Physiological and Molecular Plant Pathology xxx (2017) 1–6

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

A fungal elicitor induces *Sclerotium rolfsii sacc* resistance in *Atractylodis maceocephalae koidz*

Xinyi Zhang [a], Pinyan Qin [b], Jiahang Song [a], Fei Qi [a], Wei Tian [a, *]

[a] School of Forestry and Bio-technology, Zhejiang Agriculture and Forestry University, Lin'an 311300, Zhejiang, China
[b] School of Agriculture and Food Science, Zhejiang Agriculture and Forestry University, Lin'an 311300, Zhejiang, China

ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form
19 February 2017
Accepted 21 February 2017
Available online xxx

Keywords:
Fungal elicitor
Resistance response
Enzyme activity
Atractylenolides
Disease index

ABSTRACT

The enzymatic activities (POD, PPO, CAT, PAL), as protective enzymes in the leaves of *Atractylodis maceocephalae koidz*, were tested by treating different concentrations of polysaccharides isolated from *Sclerotium rolfsii sacc* (P. *S. rolfsii*) at 0 mg/l, 20 mg/l, 200 mg/l and 400 mg/l against *S. rolfsii*, and atractylenolides as a phytoalexin in the rhizome of *A. maceocephalae* were evaluated in compared with control. It was evident that the plant under stress by pathogen has instigated the significant synthesis and accumulation of atractylenolides and the higher enzymes activities were described on the eight day after fungal elicitor inoculation than the control group. Furthermore, the treatments of *A. maceocephalae* seedlings with P. *S.rolfsii* increased disease index development caused by *S. rolfsii*. The disease index is lowest when inoculated at a concentration as low as 20 mg/ml. In general, these results indicated that P. *S. rolfsii* may be useful as a fungal elicitor, which can enhance resistance and triggered innate immunity in *A. maceocephalae*, and had the potential to suppress the disease on *A. maceocephalae* when P. *S. rolfsii* at 20 mg/l, were used to inoculate the root of *A. maceocephalae*.

1. Introduction

The *Atractylodis maceocephalae koidz* (*A. maceocephalae*) is a traditional Chinese medicine and a perennial herbal plant [6], which is mainly planted in Zhejiang and Jiangsu. Past researches have confirmed that *A. maceocephalae* can strengthen the spleen and replenish Qi, dry dampness, and promote diuresis, hidroschesis, and tocolysis [9,30]. There are many studies on *A. maceocephalae*, including identified and isolated several compounds, a volatile oil [2], lactones [2,24], and polysaccharides [34]. The rhizome of *A. maceocephalae* has been used for thousands of years with its particular pharmacologic activities [12,32], in which research has focused on immunomodulation, diuresis, anti-tumor activity, anti-sepsis, anti-inflammation, hypoglycemic effects, and anti-senescence, with emphases on the nervous system, digestive system, and uterine smooth muscle [35]. Continuous cropping of *A. maceocephalae* has been a major issue, and has resulted in plant infections. How to enhance disease-resistance of *A. maceocephalae* is an inevitable problem [17,36,39].

Production of the Chinese herb has been lost partly because of plants infected with *Sclerotium rolfsii sacc* (*S. rolfsii*), which is Mycelia Sterilia of Deuteromycotina [16,21] which is a soil-inhabiting pathogen. *S. rolfsii* is one of the important diseases affecting grain, horticultural plants, forests, and herbs in the tropics and subtropics. Southern blight, also referred to as white mildew, mainly hurts plants close to the surface of the stems and roots. As a stem-based disease, a dark brown, moist, amorphous, concave disease spot appears; when wet, the disease part appears to be radial spun silk with a white mycelium. The scab will expand horizontally around the base of the stem, resulting in yellow leaves, and even death [28].

Fungal elicitors are specific chemical signals derived from fungal cells. Fungal elicitors, when combined with plants, can induce genes to be expressed rapidly with high specificity and selectivity, which will lead to activation of secondary metabolic pathways and accumulate secondary metabolites [22,26,31]. Fungal elicitors are the surface structure or secretions of cells, which are rich in fungal mycelia, the degradation products of fungal mycelia, fermentation broth, and fungal secretions. The fungal elicitors consist of polysaccharides, peptides, glycolipid proteins, oligosaccharides, fatty

* Corresponding author. Nurturing Station for the State Key Laboratory of Subtropical Silviculture, School of Forestry and Bio-technology, Zhejiang Agriculture and Forestry University, Lin'an, Zhejiang, China.
E-mail addresses: xin_stellar@163.com (X. Zhang), weitian2014@yeah.net (W. Tian).

http://dx.doi.org/10.1016/j.pmpp.2017.02.002
0885-5765/

acids, glycoproteins, and other substances [23]. Fungal elicitors enhance the biomass and secondary metabolites of plants, such as polysaccharides, and improve enzymatic activity inside plants [7]. Hu et al. have demonstrated that oligosaccharides from *Dothiorella gregaria* as an elicitor can induce expression of the resistance gene, increase resistant metabolism, and accumulate resistant products rapidly [28]. Not all of fungal elicitors can promote an effect on the secondary metabolites, and some fungal elicitors can break the production of secondary metabolites and inhabit the growth of the plant [5,11,18].

Research involving *S. rolfsii* has focused on attacking the growth of plants, which perpetuates as sclerotia on plant debris and in the soil [27]. Nevertheless, very little research has addressed polysaccharides from *S. rolfsii* (P. *S. rolfsii*) as fungal elicitors to induce the production of secondary metabolites that effect the growth of plants. Our previously research has showed that polysaccharides isolated from *Chrysanthemum indicum* can trigger systemic acquired resistance (SAR) with a certain concentration in *A. maceocephalae* [1]. While it is a heroic guess that is polysaccharides isolated from *S. rolfsii* may be induce SAR with a certain concentration and utilize its elicitor activities against *S. rolfsii* in *A. maceocephalae*.

Our aim was to research potential fungal elicitor activities of the polysaccharides originated from *S. rolfsii*, whether the activities of P. *S. rolfsii* can tangle together with other biomolecules in *A. maceocephalae* and their level in *S. rolfsii*- inoculated rhizome of *A. maceocephalae* in response to P. *S. rolfsii* treatment in comparison with the control. Our experiment was (i) to analyze the activities of peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), the protective enzymes in *A. maceocephalae*. (ii) to assess the level of atractylenolides -II, a phytoalexins of *A. maceocephalae.* (iii) to evaluate the disease index (DI), resistance response in *A. maceocephalae.*

2. Materials and methods

2.1. Fungal cultures and fungal elicitor polysaccharide preparation

The fungal elicitor was prepared from the solid culture of the isolate of *S. rolfsii*, which were derived from China General Microbiological Culture Collection Center called BJJ-528. Solid cultures were initiated from potato dextrosc agar (PDA) with 9 cm petri dishes. The cultures were incubated in the dark and 25 °C for 6 day. The mycelia were washed by sterile water, and the elicitor was transferred into sterile 250 ml conical flasks, sterilized by autoclaving, filtered with acetone and dried. The finally solid was used as crude extract of fungal elicitor.

Crude polysaccharides were collected from crude extract of fungal elicitor which were grind into homogenate and diluted with to 50 ml in volumetric flask. The total sugar content was measured by phenol-sulfuric acid method referring to glucose standard curve [13], which was calculated regression equation: $A = 0.0145c + 0.0039$, $r = 0.9992$ [14]. Then we figured out the conversion factor (f) of fungal elicitor by $f = W \div CD$ (W: the weight of polysaccharose (μg); C: the concentration of glucose (μg/mL); D: the dilution ratio of polysaccharose) [14]. So the conversion factor (f) was worked out 7.655. Finally, the polysaccharide content of fungal elicitor was figured out, which was 100% in the crude extract by the conversion coefficient $P\% = (C \times D \times F \div W) \times 100\%$.

2.2. The pretreatment of A. maceocephalae

A. maceocephalae seeds were purchased from cultivation base, Dafeng, Yancheng City, AnHui province, China. Seeds (50 seeds per treatment, 15 pots in total) were soaked for 24 h in 25 °C water before sowing in soil and were cultivated in a greenhouse at 25–27 °C with the maximum illumination intensity of 80 000 lx, photoperiod of 16/8 h (day/night), their nutrition substrate was peat soil and perlite (3: 1, v/v). When *A. maceocephalae* seedlings grew to a certain period of five to seven leaves, 15 pots were divided into five groups with three duplicates randomly, including 0 mg/L (CK), 20 mg/L, 200 mg/L, 400 mg/L fungal elicitor with *S. rolfsii* incubated respectively, and a blank control check (BCK) (Table 1). Fifth days after inoculation, the leaves of *A. maceocephalae* seedlings in the treatment groups and control groups were sampled separately, then the enzyme activity assay were measured and recorded the day before spray and inoculation and on 0 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h after spray and inoculation.

2.3. Enzyme extraction and assays

The true leaves from different concentrations (0 mg/L, 20 mg/L, 200 mg/L, 400 mg/L) and BCK were harvested at different times (0 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h) after P. *S. rolfsii* treatment and were stored at −80 °C. The frozen leaf sections (1 g) for each treatment were homogenized in sodium borate buffer (0.1 mol/L, pH 8.8, 1 mmol/L EDTANa2, 5 mmol/L mercaptoethanol) in a mortar and pestle. Then the mixture was centrifuged at 10,000 g for 18 min at 4 °C. The supernatant was collected under the same condition for use as crude enzyme extracts. The activities of PAL, POD, PPO, CAT were assayed according to the Moerschbacher's method [15], the Guaiacol methol [19], the colorimetry methol [42], and UV spectrophotometry [37] respectively.

2.4. Atractylenolides determined by HPLC

Sun Fire C18 (250 mm × 4.6 mm, 5 μm) chromatographic column was used for HPLC, with the column temperature maintained at 30 °C. Gradient elution was formed on a mobile phase which consisted of two solutions, methyl alcohol (A) and distilled water (B). Initially, the mobile phase consisted of a ratio of 85:15 (A: B, v/v); after 14 min, this was changed to a ratio of 95:5, which was maintained just 1min; from 15 to 22min, solvent A was reach 95%, and from 22 to 26min, A was keeping in 100%. The flow rate was 1 mL/min.

Stock solution of atractylenolides -I, -II and -III of standard substance were prepared by dissolving an accurately weighed sample in an appropriate volume of methanol to get a final concentration of 0.120 mg/ml, 0.137 mg/ml, 0.114 mg/ml, respectively. *A. maceocephalae* samples roots were accurately weighed 0.05 g in 1 ml volumetric flask, then dilute with methanol to 1 mL.

2.5. Bioassay for fungal elicitor- induced disease resistance in A. maceocephalae

A. maceocephalae seedlings were treated with fungal elicitors respectively with the three concentrations (20 mg/L, 200 mg/L, 400 mg/L), CK (0 mg/L) and BCK. Afterwards, the seedlings were

Table 1
The handing methods of different leaves.

Experiment	20 mg/L	200 mg/l	400 mg/L	Hypha suspension	water
1	+	−	−	+	−
2	−	+	−	+	−
3	−	−	+	+	−
4	−	−	−	+	−
5	−	−	−	−	+

+Spraying different concentrations of fungal elicitor extract; -no treatment.

Please cite this article in press as: X. Zhang, et al., A fungal elicitor induces *Sclerotium rolfsii sacc* resistance in *Atractylodis maceocephalae koidz*, Physiological and Molecular Plant Pathology (2017), http://dx.doi.org/10.1016/j.pmpp.2017.02.002