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Regulation of Salicylic Acid on Polyamine Synthesize under NaCl Stress in Leaves of the Yali Pear

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Abstract: The regulation of SA to the changes of polyamine metabolism under salt stress in leaves of Yali Pear were studied. In order to reduce environmental effects on results, leaf disk tests were first used to confirm whether the salicylic acid affected the metabolism of polyamines. The results show that in the leaf disk experiment, exogenous salicylic acid changes the endogenous polyamine levels and different SA concentrations have different effects. Under the NaCI treatment the ADC activity increased significantly, but the ODC activity did not have a significant increase and was lower than ADC activity. Polyamine contents increased under the NaCI treatment at the low concentrations and did not increase or decrease with the NaCl concentration increasing. SA increased ODC activity in the control and NaCI treatment, did not significantly affect ADC activity, but increased the free polyamine contents under NaCI treatment.

Keywords: ADC, NaCl stress, ODC, polyamines, salicylic acid

INTRODUCTION

Saline-alkali soil is widely distributed in the world, there is a serious phenomenon of soil salinization. Salt stress will seriously inhibit the growth of crops, reduce crop yields. The polyamines are a kind of low molecular fatty group nitrogenous base and they were reported to have various effects on germination, blossoming, fruit setting, differentiation of flower bud, sex expression, fruit growth, root system formation and resistance. With in-depth study of plant stress physiology, it was discovered that plants have undergone a variety of stress conditions with the accumulation of polyamines. Putrescine (Putrescine, Put), Spermidine (Spermidine, Spd) and Spermine (Spermine, Spm) are the three most common polyamines and are closely related to environmental stress. The use of exogenous growth regulators or compounds to improve plant salt tolerance is a commonly used method. Salicylic Acid (SA) and polyamines are endogenous signal members in plants. They have important physiological functions in plants, such as flowering, synthesis, organ differentiation, membrane stability and endogenous hormone balance. She Xiaoping found that (She *et al.*, 2002), under salt stress salicylic acid can increase seedling relative water content and lower Na^+, K^+ upward transportation choice, ease salt stress on seedling growth inhibition.

In order to study the regulation of SA to the changes of polyamine metabolism under salt stress, pear branches was used as test materials cultured with different nutrient solution concentrations of NaCl. Through this experiment, the role of salt stress relief of salicylic acid were studied and the SA biochemistry mechanism in fruit trees will be clearer and it is good for studies in salicylic acid and polyamine signal extension at the molecular level.

MATERIALS AND METHODS

Materials: Current-year branch of Yali Pear

Methods:

Leaf disks culture: Yali pear leaves were picked from the orchard inside the west campus of Agricultural University of Hebei. The surface was first wiped for dust with a clean gauze, then the main vein was removed. Round disks with a diameter of 1 cm were made and cultured at room temperature with 20, 2, 0.2 and 0.02 mmol/L, respectively exogenous salicylic acid in a petri dish. Processing times were 2, 4, 6, 8, 10 and 12 h, respectively. Then the leaf disks were cleaned with distilled water and dried with filter paper and then were quick-frozen with liquid nitrogen and stored in a low temperature refrigerator at -70ºC.

Branch culture: Branches were cultured with l/2 Hoagland's nutrient solution containing 0, 20, 50, 100, 150, 200, 300 nmol/L NaCI, respectively inside, taken out to be measured after 5 days treatment.

The activities of Arginine decarboxylase (Arginine Decarboxylase, ADC) and ornithine decarboxylase

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(Ornithine Decarboxylase, ODC) were measured according to Zhao Fugeng method (Zhao, 2000), polyamine content was determined according to Yang Jun (He *et al.*, 1988) and Zhao Fugeng method (Zhao, 2000). Each treatment repeated three times and test data were analyzed using the spss software package.

RESULTS AND ANALYSIS

Effect of exogenous salicylic acid on put content: It can be seen from Fig. 1 that, the SA 0.02 mmol/L treatment increased content of endogenous Put. Put content achieved a peak of 4.28 μmol/gFW after 2 h and was significantly higher than the control, 133.1% of control. Four hours after treatment, Put content was significantly higher than the control, 120.5% of control, but after 6 h, there were no significant differences to the control. After the SA 0.2 mmol/L treatment, endogenous Put concentration increased. After 6 h, it achieved a peak of 4.01 μmol/gFW and was significantly higher than the control, 119.7% of control. After 6 h, Put content decreased with no significant difference to the control. After 2 h of the SA 2 mmol/L treatment, the Put content reached a peak of 3.99 μmol/gFW, significantly higher than the control, 118.1% of control and decreased after 2 h with no significant difference to the control. After the SA 20 mmol/L treatment, endogenous Put concentration for the whole period was significantly lower than the control.

Effect of exogenous ssalicylic acid on spd content: From Fig. 2 it can be seen that, after the SA 0.02 mmol/L treatment, endogenous Spd content increased after 6 h achieving a peak of 3.52 μmol/gFW. This was significantly higher than the control, 122.2% of control. After the SA 0.02 mmol/L treatment, the Spd content reached a peak of 3.12 μmol/gFW, significantly higher than control and then decreased after 2 h. After 4 h, there was no significant difference to the control. After the SA 20 mmol/L treatment, the endogenous Spd content reached 1.52 μmol/gFW which was 52.8% of the control.

Effect of exogenous salicylic acid on spm content: It can be seen from Fig. 3 that, after the SA 0.02 mmol/L treatment, endogenous Spm content for the whole period was significantly higher than the control. After the SA 0.2 mmol/L treatment, endogenous Spm content increased after 2 h reaching 5.32 μmol/gFW, significantly higher than the control, 116.2% of control. After 6 h, a peak of 6.52 μmol/gFW was reached, significantly higher than the control. After the SA 2 mmol/L treatment, Spm content reached a peak of 6.05 μmol/gFW, significantly higher than the control, 132.1% of control. After 4 h, it was also significantly higher than the control. Four hours later, there was no significant difference to the control. After the SA 20 mmol/L treatment, endogenous Spm content

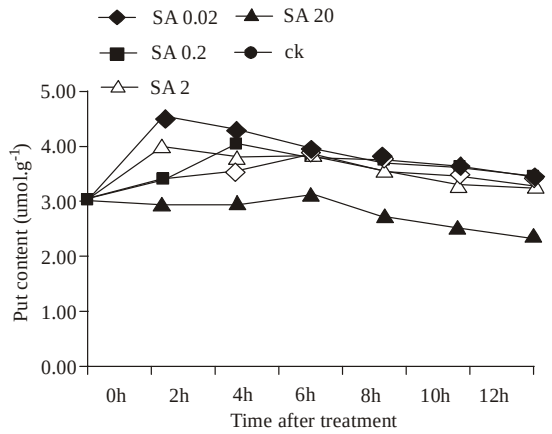

Fig. 1: Effects of salicylic acid on put in detached leaves of Yali Pear

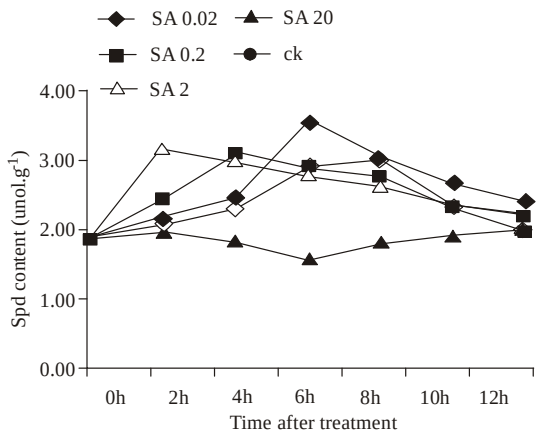

Fig. 2: Effects of salicylic acid on Spd in detached leaves of Yali Pear

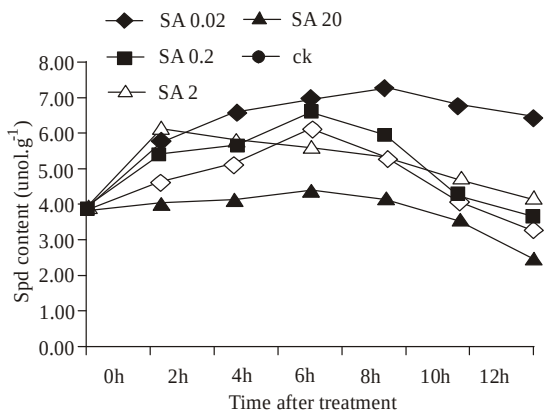

Fig. 3: Effects of salicylic acid on Spm in detached leaves of Yali Pear

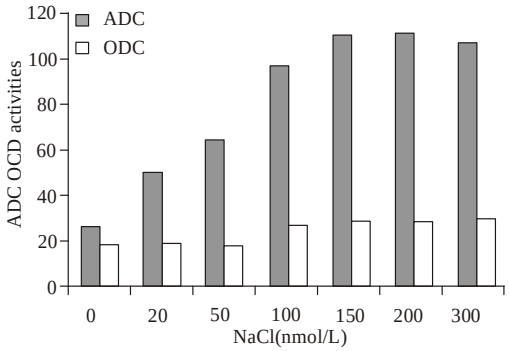

Fig. 4: Effects of NaCI on ADC, ODC in leaves of Yali Pear

Table 1: Changes of free polyamines level under NaCl stress

	NaCI (mmol/L)				
Pa nmol/L	0	20	50	100	150
Put1	14.2±4.5	129.2±6.0	135.4±6.5	230.5±12.2	330.6±16.2
Spm	90.5±4.2	94.4±5.3	100.3±4.2	91.3±3.6	80.2±4.2
Spd	94.3±6.2	121.5±7.4	104.4±8.6	64.4±4.8	52.4±4.6

Table 2: Changes of conjugated polyamines level under NaCl stress

	NaCI (mmol/L)				
PA nmol/L	0	20	50	100	150
Put	79.3±3.6	109.6±6.3	110.5±5.7	57.2±2.4	27.1±2.1
Spm	73.2±3.1	77.2±2.4	72.1±3.5	65.3±3.0	54.3±3.1
Spd	64.7±2.1	89.5±2.9	60.0±2.6	55.4±2.8	41.7±3.7

followed the same trend as the control, but was significantly lower than the control.

The changes of polyamine metabolism under salt stress: Arginine decarboxylase is the key enzyme when Arginine transformate to Put. Figure 4 showed that, as the NaCI concentration rise the ADC activity also rise, when salt concentration was too high (200 and 300 nmol/L) the ADC activity did not increase any more. Ornithine decarboxylase is the key enzyme when Ornithine transformate to Put. The ODC activity was markedly lower than ADC activity and did not change significantly after the NaCI treatment.

ADC activity and ODC activity, 0, 20, 50, 100, 150 nmol/L NaCI-treated leaves were selected to do the determination of polyamines respectively. Table 1 showed that when the NaCI treatment concentration increased, the free Put levels enhanced. Low concentrations of NaCI treatment significantly increased free Spd content, but when NaCI concentration was over 100 nmol/L, free Spd content decreased. The Spm content didn't change significantly after NaCI treatment.

Table 2 showed that polymer-bound polyamine were detected in the leaves corresponding to the same types of free polyamines. Conjugated Spm level did not change significantly before and after the salt treatment, but decreased slightly when the salt concentration increased. Conjugated Put level and Conjugated Spd level were

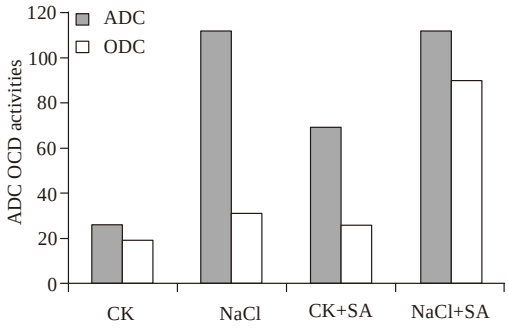

Fig. 5: Effects of SA on ADC and ODC under NaCI treatment

significantly increased at low salt concentrations and decreased rapidly at high salt concentrations. The total polymer-bound polyamine levels increased at low salt concentrations and decreased significantly at high salt concentrations.

The regulation of salicylic acid to polyamine metabolism under salt stress: Branches were cultured with l/2 Hoagland's nutrient solution containing 150 nmol/L and at the same time sprayed with 2 mmol/L SA (spraying once at each morning and evening), taken out to be measured after 5 days treatment.

Figure 5 showed that, ADC activity was significantly increased after 150 mmol/L NaCI stress, ODC activity increased slightly. Salicylic acid treatment significantly increased ADC activity, but had no significant effect on ODC activity. Salicylic acid significantly increased ODC activity after the salt treatment but had no effect on ADC activity.

Table 3 showed that, free Put content was significantly increased under 150 mmol/L NaCI stress and was higher than that of the control, but Spm level and Spd level decreased slightly. Free polyamine levels also increased after salicylic acid treatment, but did not change significantly. Salicylic acid had a significantly regulation of free polyamine content under salt treatment, the free Put content increased, more than three times the control, free Spm content and Spd content also had a significant increase of nearly 30%.

Table 4 showed that, conjugated polyamine content was significantly decreased under 150 mmol/L NaCI stress, but salicylic acid treatment increased Put content, Spm content and Spd content did not change significantly. Salicylic acid had a significantly regulation of conjugated polyamine content under salt treatment, conjugated Put content increased significantly, at a ratio of 164.5% of the control, conjugated Spm content and conjugated Spd content were significantly increased at the ratio of 26-40%.

Tale 3: Changes of free polyamines level

	Treatments			
PA (nmol/L)	ck	NaCI	Ck+SA	NaCI+SA
Put	114.2±4.5	330.6±16.2	135.4±6.5	375.5±12.2
Spm	90.5±4.2	80.2±4.2	100.3±4.2	121.3±3.6
Spd	94.3±6.2	52.4±4.6	104.4±8.6	114.4±4.8

Table 4: Changes of band polyamines level

	Treatments			
PA (nmol/L)	ck	NaCI	Ck+SA	NaCI+SA
Put	79.3±3.6	27.1±2.1	109.6±6.3	130.5±5.7
Spm	73.2±3.1	54.3±3.1	77.2±2.49	2.1±3.5
Spd	64.7±2.1	41.7±3.7	89.5±2.99	0.0±2.6

DISCUSSION

Polyamines, mainly including Putrescine (Put), Spermidine (Spd), Spermine (Spm), are ubiquitous low molecular aliphatic amines generated in organism metabolism, play an important role after the modification in protein synthesis (Wang and Zhang, 1998). Polyamine accumulation occurs under a variety of stress conditions (Zhou *et al.*, 2010; Wang *et al.*, 2010; Chun *et al.*, 2010; APTEPV, 1982; Hejx *et al.*, 1998). In this test, exogenous salicylic acid was found to significantly improve the content of endogenous polyamines in leaves of the Yali pear.

It is generally believed that the increase in free Put content under stress conditions was due to the increase of ADC activity, ODC is not involved in the reaction. In this study ADC activity was significantly increased after salt treatment and ODC activity did not change significantly, which indicate that leaves the ADC is a key enzyme of free Put synthesis, the ADC is more sensitive to salt stress than ODC.

Free Put level significantly increased after salt treatment and stopped increasing when the salt concentration is too high, while free Put level kept an upward trend, which show that the accumulation of free Put has two ways, one way is synthesis catalyzed by ADC and the other way is that PAO can degrade cell-bound Spd to generate free Put which has been proved by Federico and Angelini (1991) and Santanen and Liisa (1994).

SA improved the ODC activity both in the leaves of control and salt treatment, but had no significant effect on ADC activity, that indicate that ADC activity under NaCI stress is not sensitive to environmental stress induced by the SA. SA treatment can significantly improve the free polyamine content under NaCI treatment, but increase of the free Put content was significantly lower than that of the control, free Spd content increase is higher than that of the control, which show that the SA regulation of salt stress is to promote the transformation from Put to Spd.

CONCLUSION

SA has a regulating role of polyamine metabolism under salt stress, it eased the damage to pear leaves. Exogenous salicylic acid was found to significantly improve the content of endogenous polyamines in leaves of the Yali pear.

ADC activity was significantly increased after salt treatment and ODC activity did not change significantly, the ADC is a key enzyme of free Put synthesis, the ADC is more sensitive to salt stress than ODC.

SA improved the ODC activity both in the leaves of control and salt treatment, but had no significant effect on ADC activity. ADC activity under NaCI stress is not sensitive to environmental stress induced by the SA.

SA can ease the damage to pear is because it regulate polyamine metabolism by promoting the transformation from Put to Spd under the salt stress.

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