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RESEARCH ARTICLE

Effect of mepiquat chloride on the photosynthesis and chlorophyll fluorescence parameters in upland cotton

Zhijun Wang[1] **and Yongmei Dong**[2*]

[1]Xinjiang Academy of Agricultural and Reclamation Science, Biotechnology Research Institute, Shihezi 832000, China.
[2]Xinjiang Academy of Agricultural and Reclamation Science, Cotton Research Institute, Shihezi, 832000, China.
[*]Corresponding author (ymd_sh@sohu.com).
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ABSTRACT

Mepiquat chloride (MC) is an inhibitory plant growth regulator (PGR), pivotal to optimizing cotton (*Gossypium hirsutum* L.) growth architecture, stress resilience, and yield-quality parameters. In this research, we conducted a randomized complete block (RCB) design, evaluating four diverse cotton genotypes (SD217, XLZ74, SD1068, and AY161) under field conditions, consisting of three replicates. The treatments included various concentrations of MC (40, 80, and 120 mg L^{-1}) applied during three key growth periods: Squaring, flowering, and bolling stages. Our results indicated that the relationship between MC insensitivity and MC sensitivity was established by analysing the effects on photosynthetic characteristics, chlorophyll fluorescence, cotton yield, yield components, agronomic traits, and fibre quality. Across the four upland cotton materials, under MC treatments (40, 80, and 120 mg L^{-1}), SPAD values were significantly enhanced by 7.11%-38.05%, transpiration rate, net photosynthesis rate (P_N), stomatal conductance increase by 5.72%-35.03%, 25.56%-54.21% and 1.35%-61.58% respectively in most cases, seed index increased by 0.56%-10.16%, but Lint percent decreased by 0.44%-11.83% in most cases .Chlorophyll fluorescence parameters, including quantum efficiency of photosystem II, maximum photochemical efficiency of PSII, minimal and maximal chlorophyll fluorescence, and photochemical quenching coefficient (qP), also tended to increase in most treatments, whereas non-photochemical quenching coefficient decreased. In addition, MC treatment also improved fibre quality and single boll weight. These results collectively demonstrate that MC treatment enhances P_N and reduces the non-photochemical quenching coefficient by increasing chlorophyll content, thereby improving cotton yield.

Key words: Chlorophyll fluorescence, cotton, *Gossypium hirsutum*, mepiquat chloride, photosynthesis.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the world's leading commercial fibre crop. Its widespread cultivation is driven by the substantial economic returns from marketing its high-quality fibre (Tung et al., 2020). Due to its perennial and indeterminate growth habit (Zhao et al., 2019), cotton growth must be regulated and eventually terminated by mepiquat chloride (MC) (1,1-dimethylpiperidin-1-ium chloride). As a widely used plant growth retardant, MC is essential for regulating and ultimately terminating cotton growth. By applying MC through seed soaking or foliar spraying, it acts as an endogenous regulator to control plant development (Jameel-M et al., 2024). This application is vital for manipulating canopy structure and enhancing yield (Yan et al., 2021). The MC has been used to shorten internode elongation, reduce main stem nodes, and decrease plant height and fruiting branch length, leading to a more compact plant architecture (Li et al., 2022; Liu et al., 2025). The MC is extensively applied in China and other major producing regions to maximize cotton yield and fibre quality (Meng et al., 2023; Lou et al., 2023). The application of MC at the flowering stage effectively reduces plant height while increasing seed yield in castor (*Ricinus communis* L.) In addition to these growth regulating effects, MC enhances plant stress resistance, for example, under salt stress conditions, MC treatment in cotton significantly boosts biomass (dry and fresh weight) and the content of key osmolytes and antioxidants, such as glycine betaine, proline, and phenolic compounds (Tung et al., 2018a).

Photosynthesis is the fundamental biological process whereby plants convert light energy into chemical energy. This energy supports essential life processes and serves as the foundation for synthesizing organic matter. This process serves as the physiological foundation for plant survival and is among the most fundamental physiological mechanisms in plant production (Salisbury et al., 2018). Therefore, improving photosynthetic efficiency is a critical strategy for increasing agricultural yield and sustaining the growing global population (Smith et al., 2023; Croce et al., 2024). The application of MC has been reported to enhance the net photosynthetic rate (P_N) and light use efficiency of cotton (Luo et al., 2023), as well as to modify the partitioning of photosynthetic products and the uptake and fixation of CO_2 (Tung et al., 2018a). The application of MC as a foliar spray has resulted in a significant increase in key photosynthetic parameters, specifically the leaf CO_2 exchange rate and P_N (Zhao et al., 2019). The MC treated-cotton typically exhibits thick, “leathery” leaves with elevated chlorophyll content, resulting in a dark green appearance (Tung et al., 2018a).

Chlorophyll fluorescence parameters serve as key metrics to evaluate photosynthetic efficiency as well as plant stress responses (Faseela et al., 2019). Chlorophyll fluorescence analysis involves three primary measurable variables: Minimal fluorescence (F_o or F'_o) when photosystem II (PSII) reaction centres are open, maximal fluorescence (F_m or F'_m) when PSII centres are closed, and steady state fluorescence (F_s or F'_s). From these, key derived parameters are calculated, including variable fluorescence ($F_v = F_m - F_o$; $F'_v = F'_m - F'_o$) and the difference between maximal and steady-state fluorescence ($F_q = F_m - F_s$; $F'_q = F'_m - F'_s$). Collectively, these derived variables enable the determination of both the maximum and operating efficiencies of PSII photochemistry. These efficiencies serve as fundamental metrics for assessing photosynthetic performance and detecting plant stress (Lysenko et al., 2022).

Previous studies reported the MC effects on cotton morphology, cotton yield, functional leaf photosynthetic physiology, and fibre quality in relation to yield under the traditional planting model. However, limited information is available on the cultivation methods of drip irrigation under mulch in Xinjiang. The MC application enhances the P_N in cotton, thereby improving the source-sink relationship and promoting carbohydrate formation. This shift in carbohydrate metabolism regulates the activity of sucrose metabolizing enzymes, which in turn leads to variable yield responses (Tung et al., 2020). Therefore, this study aims to investigate the effects of MC application on leaf photosynthetic characteristics, chlorophyll fluorescence, cotton yield components, and fibre quality. A further objective is to elucidate the quantitative relationships among these key attributes under the new efficient cultivation system.

MATERIALS AND METHODS

Plant materials and treatments

Four cotton (*Gossypium hirsutum* L.) cultivars, SD217, XLZ74, SD1068, and AY161 were used in this study (obtained from the College of Agronomy, Shihezi University, Shihezi, Xinjiang, China). Experiments were carried out in 2020, from March to October. Seeds of the four cotton varieties were sown in the experimental field of Cotton Research Institute of Shihezi University (44°27’ N, 85°94’ E), Shihezi, Xinjiang, China. When the third true leaf fully expanded, seedlings were treated with foliar spraying of mepiquat chloride (MC) (1,1-dimethylpiperidin-1-ium chloride) at 40, 80, or 120 mg L^{-1} at four time points, uniformly spraying each plant with about 8 mL. Seedlings sprayed with water were used as controls. The first treatment time point was on 27 May, the second on 6 June, the third on 16 June, and the fourth on 26 June 2020. The plant height was measured from the cotyledon node to the growing point on 10 July, which was 14 d after the fourth spray. Each treatment at each time point was replicated three times, with each replicate consisting of one plot.

Experimental design

The experiments were conducted at the experimental station of the agricultural college of Shihezi University, Shihezi (86°03’ E, 45°19’ N), Xinjiang, China, during April to October 2020. The average high temperature in Shihezi from April to October 2020 is 26 °C, the air humidity is 44.7%, the average precipitation is 20.3 mm, the solar radiation is 5.9 kW m^{-2}, and the vapor pressure deficit is 1.34 kPa. Pre-planting fertility status was determined from the top soil layer (0-20 cm). The average altitude is 450.8 m. The texture of the soil from the experimental site was medium loam with 7.51 pH, 12.50 g kg^{-1} organic matter, 54.9 $mg \cdot kg^{-1}$ alkali N, 1.45$g \cdot kg^{-1}$

total N, 149 mg·kg^{-1} available K, and 23 mg·kg^{-1} available P. The region is classified as a temperate arid zone with a continental climate. The annual cumulative temperature greater than 10 °C is 3729 °C. The frost-free period is 171 d, and the sunshine duration is 2773 h. During the cotton growing season, the long-term average annual rainfall is 180-270 mm. The experiments were conducted in a randomized block design with three MC treatment levels (control, 40, 80, and 120 mg). A total of 72 plots (2.3 × 5 m² each) were established in a randomized design with three replicates. The irrigation regime consisted of eight applications at 10 d intervals throughout the growing season (March to October).

The cotton crop was implemented through a plastic film-mulched, high-density planting system. The “dry sowing and wet emergence” technique was adopted, where seeds were sown in dry soil followed by irrigation to ensure uniform germination. Four early maturing cotton cultivars (SD217, XLZ74, SD1068, and AY161) were sown on 22 April 2020. The growth period for them was 125 to 128 d. Each plot was covered with a single plastic film sheet, beneath which three drip irrigation lines were installed. A six-row planting system under each plastic film employed wide-narrow row spacing (10-66 cm) in the 2020 season. The plants were spaced 10 cm apart within rows. The subsurface drip irrigation layout was aligned with this planting arrangement.

Seed cotton yield and boll weight

A harvest area of four central rows, each 5 m in length, was manually sampled from each plot. The seed cotton from this area was weighed to determine the plot yield. Fifty cotton bolls were respectively harvested from the lower, middle, and upper portions of the plant canopy in each plot, and were subsequently used to calculate the mean single boll weight.

Yield components and fibre quality

The seed cotton from each plot was manually harvested twice during each growing season, with the harvests conducted from 30 September to 7 October 2020. After sun drying and weighing the total seed cotton, 50 fully opened bolls per plot were sampled to calculate average boll weight and lint percentage. The number of bolls (≥ 2 cm diameter) per plant was counted on 20 September 2020.

Investigation of agronomic traits at the boll stage

Cotton plant height was measured by a straight ruler, from the cotyledon node to the topping end; number of branches from bottom to top; fruit branch boll number is the number of all effective bolls on a cotton plant; first node height from the cotyledon node to the first branch height measured by ruler. The number of main stem nodes from the cotyledon node to the first fruiting branch was determined. The quality of fibre, encompassing length, strength, micronaire value, and uniformity, was quantified by a specialized laboratory at the Chinese Academy of Agricultural Sciences through high volume instrumentation.

SPAD

In the cotton squaring stage (16 June), flowering stage (9 July), and bolling stage (24 July), on the day at the end of the MC treatments, from top to bottom, the SPAD value in the second to fourth leaves on the main stem of cotton was measured using a chlorophyll meter (SPAD-502; Konica Minolta, Tokyo, Japan). In each plot, ten plant functional leaves were randomly selected and measured. The data from one plot constituted a single replicate, and each treatment consisted of three such replicates. The squaring stage refers to the period when the first bud (like a small triangle tower, about 3 mm in size) appears on 50% of cotton plants. The flowering period refers to the period when the first flower opens on 50% of cotton plants. The bolling stage period refers to the whole period from the beginning of flowering to the beginning of boll opening.

Gas exchange measurements

Meanwhile, in the cotton squaring stage (16 June), flowering stage (9 July), and bolling stage (24 July), the photosynthetic parameters of the functional leaves on the main stem namely the net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) were measured using a portable photosynthesis system (GFS-3000; Heinz Walz, Effeltrich, Germany), which was installed in an 8 cm^2 leaf area chamber. The parameters were determined under standardized conditions of 400 µmol mol^{-1} CO_2 and a light intensity of 1400 µmol m^{-2} s^{-1}. In the cotton squaring stage, leaf temperature was 34.42 °C, ambient humidity and temperature were 49% and 31.32 °C, respectively, in flowering stage leaf temperature was 33.47 °C, ambient humidity and temperature were 57.85% and 29.75 °C, in bolling stage leaf temperature was 36.20 °C, ambient humidity and temperature were 45.0% and 32.55 °C, respectively. Measurements were taken on a sunny, windless day between 11:00 and 13:00 h. When measuring, the balance time of the leaf chamber was about 2-5 min. After the reading of each leaf was stable, the leaf temperature was about 2 °C higher than the ambient temperature, generally 30-35 °C. Using five representative samples per treatment.

Chlorophyll (Chl) fluorescence

In the cotton squaring stage (24 June), flowering stage (5 July), and bolling stage (30 July), the photochemical efficiency of photosystem II (PSII) was assessed by measuring the maximum quantum yield (F_v/F_m), alongside the minimal (F_o) and maximal (F_m) chlorophyll fluorescence parameters. The F_o and F_m were measured after dark-adapting the leaves for 30 min using a dark leaf clip (DLC-8; Heinz Walz). When the saturation pulse is turned on for the light-adapted sample obtained maximum fluorescence yield (F_m') was determined under subsequent exposure to full actinic light. The quantum efficiency of photosystem II (ΦPSII) was determined as ($F_m' - F_s)/F_m'$. This parameter reflects the actual photochemical efficiency under actinic light. Additionally, the maximum photochemical efficiency of PSII was calculated as $F_v/F_m = (F_m - F_o)/F_m$. Non-photochemical quenching coefficient (NPQ) = $(F_m - F_m')/F_m'$; photochemical quenching coefficient (qP) = $(F_m' - F_s)/F_v'$.

Data analyses

Data are presented as the mean of three replicates per treatment. ANOVA was performed using SPSS 19.0 (IBM, Armonk, New York, USA). When the ANOVA indicated a significant effect, treatment means were compared pairwise using the Tukey's test ($P < 0.05$).

RESULTS

Effect of MC on the SPAD in upland cotton

Among the four cotton varieties, as compared to the control, the application of MC at 40, 80 and 120 mM increased SPAD value significantly (Figure 1). The SPAD value of 'SD217' and 'XLZ74' increased significantly compared with control, T1, T2 and T3 increased in squaring stage, flowering stage and bolling stage were 16.27%-29.81%, 14.23%-33.27%, 13.27%-22.42% and 10.67%-15.97%, 18.92%-27.23%, 12.18%-25.84%. All treatments produced a significant effect compared to the control ($P < 0.05$). The SPAD value of 'SD1068' and 'AY161' also increased significantly compared with the control. Increases of T1, T2 and T3 in three stages were 16.27%-18.75%, 13.85%-23.14%, 11.75%-28.36% and 7.11%-38.05%, 38.62%-24.32%, 18.02%-32.51%, respectively compared with control. Most of the treatments showed significant differences ($P < 0.05$).

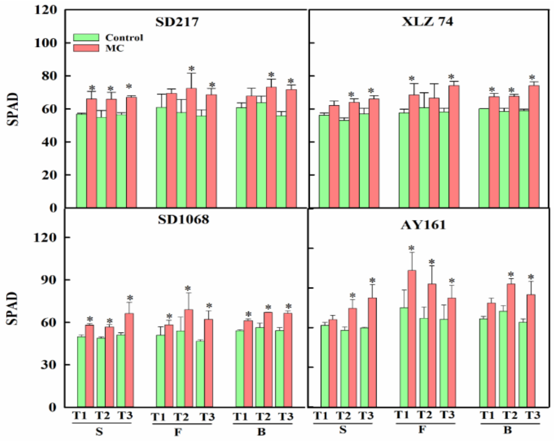

Figure 1. Effects of three concentrations of mepiquat chloride (MC) on the SPAD value of the four cotton varieties at three different growth stages. T1: 40 mg L^{-1} MC; T2: 80 mg L^{-1} MC; T3: 120 mg L^{-1} MC; S: squaring stage; F: flowering stage; B: bolling stage. Data are presented as the mean ± SD ($n = 3$). *Significant difference between the treatment and control within the same genotype ($p < 0.05$), as determined by the Tukey test.

Effect of MC on the photosynthetic parameters in upland cotton

We investigated the photosynthetic parameters to elucidate the impacts of the photosynthetic physiology on cotton under MC treatment (Figure 2); MC altered the photosynthetic characteristics of plants at different growth stages. For the groups with MC application increased the transpiration rate (T_r), net photosynthesis rate (P_N), stomatal conductance(g_s) of cotton leaves (Figure 2). Besides the application of MC with 120 mg L^{-1} for 'SD217' at squaring stage and 'XLZ74' at flowering stage, the T_r value of 'SD217' and 'XLZ74' under MC treatment was lower than control; however, the T_r value of 'SD217' and 'XLZ74' under other MC treatments increased significantly compared with control, T1 and T2 increased in the squaring stage by 12.8%-23.9%, 11.83%-19.75%, flowering stage by 29.03%-54.52%, 5.72%-10.28, and bolling stage by 14.31%-24.17%, 6.23%-35.03%, respectively. Similarly, T_r of 'SD1068' and 'AY161' in most treatments increased slightly compared to the control. In those four varieties, g_s of MC application was significantly increased in most treatments. For 'SD217', T1, T2, and T3 increased in three stages by 1.35%-61.58%, for 'XLZ 74', except at MC 40 mg L^{-1} at the bolling stage and MC 120 mg L^{-1} at the squaring stage, at the flowering stage g_s was lower compared to the control, other treatments increased by 4.70%-48.34%. 'SD1068' and 'AY161' increased by 2.54%-17.90% and 3.02%-20.93% respectively, except for minority treatments. Besides the application of MC at 40 mg L^{-1} for 'XLZ74' at bolling stage and at 120 and 80 mg L^{-1} for 'SD1068' at squaring stage and bolling stage, MC at 80 mg L^{-1} for 'AY161' at squaring stage was lower than the control, all other treatments increased MC treatment among the three growth periods by 9.33-52.10%, 2.56%-54.21% and 2.98%-37.57% and 5.99%-38.95%. The value of C_i among the four varieties did not show a regular trend.

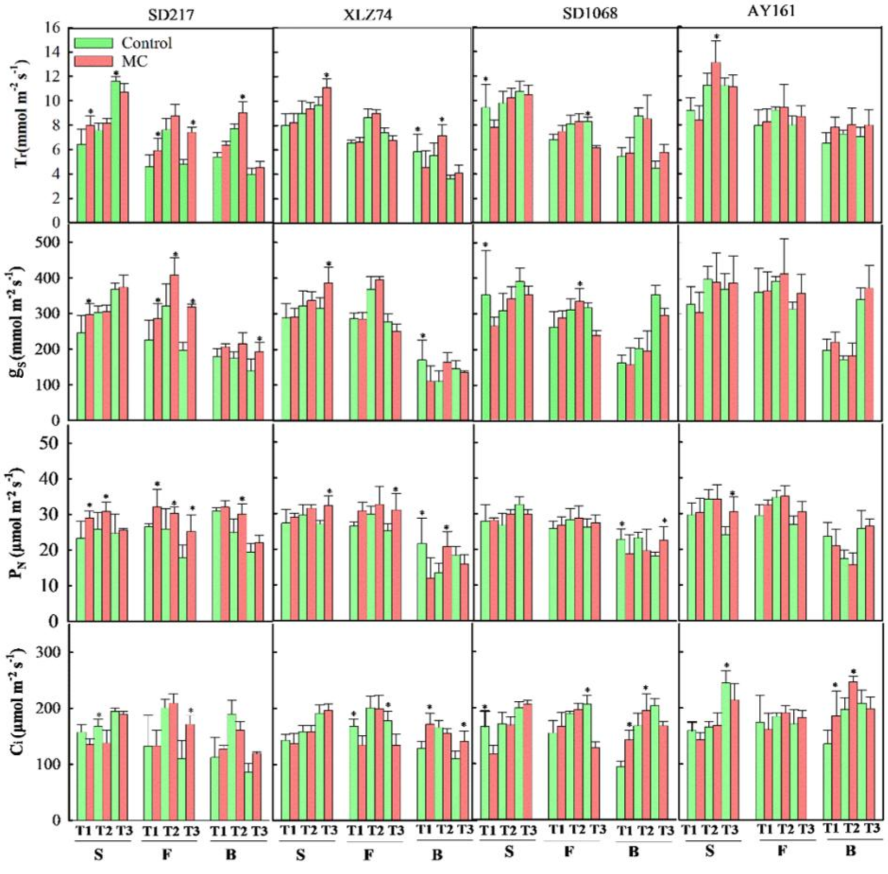

Figure 2. Effects of different concentration gradients of mepiquat chloride (MC) treatments on photosynthetic parameters of the four cotton varieties at three different growth stages. T_r: Transpiration rate; g_s: stomatal conductance; P_N: net photosynthetic rate; C_i: intercellular CO_2 concentration; T1: 40 mg L^{-1} MC; T2: 80 mg L^{-1} MC; T3: 120 mg L^{-1} MC; S: squaring stage; F: flowering stage; B: bolling stage. Data are presented as the mean ± SD (*n* = 3).

Effect of MC on the chlorophyll fluorescence in upland cotton

The effects of MC on the chlorophyll fluorescence of functional leaves on the main stem of the four cotton varieties are presented in Figure 3. For ‘SD217’ and ‘XLZ74’, the effects of MC on the (Φ PSII) did not show obvious regularity. The upland cotton varieties of ‘SD217’ and ‘XLZ74’, which dullness to MC, besides ‘SD217’ under the treatment of 40 mg L^{-1} MC concentration at the flowering stage, 120 mg L^{-1} MC concentration at the bolling stage, and ‘XLZ74’ with 120 mg L^{-1} MC treatment at squaring stage, 80 and 120 mg L^{-1} at bolling stage, less than control; however, most of the remaining treatments were higher than the control. ‘SD217’ and ‘XLZ74’ under MC treatment increased by 5.53%-17.23% and 1.43%-11.05% compared to the control, respectively. For ‘SD1068’ and ‘AY161’, which were sensitive to MC, it also shows a similar trend, but the growth rate was lower than ‘SD217’ and ‘XLZ74’. ‘SD1068’ and ‘AY161’ increased by 1.50%-8.67% and 0.91%-9.10%, respectively. The photochemical quenching coefficient (qP) is the decrease of fluorescence caused by photosynthesis. For ‘SD217’ and ‘XLZ74’, among the 18 treatments, four of them decreased compared to control, and the remaining 14 treatments increased compared to control. ‘SD217’ and ‘XLZ74’ increased by 2.51%-5.01% and 1.07%-3.88% respectively. However, for the MC sensitive ‘SD1068’ and ‘AY161’, qP value among the 18 MC treatments, of which 11 treatments were less than control, the rest seven treatments were higher than control, which increased by 0.48%-8.24% and 0.45%-5.09%. Non-photochemical quenching coefficient (NPQ), a decrease in fluorescence due to heat dissipation, in these four upland cotton varieties. In almost all MC treatments, a decrease in NPQ parameters was caused. ‘SD217’ and ‘XLZ74’ decreased by 21.53%-74.32% and 5%-69.9%; however, ‘SD1068’ and ‘AY161’ increased by 7.8%-82.87%, 7.87%-55.81%. From the overall situation, the application of MC increased minimum (F_o) and maximum fluorescence (F_m) values. Except for individual treatment, F_0 of ‘SD217’ and ‘XLZ74’ increased by 0.39%-57.70% and 2.10%-13.51% respectively, compared to the control. ‘SD1068’ and ‘AY161’ increased by 1.02%-23.11% and 0.01%-6.39% respectively; the increase was lower than that of ‘SD217’ and ‘XLZ74’. The F_m of ‘SD217’ and ‘XLZ74’ increased by 3.17%-103.08% and 2.01%-22.89% respectively, compared to the control, while ‘SD1068’ and ‘AY161’ increased by 0.45%-15.90% and 3.15%-23.24%, the rate increasing was less than ‘SD217’ and ‘XLZ74’. From the overall situation, the application of MC increased maximum quantum yield of PSII (F_v/F_m), except for individual treatment. The F_v/F_m of ‘SD217’ and ‘XLZ74’ increased by 0.43%-7.68% and 1.90%-8.6% respectively, for ‘SD1068’ and ‘AY161’ increased by 0.77%-3.94% and 1.59%-12.15% respectively.

Effect of MC on the agronomic traits in upland cotton

The effects of MC treatment on the yield and yield components are shown in Table 1. For ‘SD217’ and ‘XLZ74’, which are dull to MC, ‘SD1068’ and ‘AY161’, which are sensitive to MC, the application of MC at 40 and 80 mg L^{-1} increased the yield of ‘SD217’ and ‘XLZ74’; however, reduced the yield of ‘SD1068’ and ‘AY161’. The application of 120 mg L^{-1} significantly affects the yield, except for ‘XLZ74’; the yield of ‘SD217’, ‘SD1068’, and ‘AY161’ was reduced compared to the control. Under the MC treatment, it can increase single boll weight compared to the control, but ‘SD1068’ and ‘AY161’ showed the opposite. From the global change trend, MC treatment reduced lint percentage and increased the seed index of upland cotton.

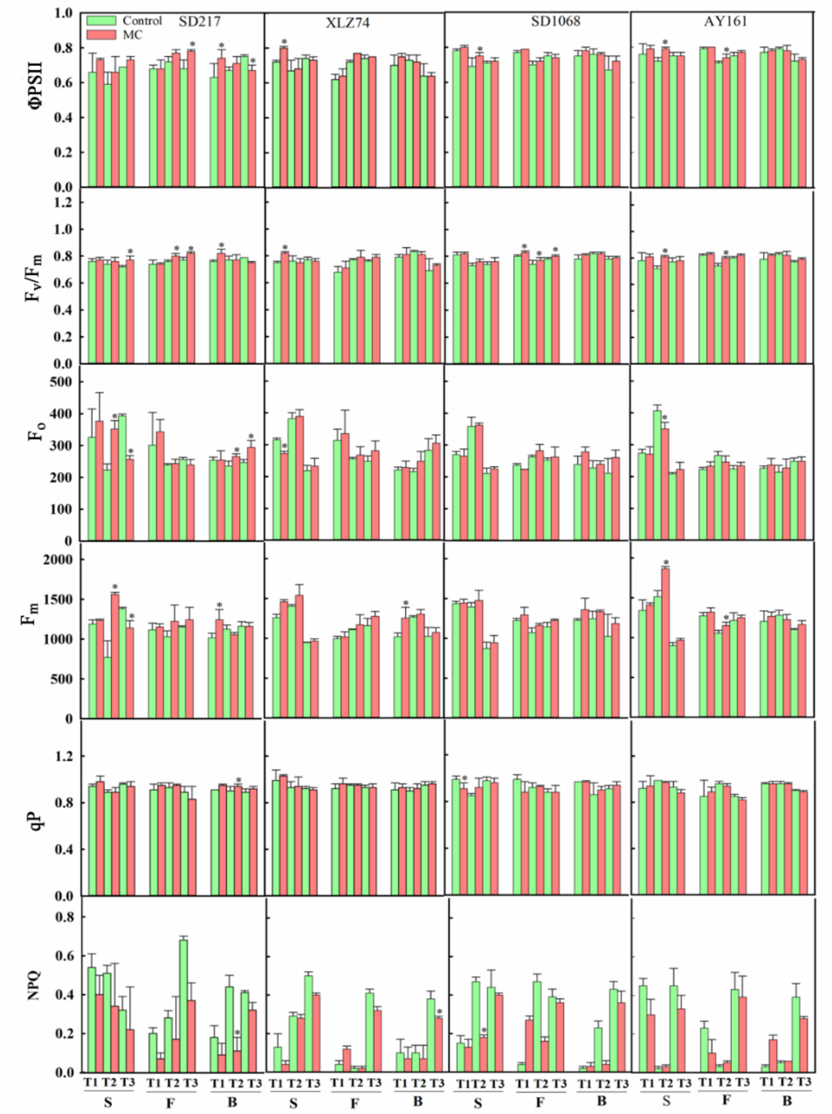

Figure 3. The effects of different concentration gradients of mepiquat chloride (MC) treatments on chlorophyll fluorescence parameters of the four cotton ecotypes at three different growth stages. ΦPSII: Quantum efficiency of photosystem II; F_v/F_m: maximum quantum yield; F_o: minimal fluorescence; F_m: maximal fluorescence; qP: photochemical quenching coefficient; NPQ: photochemical quenching coefficient; T1: 40 mg L^{-1} MC; T2: 80 mg L^{-1} MC; T3: 120 mg L^{-1} MC; S: squaring stage; F: flowering stage; B: bolling stage. Data are presented as the mean ± SD (*n* = 3). *Significant difference between the treatment and control within the same genotype ($p < 0.05$), as determined by the Tukey test.

Table 1. Effect of mepiquat chloride (MC) on the cotton yield and yield components in upland cotton. Effects of MC treatment on the yield and yield components. T1: 40 mg L^{-1} MC; T2: 80 mg L^{-1} MC; T3: 120 mg L^{-1} MC; S: squaring stage; F: flowering stage; B: bolling stage. Data are presented as the average value (*n* = 3). Different small letters indicate significant differences at $P < 0.05$ as determined by the Tukey test.

MC concentration	Variety	Treatment	Yield	Single boll weight	Lint percent	Seed index
			kg ha^{-1}	g	%	g
40 mg L^{-1}	SD217	Control	3354.00^{ab}	5.65^{bc}	44.15^{abc}	9.29^{a}
		MC	4396.05^{a}	6.17^{a}	44.65^{ab}	9.73^{a}
	XLZ74	Control	4092.90^{a}	5.18^{d}	43.52^{bc}	8.44^{cd}
		MC	5286.75^{ab}	5.46^{cd}	42.53^{c}	8.76^{bc}
	SD1068	Control	3581.25^{ab}	5.79^{b}	45.54^{a}	9.22^{ab}
		MC	4168.80^{a}	5.87^{ab}	45.27^{a}	9.37^{a}
	AY161	Control	2671.80^{ab}	5.24^{d}	45.56^{a}	7.99^{d}
		MC	2046.45^{b}	4.86^{e}	44.63^{ab}	8.14^{d}
80 mg L^{-1}	SD217	Control	3865.50^{bc}	5.69^{bc}	44.49^{ab}	9.12^{ab}
		MC	4509.75^{ab}	6.26^{a}	43.29^{ab}	9.49^{a}
	XLZ74	Control	4888.80^{a}	5.12^{de}	44.74^{ab}	8.50^{bc}
		MC	4887.80^{a}	5.55^{bcd}	40.12^{b}	8.28^{c}
	SD1068	Control	3240.30^{cd}	5.95^{ab}	43.78^{ab}	8.91^{ab}
		MC	3069.75^{d}	5.39^{cde}	44.13^{ab}	8.96^{ab}
	AY161	Control	2558.10^{d}	4.98^{e}	46.60^{a}	7.95^{c}
		MC	1356.75^{e}	4.34^{f}	42.91^{ab}	8.15^{c}
120 mg L^{-1}	SD217	Control	5571.00^{ab}	5.97^{abc}	45.07^{abc}	9.32^{b}
		MC	4168.80^{ab}	6.37^{a}	43.70^{bc}	10.11^{a}
	XLZ74	Control	5741.40^{a}	5.37^{d}	42.84^{cd}	8.43^{d}
		MC	5911.95^{ab}	5.77^{bcd}	42.65^{cd}	8.75^{cd}
	SD1068	Control	4831.95^{ab}	6.27^{ab}	47.19^{a}	8.96^{bc}
		MC	2910.60^{bc}	5.63^{cd}	45.07^{abc}	9.87^{a}
	AY161	Control	3115.20^{abc}	5.66^{cd}	45.88^{ab}	8.03^{e}
		MC	947.40^{c}	4.36^{e}	40.45^{d}	8.47^{d}

The effects of MC treatment on the agronomic traits in upland cotton are shown in Table 2. Compared with the control, MC treatment can significantly reduce the first node height and plant height of upland cotton, and reduce the number of fruit branches and bell number to varying degrees, with little effect on the initial node position.

The effects of MC treatment on the fibre quality in upland cotton are shown in Table 2. As seen from Table 2, four cotton varieties with three MC concentrations treatment give a total of 12 treatments, among them, the average length of the upper half of the fibres in 9 treatments increased compared to the control, the fibre fracture strength in 8 treatments increased compared to the control, the fibre uniformity in 7 treatments was higher than the control, and the fibre elongation in 11 treatments was higher than the control. The MC had little effect on the micronaire (MIC) value of upland cotton fibres. Overall, MC treatment is beneficial for increasing the average length of the upper half of upland cotton fibres, enhancing fibre fracture strength, increasing fibre uniformity, and increasing fibre elongation, but has little effect on the fibre MIC value.

Table 2. Effects of mepiquat chloride (MC) treatment on the fibre quality. T1: 40 mg L^{-1} MC; T2: 80 mg L^{-1} MC; T3: 120 mg L^{-1} MC; S: squaring stage; F: flowering stage; B: bolling stage. Data are presented as the average value (n = 3). Different small letters indicate significant differences at $P < 0.05$ as determined by the Tukey's test.

MC concentration	Variety	Treatment	First node height	Plant height	First node position	Number of branches	Boll number
				cm			
40 mg L^{-1}	SD217	Control	24.44^{a}	94.67^{a}	5.13^{a}	10.01^{a}	6.67^{a}
		MC	20.79^{b}	73.83^{c}	5.07^{a}	9.27^{ab}	5.80^{ab}
	XLZ74	Control	25.17^{a}	84.07^{b}	5.07^{a}	7.61^{b}	4.73^{abc}
		MC	24.67^{a}	68.67^{cd}	5.07^{a}	7.47^{b}	4.27^{bc}
	SD1068	Control	15.01^{c}	60.60^{de}	5.33^{a}	8.80^{ab}	4.80^{abc}
		MC	14.37^{c}	43.93^{fg}	5.27^{a}	7.80^{b}	4.20^{bc}
	AY161	Control	15.40^{c}	50.67^{ef}	5.60^{a}	8.86^{ab}	4.20^{bc}
		MC	13.63^{c}	37.10^{g}	5.67^{a}	7.73^{b}	3.20^{c}
80 mg L^{-1}	SD217	Control	23.33^{ab}	82.47^{a}	5.01^{bc}	10.01^{a}	5.87^{a}
		MC	19.21^{bc}	61.33^{b}	5.27^{abc}	8.67^{ab}	4.87^{ab}
	XLZ74	Control	27.23^{a}	89.77^{a}	4.93^{c}	8.73^{ab}	5.73^{a}
		MC	26.13^{a}	64.71^{b}	5.13^{bc}	7.47^{bc}	5.27^{a}
	SD1068	Control	22.37^{ab}	63.37^{b}	6.07^{a}	6.93^{c}	4.21^{abc}
		MC	14.67^{cd}	40.51^{c}	5.53^{abc}	7.87^{bc}	4.53^{ab}
	AY161	Control	14.43^{cd}	47.10^{c}	5.87^{ab}	7.93^{bc}	3.13^{bc}
		MC	13.01^{d}	30.90^{d}	5.87^{ab}	6.47^{c}	2.61^{c}
120 mg L^{-1}	SD217	Control	27.83^{ab}	100.27^{a}	5.33^{cd}	11.13^{a}	7.73^{abc}
		MC	23.13^{bc}	65.67^{bc}	4.81^{e}	9.20^{bc}	6.01^{abcd}
	XLZ74	Control	28.91^{a}	100.57^{a}	4.73^{e}	9.27^{bc}	8.27^{ab}
		MC	28.57^{a}	64.77^{c}	5.01^{de}	7.47^{de}	4.60^{cd}
	SD1068	Control	20.60^{cd}	81.20^{b}	5.67^{c}	10.73^{ab}	9.13^{a}
		MC	19.47^{cd}	43.40^{de}	5.81^{bc}	7.73^{cde}	5.07^{bcd}
	AY161	Control	20.43^{cd}	57.17^{cd}	6.61^{a}	8.33^{cd}	4.60^{cd}
		MC	16.23^{d}	34.10^{e}	6.21^{ab}	6.07^{e}	3.13^{d}

The correlation result (Figure 4) showed that plant height was highly positively associated with first node height and boll number; however, it had a highly negative correlation with first node position, T_r, g_s, and SPAD value. SPAD value has a highly positive association with T_r, g_s, and P_N, while the seed index has a highly negative correlation with T_r, g_s, fibre length, fibre strength, and has a highly positive correlation with bell weight. Fibre elongation has a highly negative correlation with SPAD value, T_r, and g_s, and has a highly positive correlation with seed index. Cotton seed yield has a highly negative correlation with qP and a highly positive correlation with seed index, elongation, and bell weight. Bell weight has a highly negative correlation with SPAD value, T_r, g_s, fibre strength, C_i, and fibre length has a highly positive correlation with elongation. Lint percentage has a highly positive correlation with qP.

The results of combined variance analysis (Table 3) showed that the main effect of growth period (Gro. P) on the SPAD value, P_N, T_r, g_s, qP, and plant height (PH), were significant at 5% probability levels under MC treatment ($p \leq 0.05$); other indicators were nonsignificant. The effects of variety (Var) on SPAD value, T_r, g_s, yield, maximum quantum yield of photosystem II (F_v/F_m), qP, PH, seed index (SI), Lint percent (lint), boll weight (BW), fibre length (FL), fibre strength (FS), MIC, fibre elongation (FE) were significant under MC treatment ($p \leq 0.05$). Other indicators were nonsignificant. The effects of MC concentration (MC. C) on SPAD value, T_r, F_m, PH, SI, lint, FS, MIC, and FE were significant under MC treatment ($p \leq 0.05$); other indicators were nonsignificant. Growth period (Gro. P), variety (Var), MC concentration (MC. C), Gro. P × MC. C have a significant effect on SPAD value. Also Gro. P × Var × MC.C had significant effects on SPAD value under MC treatment; other indicators were nonsignificant.

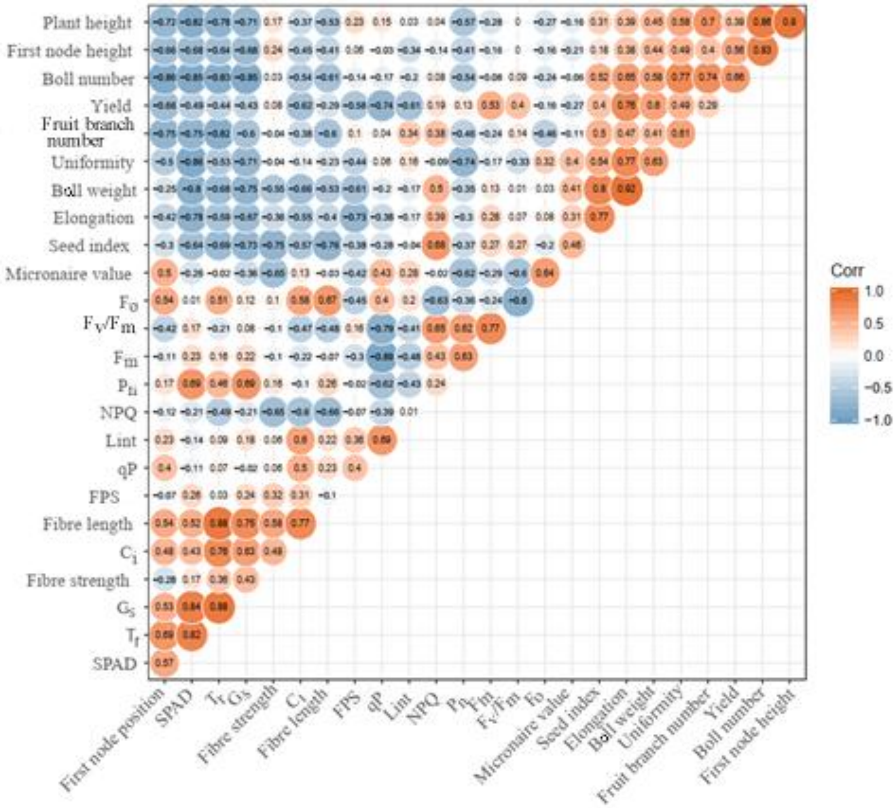

Figure 4. Correlation coefficients of the individual indices of cotton under mepiquat chloride treatment. F_o: Minimal chlorophyll fluorescence; F_v/F_m: maximum quantum yield; F_m: maximal chlorophyll fluorescence; P_N: net photosynthetic rate; NPQ: non-photochemical quenching coefficient; Lint: lint percent; qP: photochemical quenching coefficient; C_i: intercellular CO_2 concentration; g_s: stomatal conductance; T_r: transpiration rate.

Table 3. ANOVA of photosynthetic chlorophyll fluorescence, yield, agronomic traits, and fibre quality of different cotton varieties influenced by mepiquat chloride. $^*p > 0.05$, ^{ns}nonsignificant. P_N: Net photosynthetic rate; T_r: transpiration rate; g_s: stomatal conductance; C_i: intercellular CO_2 concentration; F_o: minimal chlorophyll fluorescence; F_m: maximal chlorophyll fluorescence; F_v/F_m: maximum quantum yield; qP: photochemical quenching coefficient; NPQ: non-photochemical quenching coefficient; PH: plant height; SI: seed index; Lint: lint percent; BW: boll weight; FL: fibre length; FS: fibre strength; MIC: micronaire value; FU: fibre uniformity; FE: fibre elongation; Gro. P: Growth period; Var: variety; MC. C: MC concentration.

Index	SPAD	P_N	T_r	g_s	C_i	Yield	F_o	F_m	F_v/F_m	qP	NPQ	PH	SI	Lint	BW	FL	FS	MIC	FU	FE
Gro.p	*	*	*	*	ns	ns	ns	ns	ns	*	ns	*	-	-	-	-	-	-	-	-
Var	*	ns	*	*	ns	*	ns	ns	*	*	ns	*	*	*	*	*	*	*	ns	*
MC.C	*	ns	*	ns	ns	ns	ns	*	ns	ns	ns	*	*	*	ns	ns	*	*	ns	*
Gro.p × Var	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	-	-	-	-	-	-	-	-
Gro.p × MC. C	*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	-	-	-	-	-	-	-	-
Var × MC.C	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Gro.p × Var × MC.C	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-	-	-	-

DISCUSSION

Mepiquat chloride (MC) is a plant growth regulator commonly applied to cotton to curb excessive vegetative growth and promote a more compact plant architecture (Wang et al., 2025). The MC application induced a more compact cotton canopy. Our results showed that cotton yield components, photosynthetic chlorophyll fluorescence, agronomic traits, fibre quality, morphological attributes viz, plant height, fruiting branches, boll number, lint percentage, as well as internode length were significantly reduced under MC treatments.

Furthermore, the soil and plant analyser development (SPAD) value, photosynthetic rate (P_N), transpiration rate (T_r), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), effective quantum yield of photosystem II (Φ PSII), maximum quantum yield of photosystem II (F_v/F_m), seed index was enhanced by MC treatment compared to control. Our results are in agreement with prior studies that documented reductions in both plant height and internode length (Tung et al., 2020). The MC application significantly increased chlorophyll content in cotton leaves, resulting in a darker green colour. This finding is consistent with the report by Abbas et al. (2022). An increase in SPAD values ranging 7.11%-38.62% was observed in MC-treated plants compared to the control. In our research, MC treatment obviously brought about an increase in the SPAD values by 7.11%-38.62% compared with the control. Among the four cultivars (SD217, XLZ74, SD1068, and AY161), indicates that MC treatment promoted chlorophyll synthesis in functional leaves. These findings are consistent with Tung et al. (2018a), who also found that MC application enhanced chlorophyll content in cotton leaves, as indicated by their darker green colour. They proposed that the MC application increased the specific leaf weight, which in turn led to the upregulation and accumulation of chlorophyll.

Photosynthesis (P_N) is the fundamental and dynamic process underpinning plant growth and development (Neto et al., 2021). Our results showed that P_N both increased and decreased at different growth periods among different cotton varieties, but most treatments were higher than the control. Foliar application of MC resulted in a significant increase in the leaf CO_2 exchange rate and P_N compared to the control plants (Zhao et al., 2019). They further elaborated that MC increased P_N by enlarging mesophyll cells. The increased cell surface area enhanced the CO_2 exchange rate, thereby boosting photosynthetic efficiency. Increased g_s and specific leaf weight may have also contributed to the elevated CO_2 exchange rate (Zhao et al., 2019).

However, it is noteworthy that findings on this topic are not unanimous. Some studies have reported significant reductions in the P_N of cotton under MC application at various growth stages, highlighting the potential influence of application timing and environmental conditions. Similarly, T_r and g_s mirrored this trend, whereas the C_i remained unaffected (Tung et al., 2019). The MC enhances P_N by altering leaf morphology and physiology, with increased chlorophyll content serving as a key indicator of this physiological improvement. Regulation of cotton yield components by MC, cotton yield responses to MC application have been inconsistent. Most of the previous studies reported positive yield responses (Jia et al., 2024), although others documented yield reduction (Tung et al., 2018b). Our data showed that MC application significantly increased the yield, single boll weight and seed index, but reduced lint percentage with varying responses among different MC treatments. Yield and its components are of paramount economic importance to cotton growers. A primary economic objective for cotton growers is to maximize yield and its key components. It was previously reported that yield components, including boll number, boll weight, and lint percentage, are determined by the cotton growth pattern and are significantly modulated by environmental conditions, plant density, and MC use (Cai et al., 2019).

The MC-treated plants exhibited enhanced yield in narrow row spacing relative to the control plants (Liu et al., 2025). In conclusion, the yield response to MC application is contingent upon multiple factors, including application rate, growth stage, cultivar, site-specific conditions, and unpredictable weather, all of which must be optimized to achieve the best results. Agronomic practices such as plant density and MC application offer a viable strategy for manipulating the source-sink balance and regulating cotton growth, being more readily controllable than environmental factors (Tung et al., 2018b; Murtza et al., 2022). The MC treatment decreased lint yield, boll number, and fruit number per plant. Similar to cottonseed yield, lint yield responded parabolically to rising MC application levels. This pattern can be attributed to the treatment's effect on light use efficiency, which was enhanced through greater light interception and elevated net photosynthesis (Mao et al., 2014). Our research data are consistent with Zhao et al. (2019), who also demonstrated that MC application enhanced cottonseed yield and quality, increasing seed oil, protein content, and seed index. The difference in this result may be caused by the use of different varieties and different sensitivity to MC.

While the application of MC reduced vegetative growth parameters such as plant height, first node height, first node position, and fruit branch number, but did not decrease the final boll number per unit area. Previous studies showed that application of MC reduced cotton plant height and fruit branch length and increased boll setting rate (Tung et al., 2020). The application of MC produced a lower, more compact plant canopy due to its effective suppression of vegetative growth. Maximizing cottonseed yield at an optimal plant density is facilitated by MC, which regulates vegetative growth to improve canopy structure and resource allocation (Ye et al., 2024).

The observed increase in cottonseed yield from MC application is attributed to concurrent rises in both 100-seed weight and boll number per unit area. By optimizing canopy structure and curbing excessive vegetative growth, MC application improves boll-setting percentage, thereby increasing both boll number per unit area and seed weight (Yan et al., 2019). It is important to note that these outcomes may vary due to genotypic differences among the cotton materials studied. Multiple applications of MC enhanced the coordinated development of cotton fruits across all stages, from square initiation to boll opening, though this effect was highly dependent on the growth environment (Zhang et al., 2016).

Fibre quality encompasses a set of physical and technological properties, such as strength, length, fineness, elasticity, and various indices related to spinning performance and appearance (Baytar et al., 2018). In this study, most treatments of the four cotton cultivars, fibre length, strength, and uniformity were significantly improved by applying MC. The micronaire was significantly decreased (improved) by applying MC. The F_v/F_m ratio represents the intrinsic efficiency of PSII photochemistry. As such, a reduction in this value was commonly interpreted as an indicator of photo inhibition (Takeuchi et al., 2025). The F_v/F_m of cotton 'XLZ74' and 'SD1068', in three important growth periods, the majority of treatments by MC decreased compared with the control, but 'SD1068' and 'AY161' had contrary trends.

At the square stage, for photochemical efficiency, MC treatment presented a higher effective light-adapted photochemical quantum yield (Φ PSII) compared to the control. Furthermore, the MC application caused a significant increase in Φ PSII in four cultivars. However, in the florescence and boll period, it shows the opposite change. Photochemical quenching (qP) is a fluorescence parameter, indicator of photosynthetic efficiency, indicates fluorescence quenching caused by photosynthesis. The non-photochemical quenching (NPQ) indicates the fluorescence quenching caused by heat dissipation (Wu, 2016). In our experiment, in general, qP and NPQ of the four varieties were not significantly different between the MC treatments and the control with three concentration treatments, in three key growth stages.

CONCLUSIONS

During the growth of upland cotton, plant height and initial node height were significantly reduced by mepiquat chloride (MC) treatment, while chlorophyll content (SPAD value) significantly increased, compared to the control. Most treatments exhibited elevated levels in photosynthetic parameters such as net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration. Similarly, chlorophyll fluorescence parameters, including quantum efficiency of photosystem II (PSII), maximum photochemical efficiency of PSII, minimal and maximal chlorophyll fluorescence, and photochemical quenching coefficient, also tended to increase in most treatments, whereas non-photochemical quenching coefficient decreased. In addition, MC treatment improved fibre quality, enhancing the upper half mean length, breaking strength, uniformity, and elongation, with minimal impact on micronaire value. MC treatment also increased seed index, yield, and single boll weight, but had little effect on initial node position, boll number per plant, or the number of fruit branches.

Author contribution
Conceptualization: Y.M-D., Z.J-W. Methodology: Z.J-W. Validation: Z.J-W. Formal analysis: Z.J-W. Investigation: Z.J-W. Resources: Y.M-D. Data curation: Z.J-W. Writing-original draft: Z.J-W. Writing-review & editing: Z.J-W. Visualization: Z.J-W. Supervision: Y.M-D. Project administration: Y.M-D. Funding acquisition: Y.M-D. All co-authors reviewed the final version and approved the manuscript before submission.

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