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Effects of brassinosteroids on postharvest disease and senescence of jujube fruit in storage

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ABSTRACT

The effects of brassinosteroids (BRs) against blue mould rot caused by *Penicillium expansum* and on senescence of harvested jujube fruit were investigated. Brassinosteroids at a concentration of 5 μM effectively inhibited development of blue mould rot and enhanced the activities of defense-related enzymes, such as phenylalanine ammonia-lyase, polyphenoloxidase, catalase and superoxide dismutase. However, BRs did not have direct antimicrobial activity against *P. expansum in vitro*. BRs significantly delayed fruit senescence by reducing ethylene production and maintained fruit quality. It is suggested that the effects of BRs on reducing decay caused by *P. expansum* may be associated with induction of disease resistance in fruit and delay of senescence.

1. Introduction

Jujube has been considered as a functional fruit with a high market value due to its particular nutritional qualities. However, the fruit are susceptible to postharvest losses due to fungal diseases (Tian et al., 2005b) and quality deterioration characterized by softness and decrease in soluble solids content (SSC) (Lin et al., 2004). Blue mold rot, caused by *Penicillium expansum*, is one of the most important diseases in jujube fruit (Qin and Tian, 2004), and in general, synthetic chemical fungicides are the primary means to control postharvest diseases. However, inducing resistance by biotic and abiotic factors is becoming a new potential approach to control postharvest diseases as alternatives to fungicides (Tian, 2006), particularly considering human health risks associated with the use of fungicides and the development of pathogen resistance (Droby et al., 2009).

Brassinosteroids (BRs) have recently been recognized as a new class of phytohormone occurring ubiquitously in the plant kingdom (Clouse and Sasse, 1998). Extensive research over the past two decades has revealed that BRs are essential for normal plant development and regulate a range of physiological processes, such as stem elongation, root growth, vascular differentiation, leaf epinasty and reproductive development (Brosa, 1999; Sasse, 2003). The potential of BRs to enhance disease resistance of plants has also been investigated. Field application of 24-epibrassinolide (5–15 mg ha^{-1}) to barley plants significantly decreased the extent of leaf disease induced by mixed fungal infection, along with an increase in crop yield (Pshenichnaya et al., 1997). Roth et al. (2000) found that treatment with low concentrations of a BR-containing extract of *Lychnis viscaria* L. seeds resulted in enhanced resistance of tobacco, cucumber and tomato to viral or fungal pathogens (tobacco mosaic virus, *Sphaerotheca fuliginea*, *Botrytis cinerea*). Nakashita et al. (2003) demonstrated that resistance was systemically induced by BRs in tobacco by a mechanism different from known induced resistance. However, to our knowledge, little information is available on the effects of BRs applied after harvest on physiological properties and induced resistance of fruit against pathogenic fungi.

The objectives of this study were (1) to evaluate the effects of BRs on control of postharvest disease caused by *P. expansum* in jujube fruit, (2) to determine the induction of defense-related enzymes, such as phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO), catalase (CAT) and superoxide dismutase (SOD) in fruit, (3) to measure the antifungal activity of BRs against *P. expansum in vitro*, and (4) to investigate the influence of BRs on senescence of jujube fruit.

2. Materials and methods

2.1. Plant material and chemicals

Jujube (*Zizyphus jujuba* cv. Huping) fruit were harvested in Shanxi province in China, and were immediately transported to the Institute of Botany, Chinese Academy of Sciences. The fruit were sorted based on size without physical injuries or apparent decay

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and washed in a 2% (v/v) sodium hypochlorite solution for 2 min, rinsed with tap water, and air-dried prior to use.

Brassinolide (BR) was purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd.

2.2. Pathogen

P. expansum was isolated from naturally infected jujube fruit and cultured on potato dextrose agar (PDA) at 25 °C for 14 d. Fungal spores were obtained by flooding the surface of the culture with sterile distilled water containing 0.05% (v/v) Tween-80. The suspension was filtered through four layers of sterile cheesecloth and adjusted to a concentration of 5×10^3 spores mL^{-1} using a hemocytometer.

2.3. Treatment with BR

In a preliminary experiment, we tested a series of BR concentrations, namely 2, 5, 10 μM. BR at a concentration of 10 μM significantly accelerated pericarp reddening. In contrast, BR at 2 and 5 μM could inhibit pericarp reddening and infection of *P. expansum*, and BR at 5 μM had the better effect (data not shown).

In the present study, jujube fruit were immersed in 5 μM BR solution or in distilled water as a control for 5 min, dried in air at 25 °C for 2 h and then divided into two groups. For the first group, BR-treated fruit were wounded (4 mm deep and 3 mm wide) at the equator of the fruit with a sterile nail after 24 h and inoculated with 10 μL of spore suspension of *P. expansum* (5×10^3 spores mL^{-1}) in each wound site. Fruit were put into plastic trays and covered with a plastic film to maintain a high relative humidity (95%), and stored at 25 °C. Disease incidence and lesion diameter were determined daily after treatment. Three replications for each treatment were performed, and each replicate contained 15 fruit.

For the second group, fruit were put directly into plastic trays as described above without pathogen inoculation and were observed to evaluate natural disease incidence and quality parameters at regular intervals. For each parameter assay, three replications for each treatment were performed, and each replicate contained 15 fruit. The entire experiment was repeated twice.

2.4. Enzyme assays

Samples were obtained from 15 fruit, using flesh between macerated and healthy tissue for enzyme assays, and enzyme activities were determined by a spectrophotometer (Shimadzu, Kyoto, Japan). Three replicates were performed with different storage times. Measurements of phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) activities were performed at 290 and 398 nm, according to the methods of Yao and Tian (2005) and Tian et al. (2005a), respectively. PAL activity was defined as nmol cinnamic acid h^{-1} mg^{-1} protein. PPO activity was expressed as units (U) per microgram protein. One unit was defined as ΔA_{398} of 0.1 per minute. Activities of catalase (CAT) and superoxide dismutase (SOD) were measured at 240 and 560 nm, respectively, following the method by Wang et al. (2005). CAT activity was expressed as U mg^{-1} protein. One unit was defined as 1 μmol of H_2O_2 per minute. SOD activity was also expressed as U mg^{-1} protein. One unit was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. Protein content was determined according to Bradford (1976) with bovine serum albumin (BSA) as standard.

2.5. Effects of BR on P. expansum in vitro

We investigated the effects of BR on spore germination and mycelial growth of *P. expansum in vitro* according to our previous work (Yao and Tian, 2005). Each treatment was replicated three times and the experiment was repeated twice.

2.6. Determination of ethylene production and respiration rate of fruit

For each treatment 1 kg of fruit were sealed in 5 L gas-tight jars at 25 °C. After 2 h, a 1 mL sample was removed from the headspace using a syringe and injected into a gas chromatograph (SQ-206, Beijing, China), equipped with an activated alumina column and a flame ionization detector for ethylene determination, and a thermal conductivity detector for CO_2 determination. Three replications for each treatment were performed.

2.7. Determination of fruit quality parameters

Firmness, soluble solids content, titratable acidity and vitamin C content of the fruit were determined. Flesh firmness was determined on opposite peeled cheeks of the fruit using a hand-held fruit firmness tester (FT-327, Italy), equipped with a cylindrical plunger 8 mm in diameter. Soluble solids content (SSC) was determined using an Abbe Refractometer (10481 S/N, USA). Titratable acidity (TA) was determined by titration with 0.01 mol L^{-1} NaOH. Vitamin C contents of the fruit were measured according to the method of Kampfenkel et al. (1995). Each treatment contained three replicates with 15 fruit per replicate.

2.8. Statistical analysis

Statistical analysis was performed with SPSS 13.0. Data were compared in a Student's *t*-test. Differences at $P < 0.05$ were considered as significant.

3. Results

3.1. Effects of BR on P. expansum fruit rot development in jujube fruit

BR at a concentration of 5 μM inhibited decay caused by *P. expansum* in jujube fruit. A little less disease incidence (Table 1) and

Table 1
Effect of BR on blue mould incidence and lesion diameter in jujube fruit after water or BR treatment.

Time after treatment (days)	Disease incidence (%)		Lesion diameter (mm)	
	Control	BR	Control	BR
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	95.5 ± 2.2a	88.9 ± 2.2b	6.92 ± 0.13a	6.3 ± 0.06b
4	97.8 ± 2.2a	97.8 ± 2.2a	10.63 ± 0.25a	9.44 ± 0.19b
5	100a	100a	15.43 ± 0.15a	13.77 ± 0.03b

A different letter showed significant difference at $P < 0.05$ according to Student's *t*-test. Data are accompanied by standard deviations of the means.