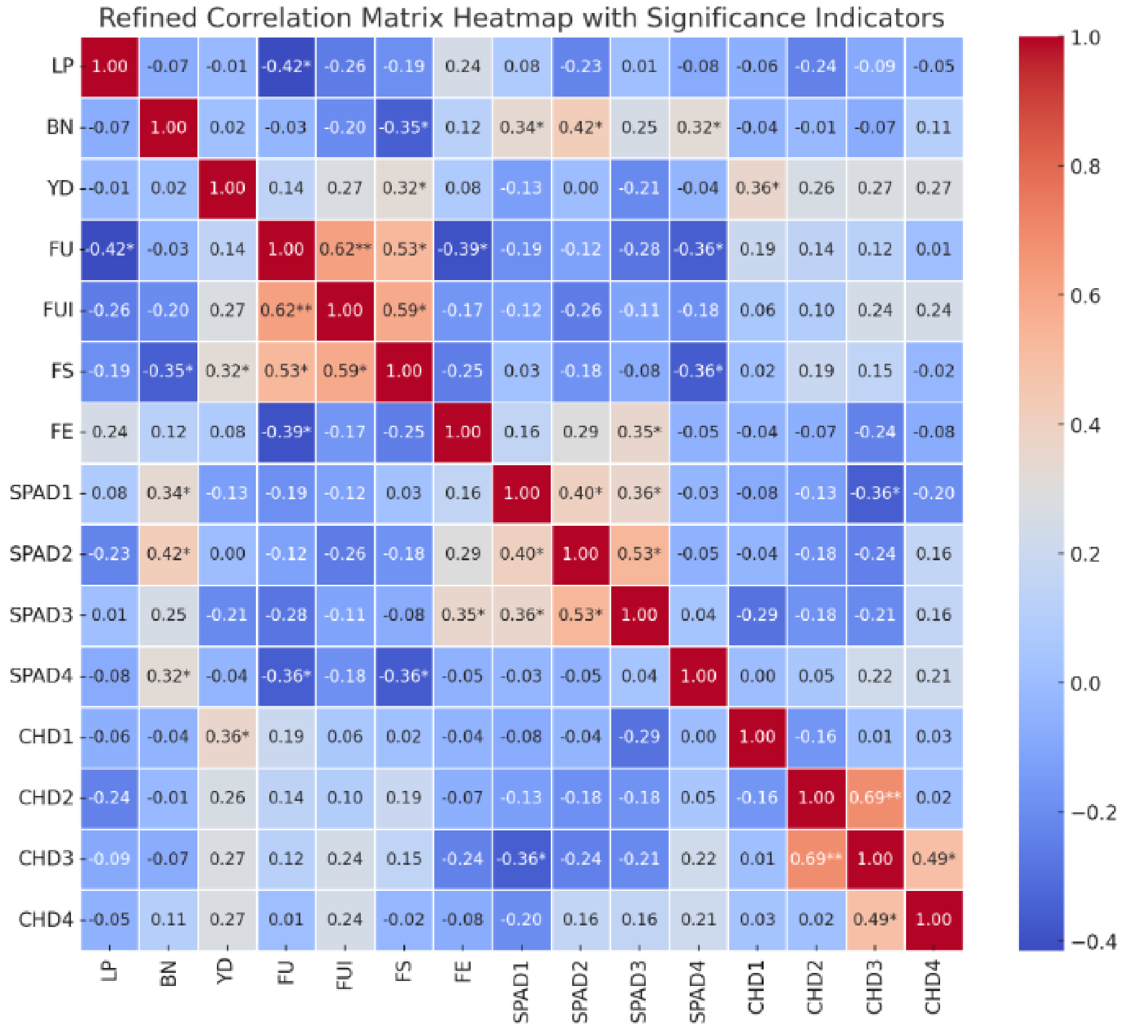

Figure 3. Correlation Matrix Heatmap for SPAD. CHD and yield and fiber parameters under deficit irrigation

*BN: Boll number. LP: Lint percentage. FU: Fiber length. FS: Fiber strength. FF:Fiber fineness. FUI: fiber uniformity index. FE: Fiber elengation. YD: Seed cotton yield. SPAD1 and CHD1: Early Growth Stage; SPAD2 and CHD2: Flowering Stage; SPAD3 and CHD3: Boll Setting Stage; SPAD4 and CHD4: Boll Opening Stage

Upon analysis of the correlations between SPAD and CHD at different time points. It was observed that SPAD1 exhibited a negative and statistically significant correlation with CHD3. while SPAD2 and SPAD3 demonstrated positive and statistically significant correlations. Additionally, SPAD2 exhibited a positive and statistically significant correlation with SPAD3. Similarly, CHD2 demonstrated a positive and statistically significant correlation with CHD3 (Figure 4).

When individual correlation heatmaps of SPAD and CHD were analysed across four different periods. It was found that there was a very high and positive correlation between BN and SPAD2. FE and SPAD3. CHD1 and YD. In contrast. SPAD4 was negatively and significantly correlated with FS and FUI.

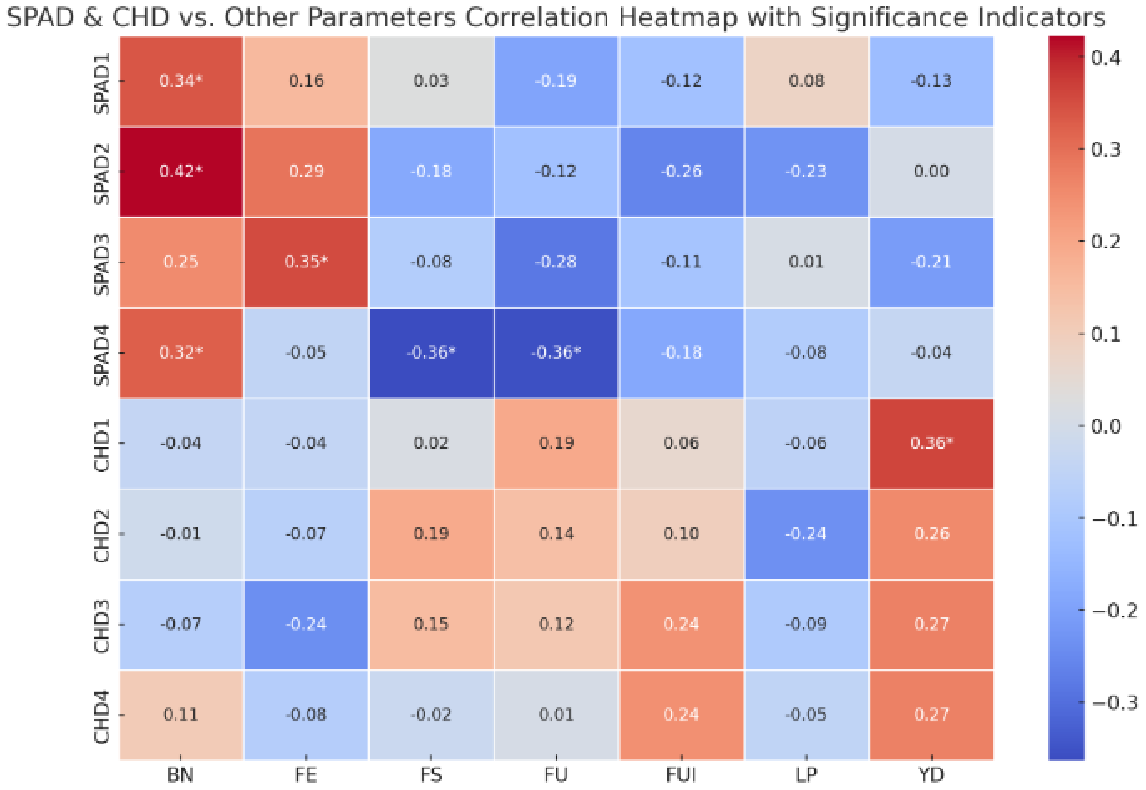

Fıgure 4. Correlation Matrix Heatmap for SPAD1. SPAD2. SPAD3. SPAD4 and CHD1. CHD2. CHD3 CHD4 under deficit irrigation

*SPAD: Leaf Chlorophyll Content. CMD: canopy temperature depression . SPAD1 and CHD1: Early Growth Stage; SPAD2 and CHD2: Flowering Stage; SPAD3 and CHD3: Boll Setting Stage; SPAD4 and CHD4: Boll Opening Stage.

4. Discussion

To determine the optimal plant development stage for assessing the SPAD parameter in cotton plants under abiotic stress conditions. It is crucial to consider the stage where the parameter can best reflect the plant's response to stress. Studies have shown that the SPAD chlorophyll meter method, is a valuable and indicative of the plant's physiological status and stress tolerance (Magwanga et al., 2020). This instrument provides a rapid and non-destructive method to measure chlorophyll content. which is indicative of plant health and stress levels In the context of cotton plants. research suggests that the SPAD parameter should be measured at specific developmental stages to effectively capture the impact of abiotic stress on the plant. For instance, in wheat. SPAD measurements were taken at the anthesis stage to assess responses to salinity and nutrient stress (Shah et al., 2019). Similarly, in rice. SPAD values were measured on the third fully opened leaf from the top under reproductive stage stress experiments (James et al., 2018). These studies highlight the importance of selecting the appropriate plant growth stage for SPAD measurements to accurately evaluate stress responses. Furthermore. the SPAD parameter has been utilized in various plant stress studies. including those involving wheat. rice. and sweet potato. to assess chlorophyll content under different stress conditions (El-Daim et al., 2019). The SPAD readings obtained from these studies have provided valuable insights into the physiological responses of plants to abiotic stressors. aiding in the identification of stress-tolerant genotypes and the development of stress mitigation strategies. When assessing canopy temperature in cotton. It is crucial to understand its significance in plant growth and development. especially in response to environmental stressors. Canopy temperature is a valuable indicator of plant stress and physiological activity. reflecting the plant's adaptation to changing conditions Monitoring canopy temperature can offer insights into the plant's water status and growth performance, as elevated temperatures may signal insufficient water availability and stress (Sarwar et al., 2019).

The timing for measuring canopy temperature and SPAD in cotton is crucial for understanding plant responses to environmental conditions and stress factors. Canopy temperature is a valuable indicator of plant water status and stress levels. reflecting the plant's physiological activity and adaptation to changing environmental conditions. Monitoring canopy temperature can provide insights into the plant's growth performance and stress tolerance. particularly in response to factors like soil salinity and water deficits (Mahan et al., 2015).

Karademir et al. (2018) in their study on advanced line and control varieties. canopy temperature values during the peak flowering period were found to be higher than the pre-flowering period. Also according to the results of their correlation analysis to determine canopy temperatures and chlorophyll content measured at different development stages and other traits associated with final yield and fiber quality traits. they found that seed cotton yield and canopy canopy at all cotton growing stages (pre-bloom, peak flowering and post-bloom/boll formation period). They observed a significant and positive relationship between temperature. However, correlation coefficients were strongest when canopy temperature was measured during the peak flowering period of the cotton growth period. Similar results have been observed by many researchers (Conaty et al., 2015; Amani et al., 1996; Bahar et al., 2008; Alam, 2014). However. the results of this study concluded that. contrary to these studies. this correlation is stronger in the boll opening stage. There are also studies parallel to these study results (Lopes and Reynolds, 2010; Mason and Singh, 2014; Bennani et al., 2016).

5. Conclusion

The results of this study demonstrate the effectiveness of Principal Component Analysis (PCA) in unraveling complex trait variations and genetic diversity in cotton under water deficit and drought stress conditions. Under 50 % water availability. PCA revealed that the first five Principal Components (PCs) explained significant trait variations. particularly in yield and fiber quality traits. Notably, traits like boll number (BN) exhibited strong negative correlations with fiber strength (FS), fiber length (FU) and fiber uniformity index (FUI). highlighting genetic associations and variability patterns.

The analysis also explored the relationship between canopy temperature depression (CTD) and yield/fiber parameters revealing positive correlations with early growth stage (CHD1) and certain fiber traits. Canopy temperature measurements during specific stages like boll setting were deemed most reliable due to trait associations. Leaf chlorophyll content (SPAD) analysis indicated SPAD4's strong correlation with fiber elongation (FE) and lint percentage (LP) emphasizing its role in trait evaluation. However. SPAD showed limited positive associations with fiber properties overall. Correlation analyses between SPAD and CHD across different time points identified significant relationships, with SPAD2 and SPAD3 exhibiting positive correlations. Additionally, CHD2 and CHD3 showed positive correlations, suggesting the importance of specific observation periods for trait assessments.

Overall, these findings underscore the significance of conducting timely measurements of SPAD and CHD to comprehend cotton traits under drought stress conditions. Specifically, SPAD3 and CHD3 (boll opening stage) emerged as crucial indicators, highlighting the boll setting stage as the most appropriate observation time for assessing these features.

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