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Effects of benzothiadiazole on disease resistance and soluble sugar accumulation in grape berries and its possible cellular mechanisms involved

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ABSTRACT

This study was conducted to investigate the effects of benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) treatments on disease resistance against *Botrytis cinerea* infection and soluble sugar accumulation in grape berries and to analyze the possible cellular mechanisms involved. In grape berries, the results indicated that BTH treatments at 0.1 or 1 mmol L^{-1} could effectively inhibit *B. cinerea* infection possibly by directly inhibiting pathogen growth and indirectly inducing disease resistance. However, an obvious change in the composition of the soluble sugars was simultaneously observed in the BTH-treated berries. In parallel, addition of BTH at 0.1 or 1 mmol L^{-1} to the medium could effectively trigger a SAR defense response in grape suspension cells, and the defense included a cellular H_2O_2 burst, *VvNPR1.1* and *PR1* genes expression, and the accumulation of stilbene phytoalexins. The 0.1 or 1 mmol L^{-1} BTH treatment induced higher activity of sucrose-hydrolyzing enzyme SS-cleavage and lower activities of sucrose-synthesizing enzymes such as SS-synthesis, SPS and SPP than the controls, contributing to the gradual increase in sucrose hydrolysis, a decrease in the glucose content and the accumulation of fructose in grape cells. Therefore, our results suggest that BTH exerts its effect on reducing fruit decay perhaps through the cellular SAR response. Moreover, the presence of costs in terms of altered soluble sugar components in grape berries may be attributed in changing activities of cellular sucrose-metabolizing enzymes after effective BTH elicitations.

1. Introduction

The grapevine (*Vitis vinifera* L.) belongs to one of the world's largest fruit crops in terms of both fresh produce and processed food industries, and it is also a natural healthy source of its special functional components such as polyphenols (Caillet et al., 2006). Nevertheless, this fruit, which is a typical non-climacteric fruit, presents severe losses during long-distance transport and storage, primarily because of its high susceptibility to pathogenic infection. *Botrytis cinerea* Pers.:Fr., which causes gray mold rot, appears to be dominant responsible for postharvest disease in grape fruit and can be effectively controlled by synthetic chemical fungicides (Feliziani et al., 2013a). Because of the negative impact of fungicides residues on environment and human health, alternative measures for controlling postharvest diseases in grape fruit have been strongly demanded. Among recent new strategies, disease resistance as induced by a low-impact environmental elicitor in horticultural crops has attracted major interest due to its ecological compatibility (Schirra et al., 2011).

Treating plants with various elicitors (*e.g.*, avirulent pathogens, cell wall fragments, plant extracts, and phytohormones such as salicylic acid, ethylene, jasmonic acid, abscisic acid and indole acetic acid) can reportedly activate a wide range of defense responses, which include an oxidative burst, the accelerated expression of defense genes, changes in the cell wall composition, and the accumulation of antimicrobial compounds such as phytoalexins (Bostock, 2005). Benzo-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) is a photostable functional analog of the plant signal molecule salicylic acid (SA). BTH has been shown to have a double action in plant protection; it inhibits the development of decay-causing fungi through its direct toxicity, and it also indirectly induces pathogenesis-related (*PR*) genes,

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leading to the establishment of systemic acquired resistance (SAR) in a variety of plants to provide broad-spectrum protection against various pathogens (Wendehenne et al., 1998; Feliziani et al., 2013a, b). At present, BTH has been applied to enhance disease resistance for the control of gray mold decay on strawberries (Romanazzi et al., 2013) and grape fruit (Iriti et al., 2005), blue mold decay on peach fruit (Cao et al., 2011), pink rot on muskmelon fruit (Ren et al., 2012) and anthracnose rot on mango fruit (Lin et al., 2011). Thus, BTH treatment after harvest has been developed as a new technology to substitute for the fungicides used to reduce fruit decay. Unfortunately, regardless of when induced resistance is expressed, there is likely to be a metabolic cost associated with the defense expression. Overwhelming ecological evidence is emerging that such costs lie with allocation of limited resources toward production of elevated level of defense and away from the plant's own primary metabolism (Cipollini et al., 2003). Few investigations using SA or BTH applications to induce resistance against pathogens or herbivores have found obvious costs in terms of reduced growth and/or seed production in wheat (Heil et al., 2000), soybean (Iriti and Faoro, 2003) and *Arabidopsis* (van Hulten et al., 2006) or of lowered leaf fresh weight in strawberries (Hukkanen et al., 2007). To date, although the above-mentioned studies highlighted the fitness costs associated with chemical induction in several plant species under field conditions, these metabolic costs have rarely been demonstrated or quantified in agronomic fruits.

Soluble sugars are not only remarkable sources of flavor and overall sensory quality in postharvest fruits, but they are also known to function as central signaling molecules that can modulate the transcript levels of a set of genes involved in defense responses and metabolic processes, consequently affecting fruit ripening and the biosynthesis of secondary metabolites (Rolland et al., 2006). Among the carbohydrates, the dominant sugar forms present in grape fruit are glucose and fructose, which are normally stocked in the cell vacuole; and sucrose plays a central role in both the distribution of carbohydrates from photosynthesis and the initiation of hexose-based sugar signals in importing structures (Koch, 2004). An investigation showed that the enhanced expression of defense-related genes was accompanied by gradually elevated soluble sugars contents during grape ripening, demonstrating a close link between the sugar metabolism and disease resistance (Salzman et al., 1998). However, relatively little is known about the relationship between the disease resistance induced by elicitors and the carbohydrate components in postharvest grape berries.

In comparison with whole plant studies, the application of cultured suspension cells as a plant model system of reduced complexity is a promising alternative for studying the inducible defense mechanism and the metabolism of secondary metabolites, because *in vitro* cultures can provide a source of homogeneous and active cells that eliminate some interference factors such as slow plant growth, seasonal and environmental variations in addition to pathogen or herbivore attacks (Liswidowati et al., 1991). In the simplified system, adding fungal cell wall fragments and various chemical inducers to grape cell cultures has been shown to augment the expression of a set of genes linked closely to the accumulation of *PR* proteins and/or stilbene phytoalexins (Martinez-Esteso et al., 2009). Therefore, the objectives of this study were to investigate: (a) the ability of BTH treatments at different concentrations to induce resistance against gray mold caused by *B. cinerea* in grape berries; and (b) the BTH effects on the cellular defense response and sucrose metabolism in a model system of grape suspension cells to gain a greater understanding of whether inducible resistance incurred costs in terms of the soluble sugar accumulation.

2. Materials and methods

2.1. Fruit material and callus tissue

Table grapes (*Vitis vinifera* L. × *V. labrusca* L. cv. 'Kyoho'), without fungicides being applied before harvest, were hand-harvested randomly at commercial maturity from a local vineyard in the Wanzhou district of Chongqing city and immediately transported to the laboratory within 2 h. The weight of a single berry was 15.74 ± 1.07 g. The freshly harvested grape berries were carefully selected on the basis of their uniform size, color and absence of visual infections and physical injuries.

Callus tissue was obtained from the table grapes of 12-year-old *Vitis vinifera* L. cv. 'Kyoho' plants, as described previously (Wang et al., 2014). The culture condition was determined according to the method of Zhang et al. (2002) with some modifications. The resulting liquid cell cultures were subcultured biweekly in the dark in 250-mL Erlenmeyer flasks in a medium (pH 5.7–5.8) with 40 mL of B5 medium supplemented with 250 mg L^{-1} casein hydrolysate, 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA), 0.2 mg L^{-1} kinetin (KT) and 30 g L^{-1} sucrose. Cell suspension cultures were maintained on a reciprocating shaker (KS4000i, IKA Inc., Germany) at 100 strokes min^{-1} at 22 ± 1 °C, and the inoculum size was set at 2.5–3.0 g wet cells of 50 mL medium.

2.2. Pathogen

A *B. cinerea* strain was isolated from decayed grape berries and cultured on potato dextrose agar media (PDA; extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g in 800 mL of deionized water). The spores of *B. cinerea* were harvested from PDA cultures of the pathogen, which had grown at 25 °C for two weeks. Five milliliter of sterile distilled water containing 0.5% (v/v) Tween-80 was gently poured into the a Petri plate culture, and then the spore suspensions were prepared by removing the spores from the sporulating edges of the cultures with a sterilized bacteriological loop and filtering through four layers of sterile cheesecloth to remove the adhering mycelia fragments. The spore concentration was determined with a haemocytometer, and adjusted to 1.0×10^5 spores mL^{-1} with sterile distilled water.

2.3. Grape berries experiment

2.3.1. Treatments and sampling

The selected grape berries were randomly divided into four groups of 300 berries each. Each group of berries was immersed in the BTH solution at the concentration of 0 (control), 0.01, 0.1 or 1 mmol L^{-1} at 20 °C for 10 min and air-dried for approximate 1 h, according to our previous method (Cao et al., 2011). These specific BTH concentrations were chosen on the basis of the previous results that showed 0.3 mmol L^{-1} BTH could effectively activate disease resistance in grapevine (Iriti et al., 2005). All the grape berries were then surface-sterilized with medical grade (75%) alcohol and drained on filter paper at room temperature. Afterwards, the berries were inoculated by spraying them with a 1.0×10^5 spores mL^{-1} suspensions of *B. cinerea* and allowed to air-dry gently for 1 h. Finally, the grape berries were stored at 22 °C for 5 d at a relative humidity of approximately 90%. Fruit samples were taken daily during the storage for disease evaluation (for those showing visible mold growth). Tissue samples from healthy pulp were mixed and frozen immediately in liquid nitrogen and stored at −80 °C until the measurement of their soluble sugar contents. Each treatment was replicated three times and the entire experiment was conducted three times.