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Growth Parameters of Cotton in Relay Strip Intercropping: Before and After Wheat Harvest

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***ABSTRACT:** To investigate cotton's adaptation to various microclimates provided by wheat height, a field experiment was conducted to observe the morphological and physiological traits of cotton seedlings before and after wheat harvest. The cotton was grown in relay strip intercropping with wheat of varying heights. The study observed canopy temperature depression (CTD), average leaf area (ALA), specific leaf area (SLA), SPAD values, net assimilation rates (NAR), total dry weight (TDW). During the shading period, intercropped cotton exhibited stress, indicated by CTD, compared to monocropped cotton (MC). This negative effect was more pronounced in short wheat-cotton intercropping (SC). Microclimates influenced leaf traits and biomass accumulation, with smaller ALA, higher SLA, higher SPAD values, and lower NAR observed in intercropped cotton, especially in SC, resulting in decreased TDW. Following wheat harvest, cotton plants, particularly in SC, exhibited significant NAR recovery by adjusting leaf structure. However, while this adjustment mitigated differences in TDW and yield compared to tall wheat-cotton intercropping (TC), disparities with MC remained. SC had a more pronounced negative impact on cotton before wheat harvest compared to TC. However, rapid recovery of cotton mitigated this negative effect in SC after wheat harvest.*

***Keywords:** Cotton, cotton growth dynamics, crop adaptation, intercropping systems, relay strip intercropping, wheat microclimate.*

Sonradan Araya Ekim Sisteminde Pamuk Büyüme Dinamikleri: Buğday Hasadı Öncesi ve Sonrası

***ÖZ:** Pamuk bitkisinin farklı buğday yüksekliklerinin sağladığı mikroklimalara adaptasyonunu araştırmak için, buğday hasadından önce ve sonra pamuk fidelerinin morfolojik ve fizyolojik özelliklerini gözlemlemek üzere bir tarla denemesi gerçekleştirildi. Pamuk, farklı yüksekliklerde buğday ile sonradan araya ekim sisteminde yetiştirilmiştir. Çalışmada, kanopi sıcaklık depresyonu (CTD), ortalama yaprak alanı (ALA), spesifik yaprak alanı (SLA), SPAD değerleri, net asimilasyon oranları (NAR) ve toplam kuru ağırlık (TDW) gibi morfolojik ve fizyolojik özellikler buğday hasadından önce ve sonra gözlemlenmiştir. Gölgeleme süreci boyunca, sonradan araya ekilen pamuk, tek ekim pamuğa (MC) kıyasla CTD de görüldüğü üzere stres belirtileri göstermiştir. Bu olumsuz etki, kısa buğday-pamuk ekimi (SC) sisteminde daha belirgin bulunmuştur. Mikroklimalar, yaprak özelliklerini ve biyokütle birikimini etkilemiş; SC'de özellikle daha küçük ALA, daha yüksek SLA, daha yüksek SPAD değerleri ve daha düşük NAR gözlemlenmiş ve bu durum TDW'de azalmaya neden olmuştur. Buğday hasadından sonra, özellikle SC'de pamuk bitkileri, yaprak yapılarını ayarlayarak NAR'da önemli bir iyileşme göstermiştir. Ancak, bu ayarlama, TDW ve verimde, uzun buğday-pamuk ekimi (TC) ile karşılaştırıldığında farkları azaltırken, MC ile karşılaştırıldığında farklılıkları tamamen ortadan kaldıramamıştır. SC, buğday hasadından önce pamuk üzerinde TC'ye kıyasla daha belirgin olumsuz bir etkiye sahip bulunmuştur. Ancak, pamuktaki hızlı iyileşme, bu olumsuz etkiyi buğday hasadından sonra SC'de hafifletmiştir.*

Anahtar kelimeler: *Pamuk, pamuk büyüme dinamikleri, bitki adaptasyonu, araya ekim sistemleri, sonradan araya ekim, buğday mikroklima.*

INTRODUCTION

Türkiye is one of the countries that is exposed to challenges such as drought, urbanization, and unsuitable lands. From 2004 to 2023, the total agricultural area in Türkiye decreased by approximately 10% according to derived data from TURKSTAT (2024). Similarly, the wheat-growing area exhibited a decreasing trend parallel to the total agricultural area (r^2=0.71). However, there was a lower correlation between the decrease in cotton-growing area and the total agricultural area (r^2=0.42) as shown in Figure 1. The changes in cotton-growing areas in

Türkiye fluctuated throughout the period from 2004 to 2023, possibly influenced by factors such as drought, government support for cotton production, and the availability of irrigation through the Southeastern Anatolia Project (GAP), and soil contamination due to the intensive use of chemicals in cotton farming. Soil contamination from the heavy use of chemicals in cotton cultivation has further exacerbated the reduction of arable lands (Cevheri and Yilmaz, 2019). In China, the competition between staple crops and cotton has become a serious issue (Dai and Dong, 2014), and a similar trend is anticipated in Türkiye, with the potential for competition between staple crops and strategic crops such as cotton for agricultural land. Thus, promoting the adoption of intercropping practices, particularly the intercropping of wheat and cotton, presents itself as a viable approach to address this challenge and optimize agricultural productivity in Türkiye.

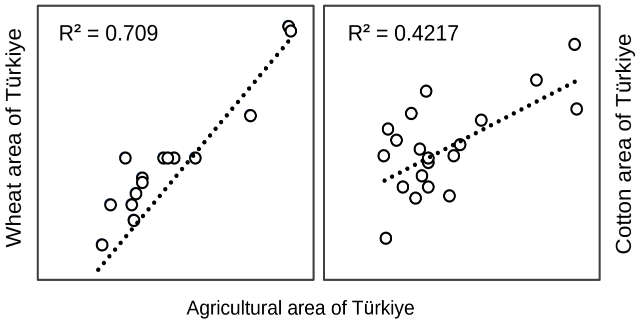

Figure 1. Correlation between the total agricultural area and wheat-growing area, and the total agricultural area and cotton-growing area in Türkiye from 2004 to 2023 (TURKSTAT, 2024).
Şekil 1. 2004-2023 yılları arasında Türkiye'deki toplam tarımsal alan ile pamuk ekim alanı ve toplam tarımsal alan ile buğday ekim alanı arasındaki korelasyon (TURKSTAT, 2024).

Intercropping is an age-old cultivation system utilized predominantly in developing countries (Wahla *et al.*, 2009; Aziz *et al.*, 2015). This approach involves the simultaneous cultivation of two or more crops within the same area and timeframe (Wezel *et al.*, 2013). There are many intercropping patterns defined which are mixed, row, strip and relay intercropping (Machado, 2009). In row intercropping, at least one crop is sown in rows simultaneously. The distance between crops is wide enough for easy differentiation yet narrow enough for interaction in strip intercropping systems. In relay intercropping, crops are grown together during different stages of their life cycle. In brief, combining relay, row and strip intercropping is referred to as relay strip intercropping (RSI). The appropriate RSI pattern should be chosen considering the crop species and their interactions with each other. Wheat and cotton crops have different cultivation times during the growing season, with their cultivation periods overlapping for a brief period. Therefore, relay strip intercropping is considered a suitable cropping pattern for cultivating wheat and cotton together.

In relay strip intercropping, cotton crops are planted in the gaps between wheat strips, allowing for their co-growth with wheat until wheat harvest, which occurs approximately seven weeks after planting (Zhang, 2008c). Typically, the later-planted crop grows under the shade of the previously planted crops, and the amount of shading depends on the height of these earlier crops. Meanwhile, the primary crop is exposed to full sunlight after the earlier crop is harvested (Wu *et al.*, 2016). While relay strip intercropping enhances land use efficiency, it also presents several disadvantages for the growth of cotton. Cotton development and maturity are delayed in the wheat-cotton intercropping systems because of shade of wheat and competition of water and nutrients, and thus cotton yield is eventually reduced (Zhang *et al.*, 2008b; Poorter *et al.*, 2019). It is well established that in intercropping systems, crops compete for above-ground radiation as well as for nutrients and water below ground (Machado, 2009). Light is the primary limiting factor in relay strip intercropping, when water and nutrients are readily available in the soil (Francis, 1989). Agronomic improvements are needed in the relay strip intercropping system to enhance land use efficiency while also maintaining the productivity and quality of the suppressed crop, which is generally a strategic crop. These improvements could include optimizing crop height, planting densities, and irrigation techniques.

Success in the relay strip intercropping system relies on achieving a harmonious balance in crop competition (Machado, 2009) and this competition can be mitigated through spatial arrangement (Aziz *et al.*, 2015). Numerous studies in the literature have focused on the best design of intercropping systems (Porter and Khalilian, 1995; Khan *et al.*, 1999; Zhang *et al*, 2007, 2008b). As mentioned in Zhang *et al.* (2008c), the primary reason for the reduced yield in intercropped cotton is the modified microclimate within the relay strip intercropping system. The cotton seedlings are exposed to the shading of tall wheat, which affects both canopy and soil temperature in the wheat-cotton relay strip intercropping system. Although the cotton plant adapts morphologically to the cultivated environment due to its indeterminate growth (Gangwar and Prasad, 2005; Siebert *et al.*, 2006; Huang *et al.*, 2017), the delay in cotton growth and development is primarily caused by the shading effect of wheat (Zhang *et al.*, 2007), and this delay cannot be completely recovered (Zhang *et al.*, 2008b). Several studies have focused on spatial arrangement to optimize light interception for cotton growth, but there is limited research on modifying light distribution within the cotton canopy by adjusting the plant height of wheat. Machado (2009) emphasized the significance of understanding the physiology, growth habits, canopy structure, root system, and nutrient utilization of intercropped crops. To optimize the advantages of intercropping, it is crucial to identify compatible species and genotypes, determine the most suitable sowing design, and adjust crop density accordingly (Yildirim and Ekinci, 2017). Based on these considerations, it is imperative to emphasize the significance of identifying the optimal conditions for the growth and development of cotton plants under the various microclimates created by wheat.

This study focused on improving relay strip intercropping systems by examining the impact of wheat height on cotton growth and development. While numerous studies have investigated the efficiency of intercropping systems, there is a lack of research specifically addressing how adjusting the height of wheat can mitigate shading effects and enhance cotton growth. Understanding these interactions is essential for optimizing the balance between crops and improving overall productivity within relay strip intercropping systems.

MATERIALS AND METHODS

Study area

The experiment was conducted in a field on the Bornova Plain (Izmir), located in Western Turkey (38°27'06.0"N 27°13'31.9"E), at an elevation of 50 meters above sea level, during 2020/21 growth season. The region has a Mediterranean climate characterized by mild, wet winters and hot, dry summers. During the experiment, the average air temperature, humidity, and total precipitation were 19.4ºC, 55.5%, and 716 mm, respectively. Detailed weather data are shown in Figure 2. The soil profile at depths of 0–20 cm and 20–40 cm consisted of silt-clay and clay-loam textures, respectively, with pH values of 8.2 and 7.8, according to soil textural classification (Gerakis and Baer, 1999).

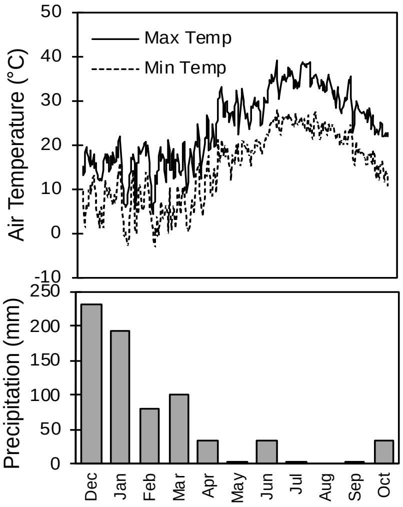

Figure 2. Daily maximum and minimum temperatures (ºC), and monthly precipitation (mm) levels during the experiment.

Şekil 2. Deneme boyunca günlük maksimum ve minimum sıcaklıklar (ºC), ve aylık yağış (mm) seviyeleri.

Plant material

Two wheat varieties, differing in plant height, were obtained from TİGEM (General Directorate of Agricultural Enterprises) to establish varying microclimates for the intercropped cotton. The taller wheat variety, Cumhuriyet-75, measured 75 cm in height. In contrast, the other wheat variety, Golia-99, is known to be shorter, approximately 30% shorter compared to Cumhuriyet-75. These wheat varieties are well adapted to coastal regions like the experimental site. Additionally, the selection of cotton varieties was informed by the growth patterns recognized by farmers in the coastal region of Türkiye. Lima was designated as full-season cotton due to its slower growth rate compared to DP 396. Conversely, DP 396 was identified as short-season cotton, typically planted as a second crop after wheat in the coastal region of Türkiye, owing to its rapid growth.

Experimental design and treatments

The trial was designed as a Strip-Split-Plot system within a Randomized Complete Blocks Design (RCBD) with three replications. The wheat and cotton were planted using the relay strip intercropping method in a 4:2 wheat-cotton design (Zhang *et al.*, 2007;

Çakaloğulları, 2023), while cotton was also sown conventionally for the control group. Each intercropping plot was comprised of three strips with four rows of wheat and two strips with two rows of cotton. The cotton planting in both RSI and traditional designs, as well as row dimensions, are illustrated in Figure 3. The study examined two main factors as treatments: cropping system and growth habit of cotton. The cropping system included monocropping of cotton (MC) as control group, tall wheat-cotton intercropping (TC) and short wheat-cotton intercropping (SC).

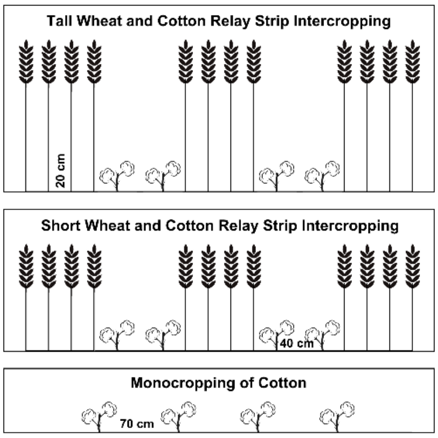

Figure 3. Illustrations of tall wheat and cotton relay strip intercropping (TC), short wheat and cotton relay strip intercropping (SC), and monocropping of cotton (MC).
Şekil 3. Uzun buğday ve pamuk (TC), kısa buğday ve pamuk (SC) sonradan araya ekim sistemleri, ve tek pamuk (MC) ekim sistemlerine ait çizimler.

Field managements

The RSI plots were established by sowing wheat on December 24, 2020. The wheat was sown at a rate of 550 plants m^{-2}, and 100 kg ha^{-1} of pure nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) were applied before wheat planting using 15-15-15 bottom fertilizer. The spaces designated for cotton in the RSI plots were cleared of weeds and prepared for planting using mechanical hoeing techniques. The cotton planting for both intercropping and monocropping plots took place on May 5, 2021. The initial fertilization, consisting of 100 kg ha^{-1} of pure nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) in the form of 15-15-15 bottom fertilizer, was applied to the cotton plots. Following cotton sowing, the drip irrigation system was immediately implemented for the first irrigation, with each cotton line equipped with one drip pipe.

The wheat plants in the RSI system were manually removed without causing any damage to the cotton plants on June 24, 2021, marking the end of the co-growth period of wheat and cotton, which lasted for 50 days. After the wheat harvest, the second fertilization was applied to the cotton plots at a rate of 100 kg ha^{-1} of nitrogen (N) in the form of ammonium sulfate (21%).

The cotton in the intercropped system received totally 370 mm of water (from both rainfall and irrigation) during the cotton growth period, while the monocropped cotton received 460 mm. Pest, disease, and weed control were implemented using both chemical methods, such as pesticides and herbicides, and mechanical methods, including manual weeding and hoeing, based on the farmers' experiences, to promote optimal crop growth. The cotton was harvested by hand on October 12, 2021.

Data collection and measurements

The climate data for the experimental site was collected using a weather data API service (Visual Crossing). The cotton measurements were conducted at various days after sowing (DAS): twice before wheat harvest (DAS 31 and DAS 50) and twice after wheat harvest (DAS 63 and DAS 73) to assess the response of cotton in the RSI systems. At each measurement time, three cotton plants were collected from each plot for measuring leaf area, dry weights and cottonseed yield. Additionally, SPAD and canopy temperature, boll number and plant height were measured without harming the cotton plants.

The collected cotton plants were separated into their components, including leaves, stems, and bolls. The leaves were placed on A4 paper and photographed immediately. The images were then processed using software (Photoshop), and leaf area of each cotton plant was estimated with a pixel counting method (Cakalogullari *et al.*, 2020a). Each component of the cotton plants was dried separately at 105ºC for one day. Subsequently, the dry weight of the leaves and the total dry weight were determined. The specific leaf area (SLA – cm^2 g^{-1}) was defined as the leaf area per unit leaf dry mass and was calculated by dividing the leaf area by the leaf dry matter. In addition, the average leaf area (ALA) was calculated as the mean of the individual leaf areas measured across all leaves on each plant. The cotton yield was evaluated by harvesting the entire plot excluding the border rows, and the cottonseed weight per plant (g plant^{-1}) was determined.

Canopy temperatures of cotton were measured using an infrared thermometer on fully expanded young leaves in all treatments. The measurement of canopy temperature was conducted at the peak temperature during the day to accurately assess the effects of stress. While measuring canopy temperature, ambient temperatures were recorded using a high-precision air temperature gauge (Tinytag Plus 2®). The Tinytag device was carried during measurements and held close to the leaf being measured to ensure accuracy. The time of each canopy temperature measurement was noted, and the corresponding ambient temperature was later retrieved from the Tinytag recordings based on synchronized timestamps. Thereafter, Canopy Temperature Depression (CTD) was calculated according to Ayeneh *et al.* (2002) as shown below:

$$CTD = AT - CT$$

where, AT and CT are the ambient air and canopy temperatures, respectively. Furthermore, the chlorophyll content of cotton was non-destructively determined using a SPAD meter (Konica Minolta – SPAD-502 Plus), conducted simultaneously with the measurement of canopy temperature.

The net assimilation rate (NAR – g m^{-2} day^{-1}) is a crucial parameter in understanding the photosynthetic efficiency and growth of plants. The calculation of the NAR was performed as follows:

$$NAR = \left(\frac{W_2 - W_1}{T_2 - T_1}\right)\left(\frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1}\right)$$

where NAR represents the net assimilation rate, W_2 and W_1 denote the total dry weights of the plant at the respective times T_2 and T_1, and LA_2 and LA_1 represent the corresponding leaf areas at times T_2 and T_1 (Díaz-López *et al.*, 2020).

Statistical analysis

Statistical analysis was performed using Python programming language, a widely used tool for data analysis and statistical computing in scientific research. A two-way analysis of variance (ANOVA) was conducted to assess the effects of the cropping systems and growth habits of cotton on the variables of interest. Post-hoc analysis was carried out using the least significant difference (LSD) method to determine specific differences between treatment groups. Additionally, p-values were calculated to evaluate the significance of observed differences. Pearson correlation analysis was employed to investigate the relationships between certain variables and to assess their strength and direction. The statistical significance level was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The study utilized two different cotton varieties, DP 396 and Lima, to assess their growth characteristics under relay strip intercropping and monocropping systems. Although previous research (Wang *et al.*, 2021) suggested that short-season cotton varieties may offer advantages in certain intercropping setups, no significant differences in early-stage growth between the cotton varieties were observed in this study, as shown in Table 1. Additionally, studies investigating different cotton genotypes under relay strip intercropping systems remain limited in the literature, which highlights the relevance of this study’s findings. As a result, these results confirmed that the effects of the different cotton varieties were minimal and thus are not further discussed in the results and discussion sections. Instead, the focus of the analysis is on comparing the effects of the different intercropping systems and the monocropping system on cotton seedling growth. By treating the two varieties as a single factor in the statistical analysis, the number of replicates effectively doubled from three to six, enhancing the reliability and robustness of the findings. This decision ensures that the presentation of the results remains clear and focused on the primary factors influencing cotton growth in the context of this study.

Table 1. Mean canopy temperature depression (CTD), specific leaf area (SLA), average leaf area (ALA), SPAD, and total dry weight (TDW) values for cotton varieties DP 396 and Lima at different days after sowing (DAS31, DAS50, DAS63, and DAS73), along with standard errors, statistical significance, and LSD values.

Çizelge 1. DP 396 ve Lima pamuk çeşitlerinin farklı ekim sonrası günlerdeki (DAS31, DAS50, DAS63 ve DAS73) ortalama canopy sıcaklık farkı (CTD), spesifik yaprak alanı (SLA), ortalama yaprak alanı (ALA), SPAD ve toplam kuru ağırlık (TDW) değerleri, standart hatalar, istatistiksel anlamlılık ve LSD değerleri ile birlikte.

	Canopy Temperature Depression (CTD)			
	DAS31	DAS50	DAS63	DAS73
DP 396	2.8 ± 0.4	5.3 ± 0.9	5.7 ± 0.4	5.7 ± 0.7
Lima	3.7 ± 0.7	5.2 ± 0.5	5.9 ± 0.5	6.1 ± 0.4
LSD	1.6	2.2	1.4	1.7
	Specific Leaf Area (SLA – cm^2 g^{-1})			
	DAS31	DAS50	DAS63	DAS73
DP 396	96.3 ± 2.5	97.4 ± 3.7 *b*	72.2 ± 4.9	63.0 ± 6.3
Lima	99.5 ± 4.3	105.9 ± 3.7 *a*	66.6 ± 1.4	63.0 ± 9.3
LSD	10.4	11.4	9.8	24.0
	Average Leaf Area (ALA – cm^2 leaf^{-1})			
	DAS31	DAS50	DAS63	DAS73
DP 396	6.3 ± 0.5 *b*	18.1 ± 0.7	25.0 ± 1.5 *b*	28.8 ± 1.3 *a*
Lima	7.3 ± 0.3 *a*	18.5 ± 1.3	28.4 ± 1.3 *a*	32.8 ± 1.5 *b*
LSD	1.3	3.1	4.4	4.2
	SPAD			
	DAS31	DAS50	DAS63	DAS73
DP 396	37.9 ± 1.1	46.6 ± 1.4	32.2 ± 0.5	37.8 ± 1.2 *a*
Lima	39.2 ± 1.3	45.5 ± 1.3	32.7 ± 1.7	35.4 ± 0.7 *b*
LSD	3.6	4.2	3.4	2.8
	Total Dry Weight (TDW – g plant^{-1})			
	DAS31	DAS50	DAS63	DAS73
DP 396	0.39 ± 0.04	2.0 ± 0.2	7.2 ± 0.9	14.4 ± 2.3
Lima	0.43 ± 0.02	1.6 ± 0.2	6.2 ± 0.7	10.6 ± 1.3
LSD	0.1	0.6	2.5	5.7

Canopy temperature depression

When compared with monocropping which was the control cropping system, the tall wheat-cotton and short wheat-cotton intercropping systems exhibited different outcomes in canopy temperature depression (CTD) prior to wheat harvest. Thanks to the shading provided by the tall wheat, the TC cotton plots attained higher CTD values (4.5) at DAS 31 (Figure 4). In contrast, the SC cotton, cultivated under short wheat which offered less shading, especially exhibited significantly lower CTD values (2.0) on DAS 31 (Figure 4). The shading provided by wheat could confer advantages in terms of reducing evaporation (Yildirim and Ekinci, 2017) and mitigating heat stress during the early growth stages of intercropped cotton. However, it is noteworthy that soil moisture content decreases as a result of wheat consumption in intercropping systems (Zhang *et al.*, 2008b). The reduction of CTD has been identified as a crucial factor leading to a decrease in transpiration in various crops such as wheat, rice, and sugar beet (Guendouz *et al.*, 2021). Canopy temperature depression is a significant trait for assessing plant water stress status and is closely linked to the transpirational status of crops (Ashfaq *et al.*, 2022). Taking all of these factors into consideration, it can be asserted that the SC system was unable to sufficiently reduce evaporation due to reduced shading as compared to the TC system and also experienced a decrease in soil moisture content due to the wheat's water consumption. Thus, the reduction of CTD in SC cotton demonstrated a decrease in transpiration, signifying that the cotton plants were experiencing water stress. However, by DAS 50, prior to wheat harvest, CTD of SC cotton (4.1) was equal to that of MC cotton (4.6), highlighting the adaptive capability of cotton. Particularly on DAS 50, TC cotton (7.0) maintained higher CTD values than even MC cotton, attributable to the shading effect of wheat. Shading has been identified as a significant factor influencing leaf temperature, as the reduction of incident solar radiation leads to lower leaf temperatures during the day (Morais *et al.*, 2006). However, following the wheat harvest, SC cotton exhibited an increase in their CTD (Figure 4). Numerous researchers have reported that cotton plants demonstrate morphological adaptations to their environment, including modifications to their canopy structure (Zhang *et al.*, 2007; Huang *et al.*, 2017; Zhi *et al.*, 2019). These findings indicated that SC cotton experienced extra environmental stress in contrast with TC cotton due to the microclimate of intercropping before wheat harvest, yet they rapidly acclimatized to new conditions following the harvest. On DAS 63 and 73, following the wheat harvest, the CTD values of the intercropped cotton were significantly higher than those of the MC cotton (Figure 4). It can be suggested that intercropped cotton demonstrated a robust adaptive capability as they increased their transpiration.

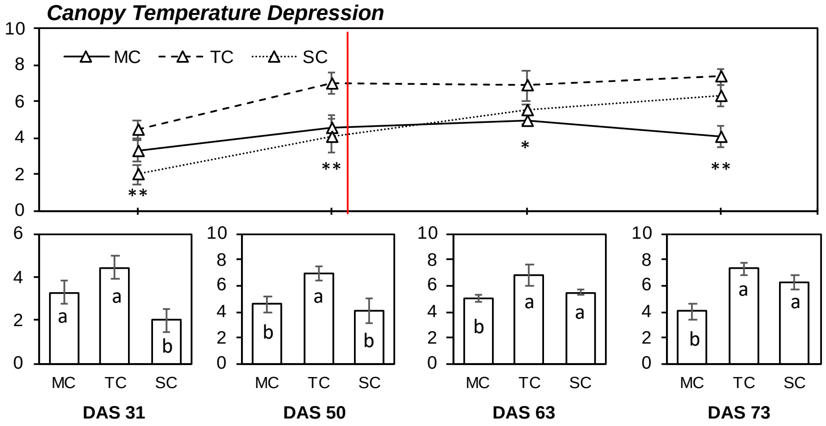

Figure 4. Comparative canopy temperature depressions (CTD) for cotton under different cultivation methods across days after sowing (DAS). MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the CTDs in different cropping systems. The red line indicates the harvest time of wheat. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 4. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı kanopi sıcaklık depresyonu (CTD) değerleri. MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki CTD'leri gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

Specific leaf area

In this study, it was observed that the leaf morphology of intercropped cotton tended to change in accordance with their CTD values prior to wheat harvest. A strong correlation was identified between CTD and morphological parameters before wheat harvest: SLA (r=0.62, p=0.031) and ALA (r=0.70, p=0.011). However, no strong correlation was observed between CTD and these morphological parameters (SLA and ALA) in MC cotton, both before and after the wheat harvest. The SLA provides insights into leaf thickness, with lower SLA values indicating greater leaf thickness (Guo *et al.*, 2022; Liu *et al.*, 2018). Several studies have shed light on the factors affecting SLA, including temperature (Rosbakh *et al.*, 2015), shading (Figueiredo *et al.*, 2019) and precipitation (Guo *et al.*, 2022). Based on our findings, it was observed that the leaf thickness of intercropped cotton significantly decreased prior to wheat harvest (Figure 5). It was reported that the specific leaf area exhibited rapid changes over short timescales in response to variations in environmental conditions (Reich, 2014; Poorter *et al.*, 2019; Liu *et al.*, 2023). In our results, after wheat harvest, the intercropped cotton rapidly increased their leaf thickness and reached the SLA levels of MC cotton, thanks to the new environmental conditions. A similar result was reported, indicating that the leaf thickness of soybeans decreased under intercropping with maize conditions. However, after maize harvest, the leaf thickness of soybeans increased and reached the same level as the leaf thickness of control soybeans (Wu *et al.*, 2016). The reduction in leaf thickness relative to MC cotton prior to wheat harvest was found to be less pronounced in SC cotton (21.1%) compared to TC cotton (28.9%). It can be attributed to the lower shading effect of the SC cropping system. This observation is consistent with reports indicating that an increase in light flux density results in a decrease in the specific leaf area (SLA) of soybean (Reddy *et al.*, 1989).

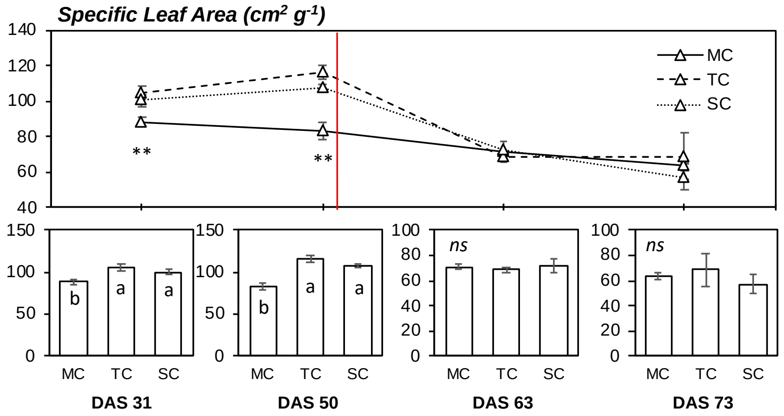

Figure 5. Comparative specific leaf areas (SLA) for cotton under different cultivation methods across days after sowing (DAS). MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the SLAs in different cropping systems. The red line indicates the harvest time of wheat. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 5. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı spesifik yaprak alanı (SLA) değerleri. MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki SLA'ları gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

Average leaf area

An increase in ALA was observed across days after sowing in all cropping systems. However, the ALA of intercropped cotton, especially in the SC system, remained consistently lower at all times compared to monocropped (MC) cotton (Figure 6). Prior to wheat harvest, there was a strong correlation (r=0.74, p=0.006) between SLA and ALA of intercropped cotton, but this correlation was not strong (r=0.33, p=0.298) after the wheat harvest. This indicates that, prior to wheat harvest, in intercropped conditions, an expansion in leaf area led to a decrease in leaf thickness, in contrast to the conditions observed in MC cotton. Similarly, Liu *et al.*, (2016) proposed that the shade-induced increases in specific leaf area (SLA) represented a plastic response to optimize radiation capture. Numerous studies have indicated that shading can result in a reduction in individual leaf area and leaf expansion, thereby impacting the overall leaf area of cotton plants. For instance, Wu *et al.* (2017) demonstrated that shade treatment significantly decreased cell numbers in developing and maturing leaves, contributing to a decrease in individual leaf area of soybean under shading conditions. Similarly, reported findings indicate that shading inhibits leaf expansion, leading to a reduction in light interception, ultimately influencing the leaf area of arabidopsis (Gong *et al.*, 2014). According to another study, limited light penetration within intercropped cotton resulted in a reduction of leaf area index during the seedling stage of cotton (Zhi *et al.*, 2019). Moreover, SC cotton generally exhibited lower ALA values throughout, but these were significantly lower than those of the MC and TC cotton at DAS 50 and DAS 73. Specifically, it was found that SC cotton had approximately 43% and 37% lower ALA compared to MC and TC cotton at DAS 50, respectively. As mentioned earlier, SC cotton experienced additional stress conditions beyond shading, in contrast to TC cotton. The CTD data corroborated that SC cotton experienced greater stress compared to TC cotton at DAS 31 (Figure 4). This finding indicated that SC cotton plants were more significantly affected by intercrop conditions at DAS 31, which is reflected in their lower ALA, particularly at DAS 50.

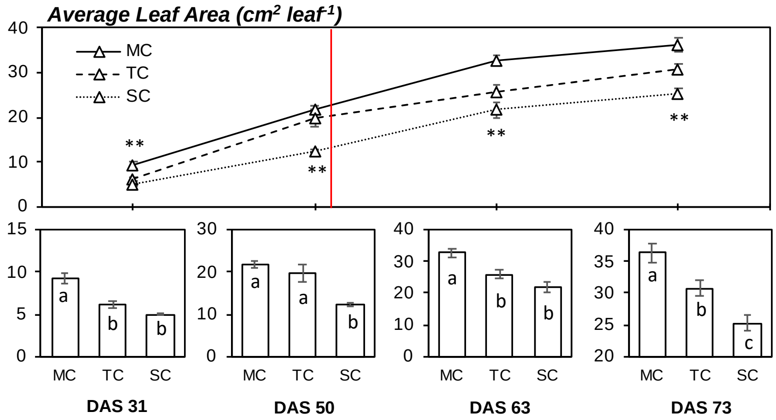

Figure 6. Comparative average leaf areas (ALA) for cotton under different cultivation methods across days after sowing (DAS). MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the ALAs in different cropping systems. The red line indicates the harvest time of wheat. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 6. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı ortalama yaprak alanı (ALA) değerleri. MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki ALA'ları gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

SPAD

The results indicated that the microclimate created by the intercropping system tended to increase the SPAD values of intercropped cotton prior to wheat harvest, followed by a significant decrease in SPAD values after the wheat harvest (Figure 7). Prior to wheat harvest at DAS 50, significant differences were observed in the SPAD values of the different cropping systems. As previously mentioned, the greater change in leaf morphology at DAS 50 was attributed to higher stress conditions for SC cropping system cotton compared to TC cotton at DAS 31. Similarly, regarding SPAD values at DAS 50, SC cotton exhibited SPAD values 31% higher than those of MC cotton, while TC cotton had values 11% higher than MC cotton. It might be considered that the increase in SPAD was due to reduced light availability in intercropping systems, yet SC cotton plants were also influenced by other environmental stress factors such as water deficit. A study has indicated that reducing the incident photosynthetic photon flux density on a leaf result in increased chlorophyll content in various plant species, including cotton (Chen *et al.*, 2015). It was also claimed that notable adjustments, such as a reduction in leaf thickness and an increase in chlorophyll content, are observed in shaded plants (Valladares and Niinemets, 2008; Wu *et al.*, 2016). Furthermore, several studies have reported an increase in chlorophyll content under limited water conditions (Martinez and Guiamet, 2004; Ahmad *et al.*, 2013), implying a reduction in leaf area and an increase in pigment density. Furthermore, according to Cakalogullari *et al.*, (2020b), the leaf tissues of cotton accumulated proline (a stress-related amino acid) to adapt to deficit water conditions, resulting in a significant increase in the SPAD of cotton leaves. Despite the wheat harvest, especially SC cotton continued to maintain their highest SPAD values at DAS 63 and DAS 73. This means that SPAD value was not only affected by chlorophyll production but also affected by leaf expansion or shrinkage. As such, the smaller reduction in leaf thickness and reduced leaf area in SC cotton led to the highest SPAD values prior to wheat harvest, and this trend continued even after the harvest.

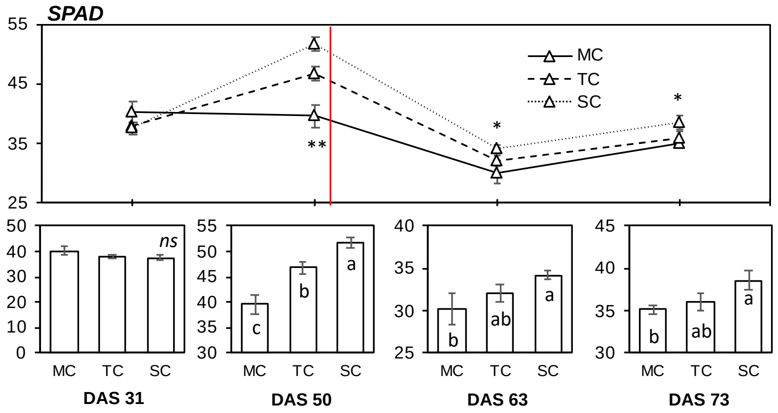

Figure 7. Comparative SPADs for cotton under different cultivation methods across days after sowing (DAS). MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the SPADs in different cropping systems. The red line indicates the harvest time of wheat. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 7. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı ortalama SPAD değerleri. MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki SPAD'ları gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

Net assimilation rate

Significant differences were observed in the Net Assimilation Rate (NAR) among the cropping systems at various times relative to the wheat harvest (Figure 8). The NAR of cotton tended to increase over time, but the outcomes varied among cropping systems. While SC cotton plants exhibited a lower NAR prior to wheat harvest (BWH) (8.4 g m^{-2} day^{-1}), they showed the highest NAR during the initial stage following the wheat harvest (AWH_1) (16.8 g m-2 day-1) and maintained their NAR increase even in the subsequent stage after the harvest (AWH_2) (47.2 g m^{-2} day^{-1}). It appeared that the adaptation of SC cotton to intercropped conditions provided a benefit to their photosynthetic activity after the wheat harvest. It was corroborated that in SC cotton, a strong correlation was found between NAR after wheat harvest and various leaf traits before wheat harvest: SLA, ALA and SPAD. However, this correlation was not found in the MC and TC cotton (Figure 9). This supports the notion that the modified leaf morphology of SC cotton before wheat harvest contributed to an increase in photosynthetic activity following the wheat harvest. Similarly, Han *et al.* (2019) demonstrated that higher area-based photosynthesis in *G. hirsutum* is primarily attributed to increased leaf thickness. It was also noted that there was rapid growth in the suppressed crop after the dominant crop was harvested (Blaise *et al.*, 2020). Furthermore, the sustained highest SPAD in SC cotton even after wheat harvest appeared to lead to an increase in their NAR compared to that of MC and TC cotton. In line with our findings, Thompson *et al.* (2022) suggest a direct relationship between leaf chlorophyll content and photosynthetic rate in upland cotton. In contrast to SC cotton, TC cotton plants displayed a distinct pattern when compared to the control group of MC cotton. They did not exhibit a significant decrease in photosynthetic activity prior to the wheat harvest (8%), but a notable reduction in NAR was observed at AWH_1 (18%) and AWH_2 (41%) as compared to MC cotton. It can be concluded that cotton plants possess different adaptive capabilities in SC and TC intercropping systems.

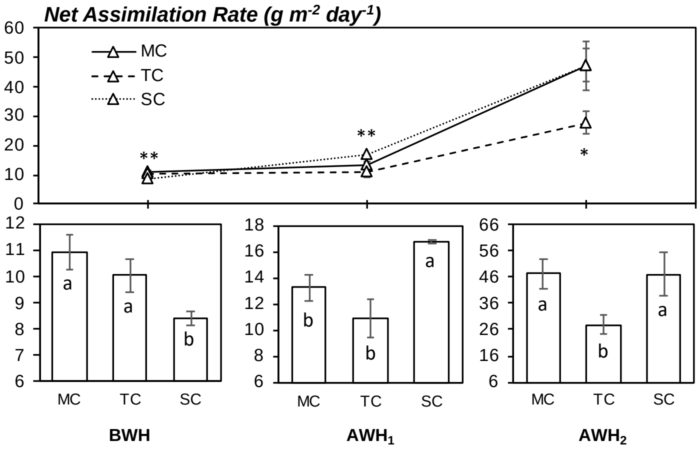

Figure 8. Comparative net assimilation rates (NARs) for cotton under different cultivation methods at various times relative to wheat harvest. MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the NARs in relay strip intercropping systems. Measurements are categorized into periods before wheat harvest (BWH) and after wheat harvest, designated as AWH1 and AWH2, respectively. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 8. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı ortalama net asimilasyon oranı (NAR). MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki NAR'ları gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

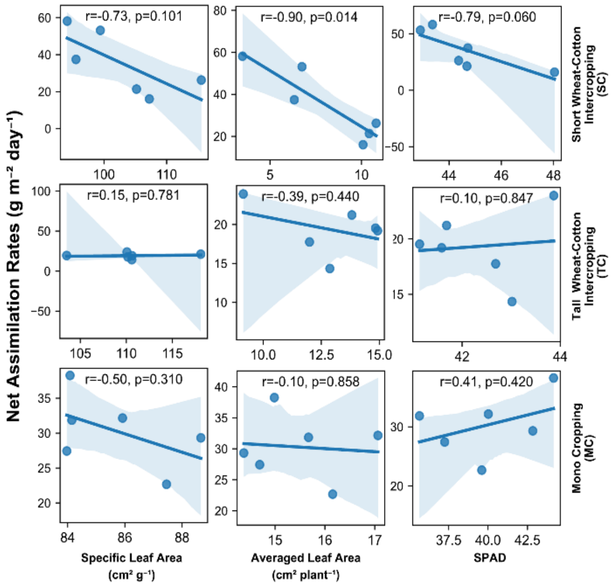

Figure 9. Correlation analysis between net assimilation rate (NAR) after wheat harvest and leaf traits before wheat harvest in short wheat-cotton intercropping (SC) cotton, compared to monocropping (MC) and tall wheat-cotton intercropping (TC) cotton. Strong correlations were observed between NAR and specific leaf area (SLA), average leaf area (ALA), and chlorophyll content (SPAD) in SC cotton, as indicated by significant correlation coefficients (r) and p-values. However, these correlations were not evident in the MC and TC cotton.

Şekil 9. Kısa buğday-pamuk araya ekimde (SC) pamuklarda, buğday hasadından sonra net asimilasyon oranı (NAR) ile buğday hasadından önce yaprak özellikleri arasındaki korelasyon analizi, tek ekim (MC) ve uzun buğday-pamuk araya ekimi (TC) pamukları ile karşılaştırılmıştır. SC pamuklarında, NAR ile spesifik yaprak alanı (SLA), ortalama yaprak alanı (ALA) ve klorofil içeriği (SPAD) arasında güçlü korelasyonlar gözlemlenmiştir ve bu, anlamlı korelasyon katsayıları (r) ve p-değerleri ile belirtilmiştir. Ancak, bu korelasyonlar MC ve TC pamuklarında belirgin değildir.

Total dry weight

The total dry weight (TDW) of intercropped cotton was consistently negatively affected by the microclimate of the intercropping system compared to MC cotton throughout the entire duration (Figure 10). This result is consistent with another study which found that the dry weight of intercropped cotton was significantly lower than that of monoculture cotton at the wheat harvest period (Zhang *et al.*, 2007). While there were no significant differences between TC and SC cotton at the initial period of stress effect on DAS 31, the TDW of SC cotton was observed to be 47% lower than that of TC cotton just before wheat harvest on DAS 50. This notable difference was found to become negligible, attributed to a significant increase in NAR shortly after wheat harvest (Figure 8). Furthermore, TDW of SC cotton was measured at 8.4 g plant^{-1}, slightly surpassing TDW of TC cotton (7.9 g plant^{-1}) on DAS 73. The rapid increase in photosynthetic activity in SC cotton following the wheat harvest appears to have led to a subsequent increase in total dry weight. Several studies have addressed the recovery and improvement capabilities of later-planted crops in relay intercropping systems (Li *et al.*, 2001; Zhang *et al.*, 2008a; Wu *et al.*, 2016). A study on intercropping systems revealed that after the dominant crop was harvested, there was a significant dry matter accumulation in the suppressed crop, attributed to direct exposure to sunlight (Li *et al.*, 2001). Additionally, a positive correlation was observed between total leaf area and total dry weight in the intercropped cotton after the wheat harvest (r=0.74, p=0.006). This suggested that the increase in photosynthetic organs and capability resulted in an increase in total dry weight. This notion was supported by Wu *et al.* (2016) who indicated that the rapid recovery of soybean after maize harvest was primarily attributed to a rapid increase in leaf area. Based on these findings, it is conceivable that the NAR capability of SC cotton after wheat harvest contributed to a more rapid increase in the plants' TDW.

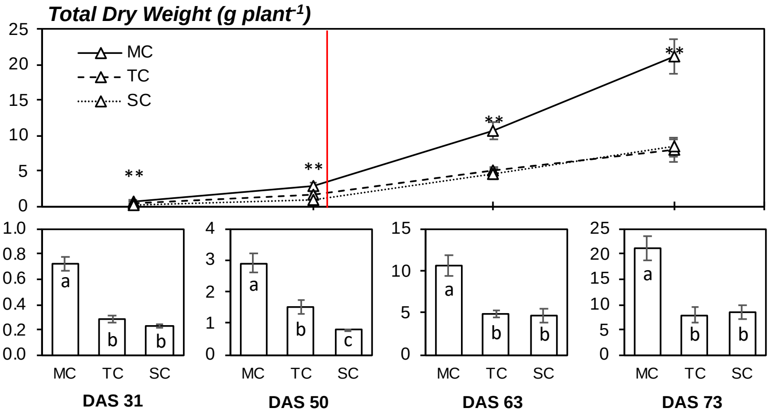

Figure 10. Comparative total dry weight (TDW) for cotton under different cultivation methods across days after sowing (DAS). MC (Mono Cropping) represents the traditional, separate cultivation of cotton. TC (Tall wheat and cotton relay strip intercropping) and SC (Short wheat and cotton relay strip intercropping) illustrate the TDWs in different cropping systems. The red line indicates the harvest time of wheat. Significance of differences between means is indicated by asterisks: a single asterisk (*) denotes $p < 0.05$, and a double asterisk (**) denotes $p < 0.01$.

Şekil 10. Pamuk için farklı yetiştirme yöntemleri altında ekimden sonraki günler (DAS) boyunca karşılaştırmalı ortalama toplam kuru ağırlık (TDW). MC (Tek Ekim) pamuğun geleneksel tek yetiştirilmesini temsil eder. TC (Uzun buğday ve pamuk sonradan araya ekim) ve SC (Kısa buğday ve pamuk sonradan araya ekim), farklı ekim sistemlerindeki TDW'leri gösterir. Kırmızı çizgi, buğdayın hasat zamanını belirtir. Ortalama değerler arasındaki farkların önemi, asteriks işaretleri ile gösterilmiştir: tek asteriks (*) $p < 0.05$ anlamına gelir ve çift asteriks (**) $p < 0.01$ anlamına gelir.

Yield and yield parameters

Traditional cropping systems of cotton were found to be superior compared to intercropped ones in terms of plant height, boll number, and cotton seed yield (Table 2). The highest plant height was observed in MC cotton (66 cm). While there were no significant differences in plant height between TC and SC cotton, TC cotton (51 cm) was slightly taller than SC cotton (46 cm). The plant height of intercropped cotton showed a strong correlation with SLA (r=0.80, p=0.002) and CTD (r=0.70, p=0.012) before wheat harvest. However, this correlation did not persist after wheat harvest for SLA (r=-0.10, p=0.749) and CTD (r=0.40, p=0.213). These results indicate that the final plant height of intercropped cotton was primarily influenced by the stress conditions before wheat harvest. In a study on the intercropping of maize and cotton, it was observed that intercropped cotton was partially shaded by the relatively taller maize plants, resulting in a significantly lower plant height compared to monoculture cotton (Liu *et al.*, 2021). This situation can be explained by the shading effect on the early growth and development of the suppressed crop in the intercropping system (Zhi *et al.*, 2019).

Table 2. Mean plant height (cm), boll number (per plant), and cottonseed yield (g plant^{-1}) across different cropping systems.

Çizelge 2. Farklı ekim sistemlerinde ortalama bitki yüksekliği (cm), koza sayısı (bitki başına) ve pamuk kütlü verimi (g bitki^{-1})

	Plant height (cm)	Boll number (per plant)	Cottonseed yield (g plant^{-1})
MC	65.9 ± 1.3 *a*	12.8 ± 1.0 *a*	54.1 ± 4.5 *a*
TC	50.7 ± 1.1 *b*	5.4 ± 0.3 *b*	17.3 ± 1.5 *b*
SC	45.6 ± 2.6 *b*	5.6 ± 0.8 *b*	20.4 ± 1.3 *b*
LSD	5.1	2.2	7.6

*Cropping systems include Mono Cropping (MC), Tall wheat and cotton relay strip intercropping (TC), and Short wheat and cotton relay strip intercropping (SC).

The generative production was also suppressed by the intercropping system. The boll number of TC and SC cotton was dramatically reduced by 58% and 56% compared with MC cotton, respectively (Table 2). In line with our findings, several studies found that the boll number of intercropped cotton was significantly lower than that of monoculture cotton (Zhang *et al.*, 2008c; Feng *et al.*, 2017). It was reported that the delay in the generative stage due to shading from wheat (Wiley, 1990; Du *et al.*, 2016) resulted in a reduction in the boll number (Zhi *et al.*, 2019). However, no significant differences were found between the boll numbers of TC and SC cotton. Consistent with the findings of boll number, intercropped cotton plants were adversely affected by the microclimate of the intercropping system, leading to a significant decline in plant yield. A notable decrease was recorded in the plant yield of TC (68%) and SC (62%) cotton compared to MC cotton (Table 2). Cotton yield is determined by biomass accumulation and its distribution among various organs (Bange and Milroy, 2004). Therefore, it is plausible to suggest that the co-growth of wheat and cotton resulted in competition for resources, thereby negatively impacting cotton yield. There are numerous studies in the literature that indicated that the yield of cotton is significantly decreased by the intercropping system, as observed in our results (Zhang *et al.*, 2008c; Feng *et al.*, 2017; Zhi *et al.*, 2019). However, although there were no significant differences, SC cotton tended to exhibit a slightly higher plant yield (18%) compared to TC cotton.

CONCLUSIONS

According to our findings, intercropped cotton demonstrated an ability to adapt to varying environmental conditions by adjusting its morphological and physiological traits. Despite these adaptations, the changes observed were insufficient to fully preserve yield and yield components. Specifically, the short wheat-cotton intercropping system provided both shading and induced water stress in cotton plants, likely due to higher evaporation rates compared to the tall wheat-cotton system. This stress effect was more pronounced in the leaves of cotton plants under short wheat-cotton intercropping conditions. However, despite the increased stress, the yield parameters were slightly higher in the short wheat-cotton system compared to the tall wheat-cotton system, indicating that the cotton plants were able to compensate for the stress to some extent.

Furthermore, it could be suggested that the morphological and physiological adjustments in cotton leaves before the wheat harvest led to better growth after the wheat harvest under shorter wheat compared to taller wheat. This study sheds light on how cotton responds to alterations in microclimate within relay strip intercropping systems. However, further investigations are warranted to fully understand their performance in different environmental contexts.

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