γ-Aminobutyric acid induces resistance against *Penicillium expansum* by priming of defence responses in pear fruit

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**Abstract**

The results from this study showed that treatment with γ-aminobutyric acid (GABA), at 100–1000 μg/ml, induced strong resistance against blue mould rot caused by *Penicillium expansum* in pear fruit. Moreover, the activities of five defence-related enzymes (including chitinase, β-1,3-glucanase, phenylalanine ammonialyase, peroxidase and polyphenol oxidase) and the expression of these corresponding genes were markedly and/or promptly enhanced in the treatment with GABA and inoculation with *P. expansum* compared with those that were treated with GABA or inoculated with pathogen alone. In addition, the treatment of pear with GABA had little adverse effect on the edible quality of the fruit. To the best of our knowledge, this is the first report that GABA can effectively reduce fungal disease of harvested fruit. Its mechanisms may be closely correlated with the induction of fruit resistance by priming activation and expression of defence-related enzymes and genes upon challenge with pathogen.

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1. Introduction

Pathogen infection is an important factor that affects fruit post-harvest physiology and metabolism (Prusky et al., 2010). *Penicillium expansum*, the causal agent of blue mould, is a widespread fungal pathogen and causes considerable pome fruit losses, in apples, cherries, pears and peaches (Cao, Yang, Hua, & Zheng, 2011; Jurick et al., 2009; Malandrakis et al., 2013; Quaglia, Ederli, Pasqualini, & Zazzerini, 2011; Venturini, Oria, & Blanco, 2002). Besides the economic impact, *P. expansum* is also regarded as the major producer of patulin (4-hydroxy-4H-furo[3,2-c]pyran-2[6H]one), mycotoxin and secondary metabolites toxic to humans (Malandrakis et al., 2013). Moreover, the intense use of synthetic fungicides has led to the increasing resistance of fungal pathogens. Therefore, there is a growing need for eco-friendly alternative strategies to inhibit the fungal decay (Droby, Wisniewski, Macarins, & Wilson, 2009).

Attempts to exploit induced resistance through the application of defence response elicitors are being pursued, to control post-harvest diseases as a safer technology (Shoresh, Harman, & Mastouri, 2010). Several chemical elicitors have been reported to induce resistance to pathogens, e.g. salicylic acid (SA) and its functional analogue acibenzolar-S-methyl (ASM) and β-aminobutyric acid (BABA) (Quaglia et al., 2011). A common feature of induced resistance caused by these elicitors is called priming (Conrath, Pieterse, & Mauch-Mani, 2002; Wang, Xu, Wang, Jin, & Zheng, 2013), which can cause a faster and stronger activation of defence response mechanisms after pathogen infection (Tonelli, Furlan, Taurian, Castro, & Fabra, 2011).

γ-Aminobutyric acid (GABA), a four carbon, non-protein free amino acid, is widespread in most prokaryotic and eukaryotic organisms (Bown, MacGregor, & Shelp, 2006). In plants, multiple signalling roles have been attributed to GABA, including involvement in pH regulation, nitrogen storage, plant development and defence against abiotic stresses, such as drought, oxidative stress, salinity and cold stress (Shelp et al., 2012). Experimental evidence shows that exogenous GABA can alleviate oxidative damage caused by H⁺ and Al³⁺ toxicities in barley seedlings (Song, Xu, Wang, & Tao, 2010) and reduce chilling injury of peach fruit by inducing proline accumulation, enhancing the enzymatic antioxidant system and maintaining energy status (Shang, Cao, Yang, Cai, & Zheng, 2011; Yang, Cao, Yang, Cai, & Zheng, 2011). However, there is little information about the effect of GABA on the post-harvest fungal diseases of fruit.

The aim of this research was to assess the effect of GABA on induction of resistance to blue mould rot caused by *P. expansum* in pear fruit and the possible action mechanisms involved.
2. Materials and methods

2.1. Fruit, pathogen and GABA

Pear fruit (Pyrus pyrifolia Nakai, cultivar “Shuijing”) were sorted on the basis of size, colour, maturity and absence of physical injury or infection. The fruit surfaces were disinfected in 0.1% sodium hypochlorite solution for 2 min, thoroughly washed in tap water and air-dried at 25 °C prior to use.

An isolate of P. expansum obtained from rotted pear fruit was maintained on potato dextrose agar (PDA) medium (containing the extract from 200 g of potato, 20 g of glucose and 20 g of agar in 1 l of distilled water) for 7 days at 25 °C. Sterile distilled water was used to flood the surface of the PDA culture and conidia were scraped by a sterile loop. The spore concentrations obtained were determined using a hemacytometer and adjusted with sterile distilled water as required.

GABA was purchased from Sangon Biotech (Shanghai, China). The stock solution of GABA was diluted to a series (1, 10, 100 and 1000 μg/ml).

2.2. Efficacy of GABA for control of blue mould rot caused by P. expansum in pear fruit

2.2.1. Effect of GABA at different concentrations on inhibition of blue mould rot in pear fruit

The fruit were wounded (5 mm wide by 3 mm deep) with the tip of a sterile border at five different sites. 30 μl of the aqueous preparation which contained GABA at 1, 10, 100 and 1000 μg/ml were injected into each wound. Wounds treated with the same amounts of sterile distilled water served as control. After 2 or 24 h, each wound was inoculated with 30 μl of a conidial suspension of P. expansum at 1 × 10⁵ spores/ml. The fruit were then air-dried and incubated in the dark at 25 °C to maintain 90% relative humidity (RH). Each treatment included 3 replicates and each replicate consisted of 9 fruit.

2.2.2. Effect of GABA treatment time on induction of disease resistance against blue mould rot in pear fruit

Five wounds were made on each pear fruit as above and each wound was treated with 30 μl of GABA, at 100 μg/ml, or sterile distilled water as the control. After 0, 6, 12, 24 and 36 h, fruit were inoculated with 30 μl of the P. expansum suspension at 1 × 10⁶ spores/ml. The fruit were then air-dried and incubated in the dark at 25 °C to retain about 90% RH. Each treatment included 3 replicates and each replicate consisted of 9 fruit.

2.2.3. Effect of GABA on blue mould rot at different spore concentrations in pear fruit

Six wounds were made on the surface of each pear fruit as described above and three of the wounds were treated with 30 μl of GABA at 100 μg/ml or with 30 μl of sterile water as control. After 24 h, 30 μl of 1 × 10², 1 × 10⁴ or 1 × 10⁶ spores/ml suspension of P. expansum were respectively inoculated into each wound. The fruits were then air-dried and incubated in the dark at 25 °C to retain about 90% RH. Each treatment included 3 replicates and each replicate consisted of 9 fruit.

2.2.4. Effect of GABA on blue mould rot development at low temperature in pear fruit

Pear fruit were wounded at two sites as above. Aliquots of 30 μl of GABA at 100 μg/ml and sterile water as a control, were pipetted onto each wound and 24 h later wounds were then inoculated with 30 μl of a 1 × 10⁵ spores/ml suspension of P. expansum. The fruits were then air-dried and incubated in the dark at 4 °C to maintain 90% RH. Each treatment included 3 replicates and each replicate consisted of 9 fruit.

In the above four tests, the percentage of wounds showing rot symptoms was assessed on a daily basis. Disease incidence was defined as decayed fruit/total fruit × 100%. Each test was performed at least twice. The discussed data were from one individual experiment and representative of the two experiments with similar results.

2.3. Effect of GABA on germination and survival of P. expansum spores in vitro

The effect of GABA on spore germination of P. expansum was tested in potato dextrose broth (PDB) with various concentrations of GABA (0, 1, 10, 100, 1000 μg/ml). Aliquots of 100 μl of pathogen suspensions were put into 10 ml glass tubes containing 2 ml of PDB to obtain a final concentration of 1 × 10⁶ spores/ml. All treated tubes were placed on a rotary shaker at 200 rpm at 25 °C. After 12 h of incubation, approximately 150–200 spores of the pathogen per replicate were measured for germination rate. Spores were considered germinated when germ tube lengths were equal to or greater than spore lengths. All treatments consisted of three replicates and the experiment was repeated twice.

Equal amounts of P. expansum spores were mixed with a solution of GABA in final concentrations of 0, 1, 10, 100, 1000 μg/ml, respectively and kept for 1 min. Then 100 μl of spore suspension were plated on PDA. The colonies per plate were counted after 72 h of incubation at 25 °C. There are three replicates per treatment with three plates per replicate and the experiment was conducted twice.

2.4. Effect of GABA on spore germination of P. expansum in pear fruit wounds

Two of the four wounded sites on each fruit were injected with 30 μl of sterile distilled water (as the control) and 30 μl of GABA, at 100 μg/ml, were placed in the other two wounds. After 2 or 24 h, each wound was inoculated with 30 μl of a conidial suspension of P. expansum at 1 × 10⁶ spores/ml. After being air-dried, pear fruit were stored in enclosed plastic trays to maintain a high relative humidity at 25 °C. After 12 h postinoculation, 150–200 spores per replicate were examined microscopically to determine germination rate. Each treatment included 3 replicates and each replicate consisted of nine fruit for each time point.

2.5. Sample treatment for enzyme activity and gene expression analysis

The wounds were inoculated with 30 μl of the P. expansum suspension at 1 × 10⁶ spores/ml 24 h after the treatment of GABA at 100 μg/ml. Tissue samples of pulp from the GABA or water-treated fruit, with or without inoculation, were collected at the same intervals (0, 12, 24, 36 and 48 h after the treatment) and immediately frozen in liquid N₂. They were then stored at −80 °C for further analysis of enzyme activity and gene expression.

2.6. Assay of enzyme activities in pear fruit

Frozen tissue samples (0.6 g) were ground in a mortar and pestle with different buffers to assay different enzymes: 1.2 ml of sodium acetate buffer (50 mM, pH 5.0); that contained 1% (w/v) polyvinyl-pyrolidone (PVP) for chitinase and 5.4 ml for β-1,3-glucanase, 1.2 ml of 200 mM sodium borate buffer (pH 8.8) for PAL and 1.2 ml of 50 mM phosphate buffer (pH 7.8) containing 1.33 mM EDTA and 1% (w/v) polyvinyl-pyrolidone (PVP) for POD and PPO. The homogenate was centrifuged at 13,000 g for 20 min
at 4 °C. The supernatants were used as the crude enzyme source to assay enzymatic activities and protein contents. Each sample, containing three replicates, was obtained from nine fruit of each treatment or inoculation and the experiment was repeated twice.

Chitinase (CHI) activity was measured according to the method of Yalpani and Pantaleone (1994) with chitin azure (Sigma–Aldrich) as the substrate. CHI activity was measured by mixing 300 μl of crude enzyme solution with 100 μl of 5 mg/ml chitin azure in 3 M AcOH. After 12 h of shaking at 37 °C, the reaction mixture was cooled and centrifuged for 5 min at 9168 g and the absorbance of the supernatant was measured at 550 nm. One unit of CHI activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 550 nm in 1 h under the assay conditions. The results were expressed as units per mg protein.

β-1,3-Glucanase (GLU) activity was assayed by measuring the amount of reducing sugar released from the substrate, following the method described by Ippolito, El-Ghaouth, Wilson, and Wisniewski (2000) with some modification. 250 μl of enzyme preparation were incubated with 250 μl of 50 mM sodium acetate buffer (pH 5.0) containing 0.5% laminarin (w/v) for 4 h at 40 °C. The blank was the crude enzyme preparation mixed with laminarin at zero time incubation. The reaction was terminated by adding 500 μl of 3.5-dinitrosalicylate and heating the sample in boiling water for 5 min. The solution was diluted with 4 ml of distilled water and the amount of reducing sugars was measured at 500 nm. The specific activity of GLU was expressed as units per mg of protein. One unit (U) was defined as the enzyme activity catalysing the formation of 1 μmol of glucose equivalents per hour.

Phenylalanine ammonia lyase (PAL) activity was assayed, referring to the method of Zhang et al. (2012) with slight modifications. The assay mixture contained 500 μl of crude enzyme and 200 μl of 50 mM L-phenylalanine in sodium borate buffer (200 mM, pH 8.8). After incubation at 37 °C for 8 h, the reaction was stopped by addition of 40 μl of 6 M HCl. One unit of PAL activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 290 nm in 1 h. The results were expressed as units per mg of protein.

Polyoxidase (POD) activity was determined, using guaiacol as substrate (Lurie, Fallik, Handros, & Shapira, 1997). The reaction mixture consisted of 100 μl of crude enzyme extract, 140 μl of 0.3% (v/v) guaiacol (in 50 mM sodium phosphate buffer, pH 6.4). The increase in absorbance at 470 nm was measured after 60 μl of 0.3% (v/v) H2O2 (in 50 mM sodium phosphate buffer, pH 6.4) were added. One unit of POD activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 470 nm per minute and the activity was expressed as units per mg of protein.

Polyphenol oxidase (PPO) activity was assayed, following the method of Mohammadi and Kazemi (2002). The reaction mixtures were 250 μl of 50 mM sodium phosphate buffer (pH 6.4) containing 10 mM pyrocatechol and 50 μl of crude enzyme. One unit of PPO activity was defined as the amount of enzyme extracts producing an increase of A398 nm by 0.01 in 1 min and the activity was expressed as units per mg of protein.

Protein content in the enzyme extracts was determined by Bradford (1976) method, bovine serum albumin (BSA) being used as a standard.

2.7. Gene expression analysis by real-quantitative PCR

Total RNA was extracted, using a modified CTAB method. First-strand cDNA was synthesised from 500 ng of total DNA-free RNA with a PrimeScript™ RT reagent Kit (TaKaRa, Japan) according to the manufacturer’s instructions. The cDNA was diluted 20-fold and 2 μl of the diluted cDNA was used as the template for real-time quantitative PCR (Q-PCR) analysis.

The reference housekeeping gene coding for actin and five target genes coding for defence proteins were chosen: PpCHI, PpGLU, PpPAL, PpPOD and PpPPO. The corresponding P. pyrifolia nucleotide sequences were obtained from the GENBANK database and used to design gene-specific primer pairs as follows, employing Primer3.0 software: PpCHI (FJ589786), forward 5’-CACACAGAGTCCTACTGC-3’ and reverse 5’-AACTTGCCGTCGGCTGAT-3’; PpGLU (JX127223), forward 5’-CTTACTCTGACATCGAATTCAAC-3’ and reverse 5’-GTA CGAGGCTTCAGAGAGAG-3’; PpPAL (JQ749640), forward 5’-ATCTTG TCCCGTGTCCCT-3’ and reverse 5’-TGAGGGTCTGAGCTTTG-3’; PpPOD (JX290377), forward 5’-CAACATGTCACACAC-3’ and reverse 5’-TGCGCCACCCCTAC-3’; PpPPO (AY338251), forward 5’-GACATTGCTATGCTGGGT-3’ and reverse 5’-CTGTGACCCTGTGATTAC-3’; PpACTIN (JN684184), forward 5’-CACTCCAGCTGTT CTCCT-3’ and reverse 5’-GCAAGTGTCGAGAGAG-3’.

PCR reactions were carried out in a 48-well system (20 μl per well). The reaction mixture contained 10.0 μl of SYBR® Premix Ex Taq™ (TaKaRa, Japan), 0.4 μl of each primer (10 μM), 0.4 μl ROX Reference Dye (50×), 2 μl of cDNA and 6.8 μl of RNase-free water. The reactions were performed on a StepOne Real-Time PCR System (Applied Biosystems) at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 30 s. Relative quantifications were then calculated, using the 2−ΔΔCt method (Livak & Schmittgen, 2001). All Q-PCR reactions were normalised, using the Ct value corresponding to the Pyrus actin gene. Efficiency of primers was analysed by the dilution method. Melting curve analysis was conducted to confirm the specificity of the primers. Each treatment included 3 biological replicate samples.

2.8. Effect of GABA on quality parameters of pear fruit

The fruit were immersed in solutions of 0, 10, 100, or 1000 μg/ml of GABA for 10 min. and then air-dried. Samples were collected after 7, 15 and 30 days of storage at 25 °C for quality evaluation. Firmness was determined at the equator of the fruit, using a hand-held penetrometer (GY-2, Fruit Firmness Tester, Zhejiang, China) and the results were expressed as percentages. Titratable acidity (TA) content was measured by titrating 10 ml of the filtered pear juice to pH 8.2 with 0.1 M NaOH. Ascorbic acid (AA) content was determined, using the 2,6-dichloro-indophenol method and the results were expressed as mg/100 g. Each treatment was replicated three times with three fruits per replicate.

2.9. Statistical analysis

Experiments were performed by a completely randomized design and conducted at least twice. The data are from one individual experiment and are representative of two independent experiments with similar results. Data were subjected to one-way analysis of variance (ANOVA univariate, SPSS 17.0; SPSS Inc., Chicago IL, USA). When the number of means in each group is two, the independent samples t test is applied for means separation. The Duncan’s multiple range test was used for separation of means.

3. Results

3.1. Effect of GABA on induction of resistance to P. expansum in pear fruit wounds

Exogenous GABA had no direct fungitoxic activity against P. expansum in pear fruit wounds when the time interval between GABA treatment and P. expansum inoculation was within 6 h (Fig. 1A and B). In contrast, the resistance to blue mould in pear
fruit pre-treated with GABA was gradually enhanced when the time interval was increased from 12 to 36 h (P < 0.05, Fig. 1A and B). Moreover, the inhibition effect was constantly increased when the concentration of GABA increased from 10 to 1000 µg/ml at the internal of 24 h (Fig. 1A). The GABA treatment of 100 µg/ml inhibited the incidence of the blue mould rot by more than 50% with respect to control at 24 h which was not statistically different from the GABA at 1000 µg/ml or the treatment of GABA at 100 µg/ml for 36 h (P < 0.05, Fig. 1A and B).

At the optimal time interval of 24 h, the treatment with GABA at 100 µg/ml was highly effective against the pathogen and reduced the level of infection caused by P. expansum at 1 × 10⁴ or 1 × 10⁶ spores/ml compared with the control. As regards P. expansum at 1 × 10⁶ spores/ml, the effect of GABA proved to be not obvious in suppressing the blue mould rot (P < 0.05, Fig. 1C).

The results in Fig. 1D indicated that the application of GABA completely inhibited the pathogen infection after storage at 4 °C for 16 days. Although the decay symptoms developed in the control and GABA treatment after 20–28 days of storage, the disease incidence in GABA-treated pear fruit was still significantly lower than that in the control (P < 0.05).

3.2. Effect of GABA on germination and growth of P. expansum spores in vitro

In general, GABA, at 1–1000 µg/ml, did not significantly influence the spore germination of P. expansum in PDB after 12 h of incubation. Similarly, there was no effect on the survival of P. expansum on PDA between the treatments with GABA at different concentrations and the control (Table 1).

3.3. Effect of GABA on spore germination of P. expansum in pear fruit wounds

Approximately 80% of spores germinated in pear wounds within 12 h of inoculation, regardless of the presence or absence of GABA at 100 µg/ml when the time interval between the treatment with GABA and P. expansum inoculation was 2 h. Conversely, germination rate was effectively inhibited in wounds inoculated with P. expansum 24 h after treatment with GABA, by 57.6% compared with that in the control fruit (P < 0.01, Fig. 2).

3.4. Effect of GABA and P. expansum on defence-related enzyme activities in pear fruit wounds

CHI activity was increased gradually by GABA as compared to the control and then sharply decreased after 36 h. Pear fruit that were treated with GABA and followed by inoculation with P. expansum showed 28.8% higher activity of CHI than with the inoculated pathogen alone after 24 h of inoculation (Fig. 3A).

GLU activity increased immediately, within 12 h after inoculation with P. expansum alone. The activity reached its highest value at 24 h in GABA-treated and inoculated fruit and then decreased gradually afterwards whereas it still maintained a relatively higher level than after other treatments (Fig. 3B). Similarly, the change
tendency of GLU activity was also obtained in GABA-treated alone fruit.

The treatment with GABA, or with pathogen inoculation alone, caused only a slight elevation in PAL activity levels compared to the respective water control within 12 h. However, PAL activity was activated quickly in GABA-treated and P. expansum-inoculated fruit and reached a maximal level at the same time, which was approximately 2.5-fold that in pathogen-inoculated fruit. After 36 h, PAL activity in pathogen-inoculated fruit was also markedly increased (Fig. 3C).

The treatment with both GABA and P. expansum resulted in the most evident inducible effect on the activities of POD and PPO in pear fruit. The two enzyme activities both reached a peak value, at 12 and 36 h, respectively (Fig. 3D and E).

3.5. Effect of GABA and P. expansum on defence-related genes expression in pear fruit wounds

Expression of the five defence-related genes was retained at very low level in fruit treated with GABA alone, while pathogen challenge triggered accelerated gene expression at different time points with the exception of PpPOD. Transcript levels of all five genes in fruit treated with GABA and inoculated with P. expansum were enhanced faster and to greater extents than after the other three treatments at 12 h (Fig. 4). A priming effect, of 19.4-, 20-, 29.5-, 2.9- and 24.1-fold, was observed in GABA-treated and P. expansum inoculated fruit for an up-regulated expression of PpCHI, PpGLU, PpPAL, PpPOD and PpPPO, respectively, in comparison with that in the control at 12 h. At 24 h, GABA induced stronger expression of PpPOD and PpPPO in pear fruit when challenged with P. expansum. Moreover, the change in expression level of PpCHI, PpGLU and PpPAL exhibited a similar tendency in pathogen-inoculated fruit that increased from 24 h and reached a higher level than after other treatments at 48 h.

3.6. Effect of GABA treatment on quality parameters of pear fruit

There were no significant differences of postharvest quality parameters, including firmness, TSS, TA and AA of pear fruit, among all treatments during 1 month of storage at 25 °C (Table 2).

4. Discussion

Most of the postharvest pathogens are incapable of penetrating directly through the fruit cuticle, depending on the wound for their invasion (Janiszewicz & Korsten, 2002). The active pathogenic process can start immediately after spores land on the wounded tissue, which results in the full spectrum of conditions conductive to pathogen development (Prusky et al., 2010). The results from this study, for the first time, showed that GABA effectively inhibited the blue mould rot caused by P. expansum in pear fruit without adverse influence on its edible quality. The use of GABA in all food commodities has been approved if it meets the statutory requirement of reasonable certainty of no harm when applied in accordance with good agricultural practises (US-EPA, 2004) and GABA may have a potential postharvest use on organic produce (Prange, Ramin, Daniels-Lake, DeLong, & Braun, 2006). The application of GABA, therefore, as a new anti-fungal biopesticide, might be developed, promisingly, into an economically feasible biocontrol agent in control of postharvest fungal rots.

To further understand the mechanisms by which GABA controlled the blue mould rot of pear fruit, we investigated the effect of GABA on P. expansum in vitro and in vivo. Our results indicated that GABA, at a concentration ranging from 1 to 1000 μg/ml, did not significantly inhibit the spore germination in PDB and the survival of P. expansum on PDA. Similarly, GABA (at 100 μg/ml) had no direct antifungal activity against P. expansum in vivo when the time interval between GABA treatment and pathogen inoculation was within 2 h. By contrast, GABA at 100 μg/ml showed clear and a strong inhibitory effect on the spore germination of P. expansum in fruit wounds when the time internal was increased to 24 h. These results imply that the induction of host-mediated resistance may be an important mechanism for GABA in reducing postharvest rots of pear fruit. It is well known that plants respond almost immediately to an inducing agent but, nevertheless, require a lag period (Yu, Chen, Lu, & Zheng, 2009). For instance, some chemical elicitors, such as chitosan, harpin, indole-3-acetic acid, or SA, were effective in developing disease resistance against postharvest pathogen attacks only after they were applied one or several days before pathogen inoculation (de Capdeville et al., 2003; Ma, Yang, Yan, Kennedy, & Meng, 2013; Yu et al., 2009).

The protection of fruit from invasion by fungal pathogens is largely due to activation of a highly coordinated defensive system that helps ward off the spread of pathogens (Tian, Wan, Qin, & Xu, 2006; Wang et al., 2013). Generally, CHI, GLU and PAL plays an important role in induced resistance of fruits (Tian et al., 2006; Yu, Shen, Zhang, & Sheng, 2011). CHI has been proved to degrade chitin, which is the major component of pathogen cell walls (Tian et al., 2007). GLU, one of the most fully characterised pathogenesis-related (PR) proteins, can also act indirectly by releasing oligosaccharide and eliciting defence reactions and then act synergistically with CHI to inhibit fungal growth (Tian et al., 2007). PAL, as the first enzyme in the general pathway of phenylpropanoid metabolism, is directly involved in the synthesis of

<table>
<thead>
<tr>
<th>GABA (μg/ml)</th>
<th>Spore germination in PDB (%)</th>
<th>Survival on PDA (CFU per plate)</th>
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<tbody>
<tr>
<td>0</td>
<td>83.7 ± 1.5 a,b</td>
<td>23.2 ± 3.2 a</td>
</tr>
<tr>
<td>1</td>
<td>77.6 ± 3.0 b</td>
<td>27.2 ± 0.3 a</td>
</tr>
<tr>
<td>10</td>
<td>79.5 ± 3.6 ab</td>
<td>24.3 ± 1.3 a</td>
</tr>
<tr>
<td>100</td>
<td>85.8 ± 1.6 a</td>
<td>26.6 ± 1.7 a</td>
</tr>
<tr>
<td>1000</td>
<td>83.5 ± 1.0 a,b</td>
<td>22.4 ± 1.6 a</td>
</tr>
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Each value is the mean of three separate determinations and the standard errors. Different letters indicate significant differences (P < 0.05) according to Duncan’s multiple range test.
active metabolites, including phenols, phytoalexins and lignin, that are associated with the localised resistance processes (Cao et al., 2008). In addition, POD and PPO are both linked to lignification of host plant cells and they participate in cell wall reinforcement. They are considered key enzymes in host defence reactions against pathogen infections (Cao et al., 2008; Ma et al., 2013; Zhang, Wang, Zhang, Hou, & Wang, 2011). Various chemical elicitors have been reported to induce resistance against fungal infection in harvested fruits by the stimulation of pathogenesis- or defence-related enzyme activities and gene expression. For example, BABA treatment induced a significant increase in the activities of CHI, GLU and POD, thereby promoting resistance against blue mould in apple fruit (Zhang et al., 2011). The induction of resistance to *Penicillium digitatum* by BABA was accompanied by activation of CHI gene expression and an increase in PAL activity in grapefruit peel tissue (Porat et al., 2003). SA, oxalic acid and calcium chloride significantly enhanced PAL and PPO activities and reduced the disease incidence caused by *Alternaria alternata* in pear fruit (Tian et al., 2006). Benzothiadiazole-induced disease resistance against anthracnose in harvested mango fruits was related to up-regulated expression of PPO and POD genes (Lin, Gong, Zhu, Zhang, & Zhang, 2011). Ma et al. (2013) reported that chitosan and oligochitosan induced the expression of GLU and POD and elevated the resistance of peach fruit against brown rot caused by *Monilinia fructicola*. Thus, the results from this present study demonstrate that GABA strongly enhanced the activities or gene expression of CHI, GLU
and PAL as well as the levels of POD and PPO in pear fruit, especially when inoculated with *Penicillium expansum*, which might contribute to the fruit resistance against *P. expansum* by the treatment with GABA.

Moreover, in view of the pronounced and rapid increase in the activities of enzymes and the expression levels of defence-related genes after treatment with GABA followed by *P. expansum* in contrast to the treatment with the pathogen alone, we infer that the mode of the action for the induced resistance by GABA might be associated with mechanisms of priming. The benefit of applying GABA is, at least, partially due to the triggering of defence responses, at enzymatic and transcript level, related to induced resistance mechanisms, which make the primed fruit less prone to disease development after infection. It is well known that the mechanisms of induced resistance appear to involve the priming of plant defences that display a more rapid response following a challenge inoculation with a virulent strain of a pathogen (Hammerschmidt, 2009). The enhanced defensive capacity by priming is correlated with a potentiated expression of defence genes and *de novo* synthesis of antimicrobial compounds, such as pathogenesis-related proteins (Solano, Maicas, de la Iglesia, Domenech, & Mañero, 2008). Priming leads, not only to an alarmed state of defence, but also offers low energy cost protection under conditions of relatively high disease pressure (van der Ent et al., 2009). This phenomenon, described as "priming", occurs in some chemical compound (BABA, BTH and ASM) – or beneficial microorganism (*rhizobacterium, Bacillus cereus AR156*) – mediated resistance (Conrath et al., 2002; van der Ent et al., 2009; Wang et al., 2013).

**Fig. 4.** Expression analysis of chitinase (PpCHI; A), β-1,3-glucanase (PpGLU; B), phenylalnine ammonialyase (PpPAL; C), peroxidase (PpPOD; D) and polyphenol oxidase (PpPPO; E) genes in wound tissue of pear fruit by qRT-PCR. Fruit wounds were inoculated with *Penicillium expansum* 24 h after the treatment of γ-aminobutyric acid (GABA) at 100 μg/ml. After various intervals, tissue samples from GABA- or water-treated fruit, with or without inoculation, were removed. Values were normalised to control at each time point, arbitrarily set to 1. *PpActin* was used as endogenous reference gene. *Indicates up-regulated expression (fold change > 2) for each time point. The vertical bars represent the standard error of three replicates.*
Nevertheless, more research into the mode of action of GABA in controlling postharvest diseases of fruit is needed, particularly to further elucidate molecular mechanisms underlying induced resistance and priming.

Acknowledgements

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Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firmness (× 10^3 Pa)</th>
<th>TSS (%)</th>
<th>TA (%)</th>
<th>AA (mg/100 g)</th>
</tr>
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<tbody>
<tr>
<td>0 day</td>
<td>12.83 ± 0.13</td>
<td>10.10 ± 0.46</td>
<td>0.09 ± 0.03</td>
<td>4.51 ± 0.06</td>
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<td>7 day control</td>
<td>12.17 ± 0.32 a</td>
<td>10.7 ± 0.41 a</td>
<td>0.11 ± 0.01 a</td>
<td>1.22 ± 0.04 a</td>
</tr>
<tr>
<td>10 μg/ml GABA</td>
<td>12.90 ± 0.69 a</td>
<td>9.97 ± 0.07 a</td>
<td>0.07 ± 0.01 a</td>
<td>0.76 ± 0.15 a</td>
</tr>
<tr>
<td>100 μg/ml GABA</td>
<td>12.62 ± 0.20 a</td>
<td>10.6 ± 0.27 a</td>
<td>0.07 ± 0.01 a</td>
<td>0.74 ± 0.19 a</td>
</tr>
<tr>
<td>1000 μg/ml GABA</td>
<td>12.62 ± 0.40 a</td>
<td>9.97 ± 0.38 a</td>
<td>0.08 ± 0.02 a</td>
<td>0.75 ± 0.15 a</td>
</tr>
<tr>
<td>15 day Control</td>
<td>12.30 ± 1.30 a</td>
<td>9.80 ± 0.40 a</td>
<td>0.09 ± 0.00 a</td>
<td>1.62 ± 0.11 a</td>
</tr>
<tr>
<td>10 μg/ml GABA</td>
<td>11.78 ± 0.18 a</td>
<td>9.85 ± 0.05 a</td>
<td>0.06 ± 0.02 b</td>
<td>1.44 ± 0.37 a</td>
</tr>
<tr>
<td>100 μg/ml GABA</td>
<td>12.45 ± 0.55 a</td>
<td>9.70 ± 0.30 a</td>
<td>0.08 ± 0.00 b</td>
<td>1.55 ± 0.22 a</td>
</tr>
<tr>
<td>100 μg/ml GABA</td>
<td>12.15 ± 1.00 a</td>
<td>10.4 ± 0.40 a</td>
<td>0.11 ± 0.02 a</td>
<td>1.49 ± 0.83 a</td>
</tr>
<tr>
<td>30 day control</td>
<td>11.75 ± 0.35 a</td>
<td>9.48 ± 0.30 a</td>
<td>0.05 ± 0.01 a</td>
<td>1.57 ± 0.44 a</td>
</tr>
<tr>
<td>10 μg/ml GABA</td>
<td>12.08 ± 0.44 a</td>
<td>9.93 ± 0.47 a</td>
<td>0.09 ± 0.01 a</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>100 μg/ml GABA</td>
<td>11.90 ± 0.18 a</td>
<td>9.23 ± 0.47 a</td>
<td>0.06 ± 0.01 a</td>
<td>0.98 ± 0.38 a</td>
</tr>
<tr>
<td>1000 μg/ml GABA</td>
<td>11.97 ± 0.07 a</td>
<td>9.10 ± 0.06 a</td>
<td>0.06 ± 0.01 a</td>
<td>0.60 ± 0.02 a</td>
</tr>
</tbody>
</table>

The fruit were immersed in solutions of 10, 100 or 1000 μg/ml of γ-aminobutyric acid for 10 min. and then air-dried. Samples were collected after 7, 15 and 30 days of storage at 25 °C for quality evaluation. Each value is the mean of three separate determinations and the standard errors. Different letters indicate significant differences (P = 0.05) according to Duncan’s multiple range test for each time point.

The references are omitted due to the page limitation.


