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RESEARCH ARTICLE

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Effects of cinnamic acid on the physiological characteristics of cucumber seedlings under salt stress

Abstract Effects of cinnamic acid on the physiological characteristics of cucumber (Shandong Mici) seedlings under salt stress were studied, and the best concentration and treatment time were ascertained. The results showed that cinnamic acid relatively increased the leaf relative water content and the chlorophyll content, decreased plasma membrane permeability, mitigated membrane damage, inhibited the accumulation of malondialdehyde (product of membrane lipid peroxidation), and promoted the activity of membrane protective enzymes such as super oxide dismutase and peroxidase, therefore improving the adaptabilities of cucumber to salt stress. It is concluded that the best treatment time of cinnamic acid is in the two euphylla period, and the best treatment concentration of cinnamic acid is 50 μmol/L.

Keywords salt stress, cucumber, cinnamic acid, physiological characteristics

1 Introduction

Soil salinization has been a widespread problem. It degrades the ecological environment and slows down the development of sustainable agriculture (Flowers and Yeo, 1995). Now, due to the farmers pursuit of high economic benefit, together with improper tillage and fertilization measures, secondary salinization in protected soils is getting serious. Salt stress induces various biochemical and physiological responses in plants. It affects almost all plant functions, including photosynthesis, growth, and development (Skriver and Mundy, 1990; Chen et al., 1994; Holappa and Walker-Simmons, 1995; Wener and Finkelstein, 1995). Cucumber, one of the important vegetables in China and the world, has important economic values. Moreover, in protected cultivation, cucumber is the primary vegetable, so the secondary salinization of protected soils has badly influenced the growth, development, and yield of cucumber (Wang, 1998).

Some scholars reported that some exogenous matters, for example, gibberellin, lycine, spermidine, fatty acid and salicylic acid, etc., can improve plant salt tolerance. Salicylic acid is especially being studied by scholars under the condition of salt stress, and has been found able to alleviate salinity injury in the course of cucumber seed germination and seedling growth (Peng et al., 2003; She et al., 2002; Zhang and Gao, 1999; Zhang et al., 1998). Lower concentration of phydroxy benzoic and cinnamic acids improved the growth of cucumber seedling, but higher concentration inhibited it (Wu, 2002). Salicylic acid is of cinnamon acid (cinnamic acid) ramification; however, we know very little about cinnamic acid in its improvement of plant resistance.

This test firstly studied the catabatic effects of salicylic ramification-cinnamic acid on cucumber seedlings under salt stress; and ascertained the best concentration and the best time of being treated. This is important for knowing the physiological action of cinnamic acid better, and for the application in its inducing of salt resistance of cucumber seedlings. Our study also provides a theoretical basis for harnessing soil salinization in protected cultivation.

2 Materials and methods

2.1 Materials

The cucumber variety 'Shandong Mici' was used in this study and cinnamic acid was supplied by Beijing Yuanpinghao Biological Technology.

2.2 Cultivation and treatments

Cucumber seeds were germinated on filter paper and kept at 27°C for six days in the dark. One week after germination, cucumber seedlings were transplanted into a nutrition bowl with vermiculite, and the culture solution was Hoagland that was replaced every four days. The cinnamic acid treatment was applied by adding cinnamic acid to the nutrient

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solution to reach a concentration of 25 μmol/L, 50 μmol/L, and 100 μmol/L in the first, second, third, and fourth euphylla periods, respectively. After cinnamic acid treatment in the fourth euphylla period, the salinity treatment was applied by adding NaCl to the nutrient solution to reach a concentration of 200 mmol/L. After nine days, physiological indexes of cucumber (Shandong Mici) seedlings under salt stress were determined. A nutrient solution with NaCl but without cinnamic acid served as a control treatment (CK1), a NaCl- and cinnamic acid-free nutrient solution served as another control treatment (CK2). The plants of both treatments were randomly arranged in the greenhouse maintaining at 28°C in the daytime and 20°C at night. Three replications for each treatment were assigned randomly. Seedlings thus obtained were used for the following experiments as shown in Table 1.

Table 1 Cinnamic acid treatment time, concentration and code in test

Treatment time	Cinnamic acid (B) concentration/μmol · L^{-1}		
	25	50	100
The first euphylla priod	B11	B12	B13
The second euphylla period	B21	B22	B23
The third euphylla period	B31	B32	B33
The fourth euphylla period	B41	B42	B43

2.3 Measurement items and methods

The water content of cucumber seedlings was measured by oven drying method; the chlorophyll content was determined directly in yield with Chlorophyll Content Indicator (SPAD-502); relative conductance was measured with conductometry; super oxide dismutase (SOD) activity was measured with NBT colorimetry; Peroxidase (POD) activity was measured with guaiacol colorimetry; determination of Malondialdehyde (MDA) was conducted with Barbituric acid colorimetry. All of these items were determined according to the method of Hao et al. (2002).

3 Results

3.1 Effects of cinnamic acid on water content and the chlorophyll content of cucumber seedlings under salt stress

From Table 2 we know that the water content of treatment CK2 was 843.5 mg/g, but 733.0 mg/g under salt stress (CK1), the water content of every treatment with cinnamic acid was higher than that of CK1 showing that cinnamic acid could increase the osmotic adjustment ability, promote the absorbability of cucumber seedling to water, raise the adaptabilities of cucumber to salt stress, and accordingly alleviate water stress from salinity injury. The treatment of B22 was better than the others, its water content reached 812.9 mg/g, very close to that of CK2, and only 3.62% lower compared with that of CK2.

Table 2 Effects of cinnamic acid on water content and chlorophyll content of cucumber seedlings under salt stress

Treatment	Water content in leaf /mg · g^{-1}	Increased or decreased value / ±Δ%	Chlorophyll content /mg · g^{-1}	Increased or decreased value / ±Δ%
CK1	733.0	−13.10	228.9	−33.90
CK2	843.5	—	346.3	—
B11	749.0	−11.20	247.0	−28.67
B12	773.4	−8.31	269.6	−22.15
B13	768.2	−8.92	226.6	−34.57
B21	772.3	−8.44	225.0	−35.03
B22	812.9	−3.62	282.5	−18.42
B23	773.8	−8.26	290.0	−16.26
B31	759.4	−9.97	235.3	−32.05
B32	744.5	−11.73	258.0	−25.50
B33	742.3	−11.99	255.3	−26.28
B41	760.1	−9.89	263.6	−23.88
B42	755.6	−10.42	279.2	−19.38
B43	773.6	−8.29	253.6	−26.77

Table 2 also shows that chlorophyll content of cucumber seedlings when salt stressed, decreased, but all the treatments with cinnamic acid, except for B13 and B21, had higher chlorophyll content than that of CK1. The chlorophyll contents of B22 and B23 (50 μmol/L and 100 μmol/L) were closer to that of CK2, with values of 282.5 mg/g and 290.0 mg/g, respectively, only 16.26% and 18.42% lower than that of CK2.

3.2 Effects of cinnamic acid on the activities of membrane protective enzymes and plasma membrane permeability of cucumber seedlings under salt stress

Table 3 shows the effects of cinnamic acid on the activities of membrane protective enzymes and plasma membrane permeability of cucumber seedlings under salt stress. From Table 3, we can find that the activities of membrane protective enzymes of every treatment were higher than that of CK1 and CK2 after 9 days of salt stress, indicating that salt stress aroused reactive oxygen species or peroxide-free radicals, damaged membrane protective system in cells. SOD and POD were general membrane protective enzymes in aerobic biology and their activities were related to plant resistance. In this study, cinnamic acid was added at different times, and activities of the two enzymes all increased. SOD and POD activity of B22 were significantly higher than those of the others. Although the treatment with cinnamic acid at a concentration of 100 μmol/L also alleviated salinity injury, its effect was not significant compared to that of 50 μmol/L, indicating that higher concentration of cinnamic acid could alleviate salinity injury, but also had inhibition effects at the same time.

When not salt stressed, the growth of cucumber seedlings was found normal, plasma membrane relative conductance was 15.32%; but under salt stress, its growth was weaker, leaf color changed to yellow, and plasma membrane relative conductance was 77.39%. Treatment at different times and at different concentrations of cinnamic acid decreased relative conductance, the B22 treatment was the best treatment,

Table 3 Effects of cinnamic acid on the activities of membrane protective enzymes and plasma membrane permeability of cucumber seedlings under salt stress

Treatment	SOD/U·g^{-1}	POD/(OD·g^{-1}·h^{-1})	REC/ %	MDA/(μmol·g^{-1})
CK1	38.25±3.11Ce	0.43±0.04 Ik	77.39±1.93 Aa	2.26±0.13 Aa
CK2	29.34±2.79Dd	0.82±0.04 Hj	15.32±0.95 Fe	0.30±0.04 Gg
B11	66.32±2.49 Aab	1.12±0.04 EFG	69.32±1.39 CDEcd	0.69±0.06 Ee
B12	66.18±2.18 Aab	1.45±0.06 B	67.23±1.74DEd	0.28±0.05 Gg
B13	57.34±0.34 Bc	1.12±0.07 EFG	74.14±1.44 ABab	0.79±0.04 DEd
B21	66.22±0.95 Aab	1.33±0.06 CD	69.54±3.50 CDEcd	0.51±0.06 Ff
B22	69.33±2.46 Aa	1.59±0.07 A	66.33±2.25 Ed	0.26±0.06 Gg
B23	57.36±2.61 Bc	1.10±0.07 FG	72.14±1.41 BCbc	0.88±0.06 CDc
B31	66.27±1.74 Aab	1.20±0.07 EF	69.34±0.94 CDEcd	0.49±0.06 Ff
B32	66.15±2.08 Aab	1.40±0.07 BC	67.25±2.12 DEd	0.32±0.01 Gg
B33	57.27±0.64 Bc	1.26±0.05 DE	72.23±2.01 BCbc	1.03±0.03 Bb
B41	66.18±1.59 Aab	1.02±0.04 G	71.23±1.74 BCDbc	0.35±0.04 Gg
B42	65.23±2.06 Ab	1.22±0.08 DEF	67.43±1.93 DEd	0.29±0.07 Gg
B43	56.34±4.25 Bc	1.13±0.06 EFG	77.51±1.85 Aa	0.95±0.05 BCbc

Notes: Capital letter stands for 0.01 level; Small letter stands for 0.05 level.

repairing ability to plasma membrane permeability was also stronger, and its relative conductance was significantly higher than that of any others.

The variation of plasma membrane permeability was related to membrane lipid peroxidation. MDA was a product of membrane lipid peroxidation, and its content could reflect the membrane lipid peroxidation degree. From Table 3 we know that, under salt stress, MDA content in cucumber leaves was 2.26. After treatment with cinnamic acid, the accumulation of MDA was reduced. The mitigative effect of B22 was better than anyone of the others at a significant level ($P<0.05$).

3.3 Suitable concentration and correct time for cinnamic acid treatment

Salt stress inhibited the growth of cucumber seedlings, decreased the chlorophyll content, damaged the plasma membrane permeability and integrality, and messed up the membrane protective enzymes system. Cinnamic acid could alleviate salinity injury to the growth of cucumber seedlings. From the results of Table 2 and Table 3 we know that cinnamic acid relatively increased the leaf relative water content and the chlorophyll content, decreased the plasma membrane permeability, mitigated the membrane damage, inhibited the accumulation of MDA, and promoted the activity of membrane protective enzymes such as SOD and POD. The mitigative effect of B22 was the best, therefore, the best treatment time of cinnamic acid is in the period of two euphylla and its best concentration is at 50 μmol/L.

4 Conclusions and discussion

Salicylic acid and analog can induce plant resistance to salt, alleviate salinity injury, and raise the adaptabilities of cucumber seedlings to salt stress (Zhang and Gao, 1999; Gaffney et al., 1993; Pean, 1993). Wu (2002) reported that lower concentration of phydroxy benzoic and cinnamic acids improved the growth of cucumber seedlings, but higher concentration inhibited it. Wang and Li (2003) treated watermelon seedlings with cinnamon acid and benzoic acid, and indicated that effects of cinnamon acid and benzoic acid were related to their concentrations; lower concentration of cinnamon acid and benzoic acid increased the activity of POD, and inhibited the watermelon fusarium wilt. But with the increasing of their concentrations, their promoting effect changed to inhibition.

Water status of plants is correlated with growth directly. Under salt stress, cinnamic acid can increase the water content of cucumber seedlings, which is beneficial to the growth of young cucumber seedlings.

Environmental stress often damages the plant organic membrane permeability and integrality, induces all kinds of water-solubility matters including electrolyte exuded, and also increases the plant conductance. Therefore, according to the relative conductance, we can understand the damaged degrees. Membrane lipid peroxidation often happens in the course of plant organ aging and environmental stressing. MDA is the product of membrane lipid peroxidation. Its content could reflect the degree of membrane lipid peroxidation (Greenway, 1980). In our test, the MDA content in cucumber seedlings under salt stress was higher than that in normal cucumber seedlings, cinnamic acid decreased the MDA content in cucumber seedlings under salt stress, and the mitigative effect was obvious.

Results of our study proved that cinnamic acid can relatively increase the leaf relative water content and the chlorophyll content, decrease the plasma membrane permeability, mitigate membrane damage, inhibit the membrane lipid peroxidation, promote the activity of membrane protective enzymes such as SOD and POD, and maintain the integrality of membrane system so as to improve the metabolism of cells, and alleviate the salinity inhibition to the growth of cucumber seedlings.

Further studies on the detailed mechanism of cinnamic acid in alleviating salinity injury and applying methods in cucumber production need to be carried out in the future.

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