Cilt 12, Sayı 2, Sayfa 111-118, 2023 Volume 12, Issue 2, Page 111-118, 2023
Araştırma Makalesi https://doi.org/10.46810/tdfd.1287892 Research Article

BİNGÖL ÜNİVERSİTESİ
Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

# Investigation of Vegetative High Temperature Tolerance of Some Cotton (*Gossypium hirsutum* L.) Varieties

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(Received: 26.04.2023, Accepted: 13.06.2023, Online Publication: 22.06.2023)

**Keywords**
Cotton,
Vegetatively High Temperature,
Cell Membrane Thermostability (CMT),
Leaf Temperature

**Abstract:** It was carried out to determine the harmful effects of high temperature stress on cotton plant during the vegetative development period in this study. The trial was established in the GAPUTAEM trial area in 2020, with 4 blocks according to the Augmented trial design. As standard; Tamcot Spnhix, SJU86, AGC208, STV468, ST474 and Carmen varieties and 88 cotton varieties registered in the National Variety List were used as trial material. In the study, the relative cell damage rate (RCI) and leaf high temperature stress index values (YYSSI) were investigated. Cell membrane damage (%RCI) varied between 41.81% and 74.84%; While the average of the standards was 68.15%, the general average was determined as 62.42%. Leaf high temperature stress index (YYSSI) values varied between 0.48 and 1.85, while the YYSSI average of the standards was 0.98, the overall YYSSI average of the experiment was found to be 1.02. It was determined that there was a wide variation among the genotypes screened for vegetatively high temperature stress. It has been concluded that YYSSI and RCI traits are important, effective, easy and applicable selection criteria for screening genotypes that are vegetatively tolerant and sensitive to high temperature stress in cotton plants. It is recommended to evaluate these two parameters together as it will provide more accurate results. It has been determined that Teksa 415 cotton variety is in the vegetatively tolerant group. Vegetatively, 31 genotypes were in the moderately tolerant group and 62 genotypes were in the susceptible group.



# Bazı Pamuk (*Gossypium hirsutum* L.) Çeşitlerinin Vejetatif Olarak Yüksek Sıcaklığa Karşı Tolerantlıklarının İncelenmesi

**Anahtar Kelimeler**
Pamuk,
Vejetatif Olarak Yüksek Sıcaklık,
Hücre Membran Termostabilitesi (CMT),
Yaprak Sıcaklığı

**Öz:** Bu çalışmada, vejetatif gelişim döneminde pamuk bitkisinde yüksek sıcaklık stresinin, zararlı etkilerinin belirlenmesi amacıyla yapılmıştır. Deneme, 2020 yılında GAPUTAEM deneme alanında, Augmented deneme desenine göre 4 bloklu olarak kurulmuştur. Standart olarak; Tamcot Spnhix, SJU86, AGC208, STV468, ST474 ve Carmen çeşitleri ve Milli Çeşit Listesinde kayıtlı 88 adet pamuk çeşidi deneme materyali olarak kullanılmıştır. Araştırmada, bağıl hücre zararlanma oranı (RCI) ile yaprak yüksek sıcaklık stres indeks değerleri (YYSSI) incelenmiştir. Hücre membran hasarı (%RCI) %41.81 ile %74.84 arasında değişim göstermiş; standartların ortalaması %68.15 iken, genel ortalama %62.42 olarak saptanmıştır. Yaprak yüksek sıcaklık stres indeks (YYSSI) değerleri 0.48 ile 1.85 arasında değişmiş olup, standartların YYSSI ortalaması 0.98 iken, denemenin genel YYSSI ortalaması 1.02 olarak saptanmıştır. Vejetatif olarak yüksek sıcaklık stresi için taranan genotipler arasında geniş bir varyasyon olduğu belirlenmiştir. YYSSI ve RCI özellikleri, pamuk bitkisinde vejetatif olarak yüksek sıcaklık stresine karşı tolerant ve hassas genotiplerin taranması için önemli, etkili, kolay ve uygulanabilir seçim kriteri olduğu kanısına varılmıştır. Bu iki parametrenin birlikte değerlendirilmesi daha doğru sonuçların elde edilmesini sağlayacağından tavsiye edilmektedir. Teksa 415 pamuk çeşidinin vejetatif olarak tolerant grubuna girdiği tespit edilmiştir. Vejetatif olarak, 31 genotip orta tolerant ve 62 genotip ise hassas grupta yer almıştır.

## 1. INTRODUCTION

The cotton plant is an important and strategic product that constitutes the raw material of approximately 50 different industrial industries, especially the textile, oil, and feed industry sectors. In the 2021 and 2022 cotton production seasons, the world's four largest cotton-producing countries are India (5.9 million tons), China (5.7 million tons), the USA (4 million tons), and Brazil 2.7 million tons, respectively. In Turkey, seed cotton production increased by 26.9% in 2021 and amounted to 2.25 million tons [1]. Considering the average data of the last 10 years in Turkey, the cotton cultivation area is 462 thousand hectares, the amount of fiber produced is 835 thousand tons, and the fiber yield is 19.3 $kg.ha^{-1}$ [2]. In our country, cotton is grown intensively, especially in the South eastern Anatolia Region, Aegean Region, Adana, and Antalya regions with the determining effect of climate factors [3]. Approximately 59.31% of the cotton produced in our country is produced in the south eastern Anatolia Region [4]. However, due to the fact that the climate conditions of the south eastern Anatolia Region are dry and hot in summer, high temperature has a negative and significant effect on the vegetative and generative periods of cotton. Cotton is frequently exposed to many biotic and abiotic stresses during its growth stages [5, 6, 45, 46]. According to the International Intergovernmental Panel on Climate Change report, air temperatures are expected to increase by 0.2 °C every 10 years, with global warming being the key factor of high temperature stress. And from 2020 to 2080, the world temperature is predicted to increase by 0.5–5.44 °C [7, 8). Temperature trends display that the global average temperature may increase by 1–4 °C by the end of the 21st century [9]. Although the temperature requirement of the cotton plant varies according to the growth stage, in conditions where it does not fall below 15 °C, leaf, bud, flower, and boll development takes place and it tends to grow continuously, and temperatures of 25–32 °C are sufficient for optimum growth [10, 11, 12]. The optimum temperature values for the first development stages of cotton (main stem elongation, leaf area development, and biomass production) are 30/22 °C day/night. Heat stress can be defined as the emergence of morphological, physiological, and biochemical changes in the plant that exceeds the thermal capacity of the plant above the desired optimum temperature in its life cycle. Accordingly, since registered commercial cultivars with little resistance to high temperature stress have a narrow genetic base with limited genetic gain, these cultivars may increase their susceptibility in a stressful environment [13, 14, 15, 45, 46]. While the cotton plant produces four times more fruit branches at 30/22 °C than at 20/12 °C, it produces fewer monopodial branches [16]. Going on of the daily maximum temperature affects the germination of cotton plants in the vegetative period, root and tiller growth, sympodial and monopodial branches, internode distance, photosynthesis, respiration, and ATP formation. In the generative period, it affects biomass, boll number per plant, boll size and weight, cellulose accumulation and fiber yield, fiber quality, fiber length, strength and micronaire value [17, 18, 18, 19, 20, 45, 46, 47]. In addition, high temperature affects the morpho-physiological properties of the cotton plant. Therefore, by discovering high temperature tolerant cultivars or genes, various techniques have been used to develop temperature tolerant genotypes. Among the many screening techniques that have been used for cotton plants, relative cell damage (%RCI) using Cell Membrane Thermostability (CMT) is a popular and rapid physiological technique for screening cotton germplasm for resistance to heat stress [21]. This method is widely used in many plants such as paddy, soybean, tomato, and cotton [22]. In cotton, the %RCI technique is widely used to screen for temperature tolerant genotypes. Because % RCI is a true indicator of cell membrane thermostability (CMT) and is simpler, more effective, and more cost-effective than other scanning techniques [23, 24, 25]. The use of canopy and leaf temperature is an important technique that has been used more and more recently in terms of germplasm temperature tolerance [22]. This technique has been used by various researchers for temperature tolerance of cotton and corn varieties [25, 26]. Our main aim in this study is to determine the response and tolerance status of some domestic and foreign origin cotton (*Gossypium hirsutum* L.) genotypes in our inventory, especially originating and registered cotton varieties in our country, to high temperatures in the vegetative period. At the same time, determining the parents with special characteristics and including them in the breeding program [27] is to facilitate the researchers in breeding studies and to minimize the environmental effects in selection.



## 2. MATERIAL AND METHOD

**2.1. Material:** In this study, 94 cotton (*Gossypium hirsutum* L.) genotypes registered in the national variety list of domestic and foreign origin, cotton varieties originating from especially our country and registered were used as plant material (Table 1).

**2.2. Experimental Design:** The field experiment was established in the trial area of the GAP International Agricultural Research and Training Center, in the cotton growing season of 2020, with 4 blocks according to the Augmented Design. Stonville474, Tamcot Sphinx, SJU86, Stonville468, AGC208, and Carmen genotypes were included as standard in the experiment. In the experiment, each of the parcels consisting of two rows is 4.0 m long and 1.4 m wide.

**Table 1.** Information on Cotton Genotypes Used as Material

| Origin of Material | Cotton Varieties |
|---|---|
| USA | Tamcot Sphinx, SJU86, AGC208 |
| BASF Turkish Chem. Inds. and Trade Ltd. Comp. | Fiona, Carla, ST498, STV468, Carmen |
| Bayer Turkish Chem. Inds. Ltd. Comp. | Claudia, Gloria, Candia, Flora |
| Birlik Seed. Inds. and Trade. Ltd. Comp. | Bir781, Bir949, Cosmos, Bir138 |
| Caso Seed. Inds. and Trade Ltd. Comp. | Caso9048 |
| EMTZARI | Furkan |
| EMARI | TYA193, Ceykot340, TYA366, ADN701, MAY355, MAY455, MAY505, TMK122, TMN18, MAY344, Nihal, ADN413, ADN710, ADN712, ADN123, ADN811, Gelincik, Sarıgelin, Çukurova 1518, Bossa 159, Teksa415, Yıldırım63, Ayzek 595, Gapkot 732, Ceykot 92 |
| GAP ARI | ZN 243 |
| GAP IARTC | Kartanesi |
| Golden West Seed Trade Ltd. Comp. | Optasia, Esperia, Bomba, GW2345, Babylon, Famosa, Fantom, Penta (Golda), Primera |
| Livagro Agr. Seed. Ltd. Comp. | Zara |
| May-Agro Seed Inds. and Trade Incorp. Comp. | Gaia, ST474, MAY404 |
| Monsanto Nutr. and Agr. Trade. Ltd. Comp. | DP332, ST478, DP396, DP499, SG125 |
| Özaltın Agr. Bus. Inds. and Trade Incorp. Comp. | Lodos, Özaltın404, Özaltın112 |
| Özbuğday Agr. Bus. and Seed Incorp. Comp. | Lider (Mig119), Diva (Teks) |
| CRI | SC2009, SC2079, Efe, Ergüven, Harem1, Harem2, ES1, ES2, Sezener76, Özbek105, İpek607, Gürelbey, Aydın110, Şahin2000 |
| Progen Seed Incorp. Comp. | Kaira, Lima, Astoria, Edessa, BA440, Carisma, PG2018, BA525, Flash |
| Tiriyo Seed. Ltd. Comp. | Zena1010, Zena1040, Zena1018 |

EMTZARI (East Mediterranean Transitional Zone Agricultural Research Institute), EMARI (East Mediterranean Agricultural Research Institute), GAPARI (GAP Agricultural Research Institute), GAP IARTC (GAP International Agricultural Research and Training Center), CRI (Cotton Research Institute)

### 2.3. Examined Features

**Leaf Temperature:** In the experimental area created under field conditions, the cotton plant was subjected to high temperature shock practice (HTSP) by being placed in a low tunnel for an uninterrupted 96 hours during the peak flowering period. With the help of the thermometer placed in the low tunnel, during the hot hours of the day (13:00-16:00), when the temperature is above 50 °C, the low tunnel was opened from the sides to reduce the temperature. Observations were taken before high temperature shock application was recorded as Control. Observations taken at the end of the high temperature shock application period were recorded as Stress. Control and Stress observations were taken separately from 3 of the same plants, which were previously coded and selected in each parcel, and the average of the observations was taken. Leaf temperature was taken separately with an Infrared device (Multifunction InfraRed Thermometer CEM DT-8811H) before and after HTSP. It was evaluated statistically over the averages of the observations taken. Leaf High Temperature Stress Index (LHTSI) was calculated according to the formula specified by Fischer and Maurer [28] and evaluated by modifying it according to Ekinci [29].

$$LHTSI:((G_N-G_S)/G_N)/((A_N-A_S)/A_N) \quad (1)$$

LHTSI: Leaf high temperature stress index
$G_S$: Leaf temperature value of the genotype under stress conditions
$G_N$: Leaf temperature value of the genotype under normal conditions
$A_S$: Average of leaf temperature values of all genotypes under stress conditions
$A_N$: The average of leaf temperature values of all genotypes under normal conditions

Regarding the evaluation of genotypes after calculating LHTSI values; If LHTSI ≤ 0.5 it was evaluated as "Tolerant", if 0.5< LHTSI ≤1 as "Medium Tolerant" and if LHTSI >1 as "Sensitive" [30].

The data obtained after control and stress were analyzed according to Augmented Design and calculated and analyzed over corrected values [31].

**Cell Membrane Injury/Damage Rate %:** Cell damage percentage is based on the measurement of electrolyte leakage from leaf discs in distilled water after heat treatment [32]. The cell membrane damage feature was calculated by utilizing the cell membrane thermostability feature. Samples were taken from 3 randomly selected and coded plants from each plot. Two sets of five 10 mm diameter leaf discs were taken from the same leaf. One of these sets was used for control and the other for stress. Leaf discs were placed in test tubes containing 15 ml of distilled water. One set was exposed to a digital water bath at 50 °C and the other set was exposed to room conditions at 25 °C for 1 hour. At the end of this period, 30 ml of distilled water was added to the test tubes and incubated at 10 °C for 24 hours. Then, the test tubes were mixed in a rinser until they reached 25 °C, and $C_1$ - $C_2$ values were measured. Both the samples were again heat treated at a temperature of 121 °C for a period of 10 minutes under a pressure of 15 lbs. Then, the test tubes were mixed in a rinser until they reached 25 °C, and $T_1$ - $T_2$ values were measured. Cell membrane injury/damage ratio (%RCI) values were obtained by formula 2 (Figure 1).

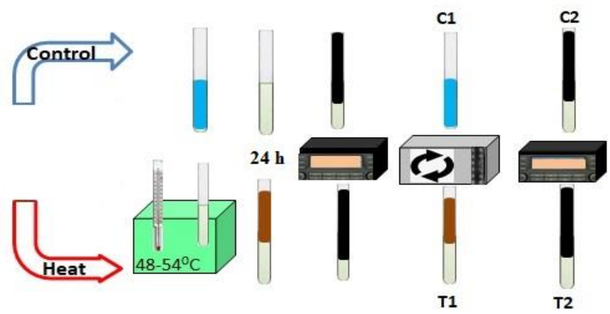


**Figure 1.** Cell Membrane Thermostability Practice Method

$$\%RCI:[1-(1-(T1/T2))/(1-(C1/C2))]X100 \quad (2)$$

%RCI= Cell membrane injury/damage ratio
$T_1$ = EC value after high temperature application
$T_2$ = EC value after autoclave
$C_1$ = EC value before control autoclave
$C_2$ = EC value after control autoclave

YGDR value was calculated with Formula 3 for the classification of RCI values.

$$YGDR= (RCIMax - RCIMin)/3 \quad (3)$$

Regarding the evaluation of genotypes after calculating the RCI values; RCI ≤ RCIMin+YGRD is "Tolerant", RCIMin+YGRD < RCI ≤ RCIMin+2*YGRD "Medium Tolerant" and RCI > RCIMin+2*YGRD "Sensitive".

## 3. RESULTS

### 3.1. Cell Membrane Injury/Damage Ratio (RCI)

The histogram of the %RCI feature is given in Figure 2a. The %RCI feature values were determined as the lowest at 41.81%, the highest at 74.84%, the genotype average at 62.78%, the standard average of 68.15%, and the YGDR value at 11.01%. The number of cotton genotypes included in the sensitive group (RCI>63.82%) in terms of RCI feature is 51 (values are in the range of 64.15-74.83%); The number of cotton genotypes in the medium tolerant group (RCI values are in the range 52.82-63.82%) is 30 (values are in the range of 53.45-63.56%) and the number of cotton genotypes in the tolerant group (RCI value <52.82%) is 13 (values are 41.80 –49.84%). Cotton varieties that are in the tolerant group in terms of their %RCI values; Caso9048 (41.81%), Flora (41.98%), Primera (42.65%), Lider (Mig119) (43.71%), Ceykot92 (43.77%), Bir138 (44.22%), Zara (46.03%), Teksa415 (46.73%), BA525 (47.23%), Flash (47.88%), TYA193 (49.32%), Gürelbey (49.42%) and Diva (Teks) (49.84%) (Table 2).

### 3.2. Leaf High Temperature Stress Index (LHTSI)

The histogram of the leaf high temperature stress index (LHTSI) feature is given in Figure 2b. In the control conditions, the average leaf temperature of the genotypes varied between 26.28 °C and 33.77 °C, while the average leaf temperature of the genotypes changed between 33.31 °C and 41.55 °C after HTSP. While the mean of the standard genotype was 30.62 °C in the control conditions, the mean of the standard genotypes was 38.73 °C after HTSP. While the average leaf temperature of all genotypes was 30.45 °C in the control conditions, it was determined that the average leaf temperature of all genotypes was 38.64 °C after HTSP. LHTSI value is greater than one, and 39 cotton varieties (LHTSI: 1.01–1.85) were found to be in the sensitive group. It has been determined that 52 cotton genotypes (LHTSI: 0.54–0.995) have an LHTSI value between 0.5<LHTSI≤1 and are in the middle tolerant group. In addition, it was determined that the LHTSI value was greater than 0.5, and 3 cotton genotypes (LHTSI: 0.48–0.495) were included in the tolerance group. It was determined that cotton genotypes in the tolerant group in terms of their LHTSI values were TMK122 (LHTSI: 0.483), ADN811 (LHTSI: 0.495), and Teksa415 (LHTSI: 0.492) (Table 2). The change of genotypes to %RCI and LHTSI values are given in Figure 3.

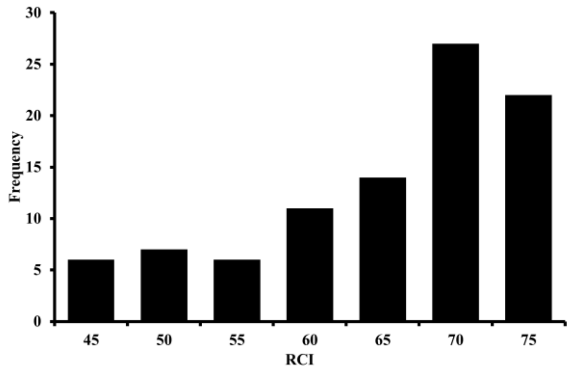


**Figure 2a**. Histogram for the %RCI

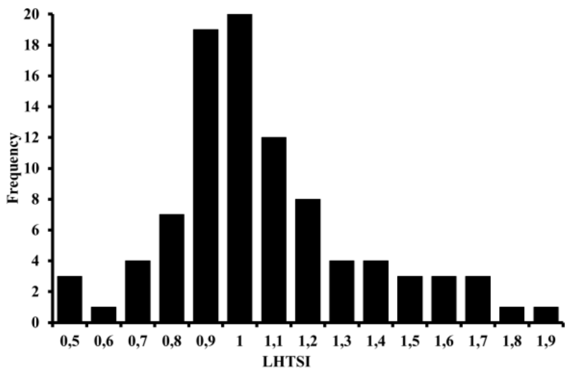


**Figure 2b.** Histogram for the LHTSI

**Table 2.** Numbers and Names of Cotton Genotypes in Sensitive, Medium Tolerant and Tolerant Groups According to %RCI and LHTSI

| Groups | In terms of %RCI | In terms of LHTSI | Joint |
|---|---|---|---|
| Sensitive | 51 | 39 | |
| Medium Tolerant | 30 | 52 | |
| Tolerant | 13 Caso9048, Flora, Primera, Lider (Mig119), Ceykot92, Bir138, Zara, Teksa415, BA525, Flash, TYA193, Gürelbey ve Diva (Teks) | 3 TMK122, ADN811, Teksa415 | Teksa415 |

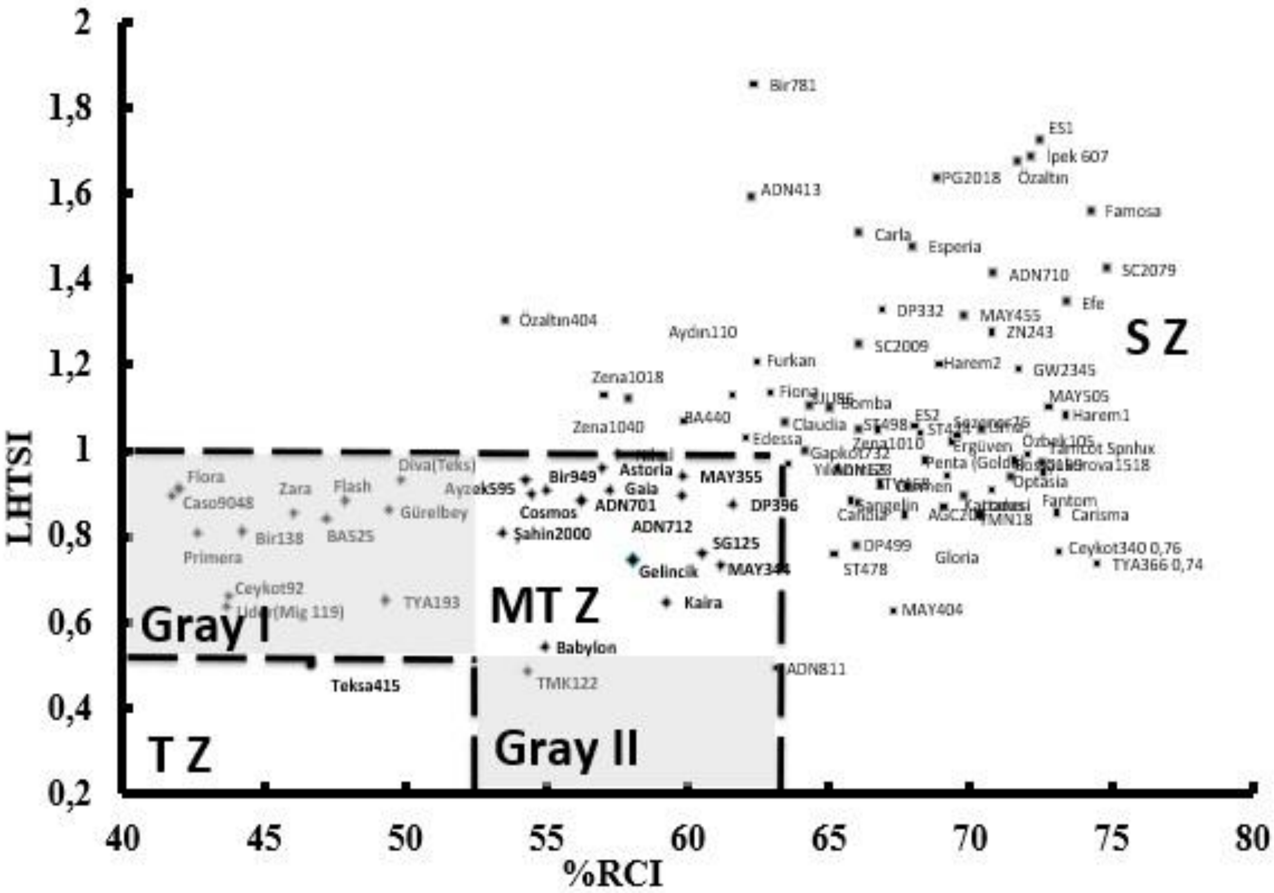


Figure 3. LHTSI and %RCI Graph

As the genotypes get closer to the origin, vegetative tolerance increases in terms of both traits (Figure 3). In terms of RCI and LHTSI characteristics examined, the Tolerance Zone (%RCI≤52.82 and LHTSI≤0.5) was marked as TZ. It attracts attention that the Medium Tolerance Zone (52.82%< RCI < 63.82% or 0.5<LHTSI≤1) consists of 3 parts (MTZ, Gray I, and Gray II). Although the Gray I region is tolerant in terms of the RCI feature, it is seen to be in the Medium Tolerant group in terms of the LHTSI feature. Similarly, although the Gray II region is tolerant in terms of the LHTSI feature, it is noticed that it is in the Medium Tolerant group in terms of the RCI feature. Therefore, Gray I and Gray II zones were included in the Medium Tolerant Zone. The Sensitivity Zone (RCI>63.82% or LHTSI>1.0) has been named SZ for both traits examined vegetatively. ADN123, ADN413, ADN710, AGC208, Aydın110, BA440, Bir781, Bomba, Bossa159, Candia, Carisma, Carla, Carmen, Ceykot340, Claudia, Çukurova1518, DP332, DP499, Edessa, Efe, Ergüven, ES1, ES2, Esperia, Fantom, Fiona, Furkan, Gloria, GW2345, Harem1, Harem2, İpek607, Kartanesi, Lima, Lodos, MAY404, MAY455, MAY505, Optasia, Özaltın112, Özaltın404, Özbek105, Penta (Golda), PG2018, Sarıgelin, SC2009, SC2079, Sezener76 , SJU86, ST474, ST478, ST498, STV468, Tamcot Spnhix, TMN18, TYA366, Yıldırım63, Zena1010, Zena1018, Zena1040 and ZN243 cotton genotypes (62 pieces) were determined to have in the sensitive region (SZ). In our study, our findings obtained by measuring different values as a result of screening genotypes in terms of vegetative tolerance or sensitivity under control and stress conditions in terms of CMT or %RCI characteristics were similar to the findings of Sajid [33], Azhar [24], Rahman [23], Farooq [34], Zafar [35], Jamil [36]. It was determined that there was a wide variation among the genotypes screened for vegetatively high temperature stress. High temperature stress reduced the vegetative growth of the cotton plant, causing the yellowing of young leaves and drying of young tillers. A high CMT value, namely a low %RCI ratio, indicates that genotypes are less vegetatively affected by high temperature. Therefore, it has been understood that genotypes with low %RCI are an important criterion for cotton breeding programs. It has been understood that plant characteristics such as plant type, leaf woolly, and cell wall thickness affect the %RCI rate depending on the severity of the temperature, the period in which the heat is experienced, and the duration of exposure to the heat. It has been concluded that the investigated RCI and LHTSI properties are two important, effective, easy, and applicable properties in revealing the vegetative performance of cotton against high temperature stress, and it is concluded that more accurate results will be obtained by examining these two properties together [18, 23, 24, 25, 33, 35, 37, 38, 39, 40, 41, 42, 43, 44].

## 4. DISCUSSION AND CONCLUSION

In this study, it was passed judgment that using CMT and LHTSI characteristics, it is an important, effective, easy and applicable selection criterion for the screening of genotypes in terms of tolerance or sensitivity to high temperature stress vegetatively in cotton plants. It is recommended that more accurate and reliable data can be obtained as a result of applying these two parameters together. As a result of examining the LHTSI and RCI characteristics together, it was determined that only the Teksa415 cotton variety was vegetatively tolerant. It was determined that 31 cotton genotypes were included in the medium tolerant group vegetatively and 62 cotton genotypes were included in the sensitive group.

**Acknowledgement**

This study was produced from the Ph.D. thesis titled " Determination of DNA Markers Associated with High Temperature Stress Tolerant / Strength in Cotton (*G. hirsutum* L.)" conducted by Yusuf Güzel DEMİRAY in the Department of Field Crops, Institute of Science and Technology, Dicle University. It was supported by Dicle University Scientific Research Projects Coordination Unit with project number ZİRAAT.20.007 and by the General Directorate of Agricultural Research and Policies with project number TAGEM/TBAD/A/20/A7/P5/1536. We thank the Scientific Research Coordination Unit and the General Directorate of Agricultural Research and Policies for their support.

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