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Effects of Exogenous Phthalic Acid on Seed Germination, Root Physiological Characteristics, and Mineral Element Absorption of Watermelon

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Abstract: To understand the effect of exogenous PA on the watermelon root system, the watermelon variety 'Zaojia 84–24' was used as experimental material. This study investigated the effects of allelochemicals DIBP and DOP at varying different concentrations (0, 0.05, 0.1, 0.5, 1, and 4 mmol·L^{-1}) on the physiological characteristics and mineral content of watermelon roots. The results revealed that proper PA treatment concentrations (0.05~0.1 mmol·L^{-1}) promoted seed germination, increased the number of RBCs and the survival rate of RBCs, and enhanced the activities of PME and dehydrogenase in watermelon roots. In addition, proper PA treatment concentrations (0.05~0.1 mmol·L^{-1}) promoted the activities of SOD, POD, CAT, and NR in watermelon roots. The contents of MDA and soluble protein were increased at 0.05~4 mmol·L^{-1} PA. In addition, proper PA treatment concentrations promoted the absorption and accumulation of P, K, Ca, Fe, Cu, and Zn elements in watermelon roots. These results indicate that PA at a concentration of 0.05~0.5 mmol·L^{-1} can promote watermelon seed germination, improve antioxidant enzyme activity of watermelon roots, and maintain normal physiological activities of watermelon by affecting absorption and accumulation of mineral elements in the root system.

Keywords: allelochemicals; phthalic acid; watermelon roots; mineral elements

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1. Introduction

Allelopathy refers to a chemical ecological phenomenon in which chemicals called allelochemicals released by various plants (including microorganisms) affect the growth and development of surrounding plants [1]. Common allelochemicals include complex polycyclic aromatic compounds, terpenes, flavonoids, polyacetylenes, and fatty acids [2]. Phenolic acids are the most important and common plant allelochemicals in the ecosystem. They are formed by the direct combination of a hydroxyl (-OH) with an aromatic hydrocarbon group. The number of known metabolites of phenolic compounds now exceeds 8000 [3]. Phenolic allelochemicals can inhibit plant root elongation and cell division, change cell ultrastructure, and ultimately inhibit plant growth [4,5]. Phenolic allelochemicals can increase the permeability of cell membrane, alter the activity of antioxidant enzymes in cells, and reduce or inactivate the physiological activity of plant hormones, thus inhibiting the normal physiological process of plants [6,7]. Phenolic allelochemicals also suppress plant respiration and affect chlorophyll content, thus reducing the rate of photosynthesis in addition to inhibiting plant nutrient uptake [8–10].

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) is an annual vine belonging to the Cucurbitaceae family and is widely cultivated in southern and northern China and is an important cash crop. Watermelons have strong allelopathic effects, and secondary metabolites released from living or residual watermelon to the environment can cause

persistent cropping disorders. Studies have indicated that watermelon root exudates can inhibit seed germination, seedling growth, and related enzyme activities in vivo [11,12]. DIBP could inhibit the growth of watermelon seedlings and reduce the photosynthetic rate [13]. In a previous study, we found that two PAs (DIBP and DOP) are potential watermelon root allelopathic substances that can promote the growth of watermelon seedlings with low concentration and inhibit growth with high concentration [14]. Zhou et al. also came to the same conclusion that a low concentration of DIBP could promote the growth of eggplant, while a high concentration of DIBP would produce toxic effects [15]. Meanwhile, PA, as a potential allelopathic substance of pepper, can reduce the glutathione content in lettuce leaves, affect the activity of antioxidant enzymes, reduce the osmotic regulation ability of cells, and thus deepen the degree of membrane lipid peroxidation [16,17].

In addition to some volatile allelochemicals acting on the aboveground part of plants, most allelochemicals enter the soil and, directly or indirectly, affect the underground part of plants; hence, the root properties of plants are among important indicators in the research of allelochemicals [18]. At present, the effect of PA on watermelon roots remains unclear. In this study, DIBP and DOP were applied to study the effects of PA on the physiological characteristics and mineral content of watermelon roots. This study also aims to better understand the allelopathic effect of PA on watermelon, identify more measures to alleviate allelopathy inhibition, and provide a certain theoretical basis for production practice.

2. Materials and Methods

2.1. Materials

The examined watermelon variety is 'Zaojia 84–24', a hybrid produced by the Shandong Shouhe seed industry, with medium plant growth and strong disease resistance, and is widely cultivated in a large area in southern China. The test reagents are diisobutyl phthalate (DIBP, $C_{16}H_{22}O_4$, analytically pure, produced by China National Pharmaceutical Group, China) and dioctyl phthalate (DOP, $C_{24}H_{38}O_4$, analytically pure, produced by China National Pharmaceutical Group, China).

2.2. Experimental Design

Evenly sized, healthy, and plump watermelon seeds were selected, washed with distilled water, diluted 100 times formalin solution and soaked for 30 min followed by rinsing with distilled water. A 10 mL of treatment solution (0, 0.05, 0.1, 0.5, 1, and 4 mmol·L^{-1} DIBP or DOP) was added into a Petri dish, and each treatment was repeated 3 times, for a total of 12 treatments. Seeds were germinated in the dark at 30 °C for 48 h in Petri dishes containing filter paper, and the germination rate of watermelon seeds was calculated. Radicles of the same length (about 25 mm) were selected, a 3 mm root tip was cut, and the root tip index was determined [19].

The seed disinfection treatment was performed the same as above. Subsequently, the seeds were incubated at 30 °C to germinate. After germination, the seeds were sowed in a hole dish (the volume ratio of perlite, vermiculite, and organic fertilizer is 2:2:1). The roots of watermelon seedlings with one leaf were treated with 20 mL treatment solution of different concentrations (DIBP and DOP of 0, 0.05, 0.1, 0.5, 1, and 4 mmol·L^{-1} were obtained with 1/3 Hoagland nutrient solution as solvent). The solution was treated every two days for 15 days. Each treatment was prepared for three biological replicates. The test site was in an artificial climate incubator of the College of Horticulture, Sichuan Agricultural University, Sichuan, China. The temperature was 25 °C, the light intensity was 5000 lx, the relative humidity was 75%, and the light–dark period was 16 h–8 h.

2.3. Determination Method

Seed germination rate was calculated by direct observation. Three root tips were randomly selected for each treatment, 15 μL distilled water was dropped on clean slides, and the root tips were immersed and gently sloped for 40 s. Then, 15 μL 0.5% trypan blue staining solution was added for 10 min in the dark, and clean cover slides were added

for observation and counting under a microscope. The number of live and dead cells observed was recorded (live cells are colorless, while dead cells are blue) [20]. The survival rate of RBCs was calculated by referring to the method of Hou et al. as follows: survival rate = (live cells/total cells) × 100% [21]. The activity of PME in the root cap was measured using spectrophotometry, and the absorbance was read at 525 nm [22]. Watermelon root length was measured with a ruler.

A total of 15 plants were randomly selected for each treatment, and the roots were washed with distilled water and dried using filter paper. Root dehydrogenase was determined by the triphenyl tetrazole chloride (TTC) method, and the absorbance was read at 485 nm [23]. SOD activity was determined by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), as described by Moradi and Ismail [24]. CAT activity was determined by the reduction of H_2O_2 at 240 nm spectrophotometrically [25]. POD activity was determined with guaiacol as the reducing substrate in a reaction mixture containing 0.2 M Na–phosphate buffer (pH 6), 3 mM guaiacol, and 4.9 mM H_2O_2, and the absorbance was read at 470 nm [26]. NR activity was assayed in 400 mL of 50 mM HEPES–KOH (pH 7.5) buffer containing 10 mM KNO_3, 2 mM EDTA, 0.2 mM NADH, and 10 mM FAD, and the absorbance was read at 540 nm [27]. MDA content was determined by the thiobarbituric acid method, and the absorbance was read at 450 nm, 532 nm, and 600 nm [28]. Soluble protein content was evaluated according to the method of Huang et al. [28], and the bovine serum albumin was used as the standard.

The plant roots are dried and crushed to determine their elements. To identify K, Ca, Mg, Fe, Zn, and Cu, 0.5 g of the sample was weighed into a Conical flask, and 4 mL perchloric acid and 16 mL nitric acid were added, then covered with plastic wrap overnight, heated, and digested on a hot plate, and then measured with an atomic absorption spectrophotometer. To estimate P, 0.5 g of samples was weighed and sealed overnight with 10 mL sulfuric acid. Hydrogen peroxide was added appropriately, heated and boiled, and filtered; the resulting volume was fixed for later use. Total P was detected using vanadium–molybdenum–yellow colorimetry [29].

2.4. Statistical Analysis

Excel 2016 was used to sort the test data, and SPSS 20.0 was utilized for statistical analysis. Origin 2019 was used for plotting, and the data in the chart are expressed as average ± standard deviation.

3. Results

3.1. Effects of Different Concentrations of Fatty Acid Esters on the Occurrence of Watermelon Root Border Cells

As indicated in Table 1, DIBP and DOP promoted the increase in the survival rate of RBCs and PME activity of watermelon seeds. The survival rate of RBCs and PME activity reached the maximum value at 0.5 mmol·L^{-1} and 0.1 mmol·L^{-1}, respectively. At high concentrations (1~4 mmol·L^{-1}), the promoting effect was weakened or even inhibited. DIBP and DOP at different concentrations can significantly promote the increase in the number of RBCs in roots. As DIBP concentration increased, the number of RBCs in watermelon root gradually increased, reaching the maximum value at 4 mmol·L^{-1}. By increasing DOP concentration, the number of RBCs increased first and then decreased, and reached the maximum value when the concentration was 0.1 mmol·L^{-1}. These results indicated that an appropriate concentration of PA could enhance the generation of watermelon RBCs.

Table 1. Effects of different concentrations of DIBP and DOP on the production of watermelon root border cells.

Treatment (mmol·L^{-1})		Survival Rate of RBCs (%)	PME Activity (μmol·h^{-1})	Number of RBCs (pcs)
DIBP	0	85.19 ± 1.30 b	0.033 ± 0.003 d	5761.09 ± 257.96 e
	0.05	81.56 ± 2.88 b	0.068 ± 0.005 c	10,920.38 ± 257.96 d
	0.1	82.66 ± 1.35 b	0.147 ± 0.003 a	13,242.03 ± 547.77 c
	0.5	91.76 ± 1.41 a	0.094 ± 0.004 b	16,853.50 ± 1240.12 a
	1	83.80 ± 0.70 b	0.077 ± 0.005 c	15,460.50 ± 1186.62 b
	4	80.5 ± 0.80 b	0.029 ± 0.002 d	17,778.13 ± 694.13 a
DOP	0	85.19 ± 1.30 ab	0.033 ± 0.003 d	5761.09 ± 257.96 d
	0.05	86.78 ± 0.27 ab	0.087 ± 0.002 b	13,465.39 ± 1256.64 a
	0.1	89.45 ± 1.86 ab	0.157 ± 0.003 a	14,904.45 ± 1302.17 a
	0.5	94.51 ± 1.68 a	0.153 ± 0.005 a	13,872.61 ± 1007.67 a
	1	84.8 ± 4.35 ab	0.075 ± 0.003 c	10,401.68 ± 650.57 b
	4	82.45 ± 5.37 b	0.034 ± 0.004 d	8159.23 ± 1004.01 c

Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

3.2. Effects of Exogenous Phthalic Acid on Germination Rate and Root Growth of Watermelon

According to Figure 1, 0.05~0.1 mmol·L^{-1} DIBP and DOP promoted watermelon seed germination rate, while 0.5~4 mmol·L^{-1} DIBP and DOP inhibited seed germination rate. Watermelon root length increased firstly and then decreased with increasing DIBP and DOP concentrations, and reached the maximum value when DIBP and DOP concentrations were 0.1 mmol·L^{-1}, which increased by 23.87% and 31.54%, compared with the controls, respectively. When DIBP and DOP concentrations were in the range of 0.5~4 mmol·L^{-1}, the growth of the watermelon root was inhibited. The results demonstrated that 0.05~0.1 mmol·L^{-1} PA enhanced seed germination and root growth of watermelon.

3.3. Effects of Exogenous Phthalic Acid on Dehydrogenase Activity of Watermelon Roots

As illustrated in Figure 2, watermelon root dehydrogenase activity increased after treatment with different DIBP concentrations than the control. The difference was significant when the concentration was from 0.5 to 4 mmol·L^{-1}, and it reached the maximum value when the concentration was 0.5 mmol·L^{-1}, which significantly increased by 172.07%, compared with the control. Under 0~4 mmol·L^{-1} DOP treatment, the root dehydrogenase activity of watermelon first increased and then decreased, then reached the maximum value at 0.1 mmol·L^{-1} DOP, demonstrating a significant increase of 56.68%, compared with the control. The results revealed that DIBP and DOP in the range of 0.1~0.5 mmol·L^{-1} had the best-promoting effect on watermelon root dehydrogenase activity.

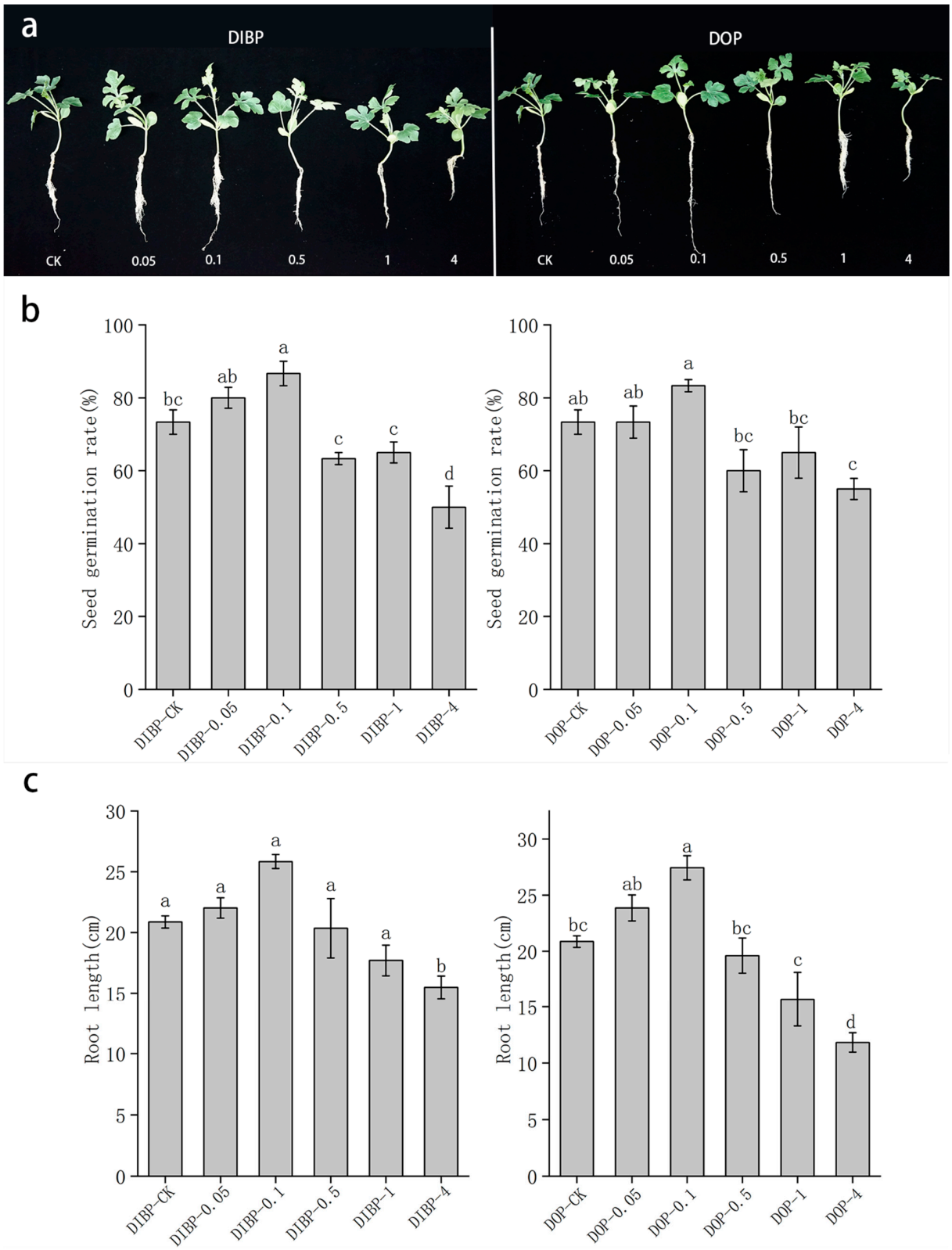

Figure 1. Effects of exogenous PA on seed germination and growth of watermelon: (**a**) phenotypes of plants following different treatments; (**b**,**c**) effects of exogenous PA on seed germination rate and root length of watermelon. Different lowercase letters between different treatments of the same variety indicated a significant difference at a level of 0.05.

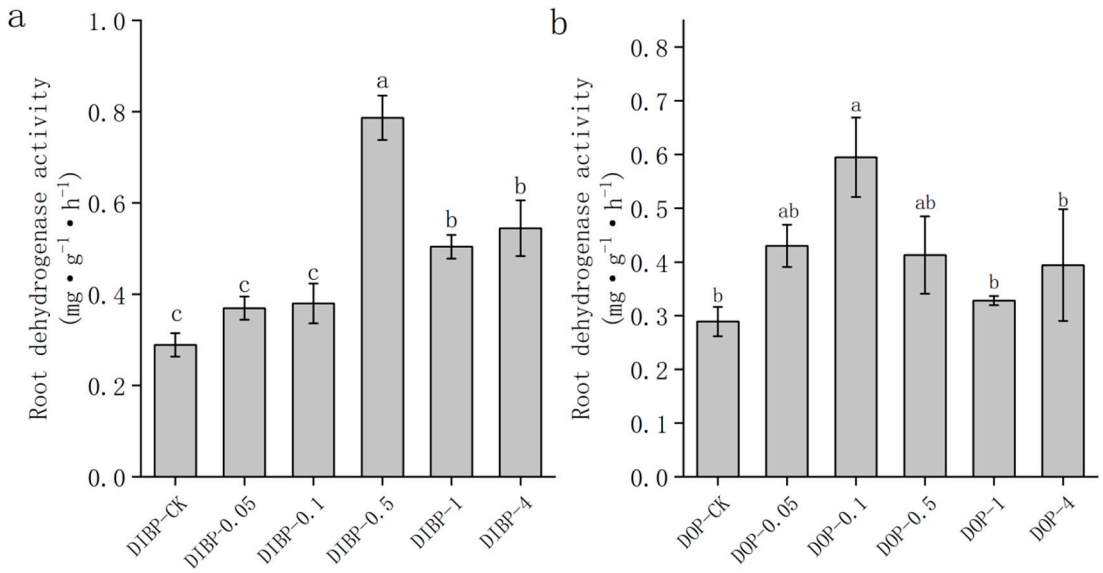

Figure 2. Effects of exogenous PA on dehydrogenase activity in watermelon roots: (**a**) effects of exogenous DIBP on dehydrogenase activity in watermelon roots; (**b**) effects of exogenous DOP in dehydrogenase activity of watermelon roots. Different lowercase letters between different treatments of the same variety indicated a significant difference at a level of 0.05.

3.4. Effects of Exogenous Phthalic Acid on Antioxidant Enzyme Activity in Watermelon Roots

As demonstrated in Table 2, the antioxidant enzyme activities of watermelon roots under two PA treatments showed different changes with increasing treatment concentration. An appropriate concentration of DIBP would enhance the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in watermelon roots. Among them, SOD activity was significantly different at DIBP concentration of 0.5 to 4 mmol·L^{-1}, compared with the controls, and reached the maximum at 0.5 mmol·L^{-1}. POD activity decreased as DIBP concentration increased and reached the maximum value at 0.05 mmol·L^{-1}. DIBP from 0.05 to 4 mmol·L^{-1} improved CAT enzyme activity, reaching a maximum value when the concentration was 0.1 mmol·L^{-1}. Under DOP treatment, SOD, POD, and CAT activities of watermelon seedling roots first increased and then decreased and reached a maximum value at 0.1 mmol·L^{-1}, with significant increases by 327.59%, 12.15%, and 1758.92%, respectively, compared with the control. These results indicated that PA in the range of 0.05~0.1 mmol·L^{-1} exhibited the most obvious promotional effect on antioxidant enzymes in watermelon roots, while a higher concentration of PA (0.5~4 mmol·L^{-1}) reduced its promoting effect or even inhibited it.

Table 2. Effects of exogenous PA on antioxidant enzyme activity in watermelon roots.

Treatment (mmol·L^{-1})		SOD Activity (U·g^{-1} FW)	POD Activity (U·g^{-1} FW)	CAT Activity (U·g^{-1} FW)
DIBP	0	28.41 ± 1.26 c	1296.28 ± 40.48 b	0.17 ± 0.07 c
	0.05	32.38 ± 2.98 c	1460.60 ± 7.45 a	0.88 ± 0.13 b
	0.1	34.62 ± 4.09 c	1387.27 ± 56.41 ab	1.77 ± 0.19 a
	0.5	96.48 ± 2.19 a	1076.11 ± 26.31 c	0.76 ± 0.05 b
	1	77.44 ± 2.74 b	1074.69 ± 27.71 c	1.09 ± 0.11 b
	4	75.06 ± 5.05 b	1015.01 ± 34.49 c	1.00 ± 0.04 b

Table 2. *Cont.*

Treatment (mmol·L^{-1})		SOD Activity (U·g^{-1} FW)	POD Activity (U·g^{-1} FW)	CAT Activity (U·g^{-1} FW)
DOP	0	28.40 ± 1.26 e	1262.95 ± 7.38 bc	0.17 ± 0.01 d
	0.05	58.46 ± 4.99 cd	1312.87 ± 14.36 b	1.45 ± 0.05 c
	0.1	121.44 ± 7.08 a	1417.76 ± 31.07 a	3.33 ± 0.18 a
	0.5	93.01 ± 2.58 b	1210.78 ± 47.16 bcd	2.26 ± 0.17 b
	1	63.85 ± 1.41 c	1196.88 ± 46.29 cd	1.30 ± 0.19 c
	4	46.73 ± 5.52 d	1115.62 ± 20.46 d	1.26 ± 0.05 c

Note: Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

3.5. Effects of Exogenous Phthalic Acid on Malondialdehyde Content in Watermelon Roots

Figure 3 displays that two exogenous PAs increased MDA content. Compared with the control, as DIBP and DOP concentrations increased, MDA content in watermelon roots increased continuously, reaching its maximum at 4 mmol·L^{-1}, demonstrating significant increases by 94.59% and 140.20%, respectively. Furthermore, it is indicated that DIBP and DOP treatments would cause root damage to watermelon, and MDA content was positively correlated with treatment concentration.

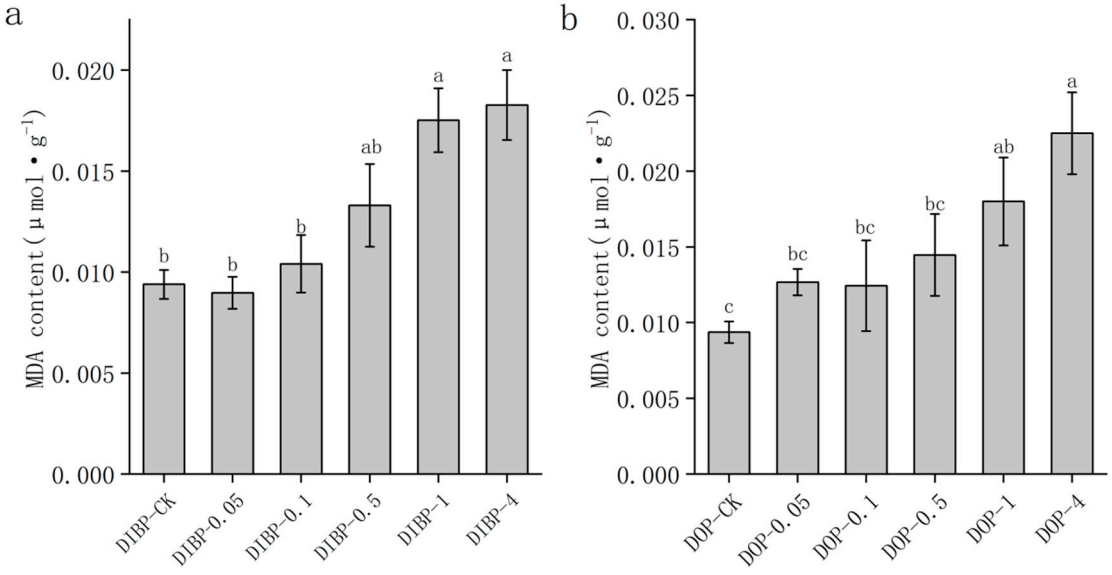

Figure 3. Effects of exogenous PA on MDA content in watermelon roots: (**a**) effects of exogenous DIBP on MDA content in watermelon roots; (**b**) effects of exogenous DOP on MDA content in watermelon roots. Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

3.6. Effects of Exogenous Phthalic Acid on the Soluble Protein Content of Watermelon Roots

Figure 4 indicates two exogenous PAs that increased soluble protein content in the roots of watermelon seedlings. By increasing DIBP and DOP concentrations, the content of soluble protein continued to increase. Among them, 0.5~4 mmol·L^{-1} DIBP significantly promoted the increase in root soluble protein content, which was increased by 63.58%, compared with the controls at 4 mmol·L^{-1}. DOP of 0.05~4 mmol·L^{-1} significantly promoted the increase in soluble protein content, and when DOP was 4 mmol·L^{-1}, it significantly increased by 94.56%, compared with the controls. These results demonstrate that PA promoted the increase in soluble protein content in roots to maintain the osmotic adjustment balance of the watermelon root system.

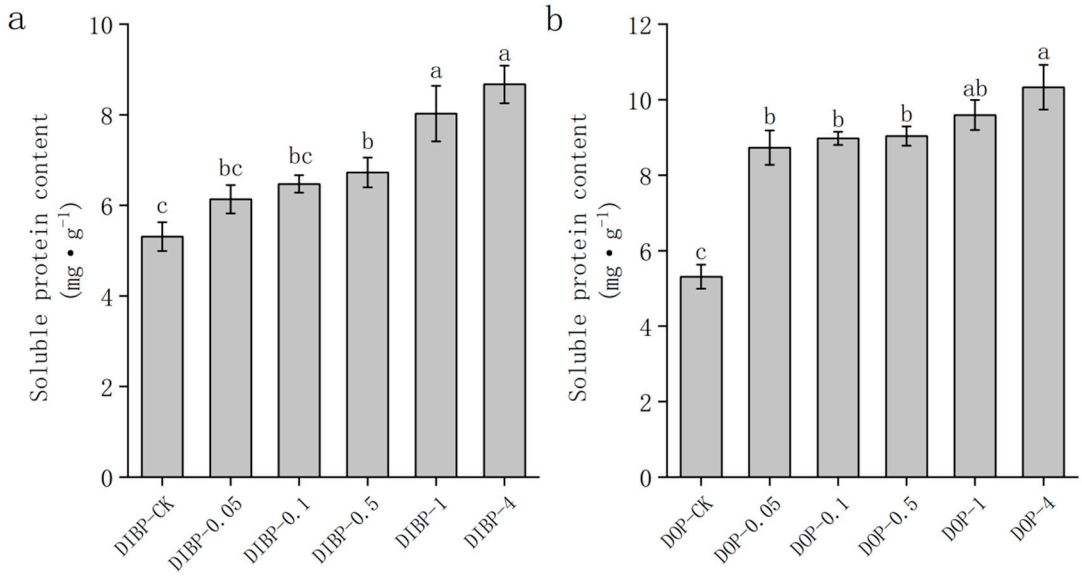

Figure 4. Effects of exogenous PA on the soluble protein content in watermelon roots: (**a**) effects of exogenous DIBP on soluble protein content in watermelon roots; (**b**) effects of exogenous DOP on soluble protein content in watermelon roots. Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

3.7. Effects of Exogenous Phthalic acid on Nitrate Reductase Activity in Watermelon Roots

Figure 5 demonstrates two exogenous PAs that promoted the increase in NR activity in watermelon roots, which first increased and then decreased with increasing treatment concentration. Among them, NR activity in roots was the highest under 0.5 mmol·L^{-1} DIBP treatment, significantly increasing by 49.43%, compared with the controls. NR activity of roots reached its maximum under 0.1 mmol·L^{-1} DOP treatment, significantly increasing by 187.96%, compared with the controls. The results indicated that 0.05~0.5 mmol·L^{-1} PA had the best promotion effect on NR activity in watermelon roots, while the elevated concentration of PA (1~4 mmol·L^{-1}) reduced the promoting effect.

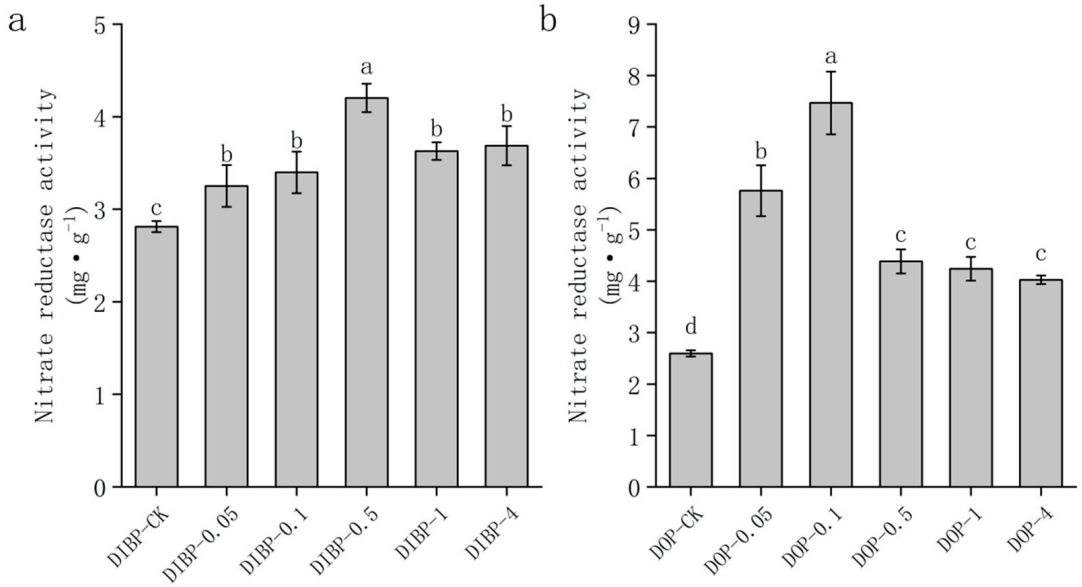

Figure 5. Effects of exogenous PA on nitrate reductase activity in watermelon roots: (**a**) effects of exogenous DIBP on nitrate reductase activity in watermelon roots; (**b**) effects of exogenous DOP on nitrate reductase activity in watermelon roots. Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

3.8. Effects of Exogenous Phthalic Acid on Mineral Content in Watermelon Roots

Table 3 presents two exogenous PAs that affected the absorption of mineral elements by watermelon roots, and different elements showed varying trends. Among them, P, Mg, and Zn first increased and then decreased with increasing DIBP concentration, while K and Fe decreased first and then increased with increasing DIBP concentration; furthermore, Cu consistently decreased with increasing DIBP concentration, while Ca demonstrated no obvious regular change. Under DOP treatment of 0~4 mmol·L^{-1}, K, Ca, Mg, and Cu in watermelon roots increased first and then decreased with increasing DOP concentration, whereas Zn decreased first and then increased with increasing DOP concentration. P increased with increasing DOP concentration, and Fe had no special change pattern. It was proven that different PA concentrations affected the absorption and accumulation of nutrient elements by watermelon roots. The overall performance revealed that low PA concentrations promoted the absorption of nutrient elements by watermelon roots, while high PA concentrations weakened or inhibited the absorption of nutrient elements.

Table 3. Effects of exogenous PA on mineral element content in watermelon root.

Treatment (mmol·L^{-1})		P Content (mg·g^{-1})	K Content (mg·g^{-1})	Ca Content (mg·g^{-1})	Mg Content (mg·g^{-1})	Fe Content (mg·g^{-1})	Cu Content (mg·g^{-1})	Zn Content (mg·g^{-1})
DIBP	0	280.21 ± 22.22 c	100.39 ± 3.06 c	1.65 ± 0.02 cd	0.68 ± 0.04 a	1.38 ± 0.07 bc	3.38 ± 0. 3 b	0.076 ± 0.005 bc
	0.05	395.89 ± 5.32 ab	194.27 ± 4.31 a	2.25 ± 0.01 ab	0.38 ± 0.02 b	1.27 ± 0.06 cd	4.70 ± 0. 04 a	0.087 ± 0.009 ab
	0.1	444.21 ± 7.19 a	161.88 ± 6.15 b	1.56 ± 0.06 d	0.36 ± 0.01 b	1.17 ± 0.04 cd	4.68 ± 0. 1 a	0.099 ± 0.001 a
	0.5	433.52 ± 52.82 a	51.58 ± 8.04 d	2.48 ± 0.22 a	0.61 ± 0.07 a	1.06 ± 0.06 bd	4.28 ± 0. 25 a	0.054 ± 0.002 d
	1	342.86 ± 9.88 bc	142.59 ± 8.81 b	1.98 ± 0.10 bc	0.63 ± 0.07 a	1.45 ± 0.12 b	3.59 ± 0. 13 b	0.061 ± 0.003 cd
	4	348.11 ± 9.77 bc	149.87 ± 7.27 b	1.41 ± 0.09 d	0.57 ± 0.04 a	2.14 ± 0.01 a	2.63 ± 0. 16 c	0.076 ± 0.003 bc
DOP	0	280.21 ± 22.22 c	100.39 ± 3.06 d	1.65 ± 0.01 b	0.68 ± 0.05 a	1.38 ± 0.08 bc	3.38 ± 0. 3 ab	0.076 ± 0.005 ab
	0.05	291.89 ± 2.78 c	183.11 ± 3.28 c	1.65 ± 0.01 b	0.43 ± 0.02 b	1.30 ± 0.06 bc	3.62 ± 0. 24 ab	0.074 ± 0.011 ab
	0.1	380.62 ± 27.68 b	227.78 ± 8.02 ab	2.55 ± 0.34 a	0.48 ± 0.04 b	1.23 ± 0.02 c	3.13 ± 0. 33 ab	0.059 ± 0.005 b
	0.5	377.81 ± 16.90 b	268.34 ± 26.7 a	1.96 ± 0.07 b	0.44 ± 0.05 b	1.58 ± 0.13 b	4.29 ± 0. 05 a	0.093 ± 0.002 a
	1	389.44 ± 17.68 b	217.11 ± 4.62 bc	1.90 ± 0.08 b	0.13 ± 0.02 c	1.47 ± 0.01 c	3.98 ± 0. 59 a	0.096 ± 0.013 a
	4	453.07 ± 1.50 a	193.05 ± 3.60 bc	2.68 ± 0.12 a	0.02 ± 0.01 c	2.08 ± 0.11 a	2.56 ± 0. 16 b	0.099 ± 0.004 a

Note: Different lowercase letters between different treatments of the same variety indicate a significant difference at the 0.05 level.

4. Discussion

As shown in Figure 6, different concentrations of DIBP and DOP treatments showed differences in watermelon morphology, and the watermelon growth was better at 0.05~0.5 mmol·L^{-1} concentration, which had a positive effect. However, at the concentration of 1~4 mmol·L^{-1}, the growth of watermelon was inhibited, which had negative effects. Root border cells (RBCs), also known as abscission root cap cells, are a special group of cells isolated from around the root cap and gathered at the root tip. The existence of RBCs can enhance the ability of plants to resist adversity and enhance the growth of plant roots by increasing the thickness of the adhesive layer [30,31]. RBC production rate is closely related to pectin methylesterase (PME), which can promote pectin decomposition through pectin demethylation, promote the expression of other pectin genes, and eventually, induce the release of RBCs from the root cap [32,33], and also affect the hardness of root cytoderm and regulate the development process of root [34]. In this study, DIBP and DOP promoted the PME activity and the number of RBCs in watermelon roots, and the changing trend of PME activity and RBCs survival rate in watermelon roots first increased and then decreased, indicating a certain correlation. As the root system of plants is the main link between plants and soil, it will directly communicate with allelochemicals in soil, which leads to allelochemicals directly inhibiting the root activity of plants and thus affecting the growth of plant stems and leaves and crop yield. Studies have shown that PA treatment reduces the pepper seed germination rate and germination index, and also inhibits the radicle of mustard of elongation and germ to grow [35,36]. In this study, the two kinds of PA had dual effects on watermelon seed germination, watermelon seedling root activity, and root length, i.e., low concentration promoted seed germination, promoted RBC generation, improved the survival rate of RBCs, and promoted watermelon root activity and root growth, while a higher concentration weakened or even inhibited these promoting effects.

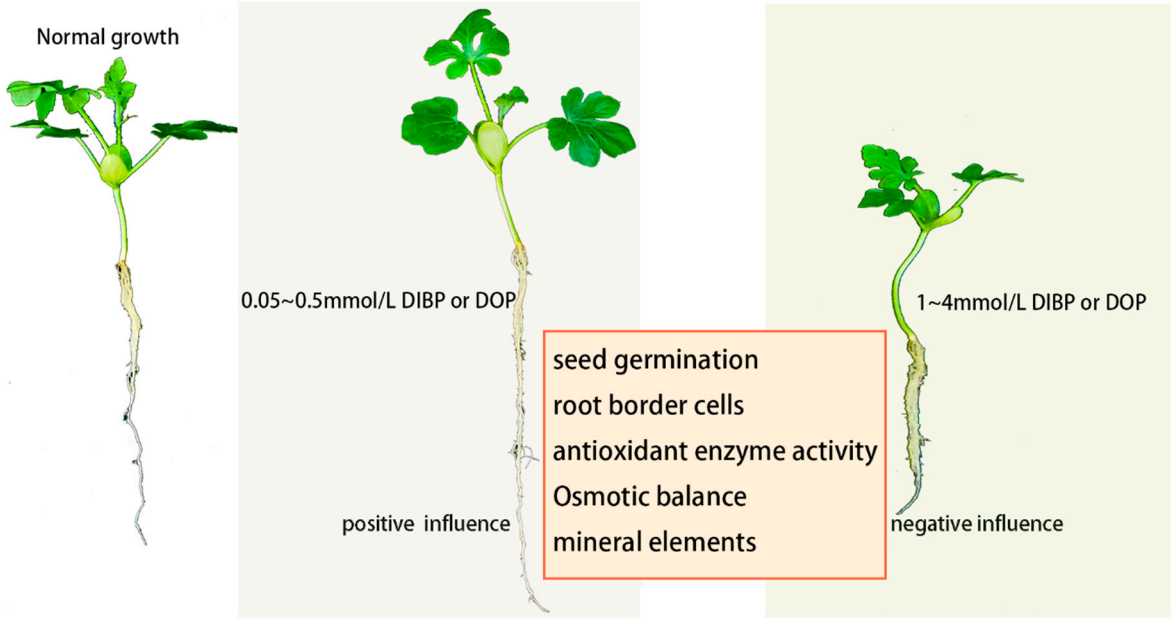

Figure 6. Schematic of the effects of DIBP and DOP on watermelon roots.

When plants encounter adversity stress, their antioxidant defense system is activated to minimize adversity-induced damage [37]. SOD, POD, and CAT play important roles in maintaining the dynamic balance with ROS [38]. If excessive ROS cannot be removed in time, it inevitably causes cell structure destruction and function loss [39]. As an indicator of membrane peroxidation, MDA content refers to the level of membrane peroxidation [40]. Studies have revealed that a low concentration of allelochemicals can increase SOD, POD, and CAT enzyme activities in cucumber, heliotrope, and mung bean roots [41–43]. In addition, there is a certain linear relationship between ROS content in roots with increasing treatment concentration, and a large amount of MDA is produced. In our study, 0.05~0.5 mmol·L^{-1} DIBP and DOP promoted the activities of SOD, POD, and CAT in the root system of watermelon, and could also effectively clear ROS and maintain endosmotic balance to enhance stress resistance. However, with the increase in DIBP and DOP concentrations, the osmotic balance of the cell membrane was damaged, indicating that a high concentration of PA would produce toxic effects, which was consistent with previous results. As a kind of osmotic regulatory substance, soluble protein is critical when plants face stress [44]. In this experiment, the activities of SOD, POD, and CAT in watermelon leaves decreased at the concentration of PA (1~4 mmol·L^{-1}), and the osmotic balance was mainly maintained by synthesizing soluble protein. Nitrate reductase (NR) is the most important rate-limiting enzyme in nitrogen metabolism in plants, directly regulating NO_3^- reduction. The level of its activity is closely related to nitrogen assimilation ability in plants; therefore, NR activity is often used to indicate nitrogen metabolism strength [45]. By increasing DIBP and DOP treatment concentrations, NR activity of watermelon root first increased and then began to decrease. These results indicate that PA treatment promoted the activity of antioxidant enzymes in watermelon roots and enhanced the activity of NR to improve the ability of nitrogen assimilation in watermelon roots, while 1~4 mmol·L^{-1} PA destroyed the antioxidant defense system of root and cause oxidative damage to root cells. Breaking the intracellular osmotic balance and reducing NR activity decreased the absorption of nitrogen nutrients in plants.

Allelochemicals can also affect the absorption of some nutrient elements in plants. For instance, phenolic acids influence the membrane function of plants, increase the permeability of the root cell membrane, and inhibit root activity, thus affecting the absorption of mineral elements and water in plants [46]. Jing et al. found that cucumber root exudates could inhibit root absorption of NO_3^-, SO_4^{2-}, K^+, Ca^{2+}, Mg^{2+} and Fe^{2+} [47], whereas

Guo et al. [48] illustrated that under PA treatment, the content of nutrient elements (N, P, K) in lettuce seedlings decreased by 6.5%~19.5%. In this study, the concentration range between 0.05 and 0.5 mmol·L^{-1} was found to be suitable to promote the absorption and accumulation of P, K, Ca, Cu and Zn in watermelon roots, while high concentrations (1~4 mmol·L^{-1}) weakened or even inhibited the accumulation of K, Ca, Cu and Zn elements. Allelopathy promotes or inhibits ion absorption but also breaks the balance of ion uptake by plant roots, thus perturbing plant nutrient requirement. In maize roots, allelochemicals can affect the Na^+ /K^+ pump activity on the protoplast membrane, thus inhibiting the nitrate absorption and H^+-ATPase activity [49]. Another study also showed that the membrane potential of barley roots is rapidly depolarized after PA treatment [50]. Geng et al. [51] demonstrated that PA treatment increased Ca^{2+}-ATPase activity, effectively regulated Ca^{2+} concentration, and maintained the normal signal transduction function of Ca^{2+} messenger. In this experiment, high concentrations of PA inhibited the uptake of some elements in watermelon roots, which may be caused by exogenous DIBP and DOP inhibiting the ion pump activity on the membrane by affecting target cell membrane sites, resulting in an imbalance of ion exchange on both sides of the membrane. Therefore, absorption and accumulation of mineral elements in roots were inhibited.

5. Conclusions

In conclusion, the application of 0.05~0.5 mmol·L^{-1} DIBP and DOP promoted watermelon seed germination, and increased RBC count and PME enzyme activity, in addition to improving root activity and promoting root growth. Likewise, it improved the activity of antioxidant enzymes, maintained the osmotic balance of cells, and promoted the absorption and accumulation of some nutrient elements in roots. However, high concentrations of DIBP and DOP had toxic effects on watermelon, inhibited root growth, and affected root absorption of nutrient elements. This study contributes to understanding the allelopathic effect of PA on watermelon roots and provides some experimental basis for improving watermelon yields.

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Abbreviations

PA, phthalic acid; DIBP, diisobutyl phthalate; DOP, dioctyl phthalate; PME, pectin methylesterase; RBCs, root border cells; SOD, superoxide enzyme; POD, peroxidase; CAT, catalase; MDA, malondialdehyde; NR, nitrate reductase.

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