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Timing Is Key: Mepiquat Chloride Application at Squaring Versus Flowering for Enhanced Bt Cotton Yields

Manpreet Singh* and Sunayana Punia

ABSTRACT

Mepiquat chloride (MC) application timing and plant spacing significantly influenced Bt cotton growth and yield parameters. MC application, irrespective of timing, reduced plant height, but the most pronounced effect was observed when sprayed at the squaring stage. This height reduction was associated with increased boll number per plant, particularly under high-density planting. Additionally, MC treatments resulted in heavier bolls compared to the control, especially in wider spacings. Seed cotton yield (SCY) was significantly impacted by the interaction of MC timing and plant spacing. The highest SCY was achieved with MC applied at squaring, particularly at closer spacing, demonstrating a substantial yield advantage over the control. Both MC timings enhanced SCY at closer spacings, whereas the control yielded best at medium densities. These findings indicate that MC application at the squaring stage is superior in reducing plant height, promoting boll development, and ultimately maximizing cotton yield, especially under high-density planting. Nevertheless, both MC timings can optimize cotton production, underscoring the critical role of tailored plant spacing for yield maximization. Future research should investigate the physiological mechanisms underpinning these responses and explore the potential of MC in conjunction with other agronomic practices to further enhance cotton productivity.

Bt cotton, genetically modified to express insecticidal proteins from *Bacillus thuringiensis* Berliner, has revolutionized cotton production in India by reducing pest damage and the need for insecticides to manage Lepidopteran pests. This has led to substantial increases in yield and profitability for farmers. However, the indeterminate growth habit of cotton, characterized by continuous vegetative growth throughout the growing season, can lead to excessive foliage, lodging, and delayed maturity, ultimately impacting yield and fiber quality. Plant growth regulators (PGRs) like mepiquat chloride (MC) are commonly used to manage vegetative growth, promote early maturity, and improve yield in cotton. MC acts by inhibiting gibberellin biosynthesis, a plant hormone responsible for stem elongation. This results in shorter plants with reduced leaf area, potentially leading to better light penetration, reduced boll rot, and improved yield (Oosterhuis et al., 1998).

The use of PGRs in cotton production is not without its challenges. The effectiveness of MC can be influenced by various factors, including application timing, dosage, cultivar, and environmental conditions (Oosterhuis et al., 1998). Previous studies have shown varying results regarding the efficacy of MC application at different growth stages (squaring vs. flowering), and the ideal plant spacing for maximizing yield can differ based on local conditions and agronomic practices (Ren et al., 2013). In Punjab, India, where this study was conducted, Bt cotton is primarily grown under wider planting geometry (67.5 x 75 cm) due to the high cost of Bt hybrid seeds. Although higher plant densities can potentially increase yields, it can also exacerbate the problems of excessive vegetative growth and lodging, making the use of PGRs like MC even more critical. The potential for yield loss due to excessive vegetative growth is particularly high in regions like Punjab with hot and humid climates, which favor such growth (Constable, 1995; Landivar and Marur, 1996; Yang et al., 2011).

Cotton lint yield is a complex trait influenced by multiple factors, primarily boll density, individual boll weight, and the percentage of lint per boll. MC and plant density are key agronomic tools that can be manipulated to optimize these yield components, offering a potential avenue for maximizing cotton

M. Singh* and S. Punia, Punjab Agricultural University Regional Research Station, Abohar, Punjab, India – 152116.
*Corresponding author: agrimanpreet@pau.edu

production (Stewart, 2005). However, the yield response to MC has been shown to be variable across different studies. Whereas some research has reported negative or no yield response to MC application (Nichols et al., 2003; Pettigrew and Johnson, 2005; Zhao and Oosterhuis, 2000), others have demonstrated a positive yield response, particularly under conditions that favor excessive vegetative growth, such as high nitrogen availability (Kerby et al., 1982), high plant density (York, 1983), late planting (Cathey and Meredith, 1988), or warm and humid climates (Gwathmey and Clement, 2010). These findings highlight the importance of considering environmental and management factors when evaluating the potential benefits of MC.

Similarly, the relationship between plant density and cotton yield has been a subject of ongoing research, with conflicting results reported across different studies. Some research has found no significant relationship between yield and plant density (Baker, 1976; Bednarz et al., 2000; Hawkins and Peacock, 1973; Jones and Wells, 1998), whereas others have shown a yield reduction at very high or low densities (Bridge et al., 1973; Smith et al., 1979). The optimal plant density for maximizing yield appears to be dependent on various environmental factors, including temperature, rainfall, and crop management practices. For example, regions with abundant rainfall and warm temperatures might require lower plant densities to avoid excessive vegetative growth and lodging (Dong et al., 2010, 2012). In contrast, drier and hotter climates might benefit from higher plant densities, which can help conserve soil moisture and maximize light interception (Chapepa et al., 2020).

These varying responses to MC and plant density underscore the need for region-specific research to determine the optimal combination of agronomic practices for maximizing cotton yield. This study aims to address this knowledge gap by investigating the interaction between MC application timing and plant spacing on the yield and growth parameters of Bt cotton in Punjab, India, a region characterized by a semiarid climate and high-density planting systems. The specific objectives were to: (1) evaluate the impact of MC application timing on plant height, boll number, and boll weight; (2) assess the influence of plant spacing on these growth parameters and yield; and (3) determine the optimal combination of MC application timing and plant spacing for maximizing seed cotton yield in the context of Bt cotton cultivation in Punjab.

We hypothesized that MC application at squaring would be more effective in controlling vegetative growth and enhancing yield compared to application at flowering. Additionally, we expected that closer plant spacing would further improve yield due to increased plant density and resource utilization. By addressing these research questions, this study aimed to provide valuable insights for optimizing the use of MC and plant spacing to maximize the yield potential of Bt cotton in Punjab, India.

MATERIALS AND METHODS

Field Experiment. This study was conducted during the kharif (monsoon) season of 2021 and 2022 at the Regional Research Station of Punjab Agricultural University in Abohar, Punjab, India. The experimental site is characterized by a semiarid climate with hot summers and cool winters. The soil type is sandy loam with good drainage.

A factorial experiment was designed with two factors: six plant spacings (67.5 x 75 cm, 67.5 x 50 cm, 67.5 x 37.5 cm, 100 x 50 cm, 100 x 35 cm, and 100 x 25 cm) in main plots and three MC application timings (squaring, flowering, no MC [control]) in subplots. Each treatment combination was replicated three times, resulting in a total of 54 experimental plots. The Bt cotton hybrid used in this study was RCH 773 BG II, a widely cultivated hybrid in Punjab known for its high yield potential and earliness. The gross plot size for each main plot was 4 m x 20 m, whereas for subplots, net plot size was 4 m x 6 m. Cotton was sown on 30 April 2021 and 07 May 2022 and was grown using Punjab Agricultural University recommended practices. Seed cotton was picked three times on monthly intervals starting the second week of September during both years.

Mepiquat Chloride Application. MC (5%) was applied as a foliar spray at 375 ml ha^{-1} initiated at squaring and flowering, according to the treatment. An additional application was made when the average internodal length of the top five internodes was more than 5 cm. Scouting was done on 7 d intervals to monitor internode length. Application timings can be found in Table 1.

Data Collection. Plant height was measured from the ground level to the tip of the main stem at the peak flowering stage. The number of bolls per plant was counted at maturity, and the average boll weight was determined by weighing a random sample of 20 bolls from each plot. Seed cotton yield (SCY) was recorded

Table 1. Mepiquat chloride application timings

Treatment	First Spray	Second Spray
MC Initiated at Squaring	11 June 2021	16 July 2021
	18 June 2022	28 July 2022
MC Initiated at Flowering	16 July 2021	30 July 2021
	17 July 2022	07 August 2022
Control (No MC)	--	--

at harvest by adding the yield obtained from three pickings for each plot and converting the yield to kg/ha.

Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) to determine the significance of main effects (MC timing and plant spacing) and their interaction. Pooled means are presented because the years-x-treatment interaction was non-significant. Means were compared using Tukey's HSD test at a 5% significance level. Regression analysis was performed to assess the relationship between plant height and boll parameters.

RESULTS AND DISCUSSION

Plant Height. MC application significantly impacted plant height, irrespective of the application timing (Table 2). This aligns with the well-documented anti-gibberellin properties of MC, which inhibit stem elongation (Oosterhuis et al., 1998; Rademacher, 2000; Zhao and Oosterhuis, 2000). The data reveal a consistent reduction in plant height across all MC treatments compared to the control group, with the most pronounced effect observed when MC was applied at the squaring stage. This early application resulted in an average plant height of 146 cm, whereas application at flowering led to significantly taller plants with an average height of 157 cm.

The observed variation in plant height across different plant spacings, ranging from 144 cm at 67.5 x 75 cm to 172 cm at 100 x 25 cm, is also statistically significant. This suggests that plant spacing significantly influences plant height, with denser planting arrangements leading to taller plants.

Furthermore, the significant interaction effect between MC application and plant spacing, indicates that the impact of MC on plant height varies depending on the planting density. This interaction highlights the importance of considering both MC application timing and plant spacing when designing agronomic strategies for Bt cotton cultivation. The data suggest that early MC application at the squaring stage consistently reduced the plant height as compared to control under all the plant spacings, whereas MC application at flowering resulted in shorter plants in all spacings except the recommended one: 67.5 x 75 cm.

Boll Number. The number of bolls per plant was significantly influenced by the interaction between MC application timing and plant spacing (Table 3). This interaction aligns with previous findings that demonstrate the complex interplay between plant growth regulators

Table 2. Effect of mepiquat chloride application and plant spacing on plant height (cm)

	------------------------ Mepiquat Chloride Application ------------------------			
Plant Spacing	Squaring	Flowering	Control	Mean
67.5 x 75 cm	135	145	153	144
67.5 x 50 cm	142	155	180	159
67.5 x 37.5 cm	152	165	185	167
100 x 50 cm	143	145	170	153
100 x 35 cm	148	157	182	162
100 x 25 cm	153	173	190	172
Mean	146	157	177	
Critical Difference (5%) for MC application: 10				
Critical Difference (5%) for Plant height: 7				
Critical Difference (5%) for interaction: 11				

Table 3. Effect of mepiquat chloride application and plant spacing on boll number per plant

	------------------------ Mepiquat Chloride Application ------------------------			
Plant Spacing	**Squaring**	**Flowering**	**Control**	**Mean**
67.5 x 75 cm	**41.3**	**43.2**	**44.3**	**42.9**
67.5 x 50 cm	**32.7**	**34.3**	**31.5**	**32.8**
67.5 x 37.5 cm	**30.1**	**29.9**	**25.2**	**28.4**
100 x 50 cm	**40.2**	**41.8**	**40.0**	**40.7**
100 x 35 cm	**38.4**	**36.8**	**36.6**	**37.3**
100 x 25 cm	**37.6**	**34.7**	**27.8**	**33.3**
Mean	**36.7**	**36.8**	**34.2**	
Critical Difference (5%) for MC application: 0.7				
Critical Difference (5%) for Plant height: 2.7				
Critical Difference (5%) for interaction: 2.8				

and agronomic practices in determining cotton yield components (Ren et al., 2013). Under high-density planting (100 x 25 cm), MC application at squaring led to a substantial increase in boll number, averaging 37.6 bolls/plant compared to 27.8 bolls/plant in the control group. This increase of 35% is statistically significant, exceeding the critical difference value of 2.8 for interaction. This result suggests that early MC application during the squaring stage can stimulate boll production, particularly when plants are grown in proximity.

The effect of MC on boll number was less pronounced at wider spacings, indicating that plant density plays a crucial role in modulating the response to MC. This is consistent with previous research showing that the efficacy of MC can be influenced by plant spacing and that closer spacing could enhance the positive effects of MC on boll production (Oosterhuis et al., 1998). The mechanism behind this interaction could involve the optimization of plant architecture and resource allocation under high-density conditions, where early MC application could help mitigate competition for resources and promote reproductive growth (Rosolem et al., 2013; Yang et al., 2011).

Although MC application at flowering also increased boll number compared to the control, the effect was less significant than that of application at squaring. This suggests that the timing of MC application is crucial for maximizing boll production, with earlier application potentially offering a greater advantage. The difference between the two MC timings was most pronounced at the highest density (100 x 25 cm), further emphasizing the importance of considering both MC timing and plant spacing in optimizing cotton yield.

Boll Weight. Both MC treatments, whether applied at squaring or flowering, significantly increased boll weight compared to the control group (Table 4). This observation is consistent with previous research indicating that MC can enhance boll weight, potentially through improved assimilate partitioning and nutrient

Table 4. Effect of mepiquat chloride application and plant spacing on boll weight (g)

	------------------------ Mepiquat Chloride Application ------------------------			
Plant Spacing	**Squaring**	**Flowering**	**Control**	**Mean**
67.5 x 75 cm	**4.07**	**4.05**	**3.99**	**4.04**
67.5 x 50 cm	**3.88**	**3.86**	**3.71**	**3.81**
67.5 x 37.5 cm	**3.53**	**3.49**	**3.42**	**3.48**
100 x 50 cm	**4.14**	**4.11**	**3.96**	**4.07**
100 x 35 cm	**3.88**	**3.74**	**3.46**	**3.69**
100 x 25 cm	**3.30**	**3.26**	**3.17**	**3.24**
Mean	**3.80**	**3.75**	**3.62**	
Critical Difference (5%) for MC application: 0.07				
Critical Difference (5%) for Plant height: 0.23				
Critical Difference (5%) for interaction: 0.23				

allocation to reproductive structures (Oosterhuis et al., 1998; Ren et al., 2013). The average boll weight of 3.80 g for MC applied at squaring and 3.75 g for MC applied at flowering are significantly higher as compared to 3.62 g for the control.

The data also reveal a significant effect of plant spacing on boll weight, with wider spacings generally resulting in heavier bolls. This trend is consistent across both MC treatments and the control group, and the differences between spacing levels are statistically significant. This effect could be attributed to reduced competition for resources under wider spacing, allowing individual plants to allocate more resources to boll development (Kerby, 1985).

The significant interaction effect between MC application and plant spacing suggests that the impact of MC on boll weight is dependent on plant density. This interaction highlights the need to consider both factors when optimizing boll weight. The data indicate that the combination of MC application (regardless of timing) and wider plant spacing tends to maximize boll weight, potentially due to a synergistic effect of reduced plant height and decreased competition for resources. However, the specific mechanisms underlying this interaction warrant further investigation.

Relationship Between Plant Height and Boll Parameters. Regression analysis revealed a significant negative correlation between plant height and boll number ($R^2 = 0.3983$), as well as between plant height and boll weight ($R^2 = 0.5131$) (Fig. 1). This indicates that as plant height increased, both the number of bolls per plant and the average weight of individual bolls decreased. The stronger correlation between plant height and boll weight ($R^2 = 0.5131$) suggests that plant height has a greater influence on boll weight than on boll number. These findings support the hypothesis that MC-induced reductions in plant height contribute to improved yield by increasing boll production and boll size.

The negative correlation between plant height and boll parameters could be attributed to several factors. Shorter plants, resulting from MC application, might be less prone to lodging, a common problem in cotton production that can reduce yield by damaging plants and hindering boll development. Additionally, shorter plants might have better light interception, allowing for more efficient photosynthesis and assimilate partitioning towards reproductive structures. This improved resource allocation could explain the increased boll number and weight observed in shorter plants. Moreover, the compact plant architecture resulting from MC application could facilitate better nutrient uptake and utilization, further contributing to enhanced boll development.

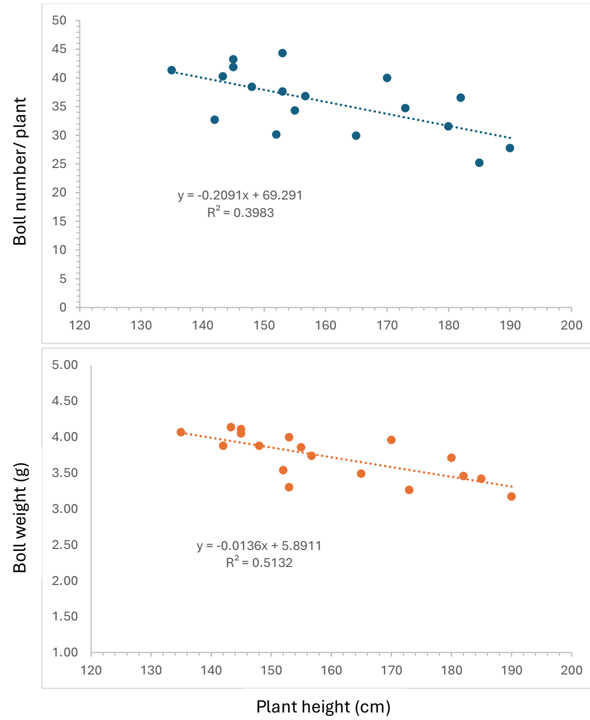

Figure 1. Relationship of plant height with boll number (a) and boll weight (b) in cotton.

These results highlight the importance of plant architecture in determining cotton yield. By reducing plant height through the application of MC, it might be possible to optimize resource allocation, minimize lodging, and ultimately improve boll production and size, leading to increased yield.

Seed Cotton Yield. SCY was significantly influenced by the interaction between MC application timing and plant spacing (Table 5). This aligns with previous research that has shown the impact of plant growth regulators and agronomic practices on cotton yield (Ren et al., 2013). Notably, the highest SCY (4,196 kg/ha) was achieved when MC was applied at the squaring stage under high-density planting (100 x 25 cm), representing a substantial 42% increase compared to the control at the same density. This result suggests that early MC application during the squaring stage, coupled with high-density planting, can synergistically enhance SCY.

The yield advantage of MC applied at squaring was more pronounced at closer spacings, particularly at 100 x 25 cm, where it led to a 42% yield increase compared to the control. This interaction between MC timing and plant spacing could be attributed to

Table 5. Effect of mepiquat chloride application and plant spacing on seed cotton yield (kg/ha)

	------------------------ Mepiquat Chloride Application ------------------------			
Plant Spacing	**Squaring**	**Flowering**	**Control**	**Mean**
67.5 x 75 cm	**2832**	**2946**	**2950**	**2909**
67.5 x 50 cm	**3175**	**3392**	**2946**	**3171**
67.5 x 37.5 cm	**3520**	**3512**	**2840**	**3291**
100 x 50 cm	**2833**	**2944**	**2617**	**2798**
100 x 35 cm	**3625**	**3393**	**3061**	**3360**
100 x 25 cm	**4196**	**3852**	**2952**	**3667**
Mean	**3364**	**3340**	**2894**	
Critical Difference (5%) for MC application: 270				
Critical Difference (5%) for Plant height: 322				
Critical Difference (5%) for interaction: 425				

the optimization of plant architecture and resource allocation under high-density conditions. Early MC application could mitigate competition for resources, such as light and nutrients, and promote a more favorable partitioning of assimilates towards reproductive growth, ultimately leading to higher yields (Oosterhuis et al., 1998; Yang et al., 2011).

Although MC application at flowering also improved SCY compared to the control, the effect was less pronounced, with a 30% increase at the highest density. This suggests that the timing of MC application is crucial for maximizing yield, with earlier application at the squaring stage potentially offering a greater advantage. The control group's highest SCY was observed at medium densities (3,061 kg/ha at 100 x 35 cm), indicating that in the absence of MC, wider spacing could be beneficial for yield. However, the use of MC appears to shift the optimal plant spacing towards higher densities, likely due to its growth-regulating effects.

These findings underscore the importance of considering both MC application timing and plant spacing when developing agronomic strategies for maximizing cotton yield. The results suggest that early MC application at the squaring stage, combined with high-density planting, can be a viable approach for increasing SCY in Bt cotton. However, further research is needed to validate these findings across different cultivars and environmental conditions, as the response to MC can vary depending on these factors (Oosterhuis et al., 1998; Ren et al., 2013; Tung et al 2018).

CONCLUSIONS

This study demonstrates that the timing of MC application and plant spacing significantly influence Bt cotton yield and growth parameters in Punjab, India. Applying MC at the squaring stage, especially under high-density planting, maximizes seed cotton yield by increasing boll number and weight. This strategy is particularly effective in mitigating competition for resources in high-density systems. However, the optimal use of MC is context-dependent and can be influenced by various factors, including cultivar and environmental conditions. Further research is needed to refine recommendations for specific scenarios and to elucidate the underlying physiological mechanisms. Nonetheless, this study provides valuable insights for optimizing Bt cotton production in Punjab, and highlights the potential of early MC application and high-density planting for maximizing yield.

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