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# Regulation of redox status contributes to priming defense against *Botrytis cinerea* in grape berries treated with β-aminobutyric acid

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ABSTRACT

This study aimed to determine the specific form of the disease resistance using BABA elicitation and to illustrate the involvement of an alteration of the redox status in the BABA-activated defense response in grape berries. The BABA treatment at 10 mmol $L^{-1}$ primed the grape berries for efficient disease resistance against *Botrytis cinerea* infection, as exhibited by the significantly enhanced levels of *PR* genes upon the *B. cinerea* challenge. In addition, the priming defense was associated with the onset of the SA-dependent SAR reaction. The BABA treatment induced higher activities of key enzymes in PPP and ascorbate-glutathione cycle, thus promoting the pools of GSH and NADPH and correspondingly elevating the [NADPH]/[$NADP^+$] and [GSH]/[GSSG] ratios, which shifted the cellular redox towards a highly reductive condition. Meanwhile, the BABA-treated fruits also showed higher contents of intercellular redox signalling molecules, such as NO and SA, than those in the controls. This increase in the redox status coincided with the enhanced expression of a series of *PR* genes and with lower disease incidence. In contrast, 6-AN completely diminished the increases in the NADPH and GSH pools elicited by BABA in grape berries in parallel with an inhibitory effect on induction of the PR genes transcript levels. Thus, these findings indicated that BABA can prohibit the oxidation of the redox state necessary for the induction of a priming response in grape berries against *B. cinerea* infection.

## 1. Introduction

Grapevine (*Vitis vinifera* L.) belongs to one of the most important fruit crops consumed around the world, and grape berries have been considered to be highly beneficial for health due to their abundant antioxidant compounds, including phenolics, procyanidins, and resveratrol, as well as their glycosides and oligomers (Doshi et al., 2015). However, the fruit is highly susceptible to pathogenic infections by diverse microbes, resulting in tremendous production decrease and economic losses unless protective strategies are used. Grey mould is caused by a necrotrophic fungal pathogen known as *Botrytis cinerea* that can proliferate rapidly under a wide temperature and humidity range, represents a serious crisis for the severe quality deterioration in grape berries and other berry fruits (Feliziani et al., 2013). Effective control of the grey mould disease depends on the persistent use of chemical fungicide, but low public acceptance of the adverse effects of chemical residuals on the environment has restricted fungicides application. Hence, several alternatives have been explored intensively in horticultural crops, minimizing the amount of chemical use (Schirra et al., 2011). One sustainable method is activating broad-spectrum resistance inside plants against pathogenic infection by applying eco-compatible natural elicitors (Romanazzi et al., 2016).

The mechanism involved in induced resistance in plants can be ascribed to the enhanced defensive levels of biochemical, structural and molecular responses to pathogens through a complex signal transduction network, which is usually activated by localized pathogen invasion or elicited with a diverse group of biotic or abiotic inducers (Spoel and Dong, 2012). The recent research on plant-pathogen interactions indicates that the induction of disease resistance occurs through two different modes: a direct induction that confers simultaneous resistance following some potent elicitations and a priming defense that can poise the defensive levels to mobilize a quicker and stronger resistant

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reaction once the plant suffers from pathogen infection (Conrath et al., 2015). Compared with direct activation, the priming defense is described as a more efficient type of induced resistance in several model plants, because the wasteful fitness costs for the constitutive expression of the defensive response is limited or prevented under low levels of disease occurrence (van Hulten et al., 2006; Huot et al., 2014). A low concentrations of some chemicals, such as MeJA, 2,6-dichloroisonicotinic acid, various vitamins, SA and its analogue BTH, and specific strains of nonpathogenic rhizobacteria can prime susceptible plant species for the augmented activation of resistance upon pathogen challenge (Wang et al., 2015; Martinez-Medina et al., 2016; Boubakri et al., 2016). In addition to these inducers, β-aminobutyric acid (BABA), known as a representative non-protein amino acid, has emerged as an effective elicitor that primes defense in *Arabidopsis* (van Hulten et al., 2006), tomato (Wilkinson et al., 2018) and grapevine (Hamiduzzaman et al., 2005). BABA has been identified to induce resistance against different fungal infections in agronomic fruits, including apple (Zhang et al., 2011; Quaglia et al., 2017), crab apple (Macarisin et al., 2009), mango (Zhang et al., 2013) and citrus (Panebianco et al., 2014). Our previous research also stated the effectiveness of BABA at triggering systemic resistance against *B. cinerea* in strawberry by stimulating an ROS burst and resultant *PR* gene expression (Wang et al., 2016a). However, the specific forms related to the BABA-mediated resistance in grape berries still require better characterization to apply this efficient disease management in practice (Table 1).

For a long time, it was commonly believed that the burst of reactive oxygen species, such as $H_2O_2$ and superoxide radicals, is a critical process after pathogen recognition, which can regulate a series of defense-related signal conduction and subsequently induce systemic resistance against additional pathogen infection (O'Brien et al., 2012). However, a few studies employing transgenic *Arabidopsis* have found that a striking alteration of the redox potential, which directly responded to a rapid production of ROS, is a prominent feature in different stress tolerances (Tada et al., 2008; Noshi et al., 2016). However, little information is available on the correlation between redox status and defense activation in horticultural crops. Therefore, this topic was investigated to a) provide a mechanistic link between BABA-induced priming and disease resistance, and b) consider the possibility that BABA elicitation could regulate the production of reductive substances and the homeostasis of cellular redox, so that the defense was activated in grape berries.

## 2. Materials and methods

### 2.1. Fruit, fungi and BABA

Organically grown grape berries (*Vitis vinifera* × *Vitis* Labrusca) cv. 'Kyoho' were harvested at a mature stage from a vineyard in Suining, Sichuan Province, southwestern China, and immediately packaged and transferred to our laboratory where the fruits were pre-cooled at 20 °C for 2 h with forced-air. Depending on the experiment that was carried out, individual berries of uniform maturity and size that were free from visual blemishes, physical damage and pathological infections were used.

**Table 1**
Sequence of primers used for real-time quantitative PCR in grape berry.

| Gene | Accession number | Primer sequence (forward/reverse) |
|---|---|---|
| *VvNPR1.1* | GSVIVT00016536001[a] | GTTCAATGCAACAATGGAGGGT/ CTGAGGAAAGGGATTCGGTGG |
| *VvPR1* | GSVIVT00038575001[a] | GGACAACACTGTGGCTGCCTAC/ GCACCCAAGACGCACTGATT |
| *VvPR2* | AY137377[b] | TGCTGTTTACTCGGCACTTG/ CTGGGGATTTCCTGTTCTCA |
| *VvChit4c* | AJ27790[b] | TCGAATGCGATGGTGGAAA/ TCCCCTGTCGAAACACCAAG |
| *VvPAL* | ABM67591.1[b] | CTGCGAGAAGGACTTGCTAAA/ CTGAGACAATCCAGAAGAGGG |
| *Vv18s rRNA* | AF321266[b] | GCTTTGCCGTTGCTCTGATGAT/ TTTGCCGATGGTGTAGGTTCCT |

[a] Genoscope *Vitis* × 8 accession number.
[b] GenBank accession number (NCBI).

The cultures of *B. cinerea* (Pers. Fr.) were separated from the decayed grape berries, preserved on PDA slants at 4 °C and cultivated on PDA plates at 26 °C until the mycelia developed. The spore suspensions were prepared by washing the spores from the sporulating edges of the PDA cultures with a bacteriological loop, suspending them in sterile distilled water containing 0.5% Tween-20, filtering them through cheese cloth and finally modifying the concentration to $1 \times 10^5$ spores $mL^{-1}$ using a haemocytometer.

BABA powder (purity ≥ 99%, Sigma Co.) was dissolved in sterile distilled water, and the solution of BABA was adjusted to the concentration of 10 mmol $L^{-1}$ to which 0.02% Tween-20 was added. This concentration was chosen for this experiment based on previous research on grape berries and strawberries (Porat et al., 2003; Wang et al., 2016a). In addition, sterile distilled water containing 0.02% Tween-20 served as controls.

### 2.2. Treatments

All of the grape berries selected were superficially sterilized with 75% medical grade ethanol, drained on filter paper and wounded (2 mm deep by 1.5 mm wide) with dissecting needles at two symmetrical sites around the equatorial region. The wounded fruits were randomly divided into two equal halves of 1200 fruits each, which were re-divided into four groups of 300 berries each. In the first half, a 20-μL aliquot of sterile water, a BABA solution at 10 mmol $L^{-1}$ or a suspension of *B. cinerea* at $1.0 \times 10^5$ spores $mL^{-1}$ were pipetted into each fruit wound site of each of the first three groups, respectively. In addition, each wounded fruit of the fourth group was pre-treated with 10 mmol $L^{-1}$ BABA and incubated at 20 °C for 6 h. Pathogenic inoculation was conducted by pipetting 20 μL of *B. cinerea* suspension into each wound. In the second half, 6-AN reagent, known as an inhibitor of PPP, was dissolved in distilled water and adjusted to 5 mmol $L^{-1}$. A 6-AN reagent was added to the BABA solution or *B. cinerea* suspension, which were each prepared at the same concentration as above to obtain a final concentration of 5 mmol $L^{-1}$ each. Subsequently, a 20-μL aliquot of 5 mmol $L^{-1}$ 6-AN, a 10 mmol $L^{-1}$ BABA solution containing 5 mmol $L^{-1}$ 6-AN or a $1.0 \times 10^5$ spores $mL^{-1}$ *B. cinerea* suspension containing 5 mmol $L^{-1}$ 6-AN were pipetted into each fruit wound site of the first three groups, respectively. In addition, each fruit wound of the fourth group was inoculated with *B. cinerea* 6 h, after pretreatment with 6-AN + BABA at 20 °C. Thus, the experiments on the first half of the fruits consisted of the four treatments: (1) control; (2) BABA treatment; (3) *B. cinerea* inoculation; and (4) BABA+ *B. cinerea* inoculation. There are four treatments for the second half of the fruits: (1) 6-AN treatment; (2) 6-AN + BABA treatment; (3) 6-AN+*B. cinerea* inoculation; and (4) 6-AN + BABA+*B. cinerea* inoculation. After being air-dried for 2 h at 20 °C, all of the treated grape berries were arranged in covered polyethylene boxes and incubated at 20 °C and 90–95 % RH for 5 d without light. Tissue samples of healthy pulp from each treatment were sampled daily and quick-frozen in liquid $N_2$. Finally, the frozen samples were immediately stored at −80 °C until analysed. Each treatment comprised three replicates, and the entire experiment was conducted three times with similar results.

### 2.3. Disease evaluation

A grape berry showing a lesion zone beyond the 1.5 mm width of the original wound was considered to be infected. All the fruits were examined for disease development on days 1, 3 and 5 during the