



Postharvest methyl jasmonate treatment inhibits blue mold decay in *Ginkgo biloba* seeds by inducing antioxidant and defense systems

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Received: 5 January 2021 / Accepted: 21 August 2021 / Published online: 17 November 2022
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Abstract

The effects of methyl jasmonate (MeJA) treatment on the mold decay and disease resistance of *Ginkgo biloba* seeds in response to pathogen attack were assessed. *G. biloba* seeds were exposed to different concentrations of MeJA vapor for 12 h before being inoculated with *Penicillium oxalicum*. Results showed that MeJA at different concentrations could markedly decrease the disease incidence and inhibit membrane damage of *G. biloba* seeds compared with the control, especially at the concentrations of 100 and 150 $\mu\text{mol L}^{-1}$. The H_2O_2 content and activities of H_2O_2 -metabolising related enzymes were induced to a higher level with MeJA. Defense related enzyme activities were also significantly enhanced in *G. biloba* seeds treated with 100 and 150 $\mu\text{mol L}^{-1}$ of MeJA. These results together indicated that MeJA stimulated the disease resistance of *G. biloba* seeds by regulating the activation of H_2O_2 accumulation and inducing activities of defense-related enzymes.

Keywords *Ginkgo biloba* seeds · Methyl jasmonate · Blue mold decay · Antioxidant enzymes · Defense related enzymes

Introduction

Ginkgo biloba seed, as a type of nut, is increasingly becoming popular food mainly due to its nutritional contents, such as carbohydrates, protein, amino acids, vitamin C, flavonoids, and lactones [1]. *G. biloba* seed is also a traditional Chinese medicine and used as a raw material for directly cooking or making processed food that benefits human health. The active ingredients of ginkgo seeds such as flavanol glycosides, terpene trilactones, ginkgolic acids and peptides were reported to treat fever, coughs, sputum and skin disease effectively [2]. Modern medical research has proven that ginkgo seeds exhibit good effects on cardiovascular and cerebrovascular system diseases; they could discourage clot formation, improve blood circulation, as

well as protect nerve cells [3–5]. Nowadays, consumption of *G. biloba* seed is increasing with the consumer's demands mainly due to its nutritional contents and functional components. However, *G. biloba* seeds are susceptible to mold infection by *Penicillium* during long-term storage.

Blue mold decay, which is caused by pathogens belonging to *Penicillium* genus, is a common and major postharvest disease that limits the storage of many horticulture crops [6–9]. *G. biloba* seeds are prone to the mold decay caused by *Penicillium oxalicum*, which is one of the pathogens isolated from decayed seeds. Various methods have been used for preserving *G. biloba* seeds, such as cold storage, SO_2 fumigation, chemical fungicide, chitosan and nanomaterial coatings [10–12]. However, these preservation treatments still have limitations, including limited antifungal effect, residue of chemical fungicides, and complicated operation, which may result in threat to human health or increased cost.

One of the possible options to reduce disease development is inducing the defense mechanisms in plants [13]. Methyl jasmonate (MeJA) is a fragrant volatile compound that is distributed ubiquitously in fruit and plants. MeJA and its free-acid jasmonic belong to jasmonates, which are synthesized in plants via the octadecanoid pathway from α -linolenic acid. As a natural plant signaling compound, MeJA is closely related to developmental processes and the defense responses against biotic and abiotic stresses

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in fruit and plants [7, 14]. Moreover, the defense-related genes in fruit, which are involved in jasmonate biosynthesis, cell wall formation, secondary metabolism, and those encoding stress protective and defense proteins, can be upregulated after MeJA treatment. Therefore, MeJA treatment has been widely used for controlling postharvest diseases, maintaining quality, and prolonging the shelf life of fruit and vegetables [15]. The treatment of MeJA vapors has been found to significantly reduce the incidence of anthracnose and gray mold diseases on avocados and table grapes [8, 16]. Moreover, it has been applied on postharvest apple, pear, and peach fruit to control blue mold decay [17–19]. The mechanism involved in the disease-resistant process may be not only related to the transient direct inhibitory effect against the pathogens, but also related to the ability to induce resistance in fruit by MeJA. The results showed that MeJA at different concentrations of 100, 150, and 200 $\mu\text{mol L}^{-1}$ could inhibit the mycelial growth of *P. expansum* and are efficient in controlling blue mold decay [17–19]. The concentrations of MeJA most commonly used in previous studies were 10, 50, and 100 $\mu\text{mol L}^{-1}$ [8]. Besides, most studies of fruit treated with MeJA focus on the defense mechanism represented by defense-related enzymes, including phenylalanine ammonia-lyase (PAL), chitinase (CHI), and β -1,3-glucanase (GLU) [17–19]. PAL is a crucial enzyme involved in the phenylpropanoid pathway. PAL could induce the production of secondary metabolites in plants to build barriers and limit the infection process. CHI and GLU, which consist of chitin and β -1,3-glucan, respectively, could hydrolyze the polymers of fungal cell walls [8].

However, to the best of our knowledge, the literature on the effect of MeJA on the blue mold disease susceptibility of *G. biloba* seeds is limited. Therefore, this research aimed to investigate the effects of MeJA vapors at different concentrations (0, 50, 100, and 150 $\mu\text{mol L}^{-1}$) on the following: (i) disease incidence, (ii) activities of antioxidant enzymes, and (iii) defense-related enzyme activities in postharvest *G. biloba* seeds kept at 25 °C, with a relative humidity (RH) 90%, for 24 d.

Materials and methods

Materials

G. biloba seeds at commercial maturity were collected from trees cultured in Nanjing Forestry University, China. The seeds without epispem were washed with tap water and air-dried until their water content was below 70%, following the process of removing the mesospem. Afterwards, seeds were selected for absence of blemishes or disease prior to MeJA treatment and pathogen inoculation.

Pathogen

P. oxalicum (Gene bank accession number: MK419111) was isolated from the infected *G. biloba* seeds and incubated on a potato dextrose agar medium (PDA: HB0233-12, Qingdao Hope Bio-Technology Co., Ltd) for 7 d. Afterwards, the spores were flushed with sterile water, and the suspensions were prepared by filtering with four layers of lens wiping paper. The desired concentration of spores (1×10^5 conidia mL^{-1}) was obtained by using a hemocytometer.

Measurement of MeJA on spore germination of *P. oxalicum* in vitro

The effects of MeJA on the spore germination of *P. oxalicum* were measured in the potato dextrose broth (PDB) according to a previous report [18]. A final concentration of 10^6 spores mL^{-1} was obtained with PDB, which contained various concentrations of MeJA (0, 50, 100, and 150 $\mu\text{mol L}^{-1}$). The PDB culture mediums were incubated at table concentrator with a speed of 100 g at 25 °C for 24 h. The germination rate was determined by observing approximately 100 spores of pathogen for each treatment, with three replications.

MeJA treatment and pathogen inoculation

A total of 500 seeds for one treatment with three replicates were placed in 40 air-tight containers. MeJAs (Sigma–Aldrich, USA) at three concentrations of 50, 100, and 150 $\mu\text{mol L}^{-1}$ were deposited on Petri dishes at the bottom of the container [20]. All treatments, including the MeJA at the concentration of 0 (control), were immediately sealed in containers and exposed to MeJA vapor for 12 h at 10.0 ± 0.5 °C. After MeJA fumigation, the containers were opened and ventilated at 25 °C for 4 h. Before pathogen inoculation, all seeds in the MeJA treatments and the control were surface disinfected with 75% ethanol for 30 s. Afterwards, they were surface wounded (1 mm diameter \times 1 mm deep) and inoculated with 10 μL of spore suspension, with the same amount of sterile water as the control. Afterward, all seeds were maintained at 25 °C with 90% RH for 24 d. Samples were collected and frozen by liquid nitrogen immediately, and then stored at -80 °C for further analysis. The quality parameters and physico-chemical characteristics were determined every 6 d.

Disease incidence determination

The disease incidence was determined by calculating the amount of diseased seeds exhibiting visible fungal infection, and the results were expressed as the percentage of infected seeds compared with total number of seeds in each treatment [20].

Measurement of relative conductivity and malondialdehyde (MDA) content

The relative conductivity was determined according to the previous method [21]. The initial conductivity (L_0) of the tissues after water bath shaking at 25 °C for 30 min and another reading (L_1) of the tissue conductivity after boiling for 20 min and then cooling down to 25 °C were measured using a conductivity meter (DDS-307, Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China). The relative conductivity was calculated using the following equation:

$$\text{Relative conductivity (\%)} = L_0/L_t \times 100. \text{ (E1)}$$

The MDA content was measured as described in a previous report [22] and the results were calculated using the following formula based on fresh weight basis: C (mmol kg^{-1}) = $6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}$. (E2).

Determination of H_2O_2 content and antioxidant enzymes activities

For H_2O_2 determination, 2 g of frozen tissues were homogenized with 1 mL of 0.1% cold trichloroacetic acid solution, and then maintained in an ice bath for 10 min. The supernatant was obtained after centrifugation at 12,000 g for 15 min at 4 °C. The reaction mixture consisted 0.5 mL of supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0), and 1 mL of 1 mol L^{-1} potassium iodide. The absorbance was measured at 390 nm, and the H_2O_2 content was extrapolated from a standard curve expressed as $\mu\text{mol g}^{-1}$ [23]. The superoxide dismutase (SOD), the peroxidase (POD), and the catalase (CAT) were measured according to previous reports [24–26].

Determination of defense related enzymes activities

The PAL activity was estimated according to the reported method [27]. The reaction system included 75 μL of crude enzyme extract and 150 μL of borate buffer (50 mM, pH 8.8) containing L-phenylalanine (20 mM). After incubated at 37 °C for 60 min, the reaction was ended with the addition of 75 μL of HCl (1 M). The absorption was measured at 290 nm, and the enzyme activity was recorded as a nmol cinnamic acid h^{-1} mg of protein $^{-1}$.

The chitinase activity was determined according to Abeles et al. [28]. The enzyme extract (600 μL) mixed with 2% (w/v) dye-labelled chitin azure (125 μL) and sodium acetate buffer (pH 5.0, 50 mM) was incubated at 40 °C for 2 h, and then the reaction system was stopped by adding 25 μL of HCl (1 mol L^{-1}). The supernatant was measured at 550 nm, and the unit of enzyme activity was defined as a nM product h^{-1} mg of protein $^{-1}$.

The β -1,3-glucanase activity was assayed according to Sellamuthu et al. [29]. The reaction system was as follows:

100 μL of enzyme extract, 100 μL of 2% (w/v) laminarin, and 25 μL of 3,5-dinitrosalicylic. The reaction was ceased after the 5-min boiling water bath, and one enzyme unit was defined as the 1 mol glucose equiv. h^{-1} mg of protein $^{-1}$.

Statistical analysis

All data presented as mean ($n=3$) \pm standard deviation (SD) were evaluated by one-way ANOVA, and the differences ($p < 0.05$) were assessed using Duncan's multiple range test using the IBM SPSS 19.0 Statistics analytical software program.

Results and discussion

Effect of MeJA at different concentrations on inhibiting blue mold decay and spore germination

Concerned to the effects of MeJA fumigation on the growth of *P. oxalicum* in vitro, we designed the in vitro test to find the changes of spore germination in response to MeJA treatment. As shown in Fig. 1, the spore germination of *P. oxalicum* of all the treatments were similar, indicating that MeJA of different concentrations had little effect on the spore germination of *P. oxalicum* ($p > 0.05$). This finding is similar to the study of Zhang et al. [18], who observed that MeJA at all tested concentrations could not suppress

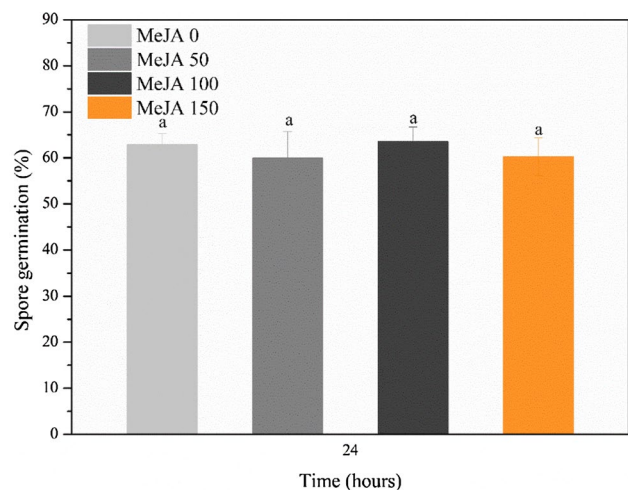


Fig. 1 Spore germination of at 25 °C for 24 h. Seeds were organized into the following treatments: (i) control (MeJA 0); (ii) treated with methyl jasmonate (MeJA) with concentrations of 50, 100 and 150 $\mu\text{mol L}^{-1}$ (MeJA 50, MeJA 100 and MeJA 150). Different letters indicate significant differences ($p < 0.05$) due to different treatments at the same storage period. Each value represented the mean of three replicates, data were presented as mean \pm standard deviation (SD)

the spore germination of *P. expansum*, which indicated that MeJA could not directly influence the growth of some fungal pathogens.

Figure 2 and Fig. 3 showed that blue mold disease occurred in control and all treated *G. biloba* seeds during postharvest storage. The disease incidence increased rapidly in the control samples, whereas significantly ($p < 0.05$) reduced in seeds fumigated with different concentrations of MeJA vapor. *G. biloba* seeds treated with 100 and 150 $\mu\text{mol L}^{-1}$ MeJA vapors exhibited significant lower disease incidences with the values of 32.99 and 33.96% compared with control (63.27%) at the end of the

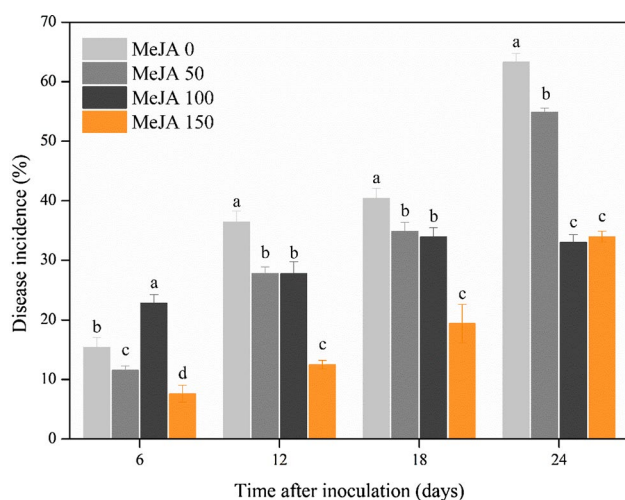


Fig. 2 Disease incidence of infected *G. biloba* seeds stored at 25 °C with RH 90% for 24 d. Seeds were organised into the following treatments: (i) control (MeJA 0); (ii) treated with methyl jasmonate (MeJA) with concentrations of 50, 100 and 150 $\mu\text{mol L}^{-1}$ (MeJA 50, MeJA 100 and MeJA 150). Different letters indicate significant differences ($p < 0.05$) due to different treatments at the same storage period. Each value represented the mean of three replicates, data were presented as mean \pm standard deviation (SD)

storage. However, no statistically significant difference was observed between the groups of MeJA 100 $\mu\text{mol L}^{-1}$ and MeJA 150 $\mu\text{mol L}^{-1}$ at the end of storage. Our result was consistent with the observation of Yao and Tian [19], who found that MeJA could control postharvest blue mold decay, but the inhibitory ability is limited. In other studies, MeJA treatment at 100 or 200 $\mu\text{mol L}^{-1}$ could significantly reduce blue mold rot lesion diameter of pear fruits, the combination of MeJA at 100 $\mu\text{mol L}^{-1}$ and antagonistic yeast was more effective to reduce the lesion diameter of blue mold decay of apples [17].

Effect of MeJA at different concentrations on the relative conductivity and malondialdehyde (MDA) content

All MeJA vapor treatments could suppress the increase of relative conductivity compared to the control during most of the storage time (Fig. 4A). The relative conductivity calculated with E1 in all treated *G. biloba* seeds showed no statistically significant differences ($p > 0.05$). However, at the end of storage, *G. biloba* seeds treated with MeJA at concentrations of 100 and 150 $\mu\text{mol L}^{-1}$ exhibited the lowest values of relative conductivities without significant differences. The values of MDA content were calculated according to E2. In Fig. 4B, the MDA content of the *G. biloba* seeds without MeJA increased approximately 1.46-, 1.99-, and 2.18-fold in 24 d compared with those with MeJA at 50, 100, and 150 $\mu\text{mol L}^{-1}$, respectively ($p < 0.05$). Changes of the relative conductivity and MDA content in *G. biloba* seeds fumigated with MeJA together indicated that MeJA could maintain the stability of cell membrane to avoid the infection of *P. oxalicum*, especially.

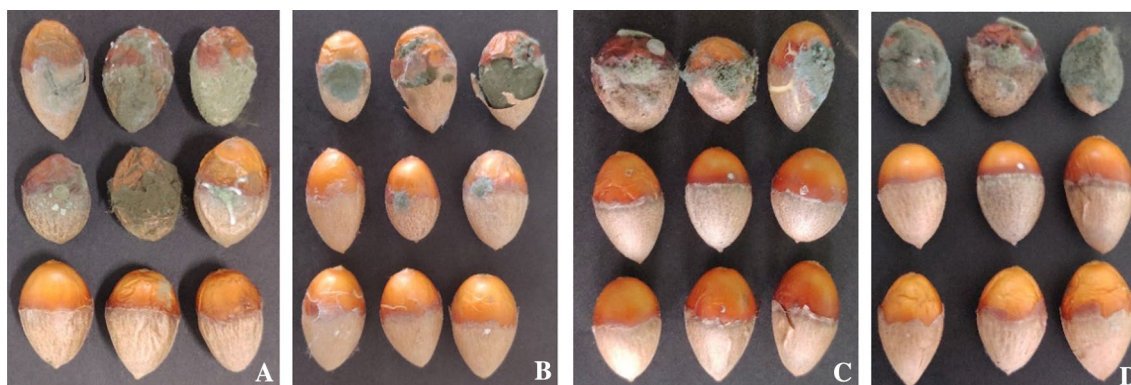


Fig. 3 Effects of MeJA on *G. biloba* seeds blue mold, 24 d after inoculation with *Penicillium oxalicum*. Seeds were organised into the following treatments: **A** control (MeJA 0); **B–D** treated with methyl

jasmonate (MeJA) with concentrations of 50, 100 and 150 $\mu\text{mol L}^{-1}$ (MeJA 50, MeJA 100 and MeJA 150)

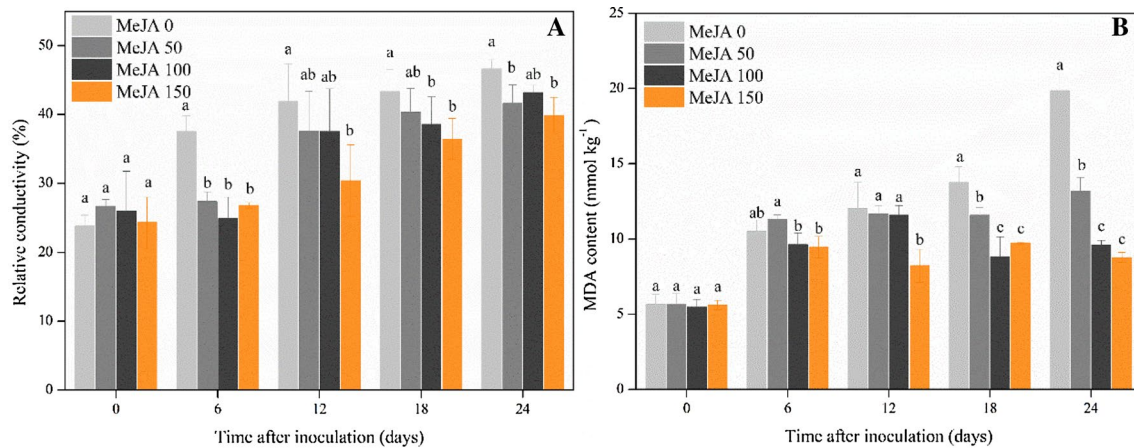


Fig. 4 Relative conductivity (A) and MDA content (B) of infected *G. biloba* seeds stored at 25 °C with RH 90% for 24 d. Seeds were organised into the following treatments: (i) control; (ii) treated with methyl jasmonate (MeJA) with concentrations of 50, 100

and 150 $\mu\text{mol L}^{-1}$. Different letters indicate significant differences ($p < 0.05$) due to different treatments at the same storage period. Each value represented the mean of three replicates, data were presented as mean \pm standard deviation (SD) of triplicate experiments

Effect of MeJA at different concentrations on H_2O_2 content and antioxidant enzymes (SOD, POD and CAT) activities

In Fig. 5A, the H_2O_2 content increased in the control and treated seeds with the extension of storage period. After 18 d of storage, *G. biloba* seeds treated with MeJA at the concentrations of 100 and 150 $\mu\text{mol L}^{-1}$ showed remarkably higher H_2O_2 contents than the control ($p < 0.05$). The difference between the 50 $\mu\text{mol L}^{-1}$ MeJA treated seeds and the control was not significant ($p > 0.05$) after 18 d of storage. Moreover, the H_2O_2 content of MeJA at 50 $\mu\text{mol L}^{-1}$ remained at a relatively lower level than those of MeJA at 100 $\mu\text{mol L}^{-1}$ and 150 $\mu\text{mol L}^{-1}$ after 18 d of storage.

The antioxidant enzymes activities varied with a similar trend of first increasing and then decreasing (Fig. 5B–D). The group of MeJA 150 showed a more rapid ascent of SOD activity during the 12 d after inoculation than all the other groups ($p < 0.05$). The highest SOD activity was noted in the seeds of MeJA 100 at day 18 ($p < 0.05$). At the end of the storage, the exposure of the seeds to MeJA at 100 $\mu\text{mol L}^{-1}$ resulted in higher SOD activity, exhibiting a significant difference from the other treatments of MeJA (50 $\mu\text{mol L}^{-1}$, 150 $\mu\text{mol L}^{-1}$) and control ($p < 0.05$). The activity of POD was markedly increased in seeds treated with MeJA at 150 $\mu\text{mol L}^{-1}$ from day 6 and maintained a high level in all treated seeds for the following storage period ($p < 0.05$). The CAT activity also showed a similar tendency as POD, showing an increase in activity from day 6 and declining from day 18 in all groups. A higher CAT activity was observed in seeds exposed to MeJA at different concentrations than those untreated from inoculation for 12 d ($p < 0.05$). The *G. biloba* seeds exposed to MeJA at 100 and 150 $\mu\text{mol L}^{-1}$ showed

significantly ($p < 0.05$) higher CAT activities than those of the untreated (control) and the other MeJA treatment during the whole storage.

Effect of MeJA at different concentrations on defense-related enzymes (PAL, Chitinase and β -1,3-Glucanase) activities

The PAL enzyme activity in all *G. biloba* seeds reached the top values over 12 d of storage, and then declined during the rest of the period (Fig. 6A). All the MeJA treated seeds exhibited significantly higher enzyme activities than those seeds without any treatment after 12 d of storage ($p < 0.05$). However, during the latter part of the storage period, MeJA at 150 $\mu\text{mol L}^{-1}$ maintained higher PAL enzyme activities than MeJA at 50 $\mu\text{mol L}^{-1}$. Moreover, at the end of storage, the PAL enzyme activity was maintained at a markedly higher level in MeJA 100-treated seeds. During most of the storage time, the CHI activity was found to be significantly increased in MeJA exposed seeds, especially at 100 and 150 $\mu\text{mol L}^{-1}$ ($p < 0.05$). Compared to the control samples, all MeJA treated seeds could maintain the CHI activity at significant high values after storage (Fig. 6B). The β -1,3-glucanase activity was higher in MeJA-treated *G. biloba* seeds than in the untreated control fruit ($p < 0.05$) from day 12 after inoculation (Fig. 6C). At the end of storage, the β -1,3-glucanase activity of MeJA at 50 $\mu\text{mol L}^{-1}$ and MeJA at 100 $\mu\text{mol L}^{-1}$ was significantly higher than that of MeJA at 150 $\mu\text{mol L}^{-1}$ and control ($p < 0.05$).

Evidently, MeJA effectively reduced the disease incidence of blue mold on postharvest *G. biloba* seeds after a long storage time, especially for MeJA at 100 and 150 $\mu\text{mol L}^{-1}$. A high level of MeJA concentration could accelerate the

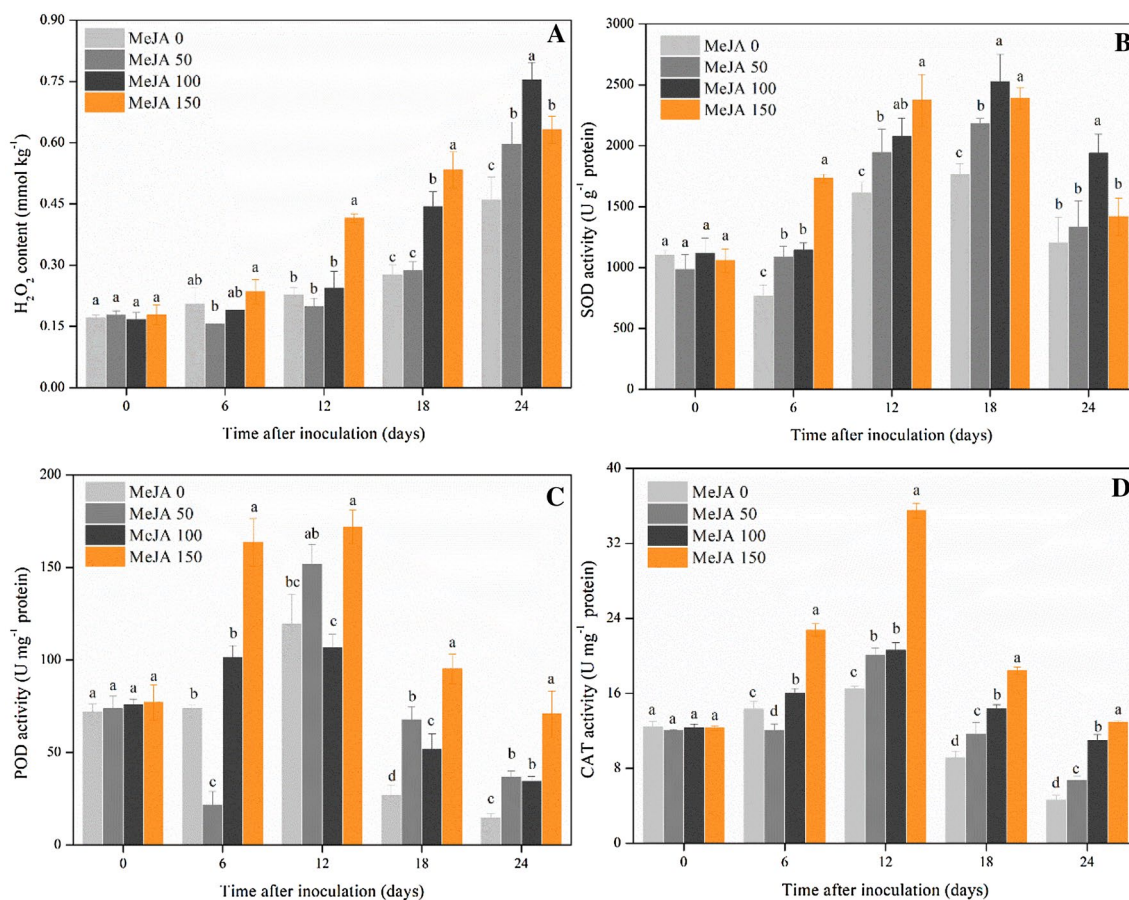


Fig. 5 H₂O₂ content (A), SOD (B), POD (C) and CAT activities (D) of infected *G. biloba* seeds stored at 25 °C with RH 90% for 24 d. Seeds were organised into the following treatments: (i) control; (ii) treated with methyl jasmonate (MeJA) with concentrations of 50, 100

and 150 μmol L⁻¹. Different letters indicate significant differences ($p < 0.05$) due to different treatments at the same storage period. Each value represented the mean of three replicates, data were presented as mean ± standard deviation (SD) of triplicate experiments

ripening and senescence of *G. biloba* seeds (data not shown). Relative research showed that the most effective concentrations focused on the range of 1–1000 μmol L⁻¹, which was dependent on many factors, including the varieties of fruit and treatment methods of MeJA [30]. Our result is consistent with those previous studies. For instance, the disease incidence could be effectively reduced by MeJA treatment in sweet cherry fruit inoculated with *P. expansum* during 5 d of storage [9] and in avocado fruit at 100 μmol L⁻¹ prior to being stored for 14 d followed by 6–7 d shelf-life, confirming the effect of MeJA treatment on enhancing the disease resistance of *G. biloba* seeds [8]. However, MeJA reportedly does not have a direct effect on delaying the spore germination of *P. expansum* and *P. digitatum* and the growth of *C. acutatum* [6, 9, 31]. Consistent with these reports, our study also demonstrated that MeJA showed no direct inhibition of the spore germination of *P. oxalicum* (Fig. 1). Therefore, the defense effect of *G. biloba* seeds was not directly achieved by inhibiting the growth of pathogens by MeJA treatment but mainly due to the induced activation of a highly coordinated

biochemical and structural defense system that could inhibit the spread of pathogens [32]. Besides, our study also showed the same phenomenon as previously reported, MeJA could alleviate the membrane lipid peroxidation and electrolyte leakage [33].

The induction of the disease resistance in postharvest horticultural crops is an important strategy to avoid infection of pathogen. They can rely on their own defense mechanisms and thus reduce the incidence of diseases. The possible induction of the disease resistance of postharvest plants involves complex reaction systems as shown in Fig. 7. It involves the activation of a series of biochemical and structural defense systems against the invasion of fungal pathogens, such as reactive oxygen species (ROS), and the phenylpropanoid pathway [34, 35]. ROS plays a role on regulating plant resistance responses to corresponding pathogen infections. It accumulates with a so-called oxidative burst as one of the earliest cellular responses following a successful pathogen recognition. ROS reportedly strengthened the host cell walls by cross-linking glycoproteins [36]. In the present

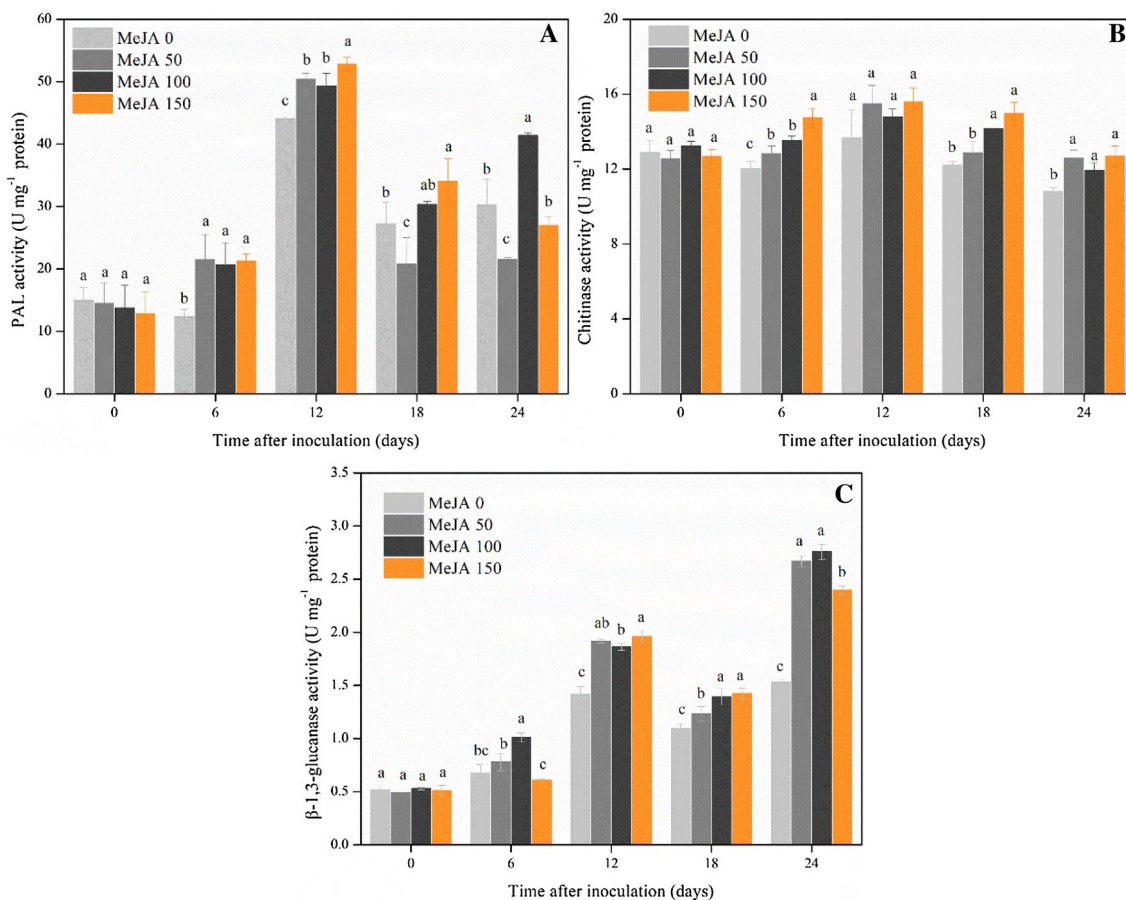
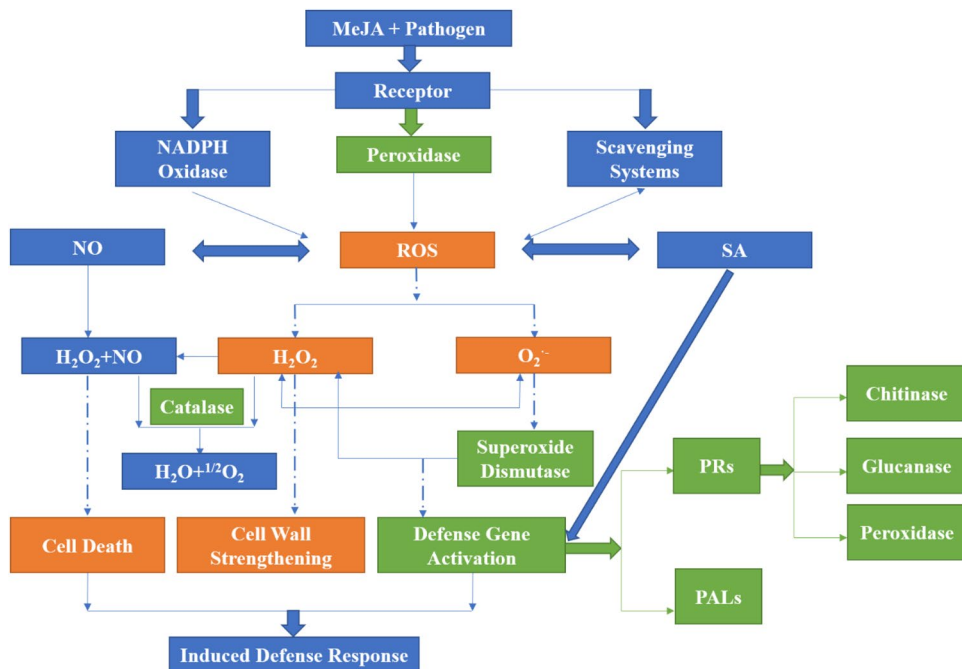


Fig. 6 PAL (A), CHI (B) and β-1,3-glucanase (C) activities of infected *G. biloba* seeds stored at 25 °C with RH 90% for 24 d. Seeds were organised into the following treatments: (i) control; (ii) treated with methyl jasmonate (MeJA) with concentrations of 50, 100

and 150 μmol L⁻¹. Different letters indicate significant differences ($p < 0.05$) due to different treatments at the same storage period. Each value represented the mean of three replicates, data were presented as mean ± standard deviation (SD) of triplicate experiments

Fig. 7 The schematic diagram of probable mechanisms of MeJA in inducing disease resistance in *G. biloba* seeds



study, the H_2O_2 content was activated to a higher level after the MeJA treatment and the inoculation by the pathogen, inducing defense responses probably by priming the tissue to promote ROS accumulation. A previous study indicated that the accumulation of ROS was closely associated with decreased fruit susceptibility to decay during postharvest storage [30]. This result was consistent with the study of the MeJA induced responses of sweet cherry against *P. expansum* [9]. Therefore, ROS might act as a central part in the MeJA primed responses against pathogen infection.

However, a substantial production of ROS burst can break the structure of host cells, resulting in cell death. Therefore, host tissues need a ROS scavenging system to protect themselves from injuries. High activities of antioxidant enzymes, including CAT, SOD, and POD, were reportedly involved in enhancing fruit disease resistance [30, 37]. Our results are consistent with those of these studies, MeJA (100 and 150 $\mu\text{mol L}^{-1}$) significantly increased the SOD, CAT, and POD activities. The CAT and POD activities were induced to high level first and then quickly declined at the later period of storage. The probable reason may be that CAT and POD were involved in the plant-derived ROS scavenging due to the accumulation of H_2O_2 . SOD can convert superoxide radicals ($O_2^{\bullet-}$) into H_2O_2 , and excessive H_2O_2 will be scavenged by intracellular CAT and POD, suggesting that the high levels of antioxidant enzymes induced by MeJA might play important roles in reducing oxidative stress and restraining the infection by the fungal pathogen. Moreover, the high activities of antioxidant enzymes induced by MeJA contribute to maintaining the balance of the redox state in the cells. Otherwise, hypersensitivity (HR) may occur due to the redox state alteration and lead to cell death. Therefore, one of the mechanisms of MeJA in inducing disease resistance in *G. biloba* seeds may be the increase of ROS and the activation of defenses following infection (Fig. 7).

In our research, the activities of defense-related enzymes, including PAL, CHI, and GLU, were all enhanced by MeJA treatment, especially at 100 and 150 $\mu\text{mol L}^{-1}$. Similar results were also obtained in peach and sweet cherry fruit [30, 32]. CHI and GLU can hydrolyze polymers in the cell wall of the pathogenic fungi to prevent fungal damage, and some metabolites can induce the accumulation of enzymes related to the disease resistance reaction. PAL is a key enzyme in the phenylpropanoid pathway associated with the biosynthesis of secondary metabolites, including phytoalexins, phenolic compounds, and lignin, which are involved in enhancing disease resistance processes [31]. These enzymes could directly or indirectly break or inhibit pathogens, contributing to the reduction of disease incidence. These defense-related enzymes exhibited various functions in the defense system, which may be collectively and coordinately induced by MeJA in *G. biloba* seeds. Hence, the activation of defense-related enzymes activities to higher levels may be

another part of the mechanisms of MeJA in inducing disease resistance in *G. biloba* seeds (Fig. 7).

Conclusion

In summary, MeJA treatment, especially at concentrations of 100 and 150 $\mu\text{mol L}^{-1}$, could effectively reduce the disease incidence in *G. biloba* seeds. Exposure to these MeJA treatments promotes the accumulation of ROS as important signals for mediating the defense system activation and enhances the activities of antioxidant and defense-related enzymes. Two probable action mechanisms of MeJA involve increased ROS and defense enzymes activities. Both results suggest that MeJA induces high disease resistances in *G. biloba* seeds, resulting in less disease infection. These results provide a reference for further application on the commercial storage of *G. biloba* seeds.

Acknowledgements This research was financially supported by National Key R&D Program of China [2019YFD1002300], North Jiangsu Science and Technology Project [XZ-SZ201929], Research Project of Jiangxi Forestry Bureau [No.202012], Postgraduate Research & Practice Innovation Program of Jiangsu Province [No. KYCX17_0836]. The authors also acknowledged the support of Priority Academic Program Development of Jiangsu Higher Education Institutions [PAPD].

Declarations

Conflict of interest All author declares that there is no conflict of interest to disclose.

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