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Effects of Mepiquat Chloride on Physiology of Soybean under Drought Stress#

Xiaomin Hou[1], Feng Yan[1], Yang Dong[1], Fuyang Zhao[1], Qingquan Li[1], Huibo Shen[1]

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ABSTRACT

Background: Drought is an important factor restricting the development of agriculture. In recent years, plant growth regulators have achieved more results in improving crop stress resistance. The physiological mechanism of exogenous application of mepiquat chloride on soybean seedlings has undergone significant changes under drought stress.

Methods: In order to study the effect of exogenous chlorine on the physiology of soybean under drought stress., 'Heinong 65' was used as the experimental material, 15% PEG-6000 was used to simulate drought stress and different concentrations of mepiquat chloride solution (100, 300, 500, 700 mg/L) were sprayed at the three-leaf stage of soybean growth by pot method. The dry matter accumulation, auxin (IAA), zeatin (ZA), gibberellin (GA_3), abscisic acid (ABA) content and superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) activity of soybean were determined.

Result: The results showed that the activities of antioxidant enzymes SOD, POD, CAT and APX in soybean leaves under drought stress were significantly increased by the treatment of appropriate concentration of mepiquat chloride solution. IAA, GA_3 and ZA endogenous hormone levels increased, dry matter accumulation increased, root-shoot ratio and ABA endogenous hormone levels decreased significantly. It can be seen that mepiquat chloride treatment can improve the antioxidant capacity, promote the growth of soybean seedlings under drought stress and effectively alleviate the inhibition of drought stress on soybean growth and development.

Key words: Antioxidant enzymes, Drought stress, Endogenous hormones, Mepiquat chloride, Soybean.

INTRODUCTION

Soybean is an important source of oil, protein and feed raw material in the world. It occupies an extremely important position in agricultural production and is inseparable from human life (Mishra *et al.*, 2021). Drought stress is an important environmental factor affecting soybean yield. Soybean has high water requirement during growth and is sensitive to water deficit. Drought has a significant negative impact on output, which has adversely affected soybean quality and yield worldwide (Kuchlan and Kuchlan, 2023). The data showed that the yield of different germplasm resources decreased by 40% ~ 70% under drought stress, which could cause a devastating disaster in soybean production (Zhang *et al.*, 2022). Studies have shown that when plants are subjected to drought stress, a unique defense mechanism is formed in the body (Xu *et al.*, 2008); The longer the stress period, the more reactive oxygen free radicals the cells will produce, which will disrupt the metabolic balance of reactive oxygen species, worsen the peroxidation of membrane lipids and impact crop morphogenesis at the seedling stage (Kachare *et al.*, 2020). Consequently, it is crucial to investigate the mechanisms underlying plant drought tolerance in order to enhance the development and growth of plants.

In order for plants to survive the damaging impacts of stress, the antioxidant system is crucial (Yadav *et al.*, 2017). By removing active oxygen free radicals created by drought stress, enzymes that produce antioxidants like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) work in concert to ensure that plants grow normally (Dadasoglu *et al.*, 2021). There has been a great deal of domestic and international research done on how drought affects the physiological traits of soybean leaves. The findings demonstrated that soybean plants' overall antioxidant capacity, osmotic adjustment, osmolyte accumulation and antioxidant enzyme activities were all increased by drought stress (Wang *et al.*, 2022). Plant endogenous hormones also have a significant impact on how plants react to drought stress at the same time. Studies have found that abscisic acid (ABA) is often accumulated under drought stress (Waadt *et al.*, 2022).

Exogenous plant growth regulators can play a regulatory role to a certain extent. Studies have confirmed that it is a more effective, simple and operable method to improve crop drought resistance, promote crop growth and development and increase yield by applying exogenous

[1]Qiqihar Sub-Academy of Heilongjiang Academy of Agricultural Sciences, Qiqihar, Heilongjiang Province, 161006, China.

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Corresponding Author: Huibo Shen, Qiqihar Sub-Academy of Heilongjiang Academy of Agricultural Sciences, Qiqihar, Heilongjiang Province, 161006, China. Email: hxm970619@126.com

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hormones (Kai *et al*., 2020). Research has indicated that the application of BA spraying during drought conditions can greatly enhance plant growth, hence facilitating the production of soybean yield (Mangena, 2020). Mepiquat chloride is a white powder, odorless, relatively stable plant growth regulator. After the solution is prepared, it can be quickly absorbed in the leaves of the plant and has a good internal absorption and conduction effect (Rosolem *et al*., 2013). It can encourage plants to reproduce and grow more roots with greater vigor (Gu *et al*., 2014). According to studies, mepiquat chloride can effectively increase stress resistance, lessen cell membrane deterioration and boost the protective enzymes' activity (SOD, POD and CAT) (Fang *et al*., 2019). Additionally, mepiquat chloride applied externally to watermelon seedlings could enhance the amount of endogenous hormones ABA and GA_3 in the tips of their shoots (Gong *et al*., 2021). It is evident that external applications of mepiquat chloride contribute significantly to plants' ability to withstand adversity stress.

Currently, mepiquat chloride is widely used in cotton and peanut, but there are few reports on drought stress alleviation in soybean. The sensitive soybean variety HN65 was used as the material to explore the effects of different concentrations of mepiquat chloride on the antioxidant enzyme activity and endogenous hormones of soybean, thereby providing a theoretical foundation for the development of novel strategies to enhance drought resistance in soybean.

MATERIALS AND METHODS

Test material

The experiment's material was the sensitive soybean cultivar "Heinong 65" and the mepiquat chloride was provided by Hebei Guoxin Nuonong Bio-technology Co., Ltd. (CangZhou, China). Purity of mepiquat chloride >99%. The polyethylene glycol used to simulate drought stress, PEG-6000 was purchased from Jinan Shuo Ding Trading Co.

Trial design

The experiment was carried out in the experimental base of Qiqihar Branch of Heilongjiang Academy of Agricultural Sciences (47°15′ 48.58 N, 123°41′ 18.64 E) from 2022 to 2023. Using sand cultivation in pots, selecting a plastic pail measuring 30 cm in height and 28 cm in diameter, drilling six holes with a diameter of 0.5 cm at the bottom of the bucket and laying a gauze mesh and sowing soybean seeds that were full of grains, keeping 4 seedlings per pot. From the time of seeding until the opposite true leaves had fully expanded, 500 ml of water was poured every days. Following the leaves' expansion, watering 500 ml Hoagland nutrient solution every day. At the three-leaf stage of soybean growth, the mepiquat chloride solution was uniformly sprayed on the leaves and drought stress was performed after 3 days. The nutrient solution mixed with 15 % PEG-6000 was irrigated every day for drought stress treatment. The experiment was set up for six treatments altogether. (1) Control group (CK): Hoagland nutrient solution was watered once a day to maintain normal water conditions (Waheed *et al*., 2019); (2) S0: Hoagland nutrition solution with 15% PEG-6000 was irrigated once a day as a drought stress therapy; (3) S100: drought stress treatment + 100 mg/L mepiquat solution; (4) S300: drought stress treatment + 300 mg/L mepiquat solution; (5) S500: drought stress treatment + 500 mg/L mepiquat solution; (6) S700: drought stress treatment + 700 mg/L mepiquat solution; after the drought treatment, samples were taken at 3, 6, 9 and 12 days for each treatment and three pots of each treatment were sampled for a total of four times.

Indicators and methods

Determination of dry matter weight

The soybean plants on the 12th day after drought stress were taken, three pots for each treatment and washed with clean water. Each pot was divided into different parts of leaves, stems, Petiole and roots into envelope bags and de-enzymed in an oven at 105°C for 30 minutes and then dried to constant weight at 65°C to measure dry weight.

Determination of antioxidant enzyme activity

With a small adjustment, the crude enzyme solution was extracted using Shamsul *et al.* (2012) methodology. The leaves weighed 0.2 g in a mortar, were ground into a powder using liquid nitrogen, combined with a tiny quantity of quartz sand, mixed with 50 mmol/L of phosphate buffer that had been chilled beforehand (including 1% polyvinylpyrrolidone and 0.1 mmol/L EDTA) and homogenized in a freezing bath. The mixture was then fixed into 5 mL centrifuge tubes, centrifuged for 30 minutes at a low temperature of 13,000 r/min and the supernatant was set aside for analysis. Nitrogen blue tetrazolium (NBT) was photochemically reduced to measure SOD activity and 50% blockage of NBT reduction was considered one unit of enzyme activity (U); The absorbance value falling by 0.01 per minute was determined as one unit of activity of the enzyme (U) by employing hydrogen peroxide reduction to measure CAT activity. The guaiacol technique was employed to quantify POD activity and a 0.01 per minute change in absorbance was considered one unit of enzyme activity (U); APX activity was measured using Krivosheeva *et al*. (1996) and the decrease of ascorbic acid (ASA) per unit of time was calculated as APX activity.

Measurement of endogenous hormones

Using high performance liquid chromatography, the contents of gibberellin (GA_3), indole acetic acid (IAA), zeatin (ZA) and abscisic acid (ABA) were ascertained (Waters ACQUITY Arc, USA) (Han *et al*., 2009).

Data analysis tools

Excel 2010 was selected for data processing and graphing, SPSS 21.0 for one-way ANOVA and significance test.

RESULTS AND DISCUSSION

Effects of mepiquat chloride on soybean dry matter accumulation under drought stress

It can be seen from Table 1 that the growth of soybean seedlings was inhibited and the application of mepiquat chloride could alleviate the inhibitory effect of drought stress on the growth of soybean seedlings to varying degrees. The dry weight of leaves, stem, petiole and roots was significantly higher than that of S0 treatment and the root-shoot ratio was significantly reduced. The dry weight of S100 treatment was significantly higher than that of S300, S500 and S700 treatments and the root-shoot ratio was the opposite.

Effect of externally applied mepiquat chloride on antioxidant enzyme activity in drought-stressed soybean seedling leaves

Drought stress significantly increased SOD activity, as Fig 1 illustrates, which rose by 39.01%, 34.19% and 37.41% at 3-9 d in the S0 treatment compared with the CK treatment, respectively, with significant differences among treatments. At 9 d, the SOD activities of S100, S300, S500 and S700 were significantly increased compared to S0 treatment, with the highest SOD activity in S100-treated leaves, which exceeded the S0 treatment's by 109.17% and the difference reached the significant level. It suggests that topical application of mepiquat chloride can enhance SOD activity and thus alleviate peroxidative damage of drought stress on soybean leaves.

As shown in Fig 2, the POD activity of S0 treatment increased significantly on the 3-9 d. On the 9 d, S100, S300, S500 and S700 were significantly higher than S0 treatment, increased by 53.45%, 23.58%, 34.76% and 7.55%, respectively and the differences between the treatments reached a significant level.

From Fig 3, it is evident that mepiquat chloride applied externally may raise the CAT activity of drought-stressed soybean leaves and that the S100 treatment's CAT activity peaked in the 3-12 d range and increased by 87.96%, 49.37%, 31.80% and 44.98%, disparities reached the significant level as compared to the S0 treatment, respectively. This indicates that topical application of mepiquat chloride can regulate the CAT activity of soybean leaves, thus enhancing the operation of the antioxidant system in soybean.

As illustrated in Fig 4, drought stress induced a significant enhancement of APX activity, with a greater increase on 3-9 d, which was 24.15%, 26.38% and 25.71%, respectively, compared with the CK treatment and a relatively greater decrease on 12 d, which was 31.29% lower. The externally applied mepiquat chloride treatment increased APX activity to different degrees, in which the APX activity of S100 treatment reached the peak at all stress days and at 9 d, S100 increased by 131.31% compared with S0 treatment and the difference reached the significant level. It indicates that spraying mepiquat chloride can increase APX activity in the leaves of soybean seedlings under drought stress.

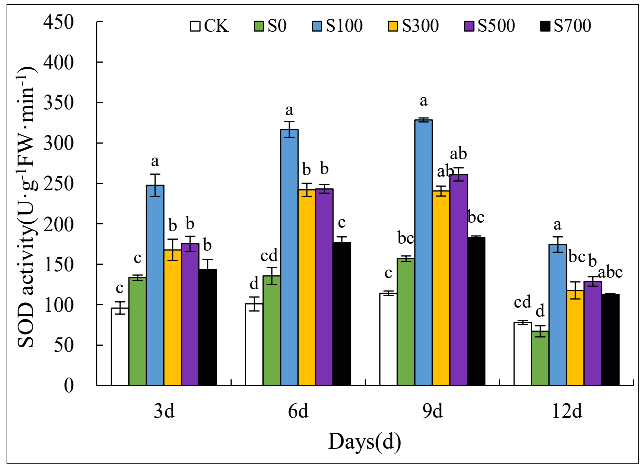

Fig 1: Effect of external mepiquat chloride treatment on soybean leaf SOD activity during drought stress.

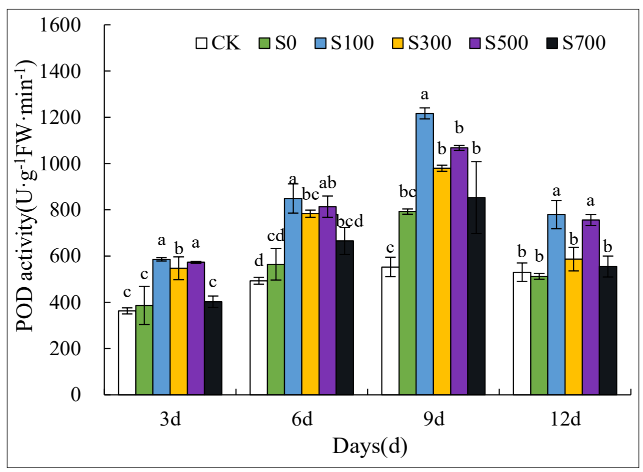

Fig 2: Effect of external mepiquat chloride treatment on soybean leaf POD activity during drought stress.

Table 1: Effects of mepiquat chloride on dry weight and root-shoot ratio of soybean seedlings under drought stress.

Treatment	Leaves (g)	Stems (g)	Petiole (g)	Root (g)	Root/Shoot ratio
CK	9.1±0.15a	7.8±0.06a	4.9±0.16a	12.8±0.37a	0.59±0.12c
S0	4.3±0.60c	3.9±0.13c	2.6±0.16d	8.0±0.74c	0.74±0.01ab
S100	6.8±0.40a	5.2±0.19d	4±0.04b	10.9±0.35b	0.68±0.03b
S300	5.1±0.70b	4.7±0.08b	2.8±0.11d	9.5±0.89bc	0.75±0.01ab
S500	4.4±0.60c	4.1±0.03b	3.2±0.11c	9.4±0.39bc	0.80±0.02a
S700	5.8±0.18b	4±0.01cd	2.6±0.09d	8.5±0.45c	0.69±0.02b

Effect of externally applied mepiquat chloride on endogenous hormone levels in drought-stressed soybean seedling leaves

From Fig 5, Under S0 treatment, the content of ABA increased significantly and there was a noticeable drop in the contents of ZA, GA_3 and IAA. The amount of ABA increased 10.53 times more under S0 treatment than it did under CK treatment; IAA, GA_3 and ZA decreased by 88.02%, 43.16% and 68.28%, respectively. The application of mepiquat chloride could regulate the dynamic balance of endogenous hormones. The contents of IAA, ABA and GA_3 increased first and then decreased with the increase of mepiquat chloride concentration. The content of IAA was the highest in S300 treatment, which was 4.85 times higher than that in S0 treatment. At the S500 treatment, the contents of GA_3 and ABA reached their peak. In comparison to the S0 therapy, there was a 62.20 percent rise in GA_3 content and a 26.8 per cent drop in ABA content. The application of mepiquat chloride had a substantial effect on the amount of IAA, GA_3 and ZA in the leaves of soybean seedlings, as evidenced by the content of ZA reaching its maximum at S100 treatment, which was 1.48 times higher than that at S0 treatment, so as to resist the damage of drought stress to its own physiological metabolism.

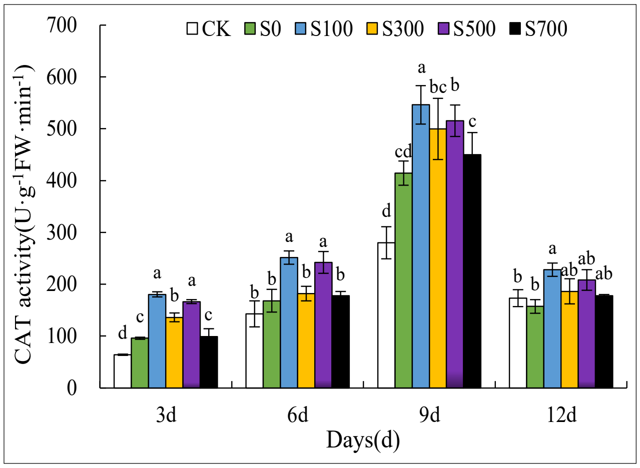

Fig 3: Effects of external application of mepiquat chloride on CAT activity in soybean leaves under drought stress.

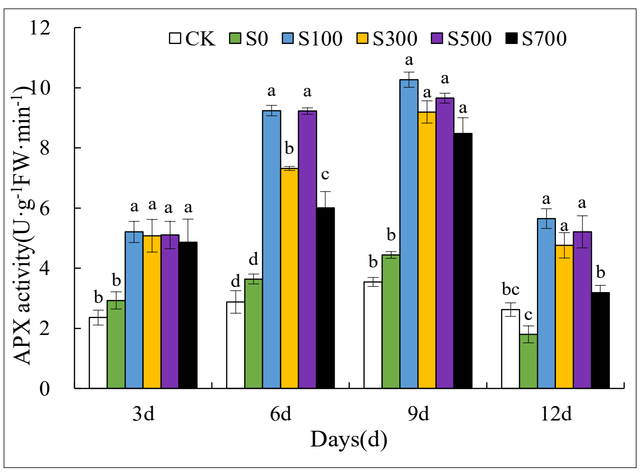

Fig 4: Effect of external mepiquat chloride treatment on APX activity in drought-stressed soybean leaves.

Examining the relationship between endogenous hormone levels and antioxidant enzyme activity in soybean leaves during conditions of drought using topical mepiquat chloride application

Table 2 illustrates the highly significant and positive ($P<0.01$) correlation between SOD activity and the activities of POD, CAT and APX in soybean leaves. The POD activity demonstrated a strong positive correlation ($P<0.01$) with both the CAT and APX activities. The relationship between CAT and APX activity was highly significant and strongly linked ($P<0.01$). There was no significant correlation between endogenous hormone content and antioxidant enzyme activity. The contents of IAA exhibited a highly significant positive correlation ($P<0.01$) with the contents of GA_3 and ZA, while all of them exhibited a strongly negative correlation ($P<0.01$) with the contents of ABA. The correlation between GA_3 and ZA content was significant ($P<0.05$) and the extremely significant level of correlation was seen between the concentration of IAA, GA_3 and ZA, suggesting that these three endogenous hormones work in concert to promote the growth and development of soybeans. The correlation between endogenous hormones GA_3 and ZA reached a significant level, which proved that they could jointly promote the growth and development of soybean seedlings.

Table 2: Correlation analysis between antioxidant enzyme activity and endogenous hormone content.

	SOD	POD	CAT	APX	IAA	ABA	GA_3	ZA
SOD	1.000							
POD	0.953**							
CAT	0.895**	0.884**						
APX	0.949**	0.867**	0.930**					
IAA	-0.117	-0.232	-0.214	-0.101				
ABA	0.368	0.290	0.413	0.323	-0.627**			
GA_3	-0.426	-0.477	-0.207	-0.237	0.706**	-0.614**		
ZA	0.081	0.078	-0.056	0.089	0.797**	-0.857**	0.531*	1.000

Note: The Pearson's correlation coefficients are shown by the numbers in the table; significant correlation at the $P<0.05$ and $P<0.1$ levels are indicated by * and **, respectively.

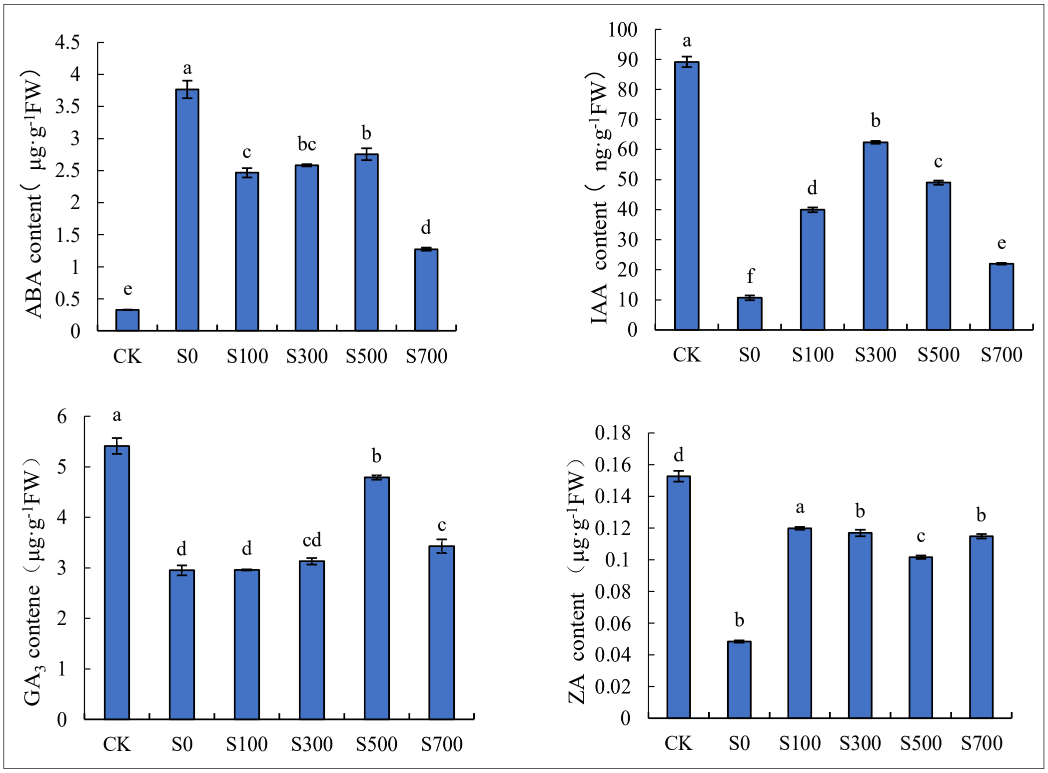

Fig 5: Effect of external mepiquat chloride treatment on endogenous hormone content in drought-stressed soybean leaves.

Dry matter quality is the product of photosynthesis and the material basis of yield formation. Water deficit will lead to the failure of normal physiological activities of crops, thus inhibiting the growth of soybean seedlings. Found that under water deficit conditions, the dry matter weight of the aboveground and underground parts of different varieties of wheat decreased significantly (Sečenji *et al.*, 2010). Consistent with the results of this experiment. In this experiment, it was found that spraying mepiquat chloride treatment could effectively increase the dry matter accumulation of aboveground and roots of soybean seedlings and reduce the root-shoot ratio. This shows that mepiquat chloride plays a certain role in the chemical regulation of soybean seedling stage, which can promote the development of lateral roots under drought stress and maintain the normal accumulation of dry matter in reproductive organs.

In order to remove too many free radicals from the body, antioxidant enzymes like SOD, POD and CAT become more active when plants are under drought stress. This increases the synthesis of reactive oxygen species in plants (Tian *et al.*, 2023). In this study, the antioxidant enzyme activities of soybean leaves under drought stress increased first and then decreased with the prolongation of stress time, they all peaked on 9 d. This indicates that under drought stress, although soybean seedlings are able to increase the activities of antioxidant system-related enzymes through their own regulatory mechanisms, they are still unable to scavenge the excessive reactive oxygen species in the body, which inevitably causes them to suffer from oxidative stress damage. Exogenous mepiquat chloride leaf spraying significantly increased the operations of SOD, POD, CAT and APX, which was consistent with previous studies on cotton seedlings (Wan *et al.*, 2021) and the explanation could be that mepiquat chloride can boost antioxidant enzyme activity by promoting the expression of genes linked to antioxidant enzymes and lowering biomolecule degradation, which increases reactive oxygen species' ability to be scavenged (Ding *et al.*, 2017). In addition, this study found that the rise of antioxidant enzyme activities in 100 mg/L of mepiquat treatment was significantly greater than that of other concentrations, in which the expressions of SOD, POD, CAT and APX were increased by 109.17%, 53.45%, 87.96% and 131.31%, respectively. This indicates that externally applied mepiquat chloride can inhibit the metabolism of antioxidant enzymes at high concentration and promote it at low concentration and the treatment of mepiquat chloride at 100 mg/L can better promote the elevation of antioxidant enzyme activities, accelerate the metabolism of reactive oxygen species and reduce the rate at which they damage plants.

Plant endogenous hormones are induced by plants in response to specific environmental signals and can have substantial physiological effects even at extremely low doses, either individually or in coordination with each other to regulate plant growth, development and in vivo metabolism (Zahedi *et al.*, 2015). IAA, GA_3 and ZA are promotional endogenous hormones that regulate plant growth and development and are involved in crop response to adversity stress (Rosenvasser *et al.*, 2006). ABA is an inhibitory hormone that slows down plant metabolism and is an important regulatory signalling molecule for plant response to drought to adapt to unfavourable environments under drought conditions (Vishwakarma *et al.*, 2017). This experiment revealed that the contents of ABA in leaves of soybeans under drought stress were considerably greater than those of the CK therapy, but the IAA, GA_3 and ZA levels were significantly lower. These results were essentially the same as those of tomato (Cohen *et al.*, 1990) and maize (Wei *et al.*, 2018). This suggests that drought stress reduces the intensity of cellular metabolism in soybean leaves and breaks the balance of endogenous hormones thereby inhibiting the growth of soybean seedlings. It has been reported to confirm that spraying mepiquat chloride can significantly increase GA_3 and IAA content and mepiquat chloride has the effect of regulating GA_3 activity in plants. The experiment's findings demonstrated that the contents of IAA, ABA and GA_3 increased as the concentration of mepiquatium increased. Additionally, spraying mepiquatium under conditions of drought significantly raised the contents of IAA, GA_3 and ZA. The contents of ABA and GA_3 peaked at 500 mg/L, the contents of ZA peaked at 100 mg/L and the contents of IAA peaked at 300 mg/L. According to these findings, mepiquat chloride may be able to control the metabolism of endogenous hormones in plants and lessen the negative effects of drought stress on soybean seedlings. Therefore, it is hypothesized that mepiquat chloride applied externally can influence IAA, GA_3, ZA and ABA levels indirectly in order to control how soybean plants respond to drought stress and enhance plant tolerance.

Applying phytohormones can modify the amount of endogenous hormones and boost the antioxidant enzymes' activity when under stress from hardship, allowing plants to continue growing and developing normally (Kundur *et al.*, 2016). The contents of GA_3 and ABA in the leaves of Lonicera japonica under adversity stress were found to have a substantial positive association and a significant negative correlation, respectively, with the SOD and POD enzyme functions (Yu *et al.*, 2022). However, the correlation analysis in this study showed that externally applied mepiquat chloride had no significant correlation with antioxidant enzymes and endogenous hormones under drought stress and we hypothesised that since crop growth is a complex process, it is related to many factors, such as plant tissue sensitivity, concentration-dependent mode, treatment time and so on. In addition, it is still controversial when topical application of mepiquat chloride promotes or inhibits plant growth and how much concentration is promotive or inhibitory and further experimental studies are needed.

CONCLUSION

Drought stress significantly inhibited the normal growth of soybean seedlings. Spraying mepiquat chloride could increase the dry matter accumulation, antioxidant enzyme activity, IAA, GA_3 and ZA content of soybean seedlings, reduce ABA content and root shoot ratio, enhance the resistance of plants to drought stress and reduce the harm of drought stress to soybean seedlings.

Conflict of interest

All authors declared that there is no conflict of interest.

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