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RESEARCH ARTICLE

Hassan Imran, ZHANG Yuxing, DU Guoqiang, WANG Guoying, ZHANG Jianghong

Effect of salicylic acid (SA) on delaying fruit senescence of Huang Kum pear

Abstract This experiment was undertaken to explore the effect of salicylic acid (SA) at different concentrations on regulating fruit senescence of Huang Kum pear. Through dipping fruits and fruit discs for a series of hours in SA solution, enzyme activities and physiological characteristics of Huang Kum pear were determined. The results revealed that SA enhanced the activity of superoxide dismutase (SOD) and peroxidase (POD) enzymes at 0.02 mmol/L and at 0.002 mmol/L with the treatment of dipping fruit discs for 4 h and 12 h, respectively. The malondialdehyde (MDA) contents were reduced at 0.002 mmol/L for 12 h, and water loss ratio was decreased at 0.5 mmol/L after 48 h of treatment. It was concluded that SA at lower concentrations could delay the senescence of Huang Kum pear fruit.

Keywords salicylic acid, enzyme activity, physiological characteristics, Huang Kum pear, fruit senescence

1 Introduction

Huang Kum pear (*Pyrus pyrifolia* Nakai) is a new variety of Rosaceae family, which was introduced from South Korea to China (Tartarini and Sansavini, 2003). The fruit is famous for its high quality such as smooth surface and good flavor. The cultivated area in Hebei Province and China was 219.1 and 1 042.4 thousand hectares (khm^2) and the fruit production was 255.2 and 930.9 metric tonnes (Mt), respectively. Hebei Province contributed 22.9% of the total production in China in 2003 (Ministry of Agriculture, China, 2003). It has a high potential to develop Huang Kum pear production in the future. Many studies have proved that salicylic acid (SA) is a kind of phytohormone, and also associated with signaling and disease resistance (Raskin, 1992; Klessig and Malamy, 1994; Tian et al., 2001; Zhang et al., 2002). It has been reported that SA is widely distributed in plants (Tian and Zhang, 2001; Hou et al., 2002; Tian et al., 2002). However, the relationship between SA and fruit senescence is not yet elaborated. This study was to explore the effect of SA on fruit senescence of Huang Kum pear so as to give an evidence of SA application on pear production.

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Hassan Imran, ZHANG Yuxing (✉), DU Guoqiang, WANG Guoying, ZHANG Jianghong
College of Horticulture, Agricultural University of Hebei, Baoding 071001, China
E-mail: jonsonzhyx@yahoo.com.cn

2 Materials and methods

This experiment was carried out at College of Horticulture, Agricultural University of Hebei (AUH), Baoding City, China from 2003 to 2004.

2.1 Materials

Fruit samples of "Asian" and "Huang Kum" pear were collected at the experimental farm of horticulture plants of AUH, China.

2.2 Methods

2.2.1 Fruit disc dipping

Fruit discs were prepared with cork borer and refrigerated at 0–5°C for further use. The SA solutions were prepared to have different concentrations viz. 0.002, 0.02, 0.2, 2.0 mmol/L. Fruits dipped in distilled water were considered as control (CK). Fruit discs were immersed immediately in the SA solution at different concentrations for 4, 12 and 24 h, respectively. Then the samples were rinsed with distilled water (DW) and were frozen at −70°C for enzyme activity analysis using electrical balance, centrifuge machine and spectrophotometer. Data were collected with three replications and their mean values for each treatment of enzyme activity were measured.

Superoxide dismutase (SOD) activity was recorded by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) as according to Dhindsa et al.

(1981). Fruit discs weighing 1.0 g after being treated with SA was homogenized in 4.0 mL of 0.01 mol/L phosphate buffer at pH 7.8, and were grinded in a grinding pot, then were centrifuged at 10 000 r/min for 20 min at 4°C. The 4 mL reaction mixture contained 0.1 mol/L phosphate buffer at pH 7.8, 104 mmol/L Methionine, 0.3 mol/L EDTA, NBT 300 μmol/L, 200 μL enzyme, and 320 mmol/L Riboflavin. Then the tubes were shaken and kept 30 min under the light of two 15 Watt fluorescent lamps for 15 min. The reaction process was terminated by covering the tubes with a piece of black cloth. Absorbance was measured at 560 nm with spectrophotometer as according to Tang and Zhang (2002). The color of reaction mixture decreased with the increase of enzyme extraction. The 50% inhibition of the reaction of enzyme extract was recorded as one enzyme unit.

For peroxidase (POD) activity, the prepared fruit discs were homogenized in 4 mL of 0.01 mol/L phosphate buffer at pH 7.0, and grinded in a grinding pot to get the crude extraction. Then samples were centrifuged at 10 000 r/min for 20 min at 4°C. The supernatant extract was used to determine POD activity as according to Reuveni et al. (1992). The 4 mL reaction mixture consisted of 0.01 mol/L Sodium phosphate buffer at pH 7.0, 1 mL enzyme supernatant, 20 mmol/L guaiacol (o-methoxyphenol) and 40 mmol/L H_2O_2. The mixture was incubated for 5 min at 37°C, and 20% TCA (trichloroacetic acid) was added to stop the reaction. Absorbance at 470 nm was recorded with spectrophotometer (Zhang, 1992; Tang and Zhang, 2002; Zhang et al., 2002).

Melondialdehyde (MDA) content measurement was conducted as follows. The level of lipid peroxidation in plant tissues was measured in terms of MDA content, which was determined by the thiobarbituric acid (TBA) reaction with minor modification of the method by Dhindsa et al. (1981). In 4.0 mL of 0.1 mol/L phosphate buffer at pH 7.8, 1.0 g sample was homogenized and grinded to get crude extraction, and then centrifuged at 10 000 r/min for 20 min at 4°C. The 4 mL reaction mixture contained 2 mL 0.1 mol/L phosphate buffer at pH 7.8, and 2 mL 10% TCA containing 0.6% TBA. The mixture was heated at 100°C for 15 min, and quickly cooled in an ice-bath. After that, it was centrifuged at 4 000 r/min for 10 min. Absorbance of the supernatant was measured at 532, 600 and 450 nm on specrophotometer. The MDA content was calculated according to Zhang (1992) and Tang and Zhang (2002).

2.2.2 Fruit dipping

Weight loss ratio of five pre-ripened harvested pears, stored at 0–5°C lower than normal, was measured by simple weighing balance for each treatment of SA concentration (0.02, 0.1, 0.5, 2.5, 12.5 mmol/L), immediately after being dipped for 48 h, fruits treated with distilled water were as CK, as according to Cheng and Zhang (2004) and Scheer (1994). Data were collected for six times at the intervals of 0, 1, 2, 4, 8, and 16 days for each sample.

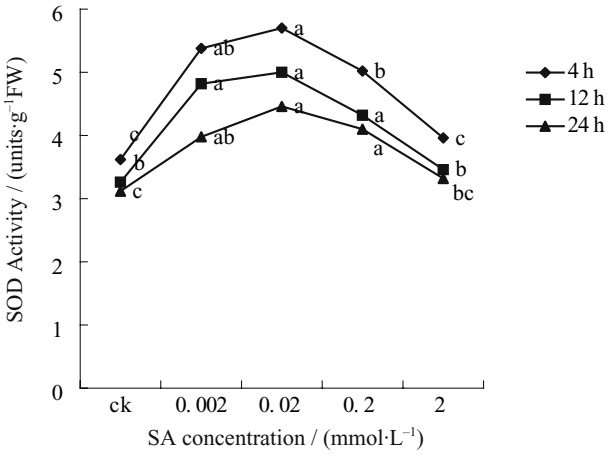
2.3 Statistical analysis

SOD, POD enzyme activities and MDA contents were subjected to statistical analysis ($P \leqslant 0.05$) by SPSS computer software with Duncan's multiple range test (DMRT). Mean values were arranged in a descending order, indicating significant differences. The statistical analysis procedures were adopted from Sokal and Rohlf (1997). The percentage differences of treatments compared with CK were measured as water loss ratio.

3 Results

3.1 Effect of SA on SOD activity in pear

Data shown in Fig. 1 suggested that the effect of SA at 0.02 mmol/L with dipping for 4, 12 and 24 h increased the SOD activity by 5.70, 5.00 and 4.46, respectively. The result showed that lower SA concentration with shorter dipping time had a better effect than others.

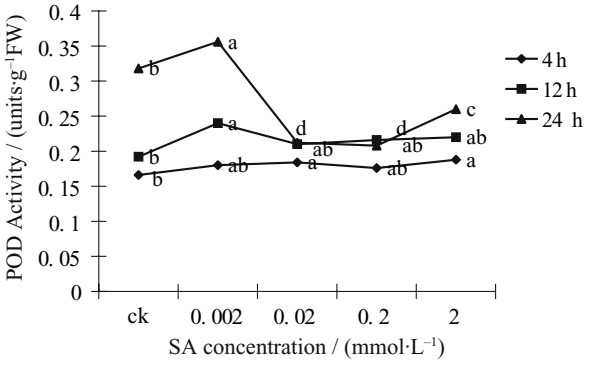


Note: (a) Enzyme activity is statistically analyzed according to Duncan's multiple range test (DMRT). Different letters showed significant differences ($P \leqslant 0.05$). (b) Treatment difference is also measured on percentage basis as compared with CK for relative dipping time.

Fig. 1 Effect of SA on SOD activity of Huang Kum pear disc

3.2 Effect of SA on POD activity in pear

The SA at 0.002 mmol/L for 12 h, 2.0 mmol/L for 4 h, and 0.002 mmol/L for 24 h increased POD activity by 25%, 13.25% and 11.94%, respectively, whereas decreased POD activity under other treatments. However, SA at 2.0, 0.02 and 0.2 mmol/L within 24 h decreased POD activity to a damaging level. The highest POD activity was 0.188, 0.24 and 0.356 with dipping for 4, 12 and 24 h, respectively at specific concentrations of SA (Fig. 2).



Note: (a) Enzyme activity is statistically analyzed according to Duncan's multiple range test (DMRT). Different letters showed significant differences and the same letters indicates non-significance ($P \leqslant 0.05$). (b) Treatment difference is also measured on percentage basis as compared to CK for relative dipping time.

Fig. 2 Effect of SA on POD activity of Huang Kum pear disc

3.3 Effect of SA on MDA contents in pear

It was evident that after the SA treatment of dipping for 12 h at 0.002 mmol/L, MDA contents were only 0.024 (10^{-6} mol/mg), which was the most effective concentration (Table 1). The SA treatment for 12 and 4 h had significant differences but the results after the SA treatment for 24 h showed no significance at different concentrations. So, at different levels of concentration and different dipping times, the MDA content showed different responses to the SA treatments. Longer dipping time with lower SA concentration was found suitable.

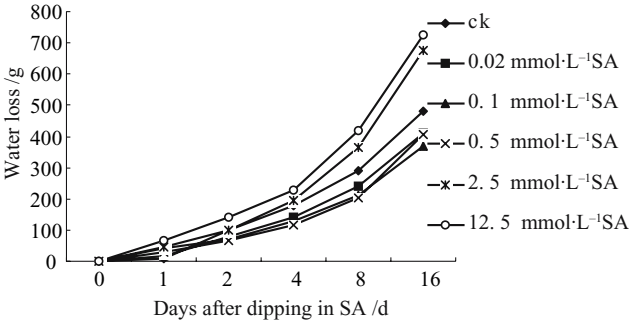
Table 1 Effect of SA on MDA contents of Huang Kum pear disc

SA concentration /mmol·L^{-1}	MDA contents /(10^{-6} mol·mg^{-1})		
	4 h dipping	12 h dipping	24 h dipping
CK	0.544 a*	0.140 a*	0.252
0.002	0.336 bc	0.024 d	0.200
0.02	0.272 bc	0.066 c	0.196
0.2	0.410 ab	0.038 d	0.132
2.0	0.190 c	0.102 b	0.174

Note: (a) Enzyme activity is statistically analyzed according to Duncan's multiple range test (DMRT); (b) * means that data with different letters indicate significant differences and data with no letters show non-significant difference in the same column at the level of $P \leqslant 0.05$; (c) treatment difference is also measured on percentage basis as compared with CK for useful mean values.

3.4 Effect of SA on water loss from pear fruit

The SA at concentrations of 0.5, 0.1 and 0.02 mmol/L showed better results from the second day to the16th day and at 2.5 mmol/L on the fourth day was also useful but later showed a trend of increasing in water loss. Higher values of water loss than CK on the second day for all SA levels were observed, and SA at 12.5 mmol/L raised water loss straightly. So SA at 0.5 mmol/L was helpful to reduce water loss, but on the 16th day, SA at 0.1 mmol/L performed better than others (Fig. 3).



Note: Treatment difference is measured on percentage basis as compared with CK from 0 to 16 days.

Fig. 3 Effect of SA on water loss of Huang Kum pear fruit

4 Discussion

4.1 Relationship between SA and different enzyme activities during fruit senescence

Exogenous application of SA at proper concentration significantly increased SOD activity and delayed senescence of pear fruit discs. SOD could convert $O_2^{\cdot-}$ to hydrogen peroxide (Dhindsa et al., 1981). The ability of $O_2^{\cdot-}$, OH* and $^1O_2$ might be reduced to cause lipid peroxidation. It was clear that activity of SOD could determine abundance of $O_2^{\cdot-}$, H_2O_2, OH* and $^1O_2$ in fruit tissues and delay lipid peroxidation. Our experiments suggested that SA delayed senescence of pear fruit discs by increasing activity of SOD by 57.46% higher than CK at 0.02 mmol/L after dipping for 4 h. Specific or lower SA concentration with shorter dipping time showed better results than others. The increase of SOD activity led to the decrease in the level of free radicals, thus reducing lipid peroxidation. Our results were in line with those of Dhindsa et al. (1981) and Zhang (1992) but contrary to that of Tang and Zhang (2002).

Results indicated that SA at 0.002 mmol/L for 12 h could increase POD activity by 25%, while at higher concentrations within 24 h decreased POD activity, and perhaps damaged the cells. Zhang et al. (2002) reported that spraying SA at 0.02 mmol/L on fruits stimulated phenylalanine ammonia lyase (PAL) activity but inhibited POD activity, lignin content, fruit spot number, stone cell density and fruit size. The decrease in lignin content was related to POD activity reduction and had nothing to do with the PAL activity.

Exogenous application of SA to regulate the levels of SA in plants may be a promising area for studying plant defense mechanism (Cao et al., 2006). Increase of endogenous SA may be achieved via enhancing transcription and translation of genes for SA biosynthesis or by blocking of the expression of genes involved in SA metabolism (Raskin, 1992). Enhanced POD activity is associated with defense mechanism such as

the lignin production, the PAL activity and the phenol accumulation (Reuveni et al., 1992; Zhang, 1992; Tang and Zhang, 2002). Delaying of senescence is probably related to the improvement of defensive mechanism in tissues as influenced by SA application.

The MDA is an important index of lipid peroxidation. Data revealed that mean value 0.024 (10^{-6} mol/mg) with 12 h dipping duration got the highest position as decreased values for MDA contents in the experiment. Whereas, the concentrations after the treatment for 12 and 4 h had significant differences, as for the results after the treatment for 24 h, they were not significant perhaps enhanced cell wall hydrolytic enzyme activities for fruit tissues. The most effective concentrations of SA to decrease MDA content were 0.002 and 2.0 mmol/L with 12 and 4 h dipping, respectively. The results implied that lower concentration for longer dipping duration was better, which was consistent with those of Dhindsa et al. (1981) and Zhang (1992) but contrary to that of Tang and Zhang (2002).

4.2 Effect of SA on fruit water loss during senescence

Data of this study revealed that SA at 0.5, 0.1 and 0.02 mmol/L showed better results from the second day to the 16th day. This might cause precarious situation, and higher values of water loss were observed on the second day for all SA concentrations, the reason may be laid in a short time for SA. Whereas, before the fourth day, SA at 2.5 mmol/L was useful but later including at 12.5 mmol/L, it began to give rise to water loss curve over CK, perhaps showing a damaging effect on fruit cells.

Water is a crucial prerequisite for plant growth (Riederer and Schreiber, 2001). The cuticle is the major barrier against water loss from leaves and fruits. The physical and chemical factors like temperature and organic compounds affecting the cuticular water permeability can be incorporated into transpiration models. Plant cuticular permeability contributes to minimize water loss at stomatal closure (Riederer and Schreiber, 2001). The mechanism by which the water and organic carbon are lost from the fruit is fruit transpiration (Hellickson and Baskins, 2000). So, a decrease in transpiration indicated the association with the reduction in hydrolytic cell wall enzymes activity (Scheer, 1994; Cheng and Zhang, 2004), which has been greatly influenced by SA at 0.5 mmol/L as compared with CK in this trial. Furthermore, the decrease in water loss may protect fruit tissues.

Therefore, it could be concluded that SA increased activities of SOD and POD, reduced lipid peroxidation, and decreased MDA contents and water loss in pear fruits. Our experiment showed that SA could delay senescence of Huang Kum pear by regulating different enzyme activities and physiological characteristics, and SA application that may play a role in maintaining the storage life of pear fruits needs to be further studied.

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