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Proteomic analysis of the interaction between *Plasmodiophora brassicae* and Chinese cabbage (*Brassica rapa L. ssp. Pekinensis*) at the initial infection stage

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ABSTRACT

The interaction between plants and pathogens that induces a defense response has been shown in previous studies. In our research, we used *Plasmodiophora brassicae* Woronin (*Plasmodiophoraceae*), the causal agent of clubroot disease, to infect the roots of *Brassica rapa* seedlings. Electron microscopic observation showed that the roots could be infected by *Plasmodiophora brassicae* on the fifth day after inoculation. We identified 438 and 414 differentially expressed proteins (DEPs) in the inoculated and control groups, respectively. Mass spectrometry was performed to identify 18 of the DEPs in which the relative protein abundance varied by > 2-fold. In general, Gene Ontology (GO) analysis revealed that the DEPs reflected a wide range of molecular functions, including "response to stimuli" (39%) and "plant defense reaction" (8%) in the Biological Process domain. Among these, two proteins (DEPs 3038 and 3129) were found to be related to salicylic acid (SA)-mediated systemic acquired resistance (SAR), two proteins (DEPs 3414 and 2358) were found to be related to jasmonic acid (JA)/ ethylene (ET)-mediated induced systemic resistance (ISR), and DEP 4043 participated in both SAR and ISR. In addition, one pathogenesis-related protein (PR-1) was found in the extracellular cell component group, and KEGG analysis indicated that a DEP participates in plant-pathogen interaction signaling pathways. KEGG analysis also confirmed that these two pathways show some degree of cross-talk, rather than acting independently.

1. Introduction

Plant growth and productivity are adversely affected by many natural biotic factors, which causes considerable yield losses worldwide, and crop production is constantly threatened by a variety of diseases caused by pathogen attack (Hatakeyama et al. 2017). These factors prevent plants from reaching their full genetic potential and thus limit productivity (Cramer et al. 2011). Plants have evolved a diverse range of complex mechanisms to respond to internal stimuli and to survive adverse conditions (Zhang et al. 2015). Plants can initiate specific defense responses by recognizing signaling molecules derived from damaged cells, and they can mount an efficient and systemic defense mechanism when infected by pathogens during growth (Jones and Dang 2006; Howe and Jander 2008). The molecular mechanisms involved in environmental stress tolerance in plants are very complex (Long et al. 2016). Defense signaling pathways include the SA, ET, and the JA signaling pathways (Thomma et al. 1998; Glazebrook, 2001; Grant and Jones, 2009; Pieterse et al. 2009; Leon et al. 2010). Of these, the SA signaling pathway results in the activation of systemic acquired resistance (SAR), while the ET/JA signaling pathway is involved in induced systemic resistance (ISR) (Ryals et al. 1996).

Six widely cultivated Brassica species (*B. carinata, B. juncea, B. oleracea, B. napus, B. nigra, and B. rapa)* are classical examples of the importance of polyploidy in plant evolution (Li et al. 2013). Cultivars of *Brassica rapa L.*. are susceptible to disease, but there are few studies describing breeding for disease resistance in this species (Yan et al. 2015). *Plasmodiophora brassicae* causes an economically important disease, known as clubroot, in cruciferous crops (Strechlow et al., 2014) including *B. rapa*. Presently, there are many studies on *Plasmodiophora brassicae* that describe the biological characteristics (Strelkov et al., 2006), physiological races (Williams, 1966; Buczacki et al. 1975), as well as the mapping of resistance genes in *B. rapa*, such as *Crr3, CRa, CRb* and *CRk* were located on A3 linkage group in *Brassica rapa L.* (Diederichsen et al. 2009; Piao et al. 2004; Sakamoto et al. 2008), which have made contributions to our understanding of the interaction between *B. rapa* and *Plasmodiophora brassicae.* In recent years, there are some reports of defense responses to *Plasmodiophora brassicae*, and the molecular mechanisms of these responses by transcriptome analysis. The transcriptome of Arabidopsis thaliana in response to *Plasmodiophora brassicae* (Zhao et al. (2017) and *Broccoli* (*Brassica*

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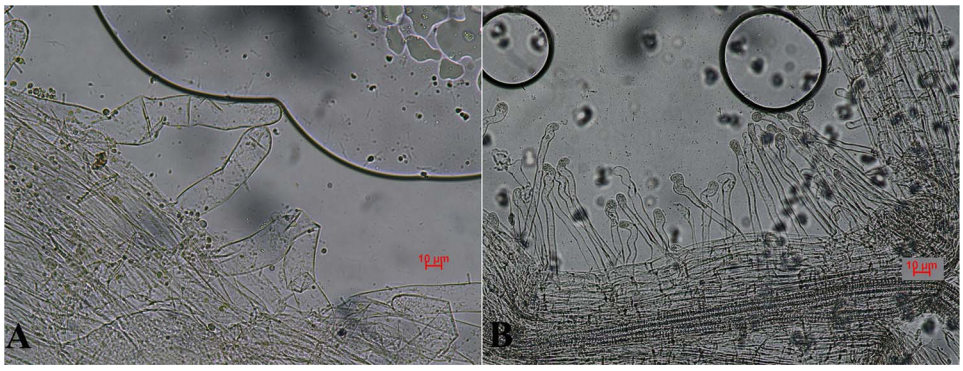

Fig. 1. Light microscopic examination of the early stage of infection of*Brassica rapa L.* roots by *Plasmodiophora brassicae*. A; uninfected root hairs. B; root hairs infected with *Plasmodiophora brassicae*. Spores are indicated by the red arrows.

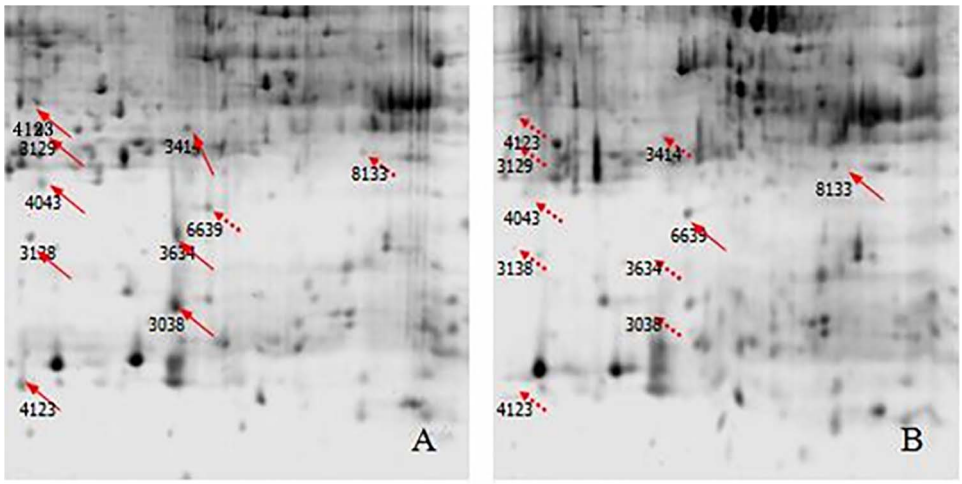

Fig. 2. Two-dimensional polyacrylamide gel electrophoresis (2-DE) of proteins isolated from roots of*B. rapa* cv. '742' seedlings for identification by mass spectrometry. A; wild-type control. B; seedlings infected with *Plasmodiophora brassicae*. The differentially-expressed proteins (DEPs) indicated by solid arrows or broken arrows were up-regulated or down-regulated, respectively, in response to infection by *Plasmodiophora brassicae*.

Table 1
Numbers of differentially-expressed proteins detected in this study in roots of CK and*Plasmodiophora brassicae*-infected seedlings of *B. rapa* cv.'742' (T).

special proteins	DEPs number	DEPS NO.
Proteins only expressed in CK	2	3138, 3634
Up-regulated proteins in CK (compared with T)	11	2358, 3008, 3038, 3129, 3139, 3202, 3414, 3439, 4043, 4123,4129
Proteins only expressed in T	1	8133
Up-regulated proteins in T (compared with CK)	5	4235, 4238, 4332, 6639, 8058

oleraceavar.italica) and Wild Cabbage (*Brassica macrocarpa Guss*) in responseto *Plasmodiophora brassicae* were analyzed (Zhang et al. 2016), but the proteomic research of *Brassica rapa L.* in response to *Plasmodiophora brassicae* is limited. The objectives of our study were to explore the interaction between *Brassica rapa L.* and *Plasmodiophora brassicae* beginning at the initial stage of infection using 2-DE to describe the molecular mechanisms that underly clubroot resistance in *Brassica rapa L.*

2. Materials and methods

2.1. Plant and pathogen

Brassica rapa L. cv. '742CK' ("CK") was provided by the Shenyang Agricultural University (SYAU) genetic breeding laboratory and was used in this study. *Plasmodiophora brassicae* was collected from a research field at SYAU, China.

2.2. Preparation of Plasmodiophora brassicae zoospore suspension and growing hydroponic plants

Plasmodiophora brassicae spores were acquired following the improved method of Castlebury et al. (1994). The concentration of zoospores was $\sim 10^7$ per milliliter, and they were put at 25°C in the dark for activation. Sterilized seeds of *B. rapa* cv. '742' were sown in petri dishes for germination. When the shoots grew to ~1.5 cm in length, they were transferred to 1.5 mL centrifuge tubes without bottoms in a box containing nutrient solution for hydroponic growth as described by Ji et al. (2014). The hydroponic boxes were placed in an incubator at 25°C with illumination for 16 h per day and 60%–70% humidity. The nutrient solution was changed every three days to maintain uniform conditions in each box. A rubber suction bulb was used to aerate the nutrient solution in the boxes to keep oxygen in the nutrient solution.

2.3. Inoculation and observation

After cultivation in nutrient solution for one week, the plants were divided into an inoculation group and a control group. The plants in the inoculation group were transfered to the prepared hypnospore liquid and the plants in the control group were continuously cultivated in nutrient solution. At least three individual inoculated plants were inspected at random and observed microscopically every three days to identify the early stage of infection by *Plasmodiophora brassicae*.

2.4. Tissue sampling and preparation of protein samples

When the root hairs became infected by *Plasmodiophora brassicae*, the roots of inoculation and control group were washed, excised from