RESEARCH ARTICLE

The Effects of Exogenous Polyamines on the Antioxidant System and Growth of Crocus Sativus L. under Aluminum Stress Based on Physiological and Biochemical Indicators

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Abstract

This study focuses on the physiological and biochemical responses of Crocus sativus L. (Crocus sativus) root growth under aluminum (Al) stress and the mitigating effects of exogenous polyamines. Measurements of root elongation, Al content, root tip cell viability, malondialdehyde (MDA) content, and antioxidant enzyme activities were conducted under Al stress and exogenous polyamine treatments. The results indicated that under Al stress, Crocus sativus L. root tip growth was significantly inhibited, with root tip Al content, Evans blue absorbance, and MDA content all gradually increasing with higher concentrations of Al^{3+}. At 0.05 and 0.2 mmol/L Al^{3+} stress, antioxidant enzyme activity increased with the concentration of Al^{3+}, but when the concentration reached 0.5 mmol/L, the enzyme activity decreased. The addition of 1 mmol/L exogenous polyamines significantly improved the growth and physiological and biochemical conditions of Crocus sativus L. under Al stress, with putrescine showing the most notable alleviating effect among the three polyamines tested.

Keywords: aluminum stress, polyamines, antioxidant enzyme activity, crocus sativus L.

1 Introduction

Crocus sativus L., or saffron, is native to Southern Europe and Asia Minor. It was introduced to China in the 1960s and has since been cultivated extensively, particularly in Zhejiang and Shanghai. The Mediterranean climate of its native region resembles the climatic conditions south of the Yangtze River in China, which are favorable for the growth of Crocus sativus L [1]. However, primary agricultural lands in these areas are generally allocated to grain and oil crops, leaving about 20% of acidic soils as potential sites for Crocus sativus L. cultivation. Acidic soils are typically rich in free aluminum, which, while insoluble under natural conditions, can transform into soluble Al^{3+} ions in acidic environments, harmful to plant health [2]. Aluminum stress inhibits crop growth,

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leading to reduced yields and quality, and is one of the main factors limiting crop cultivation in acidic soils [3].

This study examines the growth response of Crocus sativus L. under aluminum stress and seeks to identify methods to alleviate aluminum toxicity, with the aim of facilitating its broader cultivation. It has been demonstrated that the application of cations such as Ca^{2+} and Mg^{2+} can enhance aluminum tolerance in crops, possibly by reducing the uptake of aluminum ions [4]. Furthermore, recent studies have shown that polyamines—including putrescine, spermidine, and spermine—play roles not only in plant growth and differentiation but also in enhancing resistance to environmental stresses [5]. The goal of this study is to investigate the potential of polyamines in mitigating aluminum stress in Crocus sativus L., improving its aluminum tolerance, and elucidating the underlying mechanisms by which polyamines enhance stress tolerance in plants.

2 Materials and methods

2.1 Materials

The experimental material consisted of Crocus sativus L. corms, obtained from a cultivation farm in Sandu, Zhejiang, an area known for its optimal climate for saffron production [6]. The corms underwent a rigorous selection process to exclude any that exhibited signs of pathology or damage, ensuring that the experimental sample represented a homogeneous and healthy population. Prior to their use in the study, the corms were subjected to a standardized sanitation protocol. This involved gently scrubbing them with distilled water to remove soil particles and potential microbial contaminants, followed by a sterilization process to prevent the introduction of exogenous microorganisms that could influence the experimental results [7]. The sanitized corms were then maintained under controlled environmental conditions to preserve their viability and standardize the physiological state of the plants before the initiation of the experiments.

2.2 Experimental design

The greenhouse-based experimental phase commenced in October, following the blooming of the saffron flowers. At this juncture, the saffron plants were carefully transplanted into a composite growing medium comprising vermiculite and soil, and the environmental conditions were regulated to maintain a temperature of (17 ± 0.5) °C and a relative humidity level of 80%. The plants were allowed to grow until their root systems reached a length of roughly 3-4 cm, at which point they were extracted from the medium. The root tips were then cleaned of the substrate, and their lengths were measured. Subsequently, the plants underwent a 72-hour treatment in solutions with varying concentrations of Al^{3+}, specifically 0.05, 0.2, or 0.5 mmol/L. In a separate set of experiments, solutions containing 0.2 mmol/L Al^{3+} were supplemented with 1 mmol/L of either putrescine, spermidine, or spermine. A control solution, devoid of both polyamines and Al^{3+}, was also included. Throughout the experimental period, root tips were harvested every 24 hours for subsequent biochemical assays, with each treatment being conducted in triplicate to ensure experimental rigor.

2.3 Physiological index measurement

The length of the roots was determined at the outset and again after a 72-hour period following treatment, allowing for the computation of the relative growth of the roots. The relative root growth rate was ascertained by the formula: (Increase in root length in the treated group / Increase in root length in the untreated group) ×100%. The concentration of aluminum within the root tips was quantified employing the hematoxylin staining technique as referenced [8]. The viability of the cells was evaluated through the application of Evans blue staining, as described [9]. Furthermore, the level of malondialdehyde (MDA), a biomarker for lipid peroxidation, was determined by the thiobarbituric acid (TBA) assay, in accordance with the method outlined in [10].

2.4 Enzyme activity assay

A 0.3-g portion of newly harvested root tip samples was subjected to homogenization in an icy cold 50 mmol/L PBS buffer solution at pH 7.0, enriched with 1% PVP, with a total volume of 3 mL. This suspension was then spun down at 4 °C with a centrifugal force corresponding to 4,600 rpm for a period of 10 minutes to separate the solid particles from the liquid. The ensuing supernatant was carefully isolated for further analysis. The isolated supernatant was employed to quantify the enzymatic activities of SOD, POD, CAT, and APX, following the experimental procedures detailed by Jebara [11].

3 Results and Analysis

3.1 Al^{3+} Inhibition of saffron root elongation

Under conditions of aluminum stress, the growth at the tips of plant roots is notably suppressed.

Table 1. Aluminum stress impact on the growth of *Crocus sativus* root tips.

Indicator	Treatment Time/h	Control	0.05 mmol/L Al^{3+}	0.2 mmol/L Al^{3+}	0.5 mmol/L Al^{3+}
Relative root growth rate %	72	100	40.7 ± 12	25.0 ± 4.8	0
	24	4.5 ± 0.25	8.1 ± 0.45	11.07 ± 0.54	15.03 ± 0.72
Al^{3+} content (μg/g FW)	24	5.4 ± 0.27	10.26 ± 0.63	14.4 ± 0.9	24.57 ± 1.26
	48	5.4 ± 0.27	13.5 ± 0.54	18.63 ± 1.08	28.8 ± 1.35
	72	5.4 ± 0.27	13.5 ± 0.54	18.63 ± 1.08	28.8 ± 1.35
Evans Blue Absorbance (A600)	24	0.0535 ± 0.004	0.067 ± 0.005	0.0955 ± 0.005	0.135 ± 0.0095
	48	0.0615 ± 0.0045	0.096 ± 0.0065	0.13 ± 0.007	0.234 ± 0.0165
	72	0.062 ± 0.004	0.1195 ± 0.0085	0.155 ± 0.011	0.2435 ± 0.017
MDA Content (μmol/L·gFW)	24	0.053 ± 0.005	0.075 ± 0.006	0.1 ± 0.008	0.18 ± 0.015
	48	0.06 ± 0.005	0.13 ± 0.01	0.17 ± 0.014	0.27 ± 0.022
	72	0.07 ± 0.006	0.16 ± 0.013	0.23 ± 0.018	0.35 ± 0.03

Table 2. Antioxidant enzyme dynamics in *Crocus sativus* root tips in response to varied aluminum stresses.

Indicator	Treatment Time/h	Control	0.05 mmol/L Al^{3+}	0.2 mmol/L Al^{3+}	0.5 mmol/L Al^{3+}
SOD Activity (U/gFW)	24	94.4 ± 5.7	128.3 ± 7.7	156.4 ± 9.4	134.6 ± 8.2
	48	105.6 ± 6.4	145.8 ± 8.8	196.8 ± 11.9	132.2 ± 8.0
	72	113.7 ± 6.9	134.5 ± 8.1	115.4 ± 7.0	106.0 ± 6.4
POD (mmol H_2O_2/(min gFW))	24	154 ± 11	207.5 ± 14	243 ± 17	272 ± 19
	48	175 ± 13	225 ± 16	379 ± 28	316 ± 23
	72	192.5 ± 14	242.5 ± 17	353 ± 25	275 ± 20
CAT activity (μmol H_2O_2/g FW)	24	366 ± 18	416 ± 21	447 ± 22	426 ± 21
	48	421 ± 22	455 ± 23	576 ± 29	522 ± 27
	72	452 ± 24	524 ± 27	696 ± 35	399 ± 20
APX activity (mmol ascorbate/(min g FW))	24	137 ± 11	157 ± 13	166 ± 14	110 ± 9
	48	143 ± 12	180 ± 15	194 ± 16	225 ± 18
	72	139 ± 12	164 ± 13	152 ± 12	140 ± 10

As indicated in Table 1, exposure to 0.05 and 0.2 mmol/L of Al^{3+} results in a substantial reduction in root elongation, with decreases of 59.3% and 75%, respectively. Furthermore, treatment with 0.5 mmol/L Al^{3+} leads to a comprehensive impact on root growth.

Variations in the content of aluminum at the root apex, as well as the absorbance of Evans blue and the levels of malondialdehyde (MDA), all exhibit a correlation with the increasing concentrations of Al^{3+}. This suggests that plants experience membrane lipid peroxidation when subjected to aluminum stress, which can lead to alterations in membrane structure and function. These changes can ultimately result in cellular damage or even cell death.

3.2 Adaptation of Antioxidant Enzymes to Al^{3+}-Induced Stress

Exposure to Al^{3+} stress led to an elevation in the antioxidant enzyme activities within the root tips of saffron, as detailed in Table 2. At Al^{3+} concentrations of 0.05 and 0.2 mmol/L, there was a positive correlation between the Al^{3+} levels and the activities of the enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). However, at a higher Al^{3+} concentration of 0.5 mmol/L, a decline in these antioxidant enzyme activities was observed. Except for the 0.5 mmol/L Al^{3+} treatment, the activities of SOD, POD, and APX exhibited an initial rise within the initial 48 hours of stress exposure [12], followed by a subsequent decline. In contrast, the activity of CAT showed a gradual and sustained increase throughout the period.

3.3 Polyamine-Induced Tolerance to Aluminum Ions in Plant Systems

In the experimental setup, a concentration of 1 mmol/L polyamine was introduced to a solution containing 0.2 mmol/L Al^{3+}. As depicted in Table 3, the incorporation of polyamines notably enhanced the root growth of saffron crops under aluminum-induced stress, decreased the aluminum levels in the root tips, elevated the viability of root tip cells [13], and curbed the formation of malondialdehyde (MDA). Specifically, 1 mmol/L putrescine was capable of reviving the root elongation of saffron to 85% of that observed in the control group, a more substantial recovery compared to spermine and spermidine, which only managed to restore growth to approximately

Table 3. Polyamine influence on *Crocus sativus* root tip growth under 0.2 mmol/L aluminum stress.

Indicator	Treatment Time/h	0.2 mmol/L Al^{3+}	0.2 mmol/L Al^{3+} + 1 mmol/L Spd	0.2 mmol/L Al^{3+} + 1 mmol/L Put	0.2 mmol/L Al^{3+} + 1 mmol/L Spm
Relative root growth rate/%	72	25 ± 5	85 ± 7	55.0 ± 5	50.3 ± 7
	24	11.07 ± 0.54	5.67 ± 0.18	6.3 ± 0.36	6.75 ± 0.45
Al content/ (μg/gFW)	24	11.07 ± 0.54	5.67 ± 0.18	6.3 ± 0.36	6.75 ± 0.45
	48	14.4 ± 0.9	6.75 ± 0.27	8.1 ± 0.63	9 ± 0.72
	72	18.63 ± 1.08	8.1 ± 0.27	9 ± 0.54	10.8 ± 0.63
Evans Blue Absorbance (A600)	24	0.0955 ± 0.005	0.06 ± 0.003	0.07 ± 0.006	0.075 ± 0.005
	48	0.13 ± 0.007	0.075 ± 0.005	0.085 ± 0.005	0.09 ± 0.006
	72	0.155 ± 0.011	0.085 ± 0.006	0.1 ± 0.007	0.11 ± 0.008
MDA content/ (μmol/gFW)	24	0.1 ± 0.008	0.07 ± 0.005	0.08 ± 0.007	0.09 ± 0.008
	48	0.17 ± 0.014	0.1 ± 0.008	0.12 ± 0.01	0.13 ± 0.012
	72	0.23 ± 0.018	0.11 ± 0.008	0.15 ± 0.013	0.16 ± 0.015
SOD activity/ (U/gFW)	24	156.4 ± 9.4	121.2 ± 7.3	135.6 ± 5.9	148.2 ± 8.3
	48	196.8 ± 11.9	138.8 ± 8.4	181.7 ± 7.3	190.8 ± 9.5
	72	115.4 ± 7	124.7 ± 7.5	151.3 ± 7.1	165.5 ± 8.8
POD activity/ (mmol H_2O_2/(min gFW))	24	243 ± 17	175 ± 12	227.5 ± 16	240 ± 17
	48	379 ± 25	216 ± 15	242 ± 18	265 ± 19
	72	353 ± 28	203 ± 14	250 ± 17	280 ± 20
CAT activity/ (μmol H_2O_2/gFW)	24	447 ± 22	395 ± 20	420 ± 21	435 ± 22
	48	576 ± 29	450 ± 23	519 ± 26	500 ± 25
	72	696 ± 35	498 ± 26	590 ± 30	550 ± 28
APX activity/ (mmol ascorbate/(min gFW))	24	166 ± 14	123 ± 10	158 ± 13	150 ± 12
	48	194 ± 16	107 ± 9	180 ± 14	175 ± 14
	72	152 ± 12	102 ± 8	134 ± 10	146 ± 13

50% of the control levels. Following a 48-hour exposure period, the root tips of saffron treated with putrescine exhibited significantly reduced aluminum levels, Evans blue absorbance, and MDA content in comparison to those treated with spermine and spermidine [14]. Concurrently, it was observed that polyamine application markedly decreased the activity of antioxidant enzymes compared to the activity than those treated with spermine and spermidine. Notably, only the putrescine treatment resulted in a significant decrease in APX activity in the root tips when compared to the group treated with 0.2 mmol/L Al^{3+}.

4 Conclusion

The root tip of plants is highly sensitive to Al^{3+} ions. Prolonged exposure to aluminum stress results in the accumulation of reactive oxygen species (ROS) such as O^{2-} and H_2O_2, which in turn triggers oxidative damage and lipid peroxidation [15]. The activation of natural antioxidant enzymes can help to lessen oxidative harm. These enzymes include superoxide dismutase (SOD) [16, 17], catalase (CAT) [18], peroxidase (POD) [19], and ascorbate peroxidase (APX) [20]. Each of these enzymes plays a crucial role in combating oxidative stress. In saffron, treatment with Al^{3+} significantly inhibited root elongation compared to the control group. The concentration of Al^{3+} in the root tip increased proportionally with the rise in Al^{3+} levels and extended treatment duration. Additionally, the absorbance of Evans blue and malondialdehyde (MDA) content both rose with the increasing Al^{3+} accumulation, indicating lower cell viability and elevated lipid peroxidation. This damage to roots can impair nutrient uptake, hinder bulb development, and ultimately decrease stigma yield.

Antioxidant enzyme activity initially increased under low concentrations of Al^{3+} stress, but at higher concentrations, most enzymes exhibited reduced activity. This suggests that while plants may boost antioxidant defenses under moderate stress to mitigate damage, their ability to regulate these defenses diminishes under severe toxic conditions, leading to extensive cellular damage and death.

Polyamines, known for their ability to stabilize cell membranes through interaction with the

negatively charged groups of phospholipids [21], have been shown to reduce lipid peroxidation, limit ROS production, and enhance antioxidant enzyme activity, thereby alleviating oxidative damage [22]. Experimental findings demonstrated that polyamines significantly reduce aluminum toxicity, with putrescine being the most effective. The protective effect of polyamines is likely due to reduced aluminum uptake. Under 0.2 mmol/L Al^{3+} stress, the addition of putrescine decreased aluminum content in the root tip by 57%, alleviating aluminum-induced stress and lowering antioxidant enzyme activity as a result. In contrast, treatments with spermine and spermidine led to higher aluminum absorption, which caused increased lipid peroxidation, oxidative damage, and elevated antioxidant enzyme activity.

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

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