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Effects of oligosaccharides from endophytic *Fusarium oxysporum* Dzf17 on activities of defense-related enzymes in *Dioscorea zingiberensis* suspension cell and seedling cultures

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ABSTRACT

Background: Three oligosaccharides (EOS, WOS and SOS) were respectively prepared from the corresponding polysaccharides, namely exopolysaccharide (EPS), water-extracted mycelial polysaccharide (WPS) and sodium hydroxide-extracted mycelial polysaccharides (SPS) from the endophytic fungus *Fusarium oxysporum* Dzf17. In this study, the effects of EOS, WOS and SOS on the activities of the defense-related enzymes, namely phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO) and peroxidase (POD) in its host plant *Dioscorea zingiberensis* cultures were investigated.

Results: For the suspension cell cultures of D. zingiberensis, the highest PAL activity was induced by 0.5 mg/mL of WOS at 48 h after treatment, which was 4.55-fold as that of control. Both PPO and POD activities were increased to the maximum values by 0.25 mg/mL of WOS at 48 h after treatment, which were respectively 3.74 and 3.45-fold as those of control. For the seedling cultures, the highest PAL activity was elicited by 2.5 mg/mL of EOS at 48 h after treatment, which was 3.62-fold as that of control. Both PPO and POD reached their maximum values treated with 2.5 mg/mL of WOS at 48 h after treatment, which were 4.61 and 4.19-fold as those of control, separately.

Conclusions: Both EOS and WOS significantly increased the activities of PAL, PPO and POD in the suspension cell and seedling cultures of *D. zingiberensis*. The results suggested that the oligosaccharides from the endophytic fungus *F. oxysporum* Dzf17 may be related to the activation and enhancement of the defensive mechanisms of *D. zingiberensis* suspension cell and seedling cultures.

1. Introduction

Plants exhibit constitutive and inducible defenses against pathogen attacks. Differential responses of plants to the pathogens have been found in many plant–pathogen interactions, which led to an initiation of complex defense signal transductions in plant cells. The natural resistance of plants to diseases is based not only on preformed defenses, but also on induced mechanisms [1]. Most plants have developed a variety of inducible defense mechanisms against diverse biotic and abiotic stresses, such as cell wall reinforcement by deposition of lignin, necrotic hypersensitive response, biosynthesis of phytoalexins and pathogenesis-related proteins [2,3,4]. An apparently ubiquitous feature of plant defensive response to pathogen attack is the induction of defense-related enzymes such as phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO), peroxidase (POD), glucanase, and chitinase [5,6]. PAL has been considered as the key enzyme of phenylpropanoid metabolism which consists with a range of defensive roles for phenylpropanoids [7,8]. PPO, which is widely distributed in plants, can oxidize monophenol, diphenol or trihydric to their corresponding quinines with great toxicity to pathogen [9]. POD can assist superoxide dismutase (SOD) and catalase (CAT) to scavenge the excessive superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) in plant cells, which could ensure plant healthy growth [10].

The earliest described elicitor for inducing phytoalexin biosynthesis was an oligosaccharide from the cell walls of the soybean pathogen *Phytophthora sojae* [11]. Since then, more and more elicitors including live microorganisms, fragments of cell walls, crude extracts of mycelia, polysaccharides, oligosaccharides

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and proteins, have been reported from the pathogens or non-pathogens to activate plant disease resistance [10,12,13]. Plant endophytes residing in the internal organs or tissues of healthy plants cause no disease symptoms on their host plants [14,15]. In recent years, more and more attentions have been paid to endophytic fungi because of their important functions, such as producing important and novel natural bioactive compounds, modulating plant growth, improving plant resistance to stress, and enhancing biosynthesis of plant secondary metabolites [16,17,18,19,20,21]. During the long period of co-evolution, a friendly relationship has been gradually set up between each endophytic fungus and its host plant. It is possible that some components from the endophyte might be used as the elicitors to induce disease resistance of its host plant [22].

Fusarium oxysporum Dzf17 was an endophytic fungus isolated from the healthy rhizomes of *Dioscorea zingiberensis* (Dioscoreaceae), a Chinese medicinal herb [23,24]. In our previous studies, three polysaccharides were prepared from this fungus, which were respectively exopolysaccharide (EPS), water-extracted mycelial polysaccharide (WPS) and sodium hydroxide-extracted mycelial polysaccharides (SPS), and their enhancement effects on growth and diosgenin accumulation in the suspension cell and seedling cultures of *D. zingiberensis* were observed [25]. The oligosaccharides, which were further prepared from the above polysaccharides, also showed improving growth and stimulating diosgenin accumulation in *D. zingiberensis* suspension cells [26]. Moreover, excellent *in vitro* antioxidant activities of the three polysaccharides from *F. oxysporum* Dzf17 were also observed [27]. It has well demonstrated that the polysaccharides or oligosaccharides from fungi were in favor of induction of plant defensive responses [28,29]. In the present study, we aim to investigate the effects of the oligosaccharide elicitors from *F. oxysporum* Dzf17 on the activities of the defense-related enzymes in the suspension cell and seedling cultures of its host plant *D. zingiberensis*. The defense-related enzymes studied in this work included PAL, PPO and POD. By investigating the effects of oligosaccharide elicitors from plant endophyte on the defensive system of its host plant, it will provide more information for further understanding the interactions between endophytic *F. oxysporum* Dzf17 and its host *D. zingiberensis*.

2. Materials and methods

2.1. Plant materials

The *D. zingiberensis* root explants were employed to induce callus according to the method in our previous study [23]. The induced calli were subcultured in darkness at an interval of 30 d on Murashige and Skoog (MS) medium, which contained naphthalene acetic acid (1.0 mg/L) and 6-benzyladenine (1.5 mg/L) [25]. To obtain the suspension cell cultures of *D. zingiberensis*, the calli were cultured on the above medium without agar at 25°C in darkness on a rotary shaker at 120 rpm, all of which were carried out in 125-mL Erlenmeyer flasks.

D. zingiberensis seedlings were initially acquired by redifferentiation of calli on solid MS medium containing kinetin (2.0 mg/L) and 6-benzyladenine (5.0 mg/L) at 25°C under 12 h daily illumination of approximately 2000 lx. The seedlings were then subcultured on solid hormone-free MS medium at 25°C under 12 h daily illumination at an interval of 30 d [30]. After the seedlings were subcultured for five generations in 125-mL Erlenmeyer flasks, they were used as the materials in the following elicitation experiments.

2.2. Preparation of oligosaccharides from F. oxysporum Dzf17

The endophytic fungus *F. oxysporum* Dzf17 (GenBank accession number as **EU543260**) was isolated from the healthy rhizomes of *D. zingiberensis* as described in the previous report [24]. *F. oxysporum* Dzf17 was cultured in the liquid medium (300 mL) in each 1000-mL Erlenmeyer flask, which consisted of glucose (50 g/L), peptone (13 g/L), NaCl (0.6 g/L), K_2HPO_4 (0.6 g/L), and $MgSO_4 \cdot 7H_2O$ (0.2 g/L). When the flasks were kept on a rotary shaker in darkness at 150 rpm and 25°C for 14 d, the fermented broth (150 L) were collected and then centrifuged at 7741 x g for 20 min. The supernatant was employed to prepare exopolysaccharide. The mycelia were washed twice with deionized water and then lyophilized. About 600 g of the dry mycelia was gained for mycelial polysaccharide preparation.

Three polysaccharides, namely exopolysaccharide (EPS), water-extracted mycelial polysaccharide (WPS) and sodium hydroxide-extracted mycelial polysaccharide (SPS) were respectively prepared from endophytic *F. oxysporum* Dzf17 in our previous study [25]. Three crude oligosaccharides (*i.e.*, EOS, WOS and SOS) were respectively prepared by hydrolysis of their corresponding polysaccharides (*i.e.*, EPS, WPS and SPS) with 2.17 mol/L of trifluoroacetic acid (TFA) at 85°C for 4 h according to the method of Li et al. [26]. The acid hydrolyte of each polysaccharide was filtrated. And then, an appropriate volume of methanol was added into the filtrate to form an azeotrope with TFA, which eliminated the residual TFA by evaporation under vacuum. By TLC detection, the crude oligosaccharide contained a series of oligosaccharide monomers with their degrees of polymerization ranged from 5 to 12. Furthermore, the carbohydrate content of each crude oligosaccharide or polysaccharide was respectively evaluated by the method of anthrone-sulfuric acid spectrophotography using glucose as a reference [31].

2.3. Application of oligosaccharides in D. zingiberensis cultures

Crude oligosaccharides EOS, WOS and SOS were respectively dissolved in sterile distilled water as the stock solutions, and then filtered through a sterile filter membrane (pore size, 0.45 μm). The oligosaccharide solution was diluted as 0.5, 2.5 and 5.0 mg/mL with sterile distilled water.

For *D. zingiberensis* suspension cell cultures, each 125-mL flask was filled with 30 mL liquid medium with 0.3 g of fresh cell cultures as the inoculum. When the suspension cell cultures were cultured for 20 d, 3 mL oligosaccharide elicitor solution (0.5, 2.5 and 5.0 mg/mL) was separately added. Thus the final concentrations of the oligosaccharide elicitor in medium were 0.05, 0.25 and 0.50 mg/mL of carbohydrate equivalent. Addition of 3 mL sterile distilled water was used as control. The treated suspension cell cultures were respectively harvested at 12, 24, 48, 72 and 96 h after elicitation, and then collected by vacuum filtration. The obtained fresh cell cultures were immediately used for extraction of the crude defense-related enzymes. Each treatment was carried out in triplicate.

The 20-d-old seedlings were placed in a 125-mL flask containing 20 mL oligosaccharide solution (0.5, 2.5 and 5.0 mg/mL). Each 125-mL flask was inoculated three seedlings (about 1.0 g fresh weight). The seedlings immersed in 20 mL sterile distilled water were used as control. And then the seedlings were placed in the growth chamber at 25°C under 12 h daily illumination of approximately 2000 lx. The seedlings were respectively harvested at 12, 24, 48, 72 and 96 h after oligosaccharide addition. Water adhering to the surface of seedlings was removed by absorbent papers. The harvested fresh seedlings were immediately used for extraction of crude defense-related enzymes. Each treatment was carried out in triplicate.

2.4. Extraction and detection of defense-related enzymes

As the activity changes of SOD and CAT were not detected in our preliminary experiments, just three defense-related enzymes (PAL, PPO and POD) were selected for further investigation. Each treatment of the suspension cells or seedlings was respectively subjected to extraction of PAL, PPO and POD. The PAL extraction and determination were carried out according to the methods as described previously with some modifications [32,33]. The harvested suspension cells or seedlings (0.5 g in fresh weight, fw) were homogenized in

pre-cooling 5 mL of 0.05 mol/L sodium borate buffer (pH 8.8), containing 5.0 mmol/L β-mercaptoethanol. The homogenate was then centrifuged at 12,000 x g for 15 min at 4°C, and the supernatant was collected as the enzyme extract of PAL. The reaction mixture consisted of 50 μL enzymatic extract, 100 μL of 0.02 mol/L L-phenylalanine and 50 μL of 0.05 mol/L sodium borate buffer (pH 8.8). After incubation at 40°C for 60 min, 50 μL of 2 mol/L HCl was added to stop the reaction. The absorbance at 290 nm of the reaction mixture was recorded by micro-plate spectrophotometer. One unit (U) of PAL activity is defined as a change of 0.01 OD at 290 nm per minute per gram fresh weight. The results were presented as U/min·g fw.

The crude enzymes of PPO and POD were extracted using the same methods as described previously with some modifications [34,35]. The harvested suspension cells or seedlings (0.5 g fw) were homogenized in pre-cooling 5 mL of 0.05 mol/L sodium phosphate buffer (PBS) (pH 6.8) containing 1% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 12,000 x g for 15 min at 4°C and the supernatant was used for assay of PPO or POD activities.

The reaction mixture for PPO assay contained 50 μL of enzymatic solution, 100 μL of 0.05 mol/L catechol and 50 μL of 0.05 mol/L PBS (pH 8.8), and it was monitored by measuring the change of absorbance at 398 nm for 2 min. One unit (U) of PPO activity is defined as a change of 0.01 OD at 398 nm per minute per gram fresh weight. The results were presented as U/min·g fw.

For the POD activity assay, 10 μL of crude enzymatic solution was mixed with 25 μL of 1% guaiacol (w/v), 25 μL of 1% H_2O_2 (v/v) and 150 μL of 0.05 mol/L PBS (pH 8.8). After reaction for 10 min at 37°C, the absorbance of the reaction solution at 470 nm was recorded with a micro-plate spectrophotometer. One unit (U) of PPO activity is defined as a change of 0.01 OD at 470 nm per minute per gram fresh weight. The results were presented as U/min·g fw.

3. Results and discussion

3.1. Effects of oligosaccharides on PAL activity

The effects of oligosaccharides EOS, WOS and SOS on the activity of PAL in *D. zingiberensis* suspension cells are respectively graphed in Fig. 1a, b and c. PAL activity in the treated suspension cells varied with oligosaccharide category, concentration, and time after elicitation. Of the three oligosaccharides, SOS showed no obvious enhancement of PAL activity in the suspension cells at all designed concentrations (Fig. 1c). For EOS, the PAL activity reached the maximum at 48 h after treatment and then decreased (Fig. 1a). When the suspension cells were treated with EOS at 0.50 mg/mL, the highest PAL activity was obtained with the value of 375.33 U/min·g fw, which was 3.32-fold as that of control. For WOS, the maximum of PAL activity (514.22 U/min·g fw) was

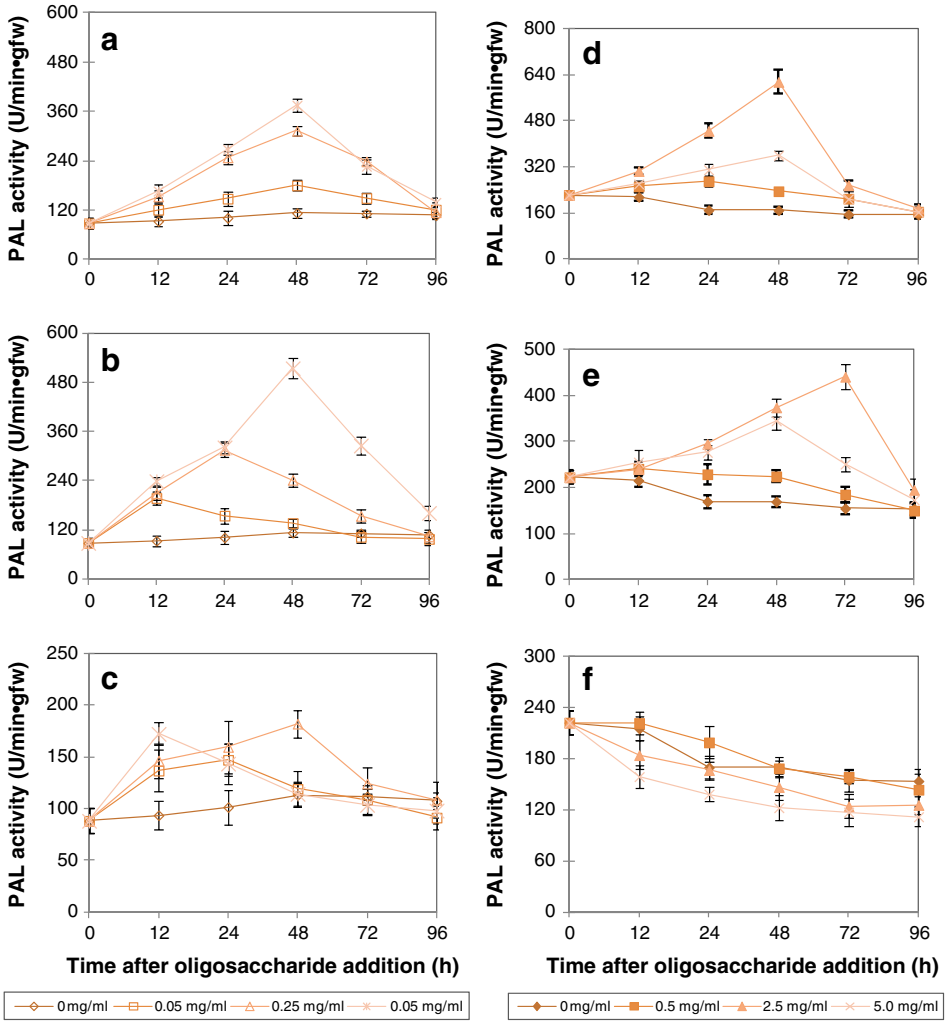

Fig. 1. Effects of oligosaccharides EOS, WOS and SOS on PAL activity in the suspension cells (a, b and c) and seedlings (d, e and f) of *D. zingiberensis* (error bars for standard deviations, n = 3), respectively.

observed when the suspension cells were treated with 0.50 mg/mL of WOS and harvested at 48 h after elicitation (Fig. 1b).

PAL activity in the seedlings of *D. zingiberensis* was higher than that in the suspension cells (Fig. 1a, b, and c vs Fig. 1d, e and f). As graphed in Fig. 1d, EOS exhibited the most significant enhancing effects on PAL activity when the seedlings were treated with 2.5 mg/mL of EOS and harvested at 48 h after elicitation. The maximum of PAL activity was 615.33 U/min·g fw which was 3.62-fold as that of control. WOS also increased PAL activity in the seedlings as shown in Fig. 1e. The maximum PAL activity (440.78 U/min·g fw) in the seedlings treated with WOS at 2.5 mg/mL was observed at 72 h after elicitation. For SOS, no dramatic enhancement of PAL activity was observed as presented in Fig. 1f.

3.2. Effects of oligosaccharides on PPO activity

The PPO activity was greatly elicited respectively by EOS, WOS and SOS in *D. zingiberensis* suspension cells, which is separately graphed in Fig. 2a, b, and c. When the suspension cells were treated with the oligosaccharide elicitors, the PPO activity was firstly increased, and then declined. As shown in Fig. 2a, the highest PPO activities in the suspension cells were observed respectively treated with 0.05, 0.25 and 0.50 mg/mL of EOS at 24, 48 and 48 h after treatment, which were respectively 3.00, 3.47 and 2.93-fold as those of control. Thus, the optimal concentration for EOS to enhance PPO activity was 0.25 mg/mL. For WOS, when it was added at 0.25 mg/mL, PPO activity reached the maximum at 48 h after elicitation which was 3.74-fold as that of control (Fig. 2b). As compared with EOS or WOS, SOS exhibited a weaker enhancing effect on PPO activity (Fig. 2c). The maximum PPO activity in the suspension cells treated with SOS at 0.50 mg/mL was obtained at 24 h after elicitation.

The effects of EOS, WOS and SOS on PPO activity in *D. zingiberensis* seedlings are respectively shown in Fig. 2d, e and f. As shown in Fig. 2d, WOS at 2.5 or 5.0 mg/mL showed a stronger effect on PPO activity than that at 0.5 mg/mL. The highest PPO activity was observed by 5.0 mg/mL of EOS at 48 h after treatment, which was 2.71-fold as that of control but showed no significant difference to that at 2.5 mg/mL. Of all treatments, WOS exhibited the most significant enhancing effects on PPO activity in the seedlings at 2.5 mg/mL and 48 h after elicitation with the maximum PPO activity which was 4.61-fold as that of control (Fig. 2e). SOS showed no obvious effects on PPO activity in the seedlings as compared with that of control (Fig. 2f).

3.3. Effects of oligosaccharides on POD activity

The effects of EOS, WOS and SOS on POD activity in the suspension cells of *D. zingiberensis* are presented in Fig. 3a, b and c, respectively. For EOS, the highest POD activity in the suspension cells was induced at 0.25 mg/mL and 48 h after elicitation, which was 2.75-fold as that of control (Fig. 3a). As compared to EOS, WOS exhibited more significant increasing effects on POD activity under the same conditions of EOS, which was shown in Fig. 3b. When the suspension cells were treated with 0.25 mg/mL of WOS and harvested at 48 h after elicitation,

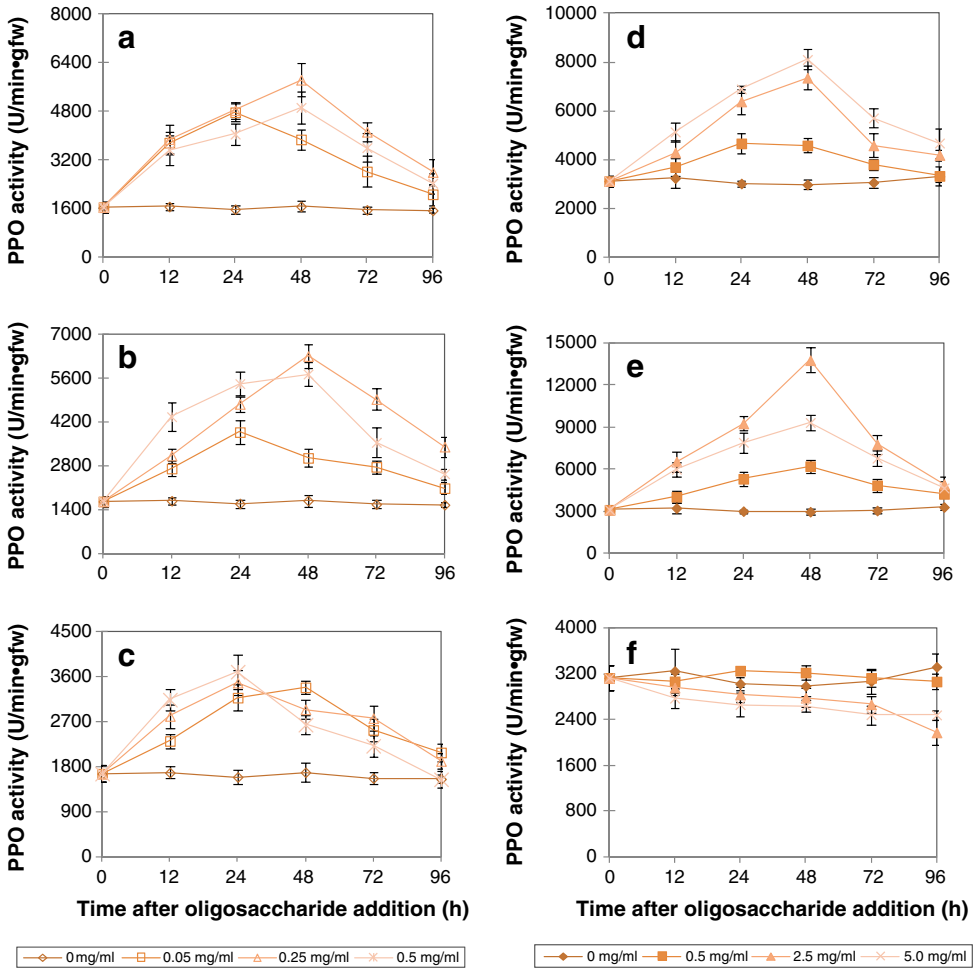

Fig. 2. Effects of oligosaccharides EOS, WOS and SOS on PPO activity in the suspension cells (a, b and c) and seedlings (d, e and f) of *D. zingiberensis* (error bars for standard deviations, n = 3), respectively.

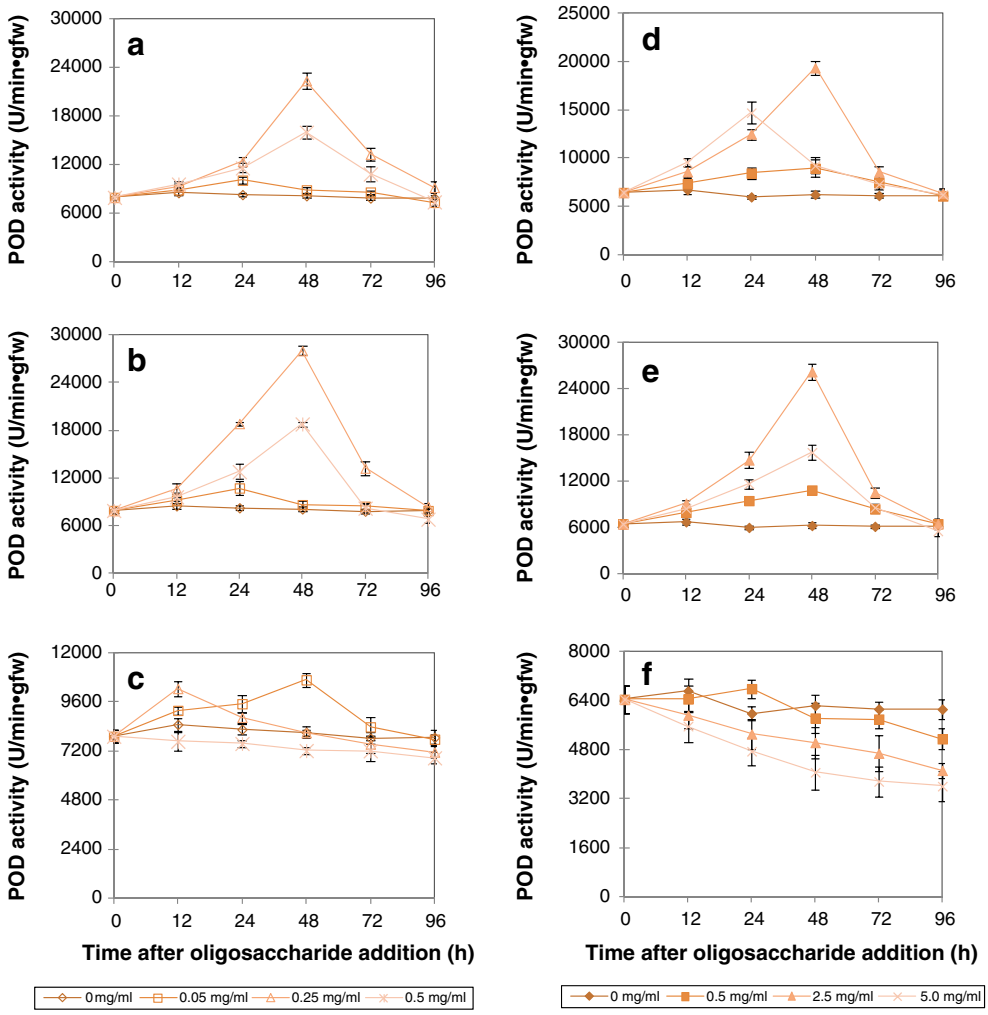

Fig. 3. Effects of oligosaccharides EOS, WOS and SOS on POD activity in the suspension cells (a, b and c) and seedlings (d, e and f) of *D. zingiberensis* (error bars for standard deviations, n = 3), respectively.

the maximum of POD activity was obtained, which was 3.45-fold as that of control. As graphed in Fig. 3c, SOS showed no dramatic enhancing effects on POD activity.

In Fig. 3d, EOS increased the POD activity in the seedlings at all designed concentrations during the period from 12 h to 72 h. When the seedlings were treated with EOS at 2.5 mg/mL and harvested at 48 h, the highest POD activity was obtained, which was 3.10-fold as that of control. For WOS, the POD activity in the seedlings showed the similar variation trend as that of EOS (Fig. 3e). However, the maximum POD activity elicited by WOS was higher than that of EOS under the same condition, which was 4.19-fold as that of control. As shown in Fig. 3f, SOS exhibited no enhancing effects on POD activity in the seedlings, it even showed inhibitory impacts.

3.4. Concluding remarks

Various fungi-originated products have been demonstrated to trigger defense mechanisms in plants [36]. Increases of PAL, PPO and POD activities have been proved to be one of the earliest defensive responses of plants against fungal stimuli [5,6]. Fungal elicitors, especially carbohydrate compounds (*i.e.*, polysaccharides and oligosaccharides), have been frequently reported to induce defensive responses in plants [37,38]. In the present work, three crude oligosaccharides (EOS, WOS and SOS) prepared by acid hydrolysis of their corresponding polysaccharides EPS, WPS and SPS from the endophytic fungus *F. oxysporum* Dzf17 were studied for their effects on the activities of the defense-related enzymes PAL, PPO and POD in the suspension cell and seedling cultures of its host plant *D. zingiberensis*. The activities of PAL, PPO and POD in the suspension cell and seedling cultures of *D. zingiberensis* were significantly increased by EOS and WOS, while the effects of SOS were not desirable. The activities of these enzymes increased quickly, reached the maximum values, and then decreased. The activity of each enzyme reached its maximum at different time after oligosaccharide treatment, which was also dependent on the category and concentration of the added oligosaccharide. The maximum values of PAL, PPO and POD activities were mostly observed at 48 h or 72 h after oligosaccharide treatment, which showed significant enhancements as compared with those of control. Enhancements of plant defense-related enzyme activities by using fungal oligosaccharides have also been reported previously, but these oligosaccharides were mostly prepared from plant pathogens [28,29]. It is rarely reported to induce defense-related enzyme activities in plants by the oligosaccharide elicitors from endophytic fungi. In this work, the oligosaccharides from *F. oxysporum* Dzf17 were observed to increase the activities of defense-related enzymes in the suspension cell and seedling cultures of its host *D. zingiberensis*, which demonstrated their abilities to activate defensive responses. However, the chemical characterizations of the oligosaccharides (*i.e.*, purification of oligosaccharide monomers, monosaccharide composition, monosaccharide linkage of each oligosaccharide monomer) as well as their structure–activity relationships and more specific defensive mechanisms are not clear and worth investigating. In addition, the disease resistance *in vivo* of the

plant *D. zingiberensis* by treatment of these oligosaccharides is also needed for further research. On the whole, the present work might be contributed to further understand the interaction between endophytic *F. oxysporum* Dzf17 and its host *D. zingiberensis*. It also provides an innovative idea for the research and development of saccharide agrochemicals.

Conflict of interest

The authors declare that there are no conflict of interest.

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Author contributions

Proposed the theoretical frame: LZ, YP; Conceived and designed the experiments: LZ; Contributed reagents/materials/analysis tools: HL, JM; Wrote the paper: PL, LZ; Performed the experiments: PL, WS, XW, SL; Analyzed the data: PL.

References

[1] Campbell SA, Kessler A. Plant mating system transitions drive the macroevolution of defense strategies. Proc Natl Acad Sci U S A 2013;110:3973–8. http://dx.doi.org/10.1073/pnas.1213867110.
[2] Nicholson RL, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol 1992;30:369–89. http://dx.doi.org/10.1146/annurev.py.30.090192.002101.
[3] Sharan M, Taguchi G, Gonda K, Jouke T, Shimosaka M, Hayashida N, et al. Effects of methyl jasmonate and elicitor on the activation of phenylalanine ammonia-lyase and the accumulation of scopoletin and scopolin in tobacco cell cultures. Plant Sci 1998;132:13–9. http://dx.doi.org/10.1016/S0168-9452(97)00260-4.
[4] Kishimoto K, Matsui K, Ozawa R, Takabayashi J. Analysis of defensive responses activated by volatile allo-ocimene treatment in *Arabidopsis thaliana*. Phytochemistry 2006;67:1520–9. http://dx.doi.org/10.1016/j.phytochem.2006.05.027.
[5] Dubery IA, Slater V. Induced defence responses in cotton leaf disks by elicitors from *Verticillium dahliae*. Phytochemistry 1997;44:1429–34. http://dx.doi.org/10.1016/S0031-9422(96)00635-8.
[6] Vurro M, Ellis BE. Effect of fungal toxins on induction of phenylalanine ammonia-lyase activity in elicited cultures of hybrid poplar. Plant Sci 1997;126:29–38. http://dx.doi.org/10.1016/S0168-9452(97)00094-0.
[7] Kevers C, Gaspar T. Soluble, membrane and wall peroxidases, phenylalanine ammonia-lyase, and lignin changes in relation to vitrification of carnation tissues cultured *in vitro*. J Plant Physiol 1985;118:41–8. http://dx.doi.org/10.1078/0176-1617-00905.
[8] Gerasimova NG, Pridvorova SM, Ozeretskovakaya OL. Role of L-phenylalanine ammonia lyase in the induced resistance and susceptibility of potato plants. Appl Biochem Microbiol 2005;41:103–5. http://dx.doi.org/10.1007/s10438-005-0019-3.
[9] Vaughn KC, Duke SO. Function of polyphenol oxidase in higher plants. Physiol Plant 1984;60:106–12. http://dx.doi.org/10.1111/j.1399-3054.1984.tb04258.x.
[10] Jetiyanon K. Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens. Biol Control 2007;42:178–85. http://dx.doi.org/10.1016/j.biocontrol.2007.05.008.
[11] Sharp JK, Valent B, Albersheim P. Host–pathogen interaction. 26. Purification and partial characterization of a beta-glucan fragment that elicits phytoalexin accumulation in soybean. J Biol Chem 1984;259:11312–20.
[12] Benhamou N. Elicitor-induced plant defence pathways. Trends Plant Sci 1996;1: 233–40.
[13] Dutsadee C, Nunta C. Induction of peroxidase, scopoletin, phenolic compounds and resistance in *Hevea brasiliensis* by elicitin and a novel protein elicitor purified from *Phytophthora palmivora*. Physiol Mol Plant Pathol 2008;72:179–87. http://dx.doi.org/10.1016/j.pmpp.2008.09.002.
[14] Strobel G. Harnessing endophytes for industrial microbiology. Curr Opin Microbiol 2006;9:240–4. http://dx.doi.org/10.1016/j.mib.2006.04.001.
[15] Porras-Alfaro A, Bayman P. Hidden fungi, emergent properties: Endophytes and microbiomes. Annu Rev Phytopathol 2011;49:291–315. http://dx.doi.org/10.1146/annurev-phyto-080508-081831.
[16] Wang C, Wu J, Mei X. Enhancement of taxol production and excretion in *Taxus chinensis* cell culture by fungal elicitation and medium renewal. Appl Microbiol Biotechnol 2001;55:404–10.
[17] Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J Nat Prod 2004;67:257–68. http://dx.doi.org/10.1021/np030397v.
[18] Li Y-C, Tao W-Y. Effects of paclitaxel-producing fungal endophytes on growth and paclitaxel formation of *Taxus cuspidata* cells. Plant Growth Regul 2009;58:97–105. http://dx.doi.org/10.1007/s10725-008-9355-7.
[19] Rodriguez RJ, White JF, Arnold AE, Redman RS. Fungal endophytes: Diversity and functional roles. New Phytol 2009;182:314–30. http://dx.doi.org/10.1111/j.1469-8137.2009.02773.x.
[20] Zhou L, Zhao J, Shan T, Cai X, Peng Y. Spirobisnaphthalenes from fungi and their biological activities. Mini-Rev Med Chem 2010;10:977–89.
[21] Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D. Anticancer compounds derived from fungal endophytes: Their importance and future challenges. Nat Prod Rep 2011;28:1208–28. http://dx.doi.org/10.1039/c1np00008j.
[22] Zhao J, Shan T, Mou Y, Zhou L. Plant-derived bioactive compounds produced by endophytic fungi. Mini-Rev Med Chem 2011;11:159–68. http://dx.doi.org/10.2174/138955711794519492.
[23] Zhang R, Li P, Xu L, Chen Y, Sui P, Zhou L, et al. Enhancement of diosgenin production in *Dioscorea zingiberensis* cell culture by oligosaccharide elicitor from its endophytic fungus *Fusarium oxysporum* Dzf17. Nat Prod Commun 2009;4:1459–62.
[24] Zhang R, Li P, Zhao J, Yin C, Zhou L. Endophytic fungi from *Dioscorea zingiberensis* and their effects on the growth and diosgenin production of the host plant cultures. Nat Prod Res Dev 2010;22:11–5.
[25] Li P, Mou Y, Shan T, Xu J, Li Y, Lu S, et al. Effects of polysaccharide elicitors from endophytic *Fusarium oxysporum* Dzf17 on growth and diosgenin production in cell suspension culture of *Dioscorea zingiberensis*. Molecules 2011;16:9003–16. http://dx.doi.org/10.3390/molecules16119003.
[26] Li P, Mao Z, Lou J, Li Y, Mou Y, Lu S, et al. Enhancement of diosgenin production in *Dioscorea zingiberensis* cell cultures by oligosaccharides from its endophytic fungus *Fusarium oxysporum* Dzf17. Molecules 2011;16:10631–44. http://dx.doi.org/10.3390/molecules161210631.
[27] Li P, Luo C, Sun W, Lu S, Mou Y, Peng Y, et al. *In vitro* antioxidant activities of polysaccharides from endophytic fungus *Fusarium oxysporum* Dzf17. Afr J Microbiol Res 2011;5:5990–3. http://dx.doi.org/10.5897/AJMR11.1342.
[28] Khan W, Prithiviraj B, Smith DL. Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. J Plant Physiol 2003;160:859–63.
[29] Meng X, Yang L, Kennedy JF, Tian S. Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. Carbohydr Polym 2010;81:70–5. http://dx.doi.org/10.1016/j.carbpol.2010.01.057.
[30] Yin C, Li P, Li H, Xu L, Zhao J, Shan T, et al. Enhancement of diosgenin production in *Dioscorea zingiberensis* seedling and cell cultures by beauvericin from the endophytic fungus *Fusarium redolens* Dzf2. J Med Plants Res 2011;5:6550–4. http://dx.doi.org/10.5897/JMPR11.921.
[31] Wang Z, Luo D, Ena C. Optimization of polysaccharides extraction from *Gynostemma pentaphyllum* Makino using uniform design. Carbohydr Polym 2007;69:311–7. http://dx.doi.org/10.1016/j.carpbol.2006.10.013.
[32] El Modafar C, Tantaoui A, El Boustani E. Differential induction of phenylalanine ammonia-lyase activity in date palm roots in response to inoculation with *Fusarium oxysporum* f. sp. *albedinis* and to elicitation with fungal wall elicitor. J Plant Physiol 2001;158:715–22. http://dx.doi.org/10.1078/0176-1617-00258.
[33] Lafuente MT, Zacarias L, Martinez-Tellez MA, Sanchez-Ballesta MT, Granell A. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biol Technol 2003;29:309–18. http://dx.doi.org/10.1016/S0925-5214(03)00047-4.
[34] Meng X, Li B, Liu J, Tian S. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. Food Chem 2008;106:501–8. http://dx.doi.org/10.1016/j.foodchem.2007.06.012.
[35] Zhang S, Zhang F, Hua B. Enhancement of phenylalanine ammonia lyase, polyphenoloxidase, and peroxidase in cucumber seedlings by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation. Agric Sci China 2008;7:82–7.
[36] Ryan CA. Oligosaccharide signaling in plants. Annu Rev Cell Biol 1987;3:295–317. http://dx.doi.org/10.1146/annurev.cb.03.110187.001455.
[37] Walker-Simmons M, Ryan CA. Proteinase inhibitor synthesis in tomato leaves: Induction by chitosan oligomers and chemically modified chitosan and chitin. Plant Physiol 1984;76:787–90. http://dx.doi.org/10.1104/pp.76.3.787.
[38] Shibuya N, Kaku H, Kuchit K, Maliarik MJ. Identification of a novel high-affinity binding site for N-acetylchitooligosaccharide elicitor in the membrane fractions from suspension-cultured rice cells. FEBS Lett 1993;329:75–8. http://dx.doi.org/10.1016/0014-5793(93)80197-3.