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Effects of Abscisic Acid on Physiology and Ecology of Pea Seedlings under Cadmium Stress

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Abstract: The effects of soaking seedlings of four abscisic acid (ABA) concentrations (0, 1, 5, 10, and 20 μmol/L) on physiology and ecology of pea under cadmium stress were studied by the pot experiment. The results showed that the soaking treatment with ABA improved the morphological indexes of pea plants. The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids of pea seedlings were increased compared with the control, and reached a maximum at ABA concentration of 5 μmol/L. Compared with the control, the POD, SOD, CAT activities and soluble protein content of pea seedlings were significantly increased, and the concentration of ABA at 5 μmol/L was the best. Therefore, soaking seeds with ABA could improve the physiological and ecological effects of pea seedlings under Cd stress.

1. Introduction

Soil contamination by heavy metals has become a widespread problem in recent decades because of anthropogenic activity and natural events [1]. Cadmium (Cd) can affect the growth and development of plants [2], leading to the accumulation of reactive oxygen species and the oxidative stress of plants [3-4], which can change the expression and activity of related enzymes in plants [5]. Abscisic acid (ABA) is a “stress hormone” involved in the regulation of stomatal movement, increases of antioxidant enzyme activity and regulation of gene expression, etc [6]. When the plant is under stress conditions, the ABA content in the plant will increase rapidly to enhance the plant's resistance [7-8]. Exogenous ABA also increase the activities of antioxidant enzymes such as SOD, POD and CAT activities in corn seedlings, rice, barley, wheat and *Potamogene crispus* [9], thus increasing resistance to Cd stress. Cd stress has a significant effect on seed germination and seedling growth of peas, reduced antioxidant enzyme activity and physiological metabolic disorders [10]. Therefore, in order to improve the physiology and ecology index of Cd stress peas, the effect of soaking seeds with ABA on the physiology and ecology of Cd stress peas was studied.

2. Materials and Methods

Materials Collection. Pea variety: Cheng Wan 8 (strong growth potential, multiple branches, good stability, and wide adaptability), purchased in Wenjiang District, Chengdu. The fluvo-aquic soil were collected from the farmland was not contaminated by heavy metals at Wenjiang, Chengdu City, Sichuan Province, China.

Experimental Design. The soil was air-dried and passed through a 6.72-mm sieve. 0.5 kg air-dried soil was weighed into each plastic pot (15 cm high, 11 cm in diameter), soaking uniformly by 10 mg/kg Cd (in the form of $CdCl_2 \cdot 2.5H_2O$) solution. Keep the soil moist and place it for 30 days. Tumble the soil to make it evenly mixed. Pea seeds were soaked in 5 concentrations (0, 1, 5, 10, and 20 μmol/L) of ABA for 24 hours, and each treatment was repeated three times. The pea seeds were planted in PVC pots that had been filled with soil and 8 pots were planted in each pot. The planting depth was shallow. Keep moist, place in the culture room, keep the temperature of the culture room around 24°C. After emergence, the PVC pots were moved to shelters in the shelters. Each pot kept 5 seedlings with consistent growth, and watered in time to keep the soil moist.

Date Determination. After 40 d cultivation, the upper mature leaves of pea were collected to determine the photosynthetic pigment, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents and the antioxidant SOD, POD and CAT activities. Determination of plant height, root length, stem base diameter, root base diameter, and root volume by conventional methods. The content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids) was determined by acetone-ethanol mixed (1:1) extraction method. Soluble protein content was determined using Coomassie brilliant blue G250 method. Antioxidant enzyme activity (POD, SOD and CAT) was analysis according to the method of "Plant Physiological and Biochemical Experimental Principles and Techniques" [11].

Statistical Analyses. Statistical analyses were performed using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with Duncan's multiple range test.

3. Results and Discussion

Morphological Index of Pea Seedlings. There were no significant differences in height and root length of pea seedlings after soaking with different concentrations of ABA (Table 1). Compared with the control, the stem base diameter and base diameter increased after ABA soaking treatments. The basal diameter and root diameter increased at first and then decreased with increasing ABA concentration, and reached the maximum at a concentration of 5 μmol/L, which was 12.11% ($p < 0.05$) and 10.45% ($p < 0.05$) higher than that of their respective controls. Thus, compared with the control, seed soaking with ABA could improve the morphological index of pea plants, and the concentration of 5 μmol/L was the best.

Table 1 Morphological index of pea seedlings

ABA concentration (μmol/L)	Plant height (cm)	Root (cm)	Base diameter (cm)	Stem base diameter (cm)
0	23.17±0.22a	15.09±0.65a	0.223±0.005b	0.220±0.006b
1	23.12±0.25a	15.38±0.61a	0.228±0.008b	0.234±0.007ab
5	23.03±0.29a	15.41±0.46a	0.250±0.008a	0.243±0.013a
10	23.06±0.28a	15.10±0.41a	0.234±0.006b	0.237±0.005ab
20	23.13±0.19a	15.15±0.39a	0.226±0.004b	0.229±0.007ab

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

Photosynthetic Pigment Content of Pea Seedlings. Compared with the control, after soaking with ABA, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids of pea seedlings all significantly ($p < 0.05$) increased (Table 2). With the increase of ABA concentration, the chlorophyll *a*, chlorophyll *b*, total chlorophyll content and carotenoid content of pea seedlings increased first and then decreased. The concentration of ABA was 5 μmol/L, the chlorophyll *a*, chlorophyll *b*, total chlorophyll content and carotenoid reached the maximum, increased by 12.89% ($p < 0.05$), 7.53% ($p < 0.05$), 11.20% ($p < 0.05$), and 34.53% ($p < 0.05$), respectively, compared with the control. Compared with the control, the ratio of chlorophyll a/b in the range of ABA concentrations of 1, 5, and 10 μmol/L was relatively increased and reached a maximum at a concentration of 5 μmol/L (Table 2). Therefore, ABA could increase the chlorophyll content of pea seedlings.

Antioxidant Enzyme Activity and Soluble Protein Content of Pea Seedlings. After soaking with different concentrations of ABA, the POD, SOD, CAT activities and soluble protein content of pea seedlings all significantly ($p < 0.05$) increased compared with the control (Table 3). The activities of POD, SOD, CAT and soluble protein in pea seedlings first increased and then decreased with the increase of ABA concentration. The peak value appeared at the concentration of ABA of 5 μmol/L and increased by 49.23% ($p < 0.05$), 84.00% ($p < 0.05$), 59.85% ($p < 0.05$), 96.43% ($p < 0.05$) compared with the respective control. Therefore, soaking seeds with ABA could increase the antioxidant enzyme activity and soluble protein content of pea seedlings, thereby increasing their stress resistance, and the best effect was ABA concentration of 5 μmol/L.

Table 2 Photosynthetic pigment content of pea seedling

ABA concentration (μmol/L)	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
0	1.481±0.004d	0.438±0.006b	1.919±0.009d	3.381	0.362±0.011c
1	1.526±0.005c	0.445±0.005b	1.971±0.004c	3.429	0.403±0.014c
5	1.663±0.003a	0.471±0.004a	2.134±0.007a	3.531	0.487±0.019a
10	1.584±0.003b	0.466±0.004a	2.050±0.006b	3.399	0.479±0.015a
20	1.519±0.007c	0.460±0.005a	1.979±0.007c	3.302	0.411±0.019b

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

Table 3 Antioxidant enzyme activity and soluble protein of pea seedlings

ABA concentration (μmol/L)	POD activity (U/g/min)	SOD activity (U/g)	CAT activity (U/g/min)	Soluble protein content (mg/g)
0	1219.38±13.83e	94.37±4.98d	39.75 ±3.27c	4.536±0.368c
1	1462.87±14.10d	119.31±4.71c	45.58±3.55bc	7.519±0.367b
5	1819.71±24.96a	173.64±3.93a	63.54±3.21a	8.910±0.471a
10	1763.29±13.52b	160.32±6.01b	53.29±4.20b	7.926±0.505b
20	1678.85±11.06c	159.98±7.09b	48.43±3.67b	7.320±0.450b

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

4. Conclusions

Under Cd stress, the different concentrations of ABA soaking treatment improved the morphological indexes of pea seedlings. The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids of pea seedlings were increased compared with the control, and reached a maximum at a concentration of 5 μmol/L. Compared with the control, the antioxidant enzyme activities and soluble protein content of pea seedlings were significantly increased, and the concentration of ABA at 5 μmol/L was the best. Therefore, soaking seeds with ABA could improve the physiological and ecological effects of pea seedlings under Cd stress.

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