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Comparative proteomic analysis of cucumber roots infected by *Fusarium oxysporum f.* sp. *cucumerium* Owen

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ABSTRACT

Cucumber Fusarium wilt (CFW), caused by the soil-borne fungus *Fusarium oxysporum f.* sp. *cucumerium*, is a serious disease in cucumber (*Cucumis sativus*) production worldwide. For the efficient control of the pathogenic fungi, a better understanding of its interaction and associated resistance mechanisms at the molecular level is required. Here, we report a comparative proteomics analysis of total root protein isolated from infected cucumber root of susceptible bulk (SB) and resistant bulk (RB) of cucumber generation F2. Two-dimensional gel electrophoresis (2-DE) coupled with MS/MS approaches identified 15 over-accumulated proteins from the RB plants. Identified proteins are mainly involved in defense and stress responses, oxidation reduction, metabolism and transport and other process. These proteins are likely to be a part of resistance-related protein network, playing different roles in cucumber disease resistance. Three vital clues regarding wilt resistance of *C. sativus* are gained from this study. First, jasmonic acid and redox signaling components were found in response to *F. oxysporum* infection in resistant plants. Second, the LRR family protein may play an important role in the defense reaction against CFW. Third, biotic and abiotic stress-related proteins were induced by the CFW fungus *F. oxysporum*, indicating the activation of common stress pathway.

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most common vegetables worldwide, which can be severely infected by *Fusarium oxysporum* f. sp. *cucumerinum* J. H. Owen. The pathogen infects the seedlings via roots or stems, especially the wounded roots, and spreads rapidly through the entire plants via vascular system, blocking the uptake of water and nutrients and ultimately resulting in plants wilt [1]. Cucumber Fusarium wilt (CFW) results in considerable losses in cucumber production [2–5], especially in areas of year-round cucumber production. The fungus can survive for several years in the soil without planting cucumber and mutate continuously to generate new species [6]. It is difficult to prevent and eliminate the disease efficiently. Some measures for controlling CFW have been studied, for example, the use of chemical substances [7], and biological control [8–11]. However, the best method for controlling the disease in cucumber is the use of resistant cultivars. Although the great efforts have been made in generation of wilt-resistant cucumber cultivars, very few varieties are resistant to CFW. Meanwhile, studies on the inheritance of CFW resistance in cucumber have been reported with different conclusions [12–15]. Understanding the molecular mechanisms and identifying the molecular components involved in the defense responses of the CFW-resistant cucumber cultivars will provide a promising approach to restraining this disease.

Many works have been done to investigate the defense mechanisms against *F. oxysporum* infection in cucumber. Through several physiological and biochemical studies [16–18], the involvement of

Abbreviations: AOC, allene oxide cyclase; CFW, Cucumber Fusarium wilt; DHAR, DHAR class glutathione transferase; GSTs, glutathione stransferases; JA, jasmonic acid; MeJA, methyl jasmonate; RB, resistant bulk; SB, susceptible bulk; TMPK, Thymidylate kinase; LRR, LRR family protein; 2 - DE, two-dimensional electrophoresis.

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phytolectin, phenolics, malondialdehyde, proline and some proteases in defense response were demonstrated. Meanwhile, progress has been made in the detection of quantitative trait locus (QTL) conferring resistance to Fusarium wilt. One major QTL, *Foc2.1*, placed in the region of SSR03084-SSR17631 within a genetic distance of 2.4 cM on chromosome 2, was detected in different years 2007, 2009 and 2012 together with seven predicted nucleotide binding site resistance genes [19]. In addition, transcript profiles in *F. oxysporum*-inoculated cucumber were explored to investigate molecular interaction between cucumber and *F. oxysporum* by construction and characterization of a suppression subtractive hybridization (SSH) cDNA library. About 80 ESTs with known functions were acquired by the study [20]. Together, these data indicate that interaction between cucumber and.

F. oxysporum is a complex process that may involve various defense pathways.

Proteins are the final executors of most biological processes. Accumulated evidence indicates that the application of proteomics for investigation of plant-pathogen interaction can provide novel insights into the molecular mechanisms involved in the defense processes [21–30]. In order to gain further insights into host resistance, we explored the proteome from cucumber root total protein with emphasis on the host during the compatible and incompatible interactions. We used a two-dimensional electrophoresis (2-DE) coupled with mass spectrometry MALDI-TOF/TOF analysis to study the root proteome of two different phenotypes (susceptible bulk and resistant bulk) of cucumber F2 in response to the infestation. The present data provide important information on the molecular mechanisms of CFW resistance in cucumber at protein level.

2. Materials and methods

2.1. Plant materials and inoculation

Cucumber generation F2 was derived from hybridization of two inbred lines, one highly susceptible (995) and the other highly resistant (F9) to CFW, which were obtained from Liaoning Academy of Agricultural Science. Cucumber seeds were soaked in water for 8 h and then surface sterilized with 75% ethanol for 30 s and 2.5% NaClO for 15 min. After being washed with sterile water at least three times, sterilized seeds were placed on sterile water soaked gauze. The seeds were allowed to germinate at 25–30 °C. When the cotyledons expanded, the seedlings were used for subsequent experiments.

Seedlings were root-dip-inoculated in *F. oxysporum* endoconidia suspension of 10^6 microconidia/ml for 30 min and transferred to Petri dishes containing sterile water. The seedlings were grown aseptically in petri dishes at 25 °C. Seven days after inoculation, lesions (brown discoloration) characteristic of *F. oxysporum* infection were observed on some seedlings as shown in Fig. 1, and cucumber root of susceptible bulk (SB) and resistant bulk (RB) of cucumber generation F2 were harvested, respectively. The tap root and hypocotyl were cut, patted dry between paper towels, and their wet weights were recorded and then stored at −80 °C. Cucumber 995 and F9 were used for gene quantification and harvested at 1, 3, 5, 7, 9, 11, 13 and 15 d respectively after *F. oxysporum* inoculation.

2.2. Protein extraction

Frozen samples were finely powdered in liquid nitrogen using a pestle and mortar. Then the powder was suspended in 5 mL/g cold acetone containing 10% TCA and 0.07% <beta>- mercaptoethanol. After being precipitated in −20 °C refrigerator overnight, the pellet was collected by centrifuged at 15,000 g for 45 min. The pellet was washed three times with 80% cold acetone containing 0.07% <beta>-mercaptoethanol and 1 mM PMSF. Proteins were freeze-dried and stored at −80 °C for subsequent tests.

2.3. Two-dimensional electrophoresis (2-DE)

The dried proteins were redissolved in lysis buffer containing 8 M Urea, 2 M Thiourea, 4% CHAPS, 1% DTT, 1% TBP and 2% IPG buffer for 3–4 h at room temperature. The samples were centrifuged at 12000 *g* for 10 min at room temperature and the pellet was discarded. Protein concentration was determined using the Bradford method [31].

IPG DryStrips (*Amersham Biosciences*) 24 cm, pH 4–7 non-linear gradients were used. Strips were passively rehydrated for 12 h with 450 μL of sample buffer, containing 250 μg protein. The IEF conditions were as follows: 100 V for 1 h, 300 V for 1 h, 500 V for 3 h, 1000 V for 2 h, 10000 V for 3 h, 10000 V for 80000Vh and 500 V 24 h (*Ettan IPGphorIII*, GH Healthcare). After IEF, the focused strips were equilibrated with equilibration solutions twice, which contained 6 M Urea, 0.05 M pH 8.8 Tris-HCl, 2% SDS and 20% glycerol adding 2% DTT and 2.5% iodoacetamide respectively. The second dimension SDS-PAGE was performed with an 10% polyacrylamide gel. Electrophoresis was carried out at 0.4 W/gel 30 min, then 15 W/gel until bromophenol blue line runoff. The gels were silver stained according to Yan method [32].

2.4. Image analysis

The silver-stained gels were digitalized using UMAX Power Look 2100XL (*Maxium Tech*). Image analyses were carried out with the PDQuest Advanced™ 2-D Analysis software (*version 8.0.1*, Bio-Rad)

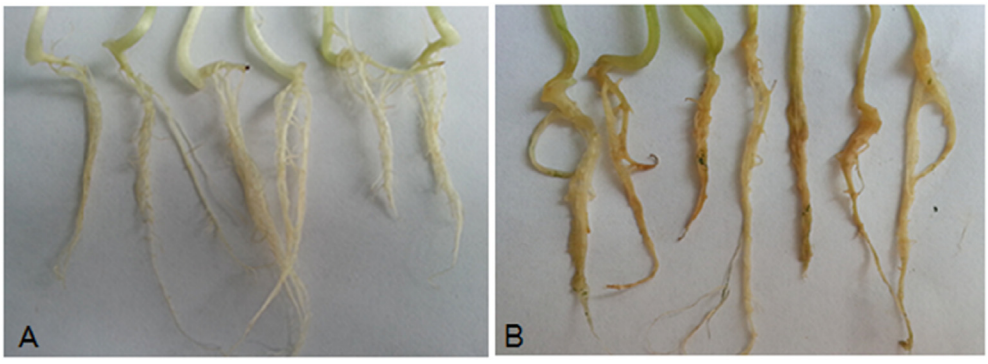

Fig. 1. Different phenotypes of resistant and susceptible plants to *F. oxysporum* infection. The plant phenotypes were observed at seven days post inoculation. (A) Resistant plants; (B) Susceptible plants.