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RESEARCH

Effects of DA-6 and MC on the growth, physiology, and yield characteristics of soybean

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Abstract

Background As a grain and oil crop, soybean presents a much lower yield than other staple crops. However, crop yields can be improved by applying modern agricultural technology, such as diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC), which are important plant-growth regulators that substantially affect crop growth and yield.

Methods This study examined the effects of DA-6 (30, 60, or 90 mg L^{-1}) and MC (100, 200, or 400 mg L^{-1}) on soybean growth, development, root structure, photosynthetic physiology, osmotic regulation, and yield via field and pot experiments.

Results The results showed that DA-6 effectively promoted the growth of soybean and increased parameters such as plant height, leaf area, and leaf dry weight in different growth stages by 21.0%, 18.7%, and 66.4%, respectively. In contrast, MC inhibited the growth and other parameters, decreasing the plant height, leaf area, and leaf dry weight in different growth stages by up to 15.7%, 11.9%, and 10.1%, respectively. Both DA-6 and MC promoted root development by increasing the dry weight, length, surface area, volume, tip number, branch number, and cross number. In terms of physiology, DA-6, and MC increased photosynthetic parameters, such as steady-state fluorescence (Fs), maximum fluorescence (Fm'), and photosynthetic system II (Phi2), increased the soluble protein contents, with maximum increases of 27.7% and 28.1% at different periods, and increased the soluble sugar contents by 38.2% and 58.3%. Regarding yield characteristics, DA-6 and MC considerably increased the yield, 100-grain weight, and number of effective pods. DA-6 increased the number of two- and three-seed pods, whereas MC increased the number of one-, two-, and three-seed pods. MC performed better than DA-6; however, they exerted different effects on the two varieties and at different concentrations. DA-6 was most effective at 30–60 mg L^{-1}, while MC was most effective at 100–200 mg L^{-1}.

Conclusions This study revealed the effects of DA-6 and MC on soybean morphology, physiology, and yield characteristics and the appropriate concentrations for application in soybean productions. Thus, these findings provide guidance for the rational application of the two regulators for soybean high-yield cultivation and stress resistance.

Keywords Soybean, Diethylaminoethyl hexanoate (DA-6), Mepiquat chloride (MC), Physiological response, Yield, Plant growth regulators, Root structure

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Introduction

Soybean (*Glycine max.* L.) is a grain crop planted worldwide that provides plant protein and oil for human use [1]. Owing to its rich composition, soybean is widely used in industry, medicine, animal husbandry, and functional foods [2–4]. Given the increasing application of soybeans in various industries, soybean production must be increased to meet human needs. Further, considering that environmental pollution, climate change, and global warming exert biotic and abiotic stress on crops, thereby reducing productivity and yields [5], soybean crop productivity and adaptability to adverse factors must be improved.

Agricultural technology can be used to improve crop adaptability to complex environments and enhance their growth and stress resistance. This is primarily achieved using plant growth regulators (PGRs), which are essential for plant growth and development [6]. Commonly used PGRs include artificial PGRs and other plant hormones and their derivatives [7]. Diethylaminoethyl hexanoate (DA-6), a high-energy PGR, exhibits revolutionary and wide-ranging effects, such as improving photosynthesis and yield, delaying leaf senescence, and improving growth under cold, drought, and other types of stress [8–11]. For example, DA-6 promotes Cd extraction and detoxification in ryegrass plants, improves Cd stress tolerance by promoting compartmentalization of cell wall regions and combinations of proteins and organic acids, and reduces metal toxicity by fixing more Cd in the cell wall and reducing Cd migration in plants [12]. Exogenous DA-6 can also activate the expression of chlorophyll synthesis- and photosystem-related genes, resulting in higher photosynthetic activity and chlorophyll production, and regulate the expression of endogenous cytokinin synthesis and decomposition genes, thereby stabilizing the chloroplast structure, reducing oxidative damage, and maintaining the photochemical activity of tomato leaves under low temperature stress [13].

Mepiquat chloride (MC) is a mild and residue-free PGR that is rapidly transferred throughout the plant by the roots, branches, and leaves. MC inhibits cell elongation, weakens terminal bud growth, delays vegetative growth, reduces gibberellin activity, controls vertical and horizontal growth, shortens internode distances, promotes a compact architecture, deepens leaf color, reduces leaf area, and increases chlorophyll synthesis, thereby preventing vigorous plant growth [14]. MC can improve cell-membrane stability and increase plant resistance [15]. MC is the most widely used plant growth regulator in cotton production, and has developed a reasonable application strategy [16]. In general, the application of MC improves the non-structural carbohydrate concentrations, carbon metabolism-related enzyme activity, and photosynthetic characteristics in cotton leaves, thereby improving the production and utilization efficiency of leaf carbohydrates and delaying leaf senescence, which increases cotton yield [17].

Different PGRs are typically used at specific growth stages. In white clover, DA-6 priming of seeds enhances seed germination during drought by affecting hormone levels, osmoregulation, and dehydrin accumulation [11]. Under natural drought in the field, the application of DA-6 can increase the antioxidant enzyme activity of pineapple at different stages and restore growth parameters weakened by drought [18]. Cotton seed priming by MC can promote Cl^- efflux under salt stress, thereby improving the seeds' salt tolerance [19]. In grapes, treatment of seedlings with DA-6 at 20 mg L^{-1} enhances seedling biomass, photosynthetic pigment content, antioxidant enzyme activity, and Se content [20]. In cotton seedlings, MC reduces internode distances by inhibiting cell division and elongation, thereby reducing plant height [21]. In soybean, DA-6 is well absorbed by the sink organs, thereby enhancing sucrose and starch distribution and accumulation in the source organs, which has crucial effects on yield, DA-6 also reduces the shedding of flowers and pods, thereby increasing yield [22]. MC is limited by crops, and different crops have different sensitivities to MC. In general, cotton is more sensitive to MC than other crops. For example, an increase in the MC dose to cotton has been reported to decrease the lint yield by 5–29% [23]. In soybean, however, MC promotes yield, although different varieties require different concentrations [24]. Studies have shown that MC treatment can reduce plant height and shoot dry weight, while promoting lateral root growth, increasing leaf protective enzyme activity, reducing malondialdehyde content and increasing total flavonoid content [25]. In addition, after MC treatment, the expression of proteins related to photosynthesis and cell wall elongation was significantly down-regulated, while the expression of key proteins related to lateral root growth and stress resistance was significantly up-regulated [25]. The study of Jaidka et al. [26] showed that with the increase of MC level, the nitrogen and protein content of soybean seeds increased, but did not reach a significant level. In addition, MC treatment had no significant effect on PAR interception, flower / plant number, seed / pod, pod length and straw yield of soybean, but significantly reduced leaf area index, increased dry matter accumulation and yield [27]. These PGRs exert different regulatory mechanisms, thereby altering crop growth and physiological characteristics and affecting yield. Nonetheless, the mechanisms underlying their effects have not been fully clarified. Therefore, this study mainly explores the following questions: How do DA-6 and MC regulate soybean growth and development (growth, root morphogenesis, and physiology) at different stages? Does the promotion of growth or regulation of plant type by different regulation methods have a more beneficial effect on yield formation?

To address these questions, this study examined the regulatory effects of DA-6 and MC during the different stages of soybean growth and development (growth, root morphogenesis, and physiology) and evaluated whether promoting growth or regulating plant architecture is more conducive to yield formation. Specifically, different concentrations of DA-6 and MC were sprayed onto the leaves of two soybean varieties at the R1 stage (start of flowering), and their effects on growth and development were evaluated in terms of the morphology, dry matter accumulation, yield, photosynthetic characteristics, and osmoregulation. The findings of this study will provide a scientific basis for the rational application of these PGRs in soybean cultivation.

Materials and methods

Experimental design

The field experiment was conducted at Xiangyang Farm, Northeast Agricultural University (126°91′ E, 45°77′ N), Harbin, China. The field site has black soil. Corn growing in the field was harvested, and the straw was crushed and returned to the field. Ditching and fertilization were performed with ammonium phosphate dibasic ($(NH_4)_2HPO_4$) at 150 kg/ ha^{-1} and potassium sulfate (K_2SO_4) at 75 kg/ ha^{-1} using a no-tillage fertilizer–planter before sowing. Plants were spaced 12 cm apart, and the seedling density was 250,000 plants ha^{-1}. Appropriate field management measures were performed at the different stages of soybean growth. The main soybean cultivars grown in Heilongjiang Province, Heinong 84 (HN84) and Heinong 87 (HN87), were used.

A total of two soybean varieties and seven treatments were included in the experiment. Each treatment contained three replicate plots (eight ridges per plot, ridge width and length of 0.65 m and 5.0 m, respectively), for a total of 42 plots. According to the randomized block design, these 42 plots were first divided according to the variety, with 21 plots for each variety, and then the 7 treatments (including three replicates) were randomly arranged in the 21 plots. Solutions of DA-6 and MC were sprayed onto the leaves using an electric sprayer (3WBD-20; Taizhou Luqiao Yijin Sprayer Factory, Taizhou City, China) at the R1 stage. To avoid the effects of factors such as rainfall, the PGRs were sprayed again one week later. DA-6 was applied at concentrations of 30, 60, and 90 mg L^{-1} (treatments A30, A60, and A90, respectively) and MC at 100, 200, and 400 mg L^{-1} (treatments M100, M200, and M400, respectively). The leaves of the control group were sprayed with water. The morphology, photosynthetic physiology, and osmoregulatory substance levels were measured at the flowering/full-bloom (R2), initial pod (R3), full pod (R4), initial grain (R5), and seed-filling (R6) stages. Yield was evaluated during harvest (R8).

In the same year (2023), a pot experiment was performed at the experimental station of Northeast Agricultural University (126°72′ E, 45°74′ N) to exclude the influence of the experimental site on the results and support the field experiment. First, the selected plot was ditched at a depth of approximately 25 cm. Black soil samples (approximately 16 kg) were placed in plastic buckets with a drainage hole at the bottom, and the buckets were buried so that the soil in the bucket was flush with the ground surface. Each ditch included two rows of buckets (one variety was planted in each row). Before sowing, the soil was fully watered, and eight seeds were sown in each pot. Four seedlings were retained in each pot when the seeds reached the V1 stage. According to the level of evaporation, watering was performed. In addition, weeds were removed from the ditches. When the plants reach the R1 stage, DA-6 (30, 60, and 90 mg L^{-1}) and MC (100, 200, and 400 mg L^{-1}) were sprayed on the leaves using an electric sprayer (3WBD-20; Taizhou Luqiao Yijin Sprayer Factory, Taizhou City, China), while water was sprayed on the control leaves. All treatments were sprayed again 7 d after the first spraying, and each treatment contained at least three pots. The experimental procedure was the same as that in the field experiment. Plant height and chlorophyll fluorescence parameters (e.g., Fs and Fm) were measured at stages R2, R3, R5, and R6. The yield characteristics were evaluated at stage R8.

Effects of DA-6 and MC on morphological characteristics of soybean

Plant height was measured using a meter. Leaf area (LA) was based on the method of Tao et al. [28] with certain appropriate modifications. Briefly, a single-hole punch with a 10 mm punch size (punch area, S) was used to punch 10 leaves, and the punched leaf tissue and remaining leaf tissue were dried to a constant weight, represented by W_1 and W_2, respectively. Leaf area was calculated as follows:

$$\text{Leaf area } = (10S \times (W_1 + W_2) \times 10^{-2})/W_1$$

To measure aboveground dry weight (DW), the leaves, stems, petioles, and pods were separated and placed in a Kraft paper bag. After 30 min of drying at 105 °C, the samples were dried at 65 °C to a constant weight and then weighed using a balance.

To reduce root damage, the roots were retrieved along with the attached soil, then rinsed with flowing water. The root DW, fresh volume, surface area, length, tip number, branch number, and crossing number were measured using a root scanner. After measurement, the roots were dried using absorbent paper, placed in a Kraft paper bag, dried at 105 °C for 30 min, and then dried to constant weight at 65 °C.

Effects of DA-6 and MC on chlorophyll fluorescence parameters of soybean

Leaf chlorophyll fluorescence was measured using a MultispeQ v2.0 multi-functional plant-measuring instrument (PhotosynQ, MI, USA). Each treatment was repeated five times under sufficient sunlight. The effective quantum yield of photosynthetic system II (Phi2), steady-state fluorescence (Fs), maximum fluorescence (Fm'), and effective photochemical quantum yield of PSII (Fv'/Fm'); the excitation-energy capture-efficiency of the open PSII reaction center under light) were measured.

Effects of DA-6 and MC on the content of osmotic adjustment substances in soybean

The Coomassie brilliant blue method [29] was used to measure the amount of soluble protein and the anthrone method [30] to measure the amount of soluble sugar using relevant kits (Norminkoda Biotechnology Co., Ltd. Wuhan, China).

Effects of DA-6 and MC on yield components of soybean

When the field plants reached the harvest stage, a 2 m^2 patch in each plot was selected, and all the plants within this patch were collected for analysis in the laboratory. In the pot experiment, all the plants in the pot were collected for analysis. Each treatment was repeated at least three times (pots). Plant height, lowest pod height, numbers of one-, two-, three-, and four-seed pods, numbers of effective pods, 100-seed weight, and yield were measured.

Data processing and statistical analysis

The data were from randomized complete block design (RCBD) field trials and pot experiments conducted in the same year (2023). Continuous variables, such as crop yield and plant height, were summarized as mean ± standard deviation. Unless otherwise stated, the changes described in the results are relative to the levels in the control (The analysis of field experiment and pot experiment was independent of each other, and compared with their respective CK groups).

Data processing was performed using Excel 2019 (Microsoft, Redmond, WA, USA). Data analysis was performed using SPSS 21.0 (IBM, Armonk, NY, USA), and Origin 2021 were used for mapping. Descriptive statistics were used to summarize the agronomic traits and physiological changes. Analysis of variance (ANOVA) was performed to assess the effects of DA-6 and MC on soybean, followed by Duncan's multiple range test for post-hoc comparisons. Correlation analysis was performed by using the Correlation plot plug-in in Origin 2021, and the correlation of different indicators was measured by Pearson correlation coefficient. The results were presented in the form of P values. A p-value of less than 0.05 was considered statistically significant. In summary, the statistical analysis section provides a comprehensive description of the data handling, and analytical methods used in this study. By detailing data collection, processing, and ANOVA, this section ensures the reproducibility and validity of the research findings. The use of advanced statistical tools and sensitivity analyses further strengthens the robustness of the results.

Results

DA-6 and MC altered growth and development

DA-6 and MC significantly affected shoot growth (Table 1). Leaf dry weight (LDW) increased over time in both varieties and peaked in R6. DA-6 increased LDW, which was greatest at stage R2 for HN84 and stage R4 for HN87. For HN84, treatment A30 increased the LDW at stages R2–R6 by 45.9%, 36.6%, 33.1%, 38.8%, and 34.6%, while for HN87, treatment A30 increased the LDW by 51.0%, 44.6%, 66.4%, 58.3%, and 21.2%. For HN84, treatment M400 strongly inhibited LDW and had the strongest inhibitory effect at stage R6, with reductions of 9.0%, 4.8%, 10.1%, 8.9%, and 6.8% at stages R2–R6. For HN87, treatment M200 had the strongest inhibitory impact at stage R6. For HN87, M200 reduced the LDW by 0.6%, 0.8%, 6.7%, 3.8%, and 4.6% at stages R2–R6. In both varieties, MC showed the greatest inhibition of LDW at stage R4.

In both varieties, stem DW (SDW) increased with growth, peaking at stage R6. For HN84, SDW was significantly elevated under treatment A60 but not under treatments A30 or A90 at stage R6 (Table 2). For HN87, SDW was significantly elevated at stage R6 under treatments A30, A60, and A90 by 16.9%, 15.2%, and 9.5%, respectively. For HN84, SDW decreased with increasing MC concentration and was lowest under treatment M400 at stage R6, which was 4.1% lower than in the control. For HN87, SDW initially decreased and then increased with increasing MC concentration, with the greatest decrease under treatment M200 (9.5%). Moreover, DA-6 further promoted increases in petiole dry weight while MC decreased of petiole dry weight (Table S1).

For HN84, pot DW (PDW) increased significantly under treatment M400, peaking at stage R6. PDW was elevated by 4.1%, 52.4%, and 5.3% at stages R4–R6. For HN87, PDW increased significantly under treatment

Table 1 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on the soybean leaf dry weight (g/plant)

Variety	Treatment	R2	R3	R4	R5	R6
HN84	CK	4.00b	5.60d	7.01c	8.23d	10.12e
	A30	6.01a	7.65a	9.33b	11.42c	15.62a
	A60	5.69a	6.66bc	8.17bc	14.05a	13.03bc
	A90	5.51a	6.57bc	7.97bc	10.44c	11.69d
	M100	5.42a	7.56a	10.88a	13.30ab	14.21b
	M200	6.66a	7.13ab	9.30b	14.17a	16.09a
	M400	5.42a	6.27c	7.69bc	11.85bc	12.18 cd
HN87	CK	3.61c	5.22c	5.56d	7.45c	10.58c
	A30	5.45a	7.55a	9.25a	11.79a	12.82a
	A60	5.43a	5.83b	8.69b	11.48a	11.53b
	A90	4.74b	5.62b	7.57c	9.01b	11.44b
	M100	3.61c	5.21c	5.55d	7.41c	10.41c
	M200	3.59c	5.18c	5.19d	7.17c	10.09c
	M400	3.60c	5.20c	5.48d	7.39c	10.24c
Analysis of variance	Variety	559.56****				
	Treatment	81.67****				
	Stage	1077.17****				
	Variety * Treatment	58.53****				
	Variety * Stage	20.54****				
	Treatment * Stage	6.34****				
	Variety * Treatment * Stage	5.90****				

In the same period, different letters for the same variety between different treatments indicate significant differences at the 5% level ($p < 0.05$). In the analysis of variance, the value represents the F value. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

Table 2 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on the soybean stem dry weight (g/plant)

Variety	Treatment	R2	R3	R4	R5	R6
HN84	CK	3.39bc	6.24a	7.57d	13.25d	17.16d
	A30	4.17ab	6.25a	10.91b	14.40 cd	18.37c
	A60	4.74a	7.53a	13.58a	16.31b	19.69b
	A90	4.68a	6.07a	11.50b	15.80bc	15.98e
	M100	3.69bc	6.41a	9.42c	14.42 cd	16.98de
	M200	3.08c	7.07a	9.09c	16.29b	18.46c
	M400	4.20ab	6.62a	10.77b	22.10a	25.20a
HN87	CK	3.56c	5.17c	12.55d	13.05d	13.94e
	A30	5.39a	5.91b	16.11a	15.20b	16.30bc
	A60	3.64c	5.59bc	13.58b	15.04bc	16.06c
	A90	4.57b	5.73bc	11.08c	13.40d	15.26d
	M100	4.59b	7.80a	13.50b	14.67bc	16.66b
	M200	5.76a	7.55a	15.47a	17.60a	19.94a
	M400	4.54b	5.54bc	12.89bc	14.47c	16.35bc
Analysis of variance	Variety	1.19 ns				
	Treatment	85.51****				
	Stage	4389.04****				
	Variety * Treatment	83.65****				
	Variety * Stage	155.73****				
	Treatment * Stage	20.23****				
	Variety * Treatment * Stage	15.28****				

Refer to Table 1 for descriptions of terms

M200, peaking at stage R6, and under treatment M400, it was elevated by 2.6%, 34.4%, and 17.7% at stages R4–R6. In both varieties, the two PGRs increased PDW the most at stage R5 (Table 3).

Through an analysis of variance found that SDW was not significantly affected by the variety but was significantly affected by the treatment, period, and their interaction. Leaf and pod dry weight were significantly affected by the variety, treatment, period, and their interaction (Tables 1, 2 and 3). These findings indicate that DA6 and MC have different effects on aboveground parts and that their regulatory effects differ. However, both PGRs promoted the formation of yield and increased the PDW. The optimal PGR concentrations varied between the varieties.

In both varieties, plant height increased during growth, peaking at stage R6 (Fig. 1a, b). DA-6 increased height and promoted growth. For HN84, plant height was highest under treatment A60 and significantly increased at stage R3. Treatment A60 increased plant height by 19.2%, 21.0%, 5.0%, 5.0%, and 4.7% at stages R2–R6. For HN87, plant height was also highest under treatment A60, with increases of 1.7%, 13.0%, 8.8%, 6.5%, and 5.9% higher at stages R2–R6.

MC inhibited plant height, and the inhibitory effect increased with its concentration. For HN84, treatment M400 reduced the plant height by 2.9%, 13.7%, 10.8%, 8.3%, and 9.2% at stages R2–R6; while for HN87, the corresponding reductions were 2.6%, 15.7%, 10.1%, 9.4%, and 8.4%.

In the pot experiments, DA-6 also promoted plant growth. However, MC exerted more pronounced concentration-dependent inhibition of plant height in the pot experiment than in the field experiment (Supplementary Material S2).

In both varieties, LA peaked at stage R6 (Fig. 1c, d). DA-6 increased LA, with HN84 and HN87 showing the greatest increases at R5 and R3, respectively. For HN84, treatment A60 achieved the highest LA, with increases of 3.7%, 16.0%, 18.0%, 18.7%, and 10.8% at stages R2–R6; for HN87, treatment A30 achieved the highest LA, with increases of 4.8%, 16.8%, 13.1%, 11.9%, and 13.5% at stages R2–R6.

The MC treatment reduced LA, with significant decreases for HN84 under treatment M400 and HN87 under treatment M200 and the greatest reductions at the R4 stage. For HN84 at stage R6, LA decreased with increasing MC concentration, reaching a reduction of 9.0% under treatment M400. For HN87, LA first decreased then increased with increasing MC concentration: the M200 treatment achieved the largest reduction in LA of 11.9%. Correlation analysis of the aboveground

Table 3 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on the soybean pod dry weight (g/plant)

Variety	Treatment	R2	R3	R4	R5	R6
HN84	CK	-	-	1.47a	2.92c	17.61b
	A30	-	-	1.53a	3.68bc	17.73b
	A60	-	-	1.48a	4.93a	19.55a
	A90	-	-	1.28a	3.77bc	18.10b
	M100	-	-	1.46a	3.22c	18.12b
	M200	-	-	1.44a	3.38bc	18.18b
	M400	-	-	1.53a	4.45ab	18.25b
HN87	CK	-	-	1.53a	6.22c	17.49d
	A30	-	-	1.83a	8.46a	19.48b
	A60	-	-	1.68a	8.25a	18.79c
	A90	-	-	1.33a	7.96ab	19.04bc
	M100	-	-	1.55a	8.28a	18.66c
	M200	-	-	1.57a	8.36a	20.59a
	M400	-	-	1.56a	7.21b	17.56d
Analysis of variance	Variety	345.67****				
	Treatment	12.58****				
	Stage	14,316.37****				
	Variety * Treatment	9.61****				
	Variety * Stage	211.86****				
	Treatment * Stage	4.21****				
	Variety * Treatment * Stage	2.84**				

Refer to Table 1 for descriptions of terms

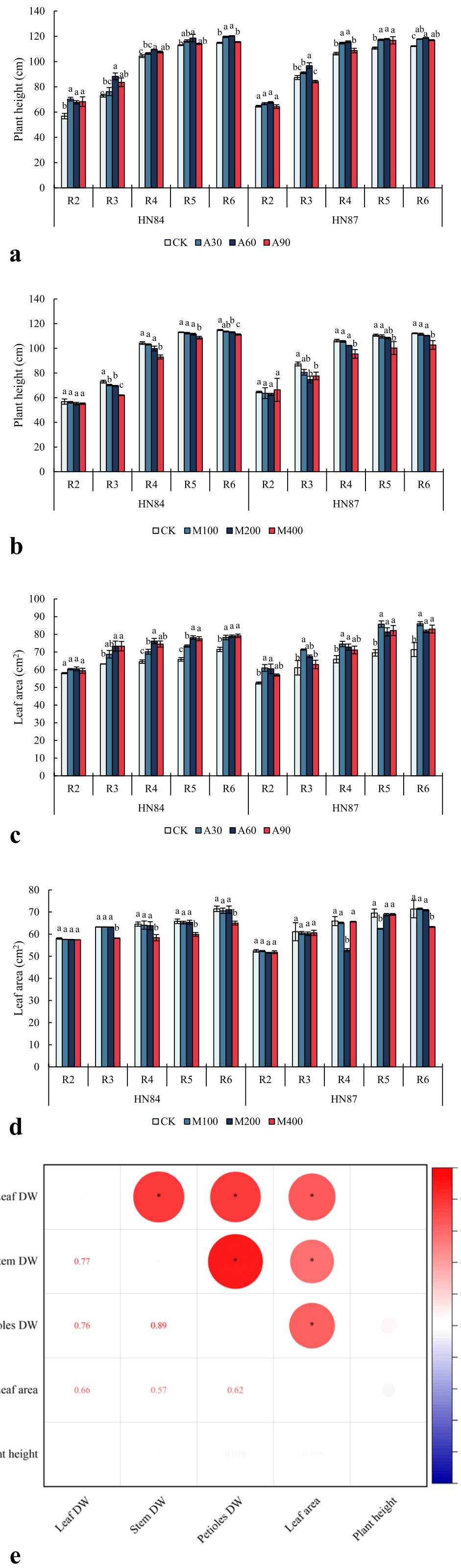

Fig. 1 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on soybean plant height and leaf area. **a**, **c** Effects of DA-6 on plant height and leaf area. **b**, **d** Effects of MC on plant height and leaf area. **e** Correlation analysis of aboveground indexes. Note: CK represents the control group; A30, A60, and A90 correspond to 30, 60, and 90 mg/L DA-6 treatments, respectively; and M100, M200, and M400 correspond to 100, 200, and 400 mg/L MC treatments, respectively. In the same period, different letters for the same variety between different treatments indicate significant differences at the 5% level ($p < 0.05$). In the correlation analysis, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

indexes revealed that leaf dry weight, which is an important index for measuring the growth and development of aboveground parts, was significantly correlated with the SDW, petiole dry weight, and leaf area. However, plant height was not significantly affected by the other indexes (Fig. 1e).

DA-6 and MC alter the root architecture

Next, the effects of DA-6 and MC on root structure were studied (Table 4). Root DW increased over time, peaking at stage R6. DA-6 promoted root DW, which increased the most under treatments A60 in NH84 and A30 in HN87. For HN84, treatment A60 increased the root DW by 18.8%, 36.6%, 6.5%, and 6.4% at stages R2, R3, R5, and R6, respectively; for HN87, treatment A30 increased the root DW by 10.3%, 19.7%, 14.9%, and 13.1% in the same stages, respectively. MC also increased the root DW, which is inconsistent with the effects on the aboveground parts: for HN84, treatment M400 significantly increased the root DW by 17.4%, 23.9%, 8.1%, and 15.3% at stages R2, R3, R5, and R6, respectively; while for HN87, treatment M200 more effectively increased the root DW by 13.8%, 26.8%, 7.3%, and 16.0% at the corresponding stages, respectively.

For HN84, treatment A60 achieved the highest root length, with increases of 14.2%, 28.8%, 17.8%, 7.2%, and 9.7% at stages R2–R6; for HN87, treatment A30 achieved the highest root length, with increases of 11.3%, 23.6%, 12.4%, and 11.0% at stages R2–R6 (Table 5). Therefore, DA-6 effectively improved soybean root length and promoted its growth, with the greatest effect in stage R3. For HN84, treatment M400 increased root length by 8.1%, 22.9%, 10.5%, and 12.4% at stages R2, R3, R5, and R6. For HN87, treatment M200 significantly improved root length, which was highest at stage R6; with increases of 11.4%, 20.3%, 12.3%, and 11.5% at stages R2, R3, R5, and R6, respectively.

For HN84, root surface area was highest for DA-6 under treatment A60, with increases of 7.0%, 17.4%, 4.1%, and 7.5% at stages R2, R3, R5, and R6, respectively; for HN87, root surface area was highest under treatment A30, with increases of 6.3%, 23.3%, 14.8%, and 14.9% at

Table 4 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on root dry matter (g/plant)

Variety	Treatment	R2	R3	R5	R6
HN84	CK	1.44a	2.13c	3.85a	4.05 cd
	A30	1.64a	2.61b	3.92a	4.10 cd
	A60	1.71a	2.91a	4.10a	4.31bc
	A90	1.55a	2.19c	3.96a	4.18 cd
	M100	1.58a	2.17c	4.03a	4.03d
	M200	1.67a	2.13c	3.99a	4.50ab
	M400	1.69a	2.34bc	4.16a	4.67a
HN87	CK	1.45a	2.13c	3.69b	4.19b
	A30	1.60a	2.39ab	4.24a	4.74a
	A60	1.50a	2.33abc	4.02ab	4.45b
	A90	1.53a	2.25bc	3.97ab	4.45b
	M100	1.54a	2.33abc	3.93ab	4.36b
	M200	1.55a	2.50a	3.96ab	4.86a
	M400	1.48a	2.28abc	3.78b	4.21b
Analysis of variance	Variety	0.019 ns			
	Treatment	8.91****			
	Stage	2287.94****			
	Variety * Treatment	5.52****			
	Variety * Stage	5.95***			
	Treatment* Stage	1.71*			
	Variety* Treatment * Stage	2.14**			

Refer to Table 1 for descriptions of terms

Table 5 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on root length (cm)

Variety	Treatment	R2	R3	R5	R6
HN84	CK	1531.84c	2131.20b	3466.26b	3555.55d
	A30	1694.02ab	2264.30a	3536.31b	3653.59 cd
	A60	1749.28a	2744.49b	3714.79a	3900.37ab
	A90	1619.55abc	2145.08b	3485.29b	3776.37bc
	M100	1541.76bc	2216.33b	3517.65b	3612.47 cd
	M200	1644.12abc	2169.88b	3475.52b	3731.26bcd
	M400	1656.13abc	2618.54a	3829.11a	3996.10a
HN87	CK	1685.17bc	2143.25c	3421.31c	3788.41d
	A30	1876.40a	2648.51a	3846.57a	4206.95a
	A60	1789.33ab	2281.23bc	3613.46b	4078.00ab
	A90	1746.49abc	2267.12bc	3718.93ab	3870.50d
	M100	1680.95bc	2205.60bc	3720.66ab	4040.38bc
	M200	1876.62a	2577.54a	3843.54a	4223.18a
	M400	1616.17c	2310.42b	3658.39b	3904.02 cd
Analysis of variance	Variety	78.26****			
	Treatment	26.25****			
	Stage	4987.14****			
	Variety * Treatment	26.60****			
	Variety * Stage	12.12****			
	Treatment * Stage	2.29**			
	Variety* Treatment * Stage	2.84****			

Refer to Table 1 for descriptions of terms

the same stages, respectively (Table 6). For HN84, root SA peaked at stage R6 at all MC concentrations, improving notably under treatment M400, with increases of 2.5%, 14.9%, 4.4%, and 12.7% at stages R2, R3, R5, and R6, respectively; for HN87, treatment M200 presented increases of 6.2%, 23.2%, 21.0%, 11.9%, and 15.0% at stages R2–R6. In both varieties, the two PGRs enhanced root SA the most at stage R3.

In both varieties, root volume peaked at stage R6. For HN84, at stage R6, root volumes were significantly higher under treatments A30 and A60 than in the control and treatment A90, with treatments A30, A60, and A90 increasing the root volume by 9.1%, 10.4%, and 3.0%, respectively, relative to the control (Table 7). For HN87 at stage R6, root volume was significantly higher under treatment A30, although the increases under treatments A60 and A90 were not significant, with A30, A60, and A90 increasing the root volume by 16.4%, 7.8%, and 9.7%, respectively. Therefore, for HN84 and HN87 treatments A60 and A30 enhanced root volume the most, respectively. MC increased the root volume. For HN84, the root volume peaked under the MC treatment at stage R6 and was significantly improved by treatment M400, with increases of 9.1%, 25.9%, 18.2%, and 9.0% at stages R2, R3, R5, and R6, respectively. For HN87, root volume peaked at stage R6 and showed the greatest increase in treatment M200, with increases of 4.6%, 20.0%, 12.6%, and 10.9% at stages R2, R3, R5, and R6, respectively. Both PGRs significantly increased the root tip number, root branching number, and root crossing number (Supplementary Material S1).

Correlation analysis of root traits revealed a significant correlation between the traits of the root system (Fig. 2). Analysis of variance showed that root traits were significantly affected by variety, treatment, period, and the interaction of Variety * Treatment and Variety * Stage (Tables 4, 5, 6 and 7). In summary, foliar spraying with DA-6 and MC altered the root structure, with MC inducing a larger root-shoot ratio and improving root growth more effectively than DA-6.

DA-6 and MC enhanced photosynthetic parameters

Both regulators improved the fluorescence parameters. For HN84, leaf Fm' was highest under treatment A60 in all stages, with increases of 8.1%, 7.8%, 10.1%, 30.5%, and 30.5% at stages R2–R6. For HN87, treatment A30 increased leaf Fm' by 8.3%, 9.7%, 11.6%, 13.6%, and 10.8% at stages R2–R6. For HN84, M400 increased leaf Fm' by 3.7%, 4.5%, 16.4%, 26.8%, and 36.4% at stages R2–R6; for HN87, M200 increased leaf Fm' by 4.0%, 17.1%, 29.8%, 21.4%, and 9.5%. Among the MC treatments, Fm' varied significantly between treatments M200 and M400 and

Table 6 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on root surface (cm^2)

Variety	Treatment	R2	R3	R5	R6
HN84	CK	346.33c	496.31d	915.06d	977.03a
	A30	355.66bc	516.17c	932.61abcd	1007.10a
	A60	370.72b	582.45a	952.48ab	1086.69a
	A90	392.21a	523.75c	947.60abc	1004.79a
	M100	345.80c	550.58b	922.81 cd	994.28a
	M200	403.13a	510.51 cd	927.13bcd	1077.15a
	M400	413.14a	570.45a	955.78a	1104.24a
HN87	CK	358.51a	487.20bc	685.94d	939.93a
	A30	380.96a	600.92a	787.27a	1080.05a
	A60	369.07a	570.75a	761.38b	1053.92a
	A90	357.65a	566.54a	709.90c	1029.00a
	M100	365.94a	510.17b	725.02c	1006.29a
	M200	380.91a	600.09a	767.26ab	1080.88a
	M400	373.96a	460.40c	747.87b	1051.69a
Analysis of variance	Variety	41.63****			
	Treatment	6.15****			
	Stage	1528.69****			
	Variety * Treatment	3.05**			
	Variety * Stage	41.45****			
	Treatment * Stage	0.946 ns			
	Variety* Treatment * Stage	0.808 ns			

Refer to Table 1 for descriptions of terms

Table 7 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on root volume (cm^3)

Variety	Treatment	R2	R3	R5	R6
HN84	CK	5.74a	7.67b	17.51b	20.7c
	A30	6.15a	8.96ab	18.36b	22.67ab
	A60	6.24a	9.94a	18.88b	22.95a
	A90	6.13a	8.99ab	17.53b	21.40abc
	M100	6.04a	7.96ab	18.21b	20.93bc
	M200	6.28a	8.44ab	18.10b	21.51abc
	M400	6.26a	9.66ab	20.69a	22.66ab
HN87	CK	4.59a	8.18a	16.04b	17.00c
	A30	4.75a	10.30a	17.99a	19.78a
	A60	4.70a	9.54a	16.30b	18.32abc
	A90	4.50a	9.27a	17.01ab	18.65abc
	M100	4.74a	8.62a	17.91a	18.18abc
	M200	4.82a	9.82a	18.06a	18.85ab
	M400	4.71a	8.55a	17.22ab	17.55bc
Analysis of variance	Variety	104.79****			
	Treatment	6.53****			
	Stage	2441.14****			
	Variety * Treatment	4.58****			
	Variety * Stage	31.62****			
	Treatment * Stage	1.38 ns			
	Variety * Treatment * Stage	0.60 ns			

Refer to Table 1 for descriptions of terms

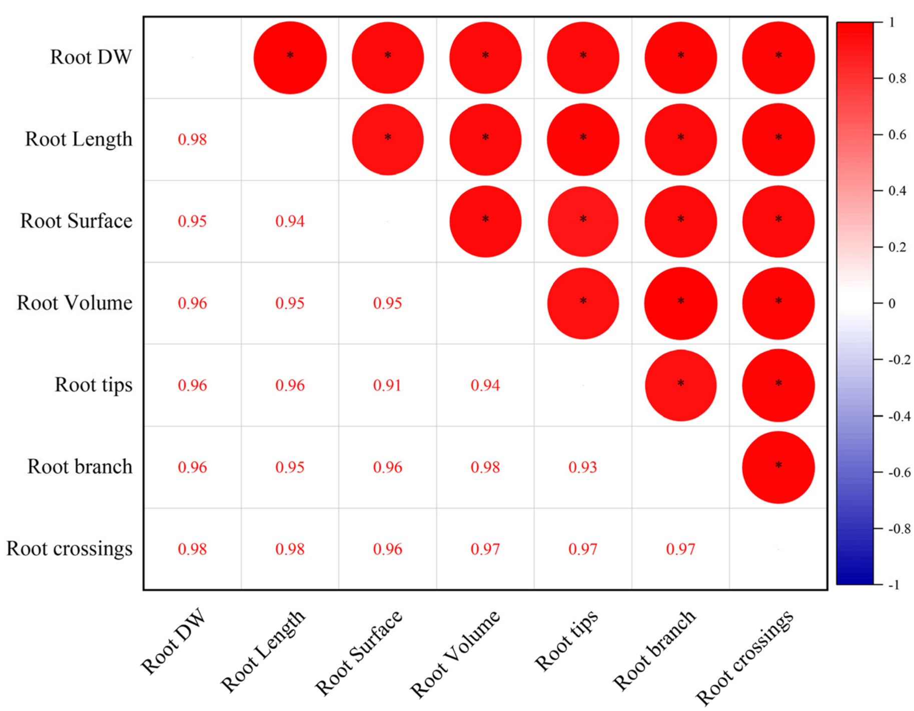

Fig. 2 Correlation analysis of root traits. Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

was significantly higher under M200, with no other significant differences (Fig. 3a, b).

Fs was significantly affected by the PGR treatments (Fig. 3c, d). For HN84 at stage R6, Fs increased the most under treatment A60 (17.0%) and was significantly higher under treatment A60 than under A30 or A90. No other significant differences were observed among the DA-6 treatments. For HN87, Fs increased the most under treatment A30, reaching 19.9% at stage R5, which was significantly different from other the DA-6 treatments. For HN84, Fs peaked at M400, increasing by 36.4% at stage R6. For HN87, the best effect on Fs occurred under treatment M200, with an increase of 25.7% at stage R5.

DA-6 treatment increased Fv'/Fm' (Fig. 3e, f), which changed over time in both varieties. For HN84 at stage R2, Fv'/Fm' changed more substantially under treatment A60, increasing by 11.4%; for HN87, and peaked under treatment A30. The effect varied significantly between stages R4 (an increase of 11.0%) and R6 (an increase of 12.0%). Furthermore, MC and DA-6 both promoted Fv'/Fm'. For HN84, it peaked under treatment M400, but the effect was apparent only at stage R2 (11.4%). This parameter varied significantly between treatment M400 and the control at stage R6. For HN87, M200 improved Fv'/Fm' more than M400, increasing it significantly by 13.1% and 13.3% at stages R4 and R6, respectively.

Phi2 reflects a plant's ability to convert light energy to ATP and NADPH and ultimately to synthesize sugars. Over time, Phi2 initially increased and then decreased (Fig. 3g, h). For HN84, Phi2 was highest under treatment A60, increasing by 15.9%, 3.0%, 13.3%, 15.2%, and 10.9% at stages R2–R6 (significantly at stages R2, R5, and R6). For HN87, under treatment A60, Phi2 increased relative to the control by 14.0%, 45.8%, 20.0%, 13.0% and 11.6% at stages R2–R6 (significantly at stages R2–R5). For HN84, Phi2 peaked under treatment M400, with increases of 16.2%, 2.8%, 10.9%, 18.0%, and 11.6% at stages R2–R6. For HN87, Phi2 was highest under treatment M200, with increases of 14.0%, 47.4%, 23.0%, 10.0%, and 18.2% at stages R2–R6. The Fs and Fm parameters under pot conditions also showed a consistent trend with the field (supplementary material S2). In conclusion, both PGRs improved the chlorophyll fluorescence parameters of the two varieties, laying the foundation for improvements in yield.

DA-6 and MC enhanced osmoregulation

DA-6 and MC significantly improved osmoregulatory capacity in both varieties, with the leaf soluble protein content initially increasing then declining and peaking at stage R4. For HN84 (Fig. 4a), among the DA-6 treatments, the osmoregulatory capacity was highest under treatment A60, with increases of 9.3%, 4.0%, 5.6%, 5.0%, and 17.4% at stages R2–R6; for HN87 (Fig. 4b), it was highest under treatment A30, with increases of 4.4%, 27.7%, 25.4%, 17.7%, and 7.8% at stages R2–R6.

Treatment M400 improved the soluble protein content more than treatment M200 in HN84, while M200 was more effective in HN87. For HN84 under treatment M400, the soluble protein content was highest at stage R4, with increases of 6.0%, 23.6%, 28.1%, 15.9%, and 26.0% at stages R2–R6. For HN87 under treatment M200, it was also highest at stage R4, with increases of 11.9%, 20.7%, 24.3%, 11.9%, and 12.1% at stages R2–R6.

Leaf soluble sugar content increased over time, peaking at R6 (Fig. 4c, d). For HN84, among the DA-6 treatments, it was highest under treatment A60, with increases of 1.9%, 38.2%, 24.2%, 10.2%, and 7.4% at stages R2–R6; for HN87, it was highest under treatment A30, with increases of 9.5%, 8.3%, 14.6%, 28.4%, and 29.9% at stages R2–R6.

For HN84, treatment M400 significantly increased the soluble sugar content by 58.3%, 15.0%, 28.4%, 27.9%, and 8.3% at stages R2–R6; for HN87, treatment M200 increased it by 11.1%, 11.6%, 35.0%, 19.2%, and 27.7% at stages R2–R6.

DA-6 and MC promoted yield traits

DA-6 and MC improved yield characteristics (Table 8). For HN84, under treatment A60, the height of the lowest pod increased by 4.4% and the effective pod number by 31.3%. For HN87, treatment A30 exerted better effects, with the height of lowest pod increasing by 9.0% and the effective pod number increasing by 29.9%. For HN84, the height of the lowest pod was lowest in treatment M400, which was 11.1% lower than that in the control, while its effective pod number was 32.3% higher than that in the control.

For HN87, the height of the lowest pod was lowest in treatment M200, which was 2.7% lower than that in the control, while its effective pod number was increased by 29.9%. DA-6 increased the height of the lowest pod, whereas MC reduced it.

DA-6 and MC significantly increased the number of two- and three-seed pods, which varied between the varieties and treatments (Table 9). For HN84, DA-6 did not significantly increase the number of pods, whereas treatment M100 significantly increased the numbers of one-, two-, and three-seed pods. For HN87, treatments A30 and M200 significantly increased the numbers of one-, two-, and three-seed pods.

For HN84, the 100-grain weight peaked under treatment A60 at an increase of 3.4%, and the yield increased by 5.4%. For HN87, 100-grain weight peaked under treatment A30 at an increase of 2.0%, and the yield increased by 14.5%. The effects of these PGRs on yield traits were

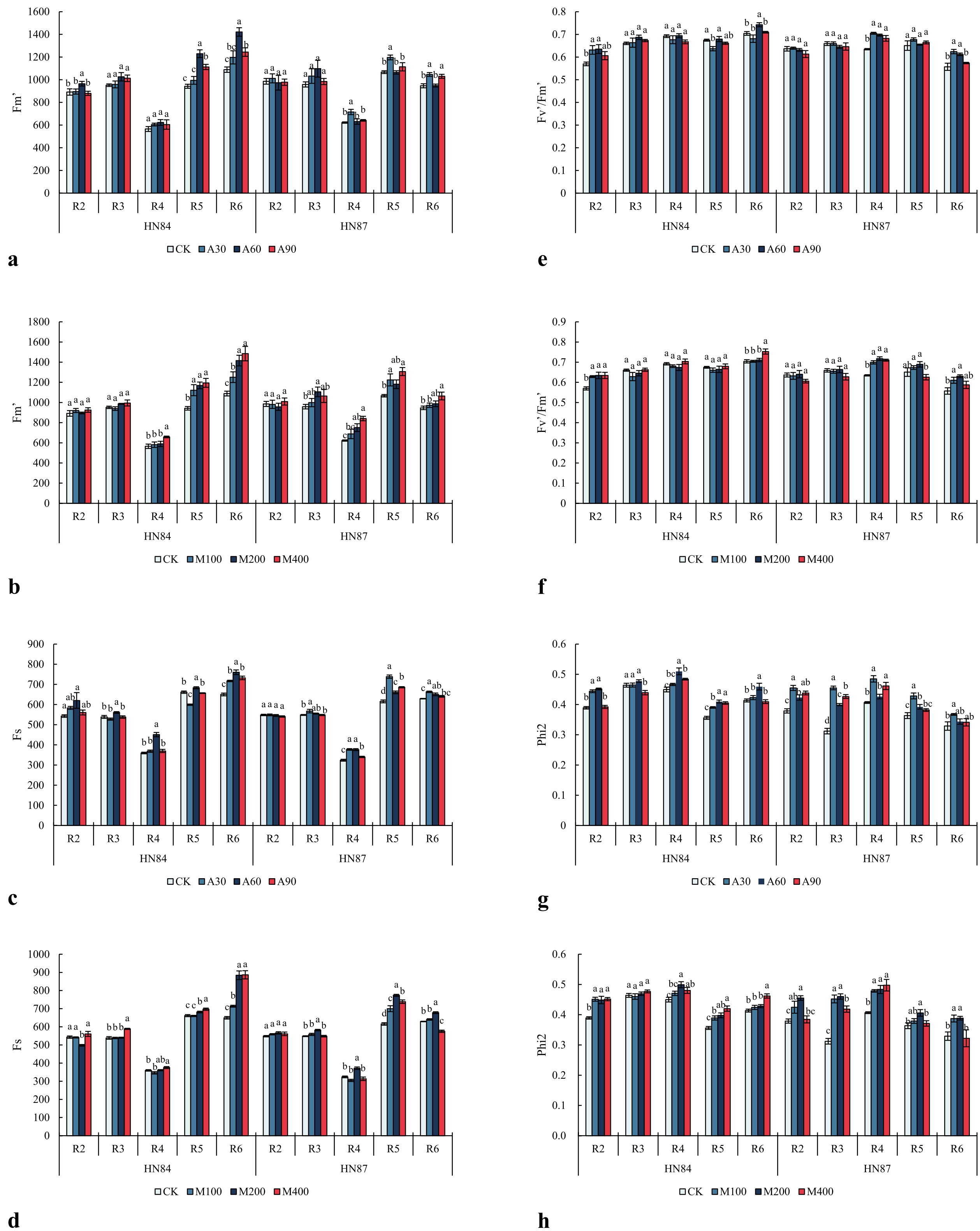

Fig. 3 Effects of diethylaminoethyl hexanoate (DA-6) and methylpiperammonium (mepiquat) chloride (MC) on chlorophyll fluorescence parameters. **a**, **c**, **e**, **g** Effects of DA-6 on Fm', Fs, Fv'/Fm', and Phi2. **b**, **d**, **f**, **h** Effects of MC on Fm', Fs, Fv'/Fm', and Phi2. Note: For an explanation of the content in the figure, please refer to Fig. 1

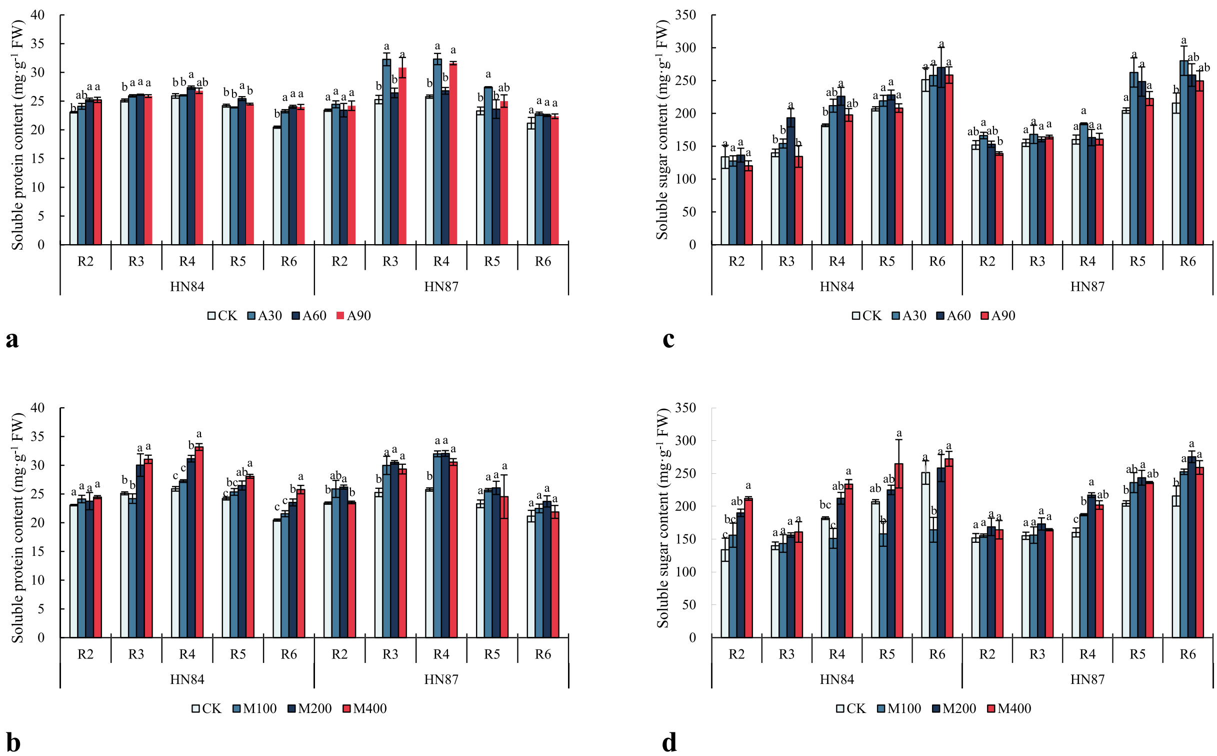

Fig. 4 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on osmoregulatory-substance levels. **a**, **b** Effects of DA-6 and MC on soluble protein content; **c**, **d** Effects of DA-6 and MC on soluble sugar content. Note: For an explanation of the content in the figure, please refer to Fig. 1

Table 8 Effects of diethylaminoethyl hexanoate (DA-6) and methylpiperammonium (mepiquat) chloride (MC) on yield characteristics

Variety*	Treatment	Plant height (cm)	Lowest pod height (cm)	Effective pod number
HN84	CK	115.74b	20.80a	28.75b
	A30	121.40a	21.30a	35.15ab
	A60	122.75a	21.72a	37.75a
	A90	117.20b	21.02a	35.50ab
HN87	CK	113.55b	20.10a	27.75c
	A30	119.70a	21.90a	36.05a
	A60	120.80a	21.55a	35.45b
	A90	118.45a	21.05a	33.40b
HN84	CK	115.74a	20.80a	28.75b
	M100	115.9a	19.86b	34.20ab
	M200	114.1a	18.80b	35.95ab
	M400	112.7b	18.50b	38.05a
HN87	CK	113.55a	20.10b	27.75c
	M100	112.30a	21.20b	34.05ab
	M200	111.45a	19.55b	36.05a
	M400	110.25b	19.65b	33.15ab

Refer to Table 1 for descriptions of terms

more pronounced in the pot experiment than in the field experiment, significantly increasing the 100-grain weight as well as the yield per plant, which varied significantly under all of the treatments in the pot experiment. The variance analysis showed that the yield and 100-grain weight were significantly affected by the interaction of variety, treatment and Variety * Treatment (Table 10).

Discussion

The application of plant growth regulators alters plant architecture and enhances plant development. This study examined the effects of DA-6 and MC on soybean growth and development, in terms of morphology, dry matter accumulation, yield, photosynthetic characteristics, and osmoregulation. DA-6 and MC affected soybean growth and structure in different ways. Foliar application of DA-6 improved plant height and LA at stages R2–R6, increasing plant size; it increased aboveground-part DW and root DW and improved root structure. MC exerted essentially the opposite effects to DA-6. Foliar application of MC reduced shoot DW, plant height, and LA, resulting in soybean plants that were compacted and smaller at every growth stage. After 2 applications at the flowering stage, the two regulators had long-term effects on soybean that extended until the plants were harvested.

Table 9 Effects of DA-6 and MC on the number of seeds per pod

Variety*	Treatment	One-seed pod	Two-seed pod	Three-seed pod	Four-seed pod
HN84	Control	2.12c	7.83bc	13.62ab	5.22a
	A30	2.20bc	7.57bc	13.40ab	5.27a
	A60	2.40bc	7.40bc	14.27ab	5.70a
	A90	2.17bc	7.88bc	13.04b	5.19a
	M100	3.98a	9.90a	15.38a	6.05a
	M200	1.72c	6.47c	13.67ab	5.42a
	M400	2.87b	8.10b	12.46b	5.90a
HN87	Control	2.37bc	6.00b	13.58c	7.14bc
	A30	3.17a	9.10a	18.92a	8.63ab
	A60	2.06c	6.32b	13.72c	5.60c
	A90	2.33bc	8.20a	16.92b	9.12a
	M100	2.15bc	6.02b	14.13c	6.73c
	M200	2.87ab	7.73a	16.30b	8.47ab
	M400	2.73abc	8.07a	15.53bc	6.75c

Refer to Table 1 for descriptions of terms

Table 10 Effects of diethylaminoethyl hexanoate (DA-6) and mepiquat chloride (MC) on yield

Variety	Treatment	100-grain weight (g) (in the field)	Field yield (kg mu^{-1})	100-grain weight (potted) (g)	Potted yield (g plant^{-1})
HN84	Control	20.3b	196.12c	19.15d	33.43d
	A30	20.6ab	197.54bc	19.30 cd	37.98b
	A60	21.0a	206.74a	19.58b	41.73a
	A90	20.6ab	199.99b	19.45bc	37.93b
	M100	/	/	19.50b	35.23c
	M200	/	/	19.80a	36.13c
	M400	/	/	19.50b	42.03a
HN87	Control	18.4b	181.18c	17.45e	30.95d
	A30	18.77a	207.37a	17.90a	37.55a
	A60	18.57b	189.99b	17.73bc	36.43a
	A90	18.50b	182.77c	17.53de	33.08c
	M100	/	/	17.68 cd	34.70b
	M200	/	/	17.85ab	37.55a
	M400	/	/	17.68 cd	37.20a
Analysis of variance	Variety	755.34****	514.68****	3753.78****	91.77****
	Treatment	6.11****	146.20****	17.15****	59.39****
	Variety * Treatment	6.82****	102.13****	5.90****	15.73****

Refer to Table 1 for descriptions of terms. For the field yield data under MC application, refer to Wang et al. [24]

In cotton, multiple applications of MC promoted whole plant fruit development from square initiation to boll opening [31].

DA-6 essentially promoted soybean plant growth. This is consistent with findings for grape seedlings, foliar application of DA-6 at 20 mg L^{-1} increased biomass, photosynthetic pigment content, antioxidant enzyme activity, and Se content, enhancing root and shoot biomass by 37.36% and 27.55%, respectively [32]. It is worth noting that although DA-6 shows a growth-promoting effect in most plants, only a concentration of 10 mg/L increased the biomass of tomato roots, stems, leaves and buds, while other concentrations either had no significant effect or inhibited the growth of tomato seedlings [33]. This suggests that DA-6 may also be selective to plants. In contrast, MC reduces or inhibits vegetative growth while promoting seedling stem strength. In cotton, MC application improves fiber length, strength, homogeneity, and micronaire. Under adequate watering, aggressive application of MC reduces vegetative development and encourages earlier physiological maturation [16]. Here, the application of MC effectively reduced soybean plant height. In sugarcane, the application of MC inhibits plant height by altering hormone signals and the synthesis of some metabolites [34]; however, this mechanism has not been revealed in soybeans. Moreover, the effects of MC on parameters related to stem strength and lodging resistance in soybean remain to be examined.

PGRs are usually sprayed directly onto the leaves; consequently, few studies have focused on their effects on root structure. Here, both PGRs increased root DW and altered root structure. Both of these PGRs are rapidly absorbed by the leaves and transported to different parts of the plant, thus affecting their growth. Root DW, length, surface area, volume, tip number, branch number, crossing number, and overall root morphology were effectively increased by applying DA-6 and MC at the flowering (R2) stage. Similarly, in purple coneflower, DA-6 application substantially increased plant height, root weight, root diameter, root number, main root length, total root length, and root-shoot ratio [35]. Following MC administration, the soybean root:shoot ratio increased, owing to the inhibition of aboveground growth. However, the effects of MC may vary among species. In all varieties of cotton, foliar application of MC during the early reproductive period (stage B3) reduces root length, starting approximately two weeks after spraying, whereas spraying at the late reproductive stage (F1) lengthens the roots [36]. In cotton, MC stimulates lateral-root production by regulating plant hormone homeostasis [37]. The responses of soybean to MC application observed here are consistent with these findings. Our findings further clarify the impact of DA-6 on soybean roots and plant architecture. Changes in root structure may also improve the water and nutrient absorption capabilities of plants. For soybean, the LA index, chlorophyll (a + b) content, and net photosynthesis were all significantly increased 56 d after foliar application of DA-6; further, 60 mg L^{-1} DA-6 improved grain-filling by enhancing the leaves' physiological function and photosynthetic capacity [38]. Our current findings regarding the effects of DA-6 on soybean are thus consistent with those of Luo et al. [38]. In this study, our results indicate that DA-6 promotes growth in both aerial parts and roots, whereas MC promotes root growth but inhibits aerial part growth. This suggests that different tissues may have varying sensitivities to these PGRs, possibly due to differences in receptor affinity or downstream signaling pathways. The authors hypothesize that DA-6 may enhance cell division and elongation in both aerial and root tissues, while MC might inhibit specific growth-related genes in aerial parts. Further studies, such as transcriptomic analysis, could provide more insights into these mechanisms. As reported in previous study [15], MC induced significant down-regulation of genes related to photosynthesis and cell wall synthesis at the transcriptional level. These findings suggest that DA-6 could be more suitable for overall plant growth promotion, whereas MC might be beneficial in scenarios where root development is prioritized over aerial growth. This could be particularly useful in in vitro cultures or stress conditions where root development is critical. The authors propose future experiments to explore the molecular mechanisms underlying these differential effects and to optimize the application protocols for these PGRs in various agricultural and biotechnological contexts.

DA-6 and MC altered soybean physiological characteristics. Plants transform light energy into chemical energy via photosynthesis. Enhancing light availability and improving plants' ability to photosynthesize increases yield; research into this important topic thus has substantial potential to enhance sustainable agricultural output and address issues related to global food security [39]. Here, DA-6 and MC application improved soybean photosynthetic parameters, improving light-reaction parameters such as the effective light quantum yield and light energy conversion ability, thus potentially facilitating conversion of light energy into metabolites during reproductive-growth stage and thereby enhancing yield. In another study on soybeans, DA-6 optimized photosynthesis and increased LA by 23%, and DA-6 treatment was most effective at 60 mg L^{-1}, consistent with our current findings. At 60 mg L^{-1}, DA-6 promotes soybean yield in different varieties and regions [9]. Here, although the application of both PGRs improved these parameters, MC improved soybean yield more than DA-6, possibly by altering features such as the angles of the branches and leaves, thereby altering plant architecture and potentially increasing light reception and light energy utilization. However, the regulation of photosynthetic parameters by MC may also be affected by factors such as the treatment period and dosage. The authors have previously examined molecular responses after applying MC to soybean seedlings, revealing that the genes involved in photosynthesis (photosystem, photosystem I, and photosystem II) were significantly downregulated by MC [15]. In cotton leaves, applying MC increased nonstructural-carbohydrate kinetic parameters, carbon metabolism-related enzyme activity, and photosynthetic parameters, however, these effects depend on higher planting density [17]. Similarly, in cotton roots, both plant population density and MC application affected nonstructural-carbohydrate content kinetics, by regulating carbohydrate metabolic enzymes activity and remobilizing carbon, thereby promoting yield formation [40], thus indicating the substantial potential of MC in regulating crop growth and development. For cotton in the Yangtze River Basin, China, MC application reduced the activity of d-ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) by 1–26%, photosynthesis by 3–37%, and lint yield by 5–27% [41]. Considering these complex findings, the effects of MC on plant photosynthetic parameters therefore remain to be clarified.

Here, both DA-6 and MC increased soybean leaf soluble sugar and protein content. Soluble sugar is the main product of photosynthesis and mediates the production of molecules that transport energy and carbon, of hormone-like signaling factors, as well as the penetration of these carriers and factors into cells, and provides the resources for protein, polysaccharide, oil, and wood production [42]. Soluble proteins play many roles, both in metabolite synthesis and photosynthesis, and RuBisCO accounts for half of the soluble protein content in plant leaves [43]. The observed increase in soluble substance content both promotes growth and development and enhances osmoregulatory capacity, thereby improving stress resistance. Under water stress, various soluble sugars protect the cell membrane and scavenge free radicals, and the accumulation of sugars can prevent cell membrane oxidation under water stress [44]. Under drought stress, soluble proteins such as late embryogenesis abundant (LEA) proteins, osmotins, and heat shock proteins (HSPs) protect cells [45]. Wang et al. [46] reported that exogenous DA-6 could effectively maintain the dry matter production and physiological effects (antioxidant enzyme activity, soluble protein and soluble sugar content increase) of two early-maturing japonica rice varieties at the booting stage under low temperature stress, thereby improving cold tolerance and yield. Under the combination of drought and high temperature stress, the application of MC can increase the content of α-ketoglutarate, soluble proteins, and amino acids [47]. Therefore, DA-6 and MC have potential applications in mediating plant-stress responses. Previously study has shown that MC positively regulates the response of soybean to drought stress by increasing SOD and POD activity and proline content [24]. In the future, the authors will further examine the roles of DA-6 and MC in the soybean stress response.

PGRs are applied to increase crop yield and improve quality. Here, foliar application of these PGRs enhanced yield in both the field and pot experiments. The authors have previously examined the effects of foliar application of MC on soybean yield [24]. Yield is known to be affected by pod DW. Here, MC significantly increased the number of effective pods, the number of two- or three-seed pods, and slightly increased the 100-grain weight, possibly by improving photosynthetic capacity and altering plant structure. Increasing photosynthetic capacity enhances the production of assimilation products; during reproductive growth, these photosynthetic products are transported continuously to the sink organs of the plant, thereby increasing yield. Similarly, the application of MC in cotton also improved yield traits, i.e., increased cotton boll opening rate and opened boll number, thereby increasing lint yield [48]. Here, DA-6 application resulted in the enlargement of the entire plant, potentially increasing the size of all components of the plant, from the source to the sink organs. Enhancing leaf area improves light absorption, while improving DW, such as that of the stems and petioles, promotes molecular transport and distribution. DA-6 maintains equilibrium between the source and sink organs, increases sucrose and starch levels in the flowers, pods, and seeds, enhances their accumulation and availability in the source organs, and helps to increase the pod-set rate and soybean yield [22]. DA-6 can enhance the photosynthetic capacity of plants, promote cell division and growth; moreover, in combination with EDAH, it can reduce the lodging rate of maize and increase the yield by 15.51%. In addition, DA-6 significantly improves the quality of corn stalks, including the mechanical properties and hemicellulose, cellulose and lignin contents [49]. Here, DA-6 enhanced soybean yield by promoting the soluble solids content, whereas MC resulted in smaller aboveground parts and larger underground parts. Although the MC application resulted in a relatively small LA, it enhanced light energy utilization efficiency by adjusting the plant's architecture and chlorophyll content, thereby improving the photosynthetic capacity. Under MC, the roots were more developed, thereby facilitating faster transport of nutrients and water to the aboveground parts, achieving faster circulation and distribution, and promoting yield.

Based on a combination of findings in the literature [9, 15, 24, 50] and the results of the experiments presented here, the authors preliminarily elucidated the regulatory effects of DA-6 and MC on the morphology, physiology, and molecular mechanisms in soybean (Fig. 5). In terms of morphology, DA-6 promoted increases in the dry weight of aboveground parts and deepening of leaf color (increased chlorophyll content). MC promoted an increase in the pod dry weight but inhibited the dry weight of leaves, stems and petioles. Both regulators increased the root dry weight and significantly improved root traits. In terms of physiology, both regulators enhanced the resistance to stress. DA-6 promoted the activity of antioxidant enzymes (SOD, POD, and CAT) and the content of osmotic adjustment

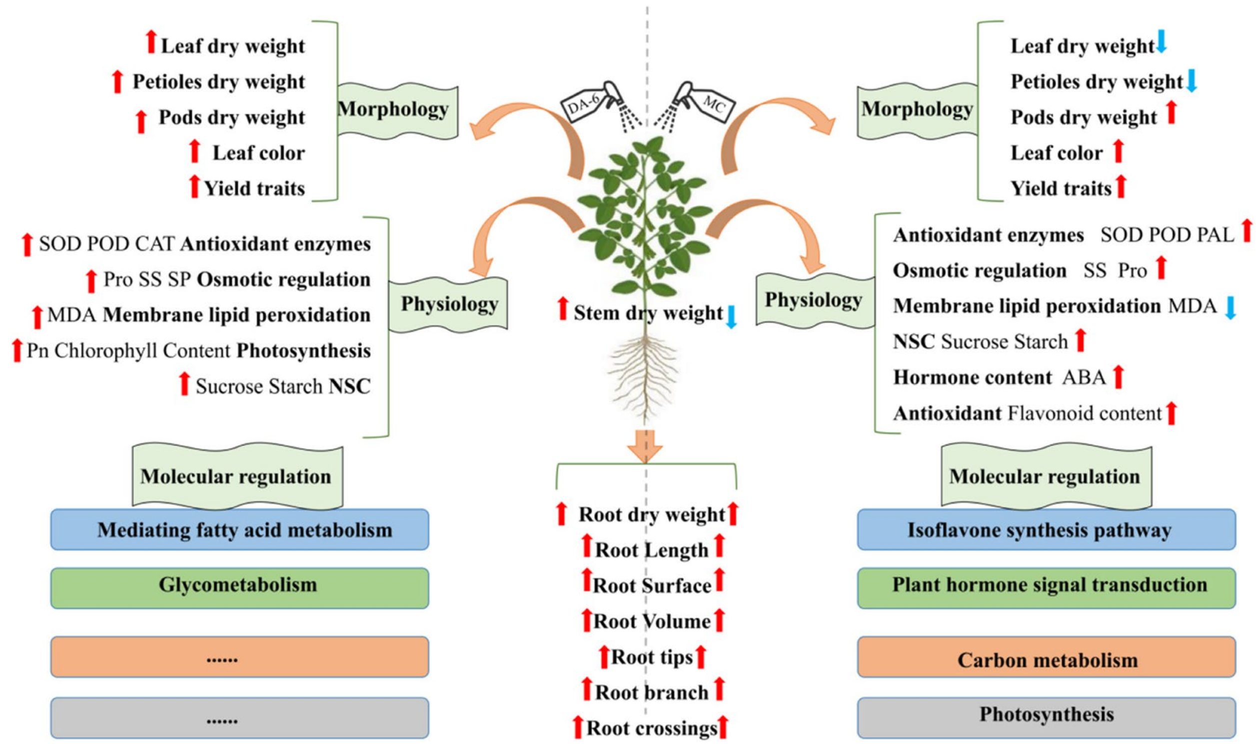

Fig. 5 Some regulatory mechanisms of DA-6 and MC on soybean. Note: The red arrows represent promoting effects, and the blue arrows represent inhibiting effects

substances (SS, SP, and Pro). The accumulation of some non-structural carbohydrates such as sucrose and starch increased, and the degree of membrane lipid peroxidation decreased. DA-6 can also improve photosynthesis, showing an increase in net photosynthetic rate. MC can better improve the physiological status of soybeans, including antioxidant enzymes (SOD, POD, and PAL), osmotic regulation (SS and Pro), non-structural carbohydrates, plant hormones (ABA), and antioxidants. At the molecular level, there is still a lack of in-depth studies on DA-6. DA-6 can mediate fatty acid metabolism and sugar metabolism to promote the germination and seedling establishment of aged soybean seeds. MC has been shown to regulate soybean growth and development through isoflavone biosynthesis, plant hormone transduction, carbon metabolism, and photosynthesis. In addition, the effect of MC on soybean under drought stress has been widely studied in previous studies, including alleviating oxidative damage caused by drought through isoflavones, GSH-ASA cycle and Calvin cycle [51, 52]. Although this study did not directly investigate stress, the findings in stress resistance physiology are consistent with these early reports. In the future, the physiological and molecular mechanisms of DA-6 and MC regulating soybean growth and development and stress response will be further studied.

Conclusions

The findings presented here elucidate the effects of DA-6 and MC on soybean shoot morphology, root structure, photosynthetic physiology, osmotic regulation, and yield characteristics, thus confirming their positive regulatory effects. Although both PGRs promoted soybean yield, they achieved this via different mechanisms, with DA-6 promoting growth and MC inhibiting it. Both PGRs improved photosynthetic parameters and levels of osmoregulatory substances. DA-6 achieved better effects at 30–60 mg L^{-1}, and MC achieved better effects at 100–200 mg L^{-1}, with the different varieties requiring different concentrations of MC. Further research is required to elucidate the mechanisms whereby these PGRs alter assimilation, transportation, and distribution to increase yield, as well as the molecular mechanisms regulating changes in plant and root structure. In summary, the application effect of MC in soybean is better than that of DA-6, and the regulation of plant type may be more conducive to yield formation. These findings provide a scientific basis for the rational application of these PGRs in soybean and support soybean crop cultivation.

Abbreviations

DA-6 Diethylaminoethyl hexanoate
DW Dry weight
LA Leaf area
LDW Leaf dry weight
MC Mepiquat chloride
PDW Pod dry weight
PGR Plant-growth regulator
SDW Stem dry weight

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06310-6.

Supplementary Material 1: Supplementary Material S1. Effects of DA-6 and MC on morphological traits of soybean

Supplementary Material 2: Supplementary Material S2. Plant height and Fs and Fm parameters of soybean under pot conditions

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Authors' contributions
X.W. wrote the manuscript, J.Z., X.W. conducted experiments and data analysis. Y.Z., X.L., Z.J., S.D. edited and reviewed the manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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