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

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Article info

Article history:
Received 31 August 2009
Accepted 28 November 2009

Keywords:
Brassinosteroids
Jujube fruit
Postharvest disease
Fruit senescence

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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doi:10.1016/j.postharvbio.2009.11.014
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 × 10⁴ 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 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 *P. expansum* spores (5 × 10⁴ 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 replications were performed with different storage times. Measurements of phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) activities were performed at 290 and 254 nm, respectively. PAL activity was defined as nmol cinemul of H₂O₂ 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 CO₂ 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 | 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.
Fig. 1. Symptoms of blue mould in jujube fruit stored for 4 d after water or BR treatment.

Table 2
Natural disease incidence in fruit stored at 25 °C after water or BR treatment.

<table>
<thead>
<tr>
<th>Time after treatment (days)</th>
<th>Disease incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3.3 ± 1.7a</td>
</tr>
<tr>
<td>6</td>
<td>10.0 ± 2.9a</td>
</tr>
<tr>
<td>9</td>
<td>35.0 ± 2.9a</td>
</tr>
<tr>
<td>12</td>
<td>58.3 ± 1.7a</td>
</tr>
<tr>
<td>15</td>
<td>73.3 ± 3.3a</td>
</tr>
<tr>
<td>18</td>
<td>100 ± 3.3a</td>
</tr>
</tbody>
</table>

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.

Fig. 2. Effects of BR on the spore germination and germ tube length of P. expansum at 25 °C. Data represent standard deviations of the means. Data followed by different letters are significantly different between samples according to Student’s t-test at P < 0.05.

3.3. Effects of BR on PAL, PPO, CAT and SOD activities in inoculated jujube fruit

PAL activity in BR-treated fruit showed a peak on the first day after treatment, and was considerably higher than that of the controls throughout the experiment (Fig. 4A). PPO activity in control fruit quickly reached the maximum level on the second day and subsequently dropped (Fig. 4B). A similar trend was found in BR-treated fruit, whereas PPO activity increased to a greater extent during the whole storage time as compared to the controls.

The control fruit had an initial increase in CAT activity and a constant decrease afterwards (Fig. 4C). Compared with the control, the increase in CAT activity was higher in BR-treated fruit, and was maintained at a relatively high level. SOD activity declined continuously in control fruit (Fig. 4D), but there was a slight decrease in SOD activity in BR-treated fruit.

3.4. Effects of BR on ethylene production and respiration rate

Ethylene production in control and BR-treated jujube fruit increased and reached a peak after 4 d of storage, then decreased (Fig. 5A). However, compared to control fruit, BR-treated fruit showed a significantly lower level of ethylene during storage. Similarly, respiration in all fruit reached a climacteric peak on the third day, but the respiration rate was significantly inhibited after BR treatment (Fig. 5B).

Fig. 3. Effect of BR on mycelial growth of P. expansum. Bars represent standard deviations of the means. Values followed by different letters are significantly different between samples according to Student’s t-test at P < 0.05.
3.5. Effects of BR on fruit quality

Although firmness and titratable acidity of jujube fruit decreased gradually with storage time increase, BR treatment significantly delayed decreases in firmness (Fig. 6A) and titratable acidity (Fig. 6C). SSC and vitamin C content in all fruit showed an increase early in storage, and then considerably decreased. BR-treated fruit had higher SSC levels and vitamin C contents compared with control fruit (Fig. 6B and D). These results indicated that a BR treatment had beneficial effects on maintaining quality of jujube fruit in storage.

4. Discussion

Decay is a major factor that reduces quality and limits storage life of jujube fruit. To prevent decay development and extend shelf-life of the fruit, a number of strategies have been evaluated instead of fungicides, such as the usage of controlled atmosphere storage (Lin et al., 2004) and application of antagonists combined with chemical substances (Wan et al., 2003; Tian et al., 2005b). In this study, the plant hormone BR was applied and the results indicated that BR can significantly reduce postharvest decay and restrain the pathogenic development of *P. expansum* in jujube fruit (Tables 1 and 2; Fig. 1). Our results were consistent with previous reports that BR could protect cucumber (Churikova et al., 1999) and tobacco plants (Nakashita et al., 2003) against pathogenic fungi in field trials. To our knowledge, this is the first report in which BR has been implicated in achieving beneficial effects against postharvest diseases of fruit.

BR has been considered to be involved in a network of interacting signal transduction pathways which regulate defense responses to pathogen stress (Dong, 1998). Nakashita et al. (2003) demonstrated that BR-mediated disease resistance (BDR) took part in plant defense response independently from SA-mediated defense response. The mechanism by which BR induced resistance against postharvest diseases of fruit was investigated in this study. Generally, defense-related enzymes including PAL and PPO are considered potentially important in induced resistance of plants (Petruzzelli et al., 1999). Our results indicated that BR significantly induced the activities of PAL, PPO in jujube fruit (Fig. 4). We also found induction of activities of CAT and SOD, which are the main detoxifying enzymes in plant cells (De Gara et al., 2003). Previous studies have shown that PAL, PPO, CAT and SOD are involved in the defense response against fungal pathogens in other fruit (Yao and Tian, 2005; Chan et al., 2007; Wang et al., 2009). Fur-
thermore, in assays of spore germination and mycelial growth, no direct antifungal effect of BR on *P. expansum* was observed (Figs. 2 and 3). This indicated that the effect of BR against *P. expansum* in jujube fruit may be attributed to its ability to induce resistance in fruit rather than a direct fungitoxicity effect on the pathogen.

Jujube is categorized as a climacteric fruit on the basis of its ethylene production and respiratory activity, with the respiration peak preceding the ethylene burst by 1 d (Fig. 5). It is possible that the high rates of respiration and ethylene production during jujube fruit ripening are responsible for the short storage life of the fruit under room temperature conditions (Abbas and Fandi, 2002). Our data indicated that application of 5 μM BR delayed postharvest senescence processes by significantly reducing ethylene production and the respiration rate (Fig. 5). It has been reported that disease resistance is bound up with the maturity stages of the fruit (Labavitch, 1998; Chan et al., 2008). Recently, some data has suggested that the application of BR could accelerate the ripening of tomato fruit with increases in ethylene production (Vardhini and Rao, 2002). Symons et al. (2006) indicated that exogenous BR promoted grape fruit ripening with a dramatic increase in endogenous BR levels. Results of the present study were not consistent with those reports. This may be attributed to the difference in fruit species and concentrations of BR used. In a preliminary study, we also found that BR could promote ripening of jujube fruit when the concentration reached 10 μM (data not shown). In addition, BR showed positive effects on fruit quality and nutritional aspects. The typical softening and vitamin C losses of jujube fruit were impaired (Fig. 6A and D); the declines in SSC and TA content were also delayed or inhibited (Fig. 6B and C). The efficiency of BR in maintaining good quality could not be linked to inhibition of ethylene production.

In conclusion, application of BR at a concentration of 5 μM reduced postharvest decay caused by *P. expansum* in jujube fruit and delayed fruit senescence by inhibiting ethylene production and respiration rate. The mechanism by which BR induced resistance against fungal pathogens may be due to stimulation of defense-related enzymes and delay of fruit senescence.

**Acknowledgements**

The study was supported by the CAS/SAFEA International Partnership Program for Creative Research Teams, National Natural Science Foundation of China, (U0631004), the Ministry of Science and Technology of China (2006BAD22B02), and the CAS/SAFEA International Partnership Program for Creative Research Teams.

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