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Effect of Germination Temperature on Germination and Seedling Growth Parameters in Cotton (Gossiypium Hirsutum L.) Varieties

Çimlenme Sıcaklığının Pamuk (Gossiypium Hirsutum L.) Çeşitlerinde Çimlenme ve Fide Büyüme Parametrelerine Etkisi

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EFFECT OF GERMINATION TEMPERATURE ON GERMINATION AND SEEDLING GROWTH PARAMETERS IN COTTON (GOSSIYPIUM HIRSUTUM L.) VARIETIES

ABSTRACT

Germination and seedling growth periods have a very important function in plant life. Especially in cases where abiotic stress conditions occur, these periods are of vital importance for the sustainability of plant life. Taking this into account, this research was conducted to determine the most suitable temperature for germination and seedling growth parameters of cotton varieties. In this research conducted under controlled conditions, germination and seedling development parameters of two cotton varieties (May-344 and Candia) were tested at 8 different temperature levels (8, 12, 16, 20, 24, 28, 32 and 36°C). It was determined that the germination percentage was 45.17-16.17%, the germination time was 4.06-3.94 days and the emergence rate index was 1.89-0.58 in May-344 and Candia cotton varieties, respectively. Moreover; root length 37.89-16.43 mm, stem length 44.69-41.00 mm, root fresh weight 22.40-19.51 mg, stem fresh weight 90.37-131.80 mg and the root/stem ratio was 0.26-0.16 mg/mg in May-344 and Candia cotton varieties, respectively. The results of the experiment revealed that most of the tested parameters were significantly affected by temperature. The highest germination rate, as the average of the two varieties and for both varieties separately, was obtained at 20 $^{\circ}$ C and no germination occurred at 8 °C. Except root to shoot ratio, other seedling growth parameters were maximum between 20-32 °C and decreased at higher temperatures.

Keywords: Cotton, Temperature Treatment, Germination Parameters, Seedling Growth.

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ÇİMLENME SICAKLIĞININ PAMUK (GOSSIYPIUM HIRSUTUM L.) ÇEŞİTLERİNDE ÇİMLENME VE FİDE BÜYÜME PARAMETRELERİNE ETKİSİ

ÖZ

Bitki yaşamında çimlenme ve fide büyüme periyodu çok önemli bir fonksiyona sahiptir. Özellikle abiyotik stress koşullarının oluştuğu durumlarda bu periyotlar bitki yaşamının sürdürülebilirliği için hayatı derecede öneme sahiptirler. Bu durum dikkate alınarak pamuk çeşitlerinin çimlenmesi ve fide büyüme parametreleri açısından en uygun sıcaklık derecesinin tespit edilmesi amacıyla bu araştırma yürütülmüştür. Kontrollü koşullar altında yürütülen bu araştırmada, iki pamuk çeşi-

dinin (May-344 ve Candia) çimlenme ve fide gelişim parametreleri 8 farklı sıcaklık seviyelerinde (8, 12, 16, 20, 24, 28, 32 ve 36°C) test edilmiştir.

Araştırma sonucu May-344 ve Candia pamuk çeşitlerinde sırasıyla çimlenme yüzdesinin %45,17-16,17, çimlenme süresinin 4,06-3,94 gün ve çıkış oranı indeksinin 1,89-0,58 olduğu belirlenmiştir. Ayrıca; May-344 ve Candia pamuk çeşitlerinde sırasıyla kök uzunluğu 37,89-16,43 mm, gövde uzunluğu 44,69-41,00 mm, kök taze ağırlığı 22,40-19,51 mg, gövde taze ağırlığı 90,37-131,80 mg ve kök/gövde oranı 0,26-0,16 mg/mg olarak bulunmuştur. İlave olarak test edilen parametrelerin çoğunun sıcaklıktan önemli ölçüde etkilendiğini belirlenmiştir. Her iki pamuk çeşidinin ortalaması ve her iki çeşit için ayrı ayrı en yüksek çimlenme oranı 20 °C'de elde edilmiştir. 8 °C'de ise çimlenme kaydedilmemiştir. Kök/sürgün oranı dışında diğer fide büyüme parametreleri 20-32 °C arasında maksimuma ulaşırken, daha yüksek sıcaklıklarda azalmıştır.

Anahtar Kelimeler: Pamuk, Sıcaklık Uygulaması, Çimlenme Parametreleri, Fide Büyüme Parametreleri.

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1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important industrial plants that can be used for multiple purposes. For example, it is used in the textile industry as fibers, its seeds pulp is used as nutrients in animal feeds and in oil and biodiesel industries, and plant stems are used in paper industries (Demiray et al., 2023). Cotton provides income and prosperity to more than 250 million farmers worldwide (Anonymous, 2017).

Cotton is successfully cultivated in the tropical and temperate regions, where the frost-free period is less than 180 days (Anonymous, 2022a). It can be grown in regions where abiotic stresses such as high temperature, drought, salinity and chemical toxicity caused by heavy metals affect physiological growth and final yield which limit plant cultivation (Jingxiang et al., 2023). However, it could tolerate stresses such as salinity and drought (Abdelraheem et al., 2019).

Temperature is among the important environmental factors affecting the germination, growth, development and maturation of cotton (Bibi et al., 2008). Depending on genetic and environmental interactions, temperature directly affects plant life by hindering chemical reactions, including respiration and photosynthesis, (Khetran et al., 2015). The temperature need of the plant throughout its life varies depending on the genetic, morphological and physiological structure of the plant, time and duration of heat formation (Ekinci et al., 2017).

High yield depends on healthy growth and development after high germination and emergence percent (Raphael et al., 2017). Seed germination rate and seedling growth capacity can be determined as a standard by studies conducted under laboratory conditions (Anonymous, 2022b). Many studies have been conducted to determine the effect of temperature on the germination of cotton seeds. These studies have reported that the optimum germination temperature of cotton varies among varieties (Khetran et al., 2015; Raphael et al., 2017; Ahmad et al., 2020). These findings indicated that the variation of germination test results among cottonseeds in a constant temperature is due to genotypic variability.

In recent years, cotton producers in the tropical region have been facing a problem in their planting schedule and seedling emergence resulting in reduced fiber and seed quality. The existing germination and emergence studies conducted with cotton varieties have revealed that cold temperatures cause chilling damage in seedlings and reduce stand establishment (Bradow and Bauer, 2010). Initial injury begins with the absorption of cold water during the germination process, and secondary injury may occur after the start of germination at below 18°C temperature (Duesterhaus et al., 2000). Determining the genotype and the optimal germination and seedling development temperature over a wide temperature range can provide a more accurate understanding of the performance of new varieties. Therefore, this research was conducted to determine whether genotypes are affected by temperature changes that may occur during the germination and seedling growth period in cotton.

2. MATERIAL AND METHOD

2.1. Experiment Area Properties

The experiment was carried out at the Department of Field Crops, Faculty of Agriculture, Ondokuz Mayıs University. Ondokuz Mayıs University is located at latitude 41° 22' 6" north and longitude 36° 11' 54" east.

2.2. Seed Imbibition and Sterilization of Petri Dishes

A total of 150 seeds of each variety were placed in petri dishes (147 mm in diameter and 22.4 mm in depth) and then kept in distilled water for 60 minutes. The seeds imbibed were removed from the distilled water and transferred into the sterile cabinet for sowing. Before sowing, petri dishes (9 cm diameter, 19 mm depth) containing two filter papers (Whatman 541) were placed in the sterilizing oven at $180 °C$ for three hours.

2.3. Germination Test

The experiment was laid in an 8x2 Factorial Experimental Design in Randomized Blocks (CRD) including factors namely temperature and varieties. In the research, eight different temperature regimes (8, 12, 16, 20, 24, 28, 32 and 36 °C) and two cotton varieties (May- 344 and Candia) were used. Seeds of each cotton variety were planted in ten petri dishes to provide ten replications.

Fifteen cottonseeds imbibed were evenly distributed on double layer of Whatman filter paper in each sterilized Petri dish. Before sowing, filter papers were moistened with 5 ml of sterilized water. Sowing was carried out on moistened filter papers. After sowing, the lids of the petri dishes were closed and the petri dishes were transferred to the incubator with a sensitivity of ± 1 °C and germination was started at the germination temperature to be tested. The number of germinated seeds was determined by counting on the 2nd and 12th days from the beginning of the test. The germinated (plumule emergence) seedlings were recorded after 48 hours. When measured with an electronic digital calliper, the seeds were considered germinated if the rootlets reached 2 mm. During counting some petri dishes were kept moistened with distilled water. Diseased seeds or abnormal seedlings were removed once observed (Raphael et al., 2017).

2.4. Germination Parameters

For each treatment, germination measurements were calculated and organized into the excel spreadsheets for statistical analysis. Germination percentage (%), germination rate, root and stem dry weight were calculated based on methodology outlined in Ranal et al., (2009). To calculate the weight of shoots and roots; after germination completed seedlings were collected and the weights of the shoots and roots were weighed separately on an electronic scale with a sensitivity of 0.001 g and their dry weights were recorded.

Germination percentage (%) = (N_T/N_s) * 100. (N_T = the total number of seeds that germinated on the 12th day and N_s = the number of seeds planted in the petri dish).

Germination time (day) = $\sum n_i$, $t_i / \sum n_i$. (t_i = the time (day) from the beginning of the experiment to the nth observation and n_i = the number of seeds germinated over time).

Emergence rate index= $(G_1/N_1) + (G_2/N_2) + ... + (G_n/N_n)$. $(G_1, G_2, G_n$ = percentage of seedling emergence in the first, second and last count, respectively, N_{1} , N_{2} , N_{n} = number of days until the first, second and last count, respectively (Maguire, 1962)).

After the last count (12 days); After the root and shoot lengths were measured separately, the roots and shoots were weighed separately on a scale with an accuracy of 0.001g. Thus, root and shoot lengths and weights were determined. Then, the root/shoot ratio was determined using the calculated root and shoot weights (Ketran et al., 2015).

2.5. Statistical Analysis

All evaluated parameters were analyzed using the SPSS (Version 17) computer package program. LSD value was used to compare the means (Gomez and Gomez, 1984).

3. RESULT AND DISCUSSIONS

3.1. Germination Parameters

Germinability was reduced for seeds from MAY-344 and CANDIA at low and higher temperature levels. Temperatures, genotypes and their interaction all had significant (P≤0.01) effects on cotton germination (Table 1). Germination at different temperatures showed different patterns; the highest germination rate (66.33%) was recorded at 20 °C and the lowest germination rate $(1.67%)$ was recorded at 12
°C as the temperature was lowered, final germination decreased and seeds failed C. As the temperature was lowered, final germination decreased and seeds failed to germinate at 8 °C. It has been observed that the seeds of cotton varieties germinate within a certain temperature range. The results revealed that the upper limit seed germination (45.17%) was found in the May-344 cotton variety and the lower seed germination (16.17%) was recorded in the Candia variety (Table 1). When the interaction of temperature and genotype was evaluated; It was determined that both genotypes within the scope of the research had a maximum germination rate at 20 °C. The highest germination percentage at 20 °C was recorded as 91.30% in the May-344 cotton variety and 41.33% in the Candia variety (Table 1; Figure 1).

Figure 1. Effect of genotype and temperature interaction on germination percentage (%)

In this research, it was noted that there were significant differences (P≤0.01) between temperature regimes in terms of germination time, whereas the effect of genotypes and genotype x temperature interaction on germination time did not make a significant difference (Table 1). It was noted that the average germination time varied between 0.00 and 6.91 days, depending on the temperature regimes applied. The shortest germination time was obtained at 36 °C (1.93 days). At 0/8 °C, no germination was observed. In other words, cottonseeds were unable to germinate under 0/8 °C temperature range, but germination was completed in approximately two days (1.93 days) at 36 °C. The shortest germination time was recorded at 36 °C in both cotton varieties (1.91 days in May-344 and 1.95 days in Candia), while the longest germination time was recorded at 16 °C with 8.48 days in May-344 variety and 6.24 days at 28 °C in Candia variety (Table 1).

Temperature is vital for manipulating several features of plant growth and development. The result reveled that temperature effect in optimizing cottonseed germination increases linearly as a sigmoid growth curve (i.e., quantitatively the percentage increases rapidly until they reach some limit) (Raphael et al., 2017). The highest germination percent was recorded at 20 $^{\circ}$ C. The temperature signifies the optimal temperature for the biochemical and physiological responses in germination. The result of increased germination percent at 20 °C was similar to previous research teams such as in cotton (Smith and Varvil, 1984), wheat (Sharma et al., 2022) and rapeseed (Sghaier et al., 2022). The minute temperatures exceeded or lagged the upper limit triggered the germination potential of cottonseeds to decline. The embarrassment of seed germination as temperatures went beyond 20 °C happened due to various factors, including enzyme denaturation, cellular damage and metabolic imbalances (Iloh et al., 2014). In addition to that, some earlier studies concluded that temperatures >35 °C tend to reduce the effectiveness of physiological processes such as seed membrane imbibition and increase content of stress-induced phytohormone (e.g. Abscisic acid) (Ikram et al., 2022). Conversely, cotton varieties exhibited genetic potential at different temperature regimes during germination. Indeed, genetic variation among varieties when exposed to temperatures might be due to differences in tolerance to temperature instabilities, induction of secondary dormancy and seed reserve mobilization (Hasan et al., 2013). Genetic discrepancies in seed germination under different temperature levels have been reported in cotton (Chu et al. 2016; Fernando and Aníbal, 2018).

3.2. Seedling Characteristics

Emergence rate index (ERI) showed significant effects ($p \le 0.01$) with temperature levels, genotypes and their interaction (Table 1). There was no seedling emergence at 8 °C. At 12 °C, 16 °C and 36 °C, lower ERI values were found for May-344 and Candia. Both Varieties were associated with high ERI at 20 $^{\circ}\textrm{C}$ (Table 1). May-344 genotype had higher ERI $(1.89\% \, d^{-1})$ than Candia with ERI (0.58 % d^{-1}). The differences between temperatures, 20 °C resulted in the highest values of ERI (2.77% d^{-1}). As a genotype and temperature interaction, the highest emergence index was obtained at 20 °C with 4.12% d-1 in May-344 and 1.42% d-1 in Candia. However, at 12 °C, May-344 resulted in lower ERI (0.02% d $^{\text{-}1)}$ than Candia with ERI (0.03% d^{-1}) Table 1; Figure 2a).

Cotton varieties showed significant differences ($p \le 0.01$) in terms of seedling root length at all temperature levels and genotype x temperature interaction. In contrast only the temperature regime created a significant difference (P≤0.01) in terms of seedling root fresh weight (Table 1). The maximum root length (50.00 mm) was recorded at 28 °C followed by 24 °C and 32 °C (49.33 and 49.23 mm respectively) temperatures. In terms of genotype and temperature interaction, the longest root length of May-344 was 75.45 mm at 28 °C, while in Candia it was 34.07 mm at 20 °C. The shortest root for May-344 (9.91 mm) and Candia (6.25 mm) varieties was observed at 16 °C (Table 1; Figure 2b). The weights of the rootlets of the seedlings increased as the temperature level increased and reached a peak (49.93 mg) at 24 °C and then decreased slightly despite the increasing temperature level.

Figure 2. Effect of genotype and temperature interaction on emergence rate index (a) and root length (b)

630 Effect of Germination Temperature

Although fresh shoot length was statistically significantly ($p \le 0.01$) affected only by changing temperatures, fresh shoot weight was statistically significantly (p≤0.01) affected by genotype, temperature and their interactions (Table 1). Shoot length increased continuously depending on increasing temperatures and reached its maximum (86.79 mm) at 32 °C. On comparing the seedlings, at 32 °C all the genotypes under study produced longest shoots (92.28 mm and 81.31 mm) for May-344 and Candia, respectively (Table 1). The range of values widened for seedling fresh shoot weight accumulation among the temperature levels. Fresh shoot weight; the maximum (222.52 mg) was recorded in the 28 °C temperature regime and the minimum (35.13 mg) was recorded in the 16 °C temperature regime (Table 1). Candia had higher fresh shoot weight (131.80 mg) than May-344 (90.37 mg). On the other hand, the highest fresh shoot weight was recorded at 28 °C temperature regime in both May-344 and Candia genotypes, with 188.31 mg and 256.72 mg, respectively (Table 1).

The ratio of root to shoot biomass varied, unsurprisingly, significantly ($p \le 0.01$) with temperature and genotypes (Table 1). The analyzed results showed the highest ratio (0.29 mg/mg) at 16 °C. At all temperature levels, the ratio of root to shoot of the May-344 was higher (0.26 mg/mg) than Candia (0.16 mg/mg). The interaction between varieties and temperature levels did not show a strong relationship between root and shoot biomass (95% confidence interval of slope ranged from 0.05 to 0.33 mg/mg). The highest ratio of root to shoot for cotton varieties under study was recorded at 16 °C whereas the ratio of root to shoot varied from 0.33 mg/mg (May-344) to 0.24 mg/mg (Candia) after 12 days of growing (Table 1).

Table 1. The effects of genotype and temperature interaction on germination parameters and seedling growth parameters of the studied cotton varieties

*Averages followed by the same letters, in the columns, do not differ by the LSD test at 5% probability. *Significant at P≤0.05; **Significant at P≤0.01; ns=not statistically significant at p≥0.05, SEM: Standart error of means

The values for emergence rate index to some extent were higher at 20 $^{\circ}\textrm{C}$, indicating that under this temperature regime water uptake is greater in regulating seed reserve utilization, cell division and elongation, and finally growth of the radicle and plumule. The outcome, the hypocotyl elongates more rapidly while pushing the cotyledons and epicotyl above the soil surface (Rajjou et al., 2012). Comparing these two cotton varieties, May-344 had the highest emergence rate index values. The differences were possible because different varieties respond differently to temperature levels based on variations of seed parameters, viz., seed weight, seed vigour, seed size, dormancy, seed storage reserve mobilization, seed reserve utilization, seed depletion ratio, seed coating, radicle length and dry weights (Maleki et al., 2023; Mašková and Herben, 2018; Singh et al., 2018; Bradow and Bauer, 2010).

Plant growth is mainly in need of several abiotic factors including temperature. The growth patterns vary as the temperature increases or decreases from the optimal level. In the temperature range, 32/20 °C, the radicle growth pattern was higher than at other temperatures (Table 1). This was possible because high temperature inhibits the synthesis of endogenous hormones, such as brassinolide and auxin, in roots. The result corroborates previous studies (Khaeim et al., 2022) that were renowned shortest root when temperature exceeded the optimal range. Conversely, shoot growth was highest when the temperature range, 32/24 °C, implying that stem elongation requires higher temperature than roots. The results of higher temperatures being conducive for stem growth were consistent with previous studies (Reddy et al., 2017; Raphael et al., 2017).

At 36 °C seedling development was diminished; the impaired development can be allied to errors in metabolism that result in an enzyme imbalance. Jackson (1967) noted similar effects and confirmed that growing cotton at temperatures beyond 35 °C has damaging effects on seedling growth traits. However, in the temperature range, 12/8 °C, no difference in seedling development in both varieties was observed. In each of the two temperature levels, no seedling managed to emerge, signifying those lower temperatures involved bringing chilling injury and dipping seedling formation (Brand et al., 2016). On the other hand, emergence rate index revealed genotypic variation within cotton varieties for stand establishment. overall, the May-344 showed the longest roots and shoots than the Candia. The discrepancies between varieties seemed attributed to activity of enzymes, reactive oxygen species and vascular tissue (Sharma, et al, 2022; Khaeim et al., 2022; Sghaier et al., 2022). There is emerging evidence that differences exist among cotton cultivars for seedling growth at different temperature levels (Singh et al., 2018; Brand et al., 2016).

Each variety reacted slightly differently to temperature levels, with variety May-344 displaying the highest root fresh weight compared to variety Candia (Table 1). Genotypic variability for root fresh weight is likely due to alterations in varieties of root length, root diameter, root surface area, root tips, root forks, root crossings and speed to emergence. These findings corroborate previous research (Fan et al., 2022; Walne and Reddy, 2022; Virk et al., 2021; Singh et al., 2018) which concluded that plant roots perceive conditions in the soil and adapt their architecture accordingly. On the other hand, the Candia presented the highest shoot fresh weight than May-344. This is supported by the observation that variety Candia took shortest time to germinate than variety May-344 (Table 1). The highest values for shoot fresh weight shown by Candia variety might be due to differences in morphological, physiological and chemical reactions compared to other varieties. The genotypic disparity in response to shoot fresh weight was reported by earlier researchers (Singh et al., 2018; Reddy et al., 2017; Raphael et al., 2017).

In this study, both varieties conquered the highest root fresh weight at 24 $^{\circ}\textrm{C}$ (Table 1). The highest root fresh weight was possibly because the root system elongated while protecting the meristem which can probably accelerate cell division, increasing the number of roots and hence decreasing the length of lateral roots (Mahmud et al., 2019; Martins et al., 2017; Ribeiro et al., 2014). However, at temperatures (24 $\rm{°C}$ and 28 $\rm{°C}$) there were fewer differences in root length (Table 1). The decrease in root length may result in to increase in root branching strength, vigor and average root diameter which may change the acquisition of root nutrients, water and hence maximum biomass (Gavelienė et al., 2022; Alsajri et al., 2019; Mahmud et al., 2019). Also, shoot fresh weight was maximized at 28 °C and slightly abridged at 24 °C (Table 1). The highest shoot fresh weight at 28 °C might be accredited by higher water uptake resulting in vigorous shoot. This finding corroborates previous research (Sghaier et al., 2022; Fiaz et al., 2020; Sainju et al., 2017; Wanjura and Buxton, 1972) that noted the effect of increasing water accessibility, flow and assimilation by the hypocotyl in 32/15 °C. Overall, seedling fresh weight was smaller at 16 and 36 °C, signifying that under these temperatures, seed metabolic processes, germination and emergence speed were negatively affected. These findings corroborate the aforementioned studies (Fan et al., 2022; Khaeim et al., 2022; Walne and Reddy, 2022; Virk et al., 2021; Singh et al., 2018) which concluded that seedling growth is consistent with the situations in the surrounding.

The values of root to shoot ratio for the health of plants in all temperature levels were slightly higher under 16 °C. In this study, the detected trend, overall, root: shoot ratio was higher in variety May-344 than that in variety Candia in each temperature level, excluding 12/8 °C (Table 1). In our experiment, the interaction of these two parameters, the results revealed the highest root-shoot ratio in the 16 ^oC, indicating the top is 29 times heavier than the roots. Working across terrestrial plants, earlier studies have found a similar observation of highest root/shoot ratio in cool temperate than in tropical climates (Whitford and Duval, 2020; Qi et al., 2019), but the results indicated that genetic dynamics and plant development plays an additional important role of root biomass. Contrary to our results, former studies have documented temperatures, viz., $28/24$ °C in protecting the meristems for healthier roots (Sghaier et al., 2022; Khaeim et al., 2022; Virk et al., 2021). The studies went further and noted that, under low temperatures, root parameters such as root diameter and xylem size, possibly can condense and hence end up with little water uptake. Also, the preceding studies recognized the change in root hydraulic conductivity under low temperatures even under well-water conditions and finally lowest root: shoot ratio (Brand et al. 2016). Moreover, increasing or decreasing degree of temperature beyond the optimal level in the seeds - affects the biomass distribution comparably. Higher temperatures hinder root development and change root architecture, thus, decreasing the absorption and transportation of water and nutrients to the other parts. Decrease in root mass and later lower root: shoot ratio due to higher temperatures have been pointed out by other research groups; in cotton (Fan et al., 2022; Virk et al., 2021; Reddy et al., 2017), wheat (Sharma et al., 2022; Benlloch-Gonzalez et al., 2014), Maize (Walne and Reddy, 2022; Hatfield and Prueger, 2015), rapeseed (Sghaier et al., 2022), Brassicaceae (Maleki et al., 2023) and potato (Taranet et al., 2018).

4. CONCLUSIONS

Temperature has a direct control over the rate of many chemical reactions, including respiration and photosynthesis. The findings of this research highlighted the critical factors affecting cottonseed germination and established the optimal range for successful germination and seedling growth. The optimal temperature for cottonseed germination and seedling growth was within a more comprehensive range from 20 °C to 24 °C. Maximum germination speed was obtained at 36 °C, and seedling growth in terms of length and dry matter accumulation was observed at 24 °C. Genotypic variability was observed in the response towards heat stress given during the germination and seedling growth stage. These findings have the potential to optimize the sowing time in different agroclimatic regions and breeding programs for the development of modern cotton cultivars tolerant to early heat stress.

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Conflict of Interest

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Contributions

Design of Study: OK(%50), MCM(%50)

Data Acquisition: OK(%25), MCH(%75)

Data Analysis: OK(%25), MSH(%75)

Writing Up: OK(%25), MCM(%75)

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