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Comparative proteomic analysis of apple branches susceptible and resistant to ring rot disease

Zhang Cai-xia · Tian Yi · Zhang Li-yi · Zong Ze-ran · Cong Pei-hua

Abstract Ring rot disease, caused by the *Botryosphaeria berengeriana* f. sp. *piricola* pathogen, is a destructive disease for apple production. To gain further understanding about the defense mechanisms of apple branches against ring rot disease, a comparative proteomic analysis was conducted in our study. We selected two different host responses to *B. berengeriana* f.sp. *piricola* infection or challenge, and compared the different proteomes of susceptible and resistant apple branches that had or had not been inoculated with the pathogen. By using 2-DE and MALDI-TOF-TOF MS analysis, 27 differentially expressed proteins were identified in two inoculation assays. According to their function, the proteins were categorized into five classes. In total, according to these two inoculation assays, there were six differentially expressed defense-related proteins identified in the bark of susceptible and resistant hosts, including Mal d1, ASR, and SAMS, which may play key roles for the resistance mechanisms of each host against ring rot disease. We speculated that the only up-regulation of the ASR protein and the dramatic decrease of SAMS in the resistant host may be related to its better disease resistance. In addition, a total of 10 proteins exhibited opposite expression patterns in the bark of susceptible and resistant branches, and they may also be related to the different disease resistances of the two hosts. Due to the complexity of antifungal mechanisms of apple branch hosts against ring rot disease, to obtain more valuable insights about the interaction between the apple host and *B. berengeriana* f. sp. *piricola* pathogen, many further investigations will be conducted.

Keywords Apple branches · 2-De · Comparative proteomics · Disease-resistant · MALDI-TOF-TOF/MS

Abbreviations
2-DE	Two-dimensional electrophoresis
PR	Pathogenesis-related
SAMS	S-adenosylmethionine synthetase
APX	Ascorbate peroxidase
ASR	Abscisic stress ripening-like

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Introduction

Host plants generally express a wide range of resistance-related proteins against pathogen attack, including pathogenesis-related (PR) enzymes (Choi et al. 2008; Li et al. 2011). The apple tree (*Malus × domestica*) is a model fruit plant because of its worldwide economic importance (Zhuang et al. 2011). However, the cultivation of apples has been severely limited by many types of diseases (Norelli et al. 2009; Jurick et al. 2011; Fan et al. 2011). Among the fungal diseases, ring rot disease,

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caused by *B. berengeriana* f. sp. *piricola*, is a destructive disease in the world, which is on trunks, branches and fruits of apple tree. It occurred in the major apple-producing areas in China, usually resulting in serious damage to apple production (Ogata et al. 2000; Xu et al. 2015). When infected with *B. berengeriana* f. sp. *piricola* pathogen, the pathogenic symptoms of the branches were obviously different in resistant and susceptible cultivars, the resistant seedling just exhibited a little turned brown around the inoculation positions, and the susceptible seedlings usually shows more seriously pathogenic symptoms, including the rounded protuberances commonly increased, darkened in color and eventually cracked (Zhou et al. 2010; Xu et al. 2015).

The secretion of some specific proteins may play key roles in plant–pathogen interactions, especially some resistance-related proteins in host plants (Mehta et al. 2008; Lee et al. 2006). Thus, intensive efforts have been made to increase the resistance of apples to various diseases. Many genes isolated from model plants are related to disease resistance (Fan et al. 2011; Bednarek and Osbourn 2009; Soh et al. 2012), and some PR proteins were found. The PR proteins are reported to be plant species-specific proteins (Upadhyay et al. 2014), and their production and accumulation in plants play an important role in the defense capacity of plants challenged by pathogens (Soh et al. 2012). However, many of the previous studies on plant pathogens focused on other model plants and few have been performed on apple trees.

In recent years, proteomic analyses have been widely used to identify stress-related proteins involved in pathogen–crop interactions (Rampitsch and Bykova 2012; Liao et al. 2009). In apples, the disease resistance mechanisms against *B. berengeriana* f. sp. *piricola* have not been elucidated, and many studies have only focused on the identification of ring rot disease (Slippers et al. 2007; Pitt et al. 2010). To date, few proteomic analyses have been performed in the study of apple host–pathogen interactions. To elucidate the different defense mechanisms in apple branches, we compared the defense-related proteins of different susceptible and resistant apple hosts that were induced by the *B. berengeriana* f. sp. *piricola* pathogen and aimed to obtain a comprehensive understanding of apple host–pathogen interactions. The findings may provide novel clues to apples' resistance to the *B. berengeriana* f. sp. *piricola* pathogen and provide a theoretical basis for accelerating the process of apple molecular breeding.

Materials and methods

Plant materials and fungal pathogen

The plant material used in this study was selected from 115 progeny, which were hybridized from 'Jinhong' (resistant cultivar) and 'Gala' (susceptible cultivar) in 2004, and the seeds were planted in the orchard at the Institute of Pomology (Chinese Academy of Agricultural Sciences; Xingcheng, China) in 2005. The ring rot disease was introduced, by field inoculations in 2012 and 2013, and evaluated as described by Zhou et al. (2010). According to the evaluation of 12 highly resistant and nine highly susceptible seedlings, four (two highly resistant and two highly susceptible) were selected as different hosts based upon tree vigor and the consistency of phenotype upon challenge with the pathogen The aggressive strain of *B. berengeriana* f. sp. *piricola* (numbered **LW-xc102**) used in this study was provided by the Fruit Plant Protection Research Center at the Institute of Pomology at the Chinese Academy of Agricultural Sciences. To culture the *B. berengeriana* f. sp. *piricola* pathogen for our in vitro assays, the mycelia of *B. berengeriana* f. sp. *piricola* were incubated on potato dextrose agar petri plates at 25 °C for approximately 7 days, and mycelial plugs (5 mm in diameter) were harvested from the leading edge of the culture for use as inoculum.

The pathogen inoculation assays on host plants

The inoculation assays were conducted as previously reported by Zhou et al. (2010), with some modifications. The 1-year-old branches of susceptible and resistant seedlings were harvested and cut into several 20-cm long segments (diameter = 6.5 mm). The branches were rinsed with distilled water and 75 % alcohol, then rinse the branch segments in water after surface sterilization in alcohol, and then placed on four layers of moist filter paper in a square box with two moist cotton balls placed at each end of the segments. Four to five segments were kept in each box. Each susceptible and resistant branch segment was inoculated with three mycelial discs and then covered with distilled sealing film. The films were removed after 7 days. The boxes were kept at 25 °C and 60 % relative humidity with a 12-h photoperiod. For each susceptible and resistant seedling, 10 branch segments were inoculated, and the control samples were treated

with blank potato dextrose agar. Three replications for each treatment were preformed, and the entire inoculation experiment was repeated three times to ensure reliable results. Infected, challenged, and control samples were harvested 35 days after inoculation. Then, equal numbers of branch segments from the two susceptible and two resistant seedlings, independently, were mixed and the bark was immediately peeled off. The samples were immediately frozen in liquid nitrogen and ground to a fine powder using a tissue-grinding apparatus (Heros-mole TL-2020). Then, the powdered samples were kept at −80 °C until protein was extracted.

Protein extraction and 2-DE

The protein extraction of the branch bark was performed as introduced by Petriccione et al. (2013), with some modifications. In short, five grams of frozen lyophilized tissue powders was re-suspended in 10 mL of ice-cold extraction buffer (30 % sucrose w/v, 0.1 M Tris-HCl (pH = 8.0), 0.5 M EDTA, 1 mM DTT, 1 % Triton X-100 v/v, 0.1 M KCl, 5 % β-mercaptoethanol v/v, 1 mM PMSF, and 2 % PVPP). After being vortexed for 10 min at 4 °C, an equal volume of Tris–HCl (pH = 8.0) saturated phenol was added, and then, the samples were further vortexed for 15 min at 4 °C. After centrifugation (4 °C, 15,000 g, 20 min), the upper phase was collected. The protein samples were precipitated from the phenol phase with five volumes of 100 mM ammonium acetate in methanol overnight at −20 °C. The protein pellets were then subsequently rinsed three times with cold acetone containing 13 mM DTT. After centrifugation, the rinsed pellets were air-dried and re-suspended in lysis buffer (7 M urea, 2 M thiourea, 4 % (w/v) CHAPS, 0.5 % (v/v) IPG buffer, and 1 % (w/v) DTT). The protein solution was then used for 2-D electrophoresis. The concentration of the sample was determined using the 2-D Quant Kit (GE Healthcare).

Two-dimensional electrophoresis (2-DE) was then performed according to Petriccione et al. (2013), with some modifications. The sample containing 800 μg of total protein was loaded into an immobilized pH gradient (IPG) strip (18 cm, pH = 4–7 linear, GE) and rehydrated for 12 h at room temperature. Then, the strips were subject to IEF in an Ettan IPGphor system according to following procedures: 500 V for 1 h, 1000 V for 1 h, 5000 V for 1 h, and 10,000 V for 6 h. After the IEF, the strips were then transferred to perform the SDS-PAGE.

Prior to the analysis of the second dimension, the strips were equilibrated for 15 min in 10 mL of equilibration solution (Petriccione et al. 2013), and then, they were alkylated with 2.5 % iodoacetamide w/v in the same solution for 15 min. The separation of proteins in the second dimension was performed with SDS polyacrylamide gels (12.5 %) on the Ettan DALT System (GE) under the following conditions: 1 w/gel for 30 min and 10 w/gel for 5 h. After electrophoresis, the gels were stained with Coomassie Brilliant Blue (CBB) R-350 (GE).

Image and data analysis

The 2-D gels were scanned at 600 dpi, and analysis was performed using Image Master 2D Platinum Version 7.0 software (GE Healthcare). The M_r values of the protein spots were determined by referencing protein markers. For each treatment, three images were obtained representing 3 independent experiment replicates, and they were grouped as a class to calculate the averaged volume of all of the protein spots. The standard values of the protein spots of each treatment were exported to SPSS Version 13.0 (Lead Technologies, Chicago, Illinois, USA) for statistical analysis. Only those with significant and consistent changes were counted as differentially accumulated proteins (>1.5-fold, $p < 0.05$).

In-gel digestion and protein identification

Proteins were identified based on a previous report (Rocco et al. 2008). In brief, the spots were manually excised from gels, alkylated and digested with trypsin. After digestion, the peptides were desalted using ZipTipC18 pipet tips (Millipore, Bedford, USA). For matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF-TOF/MS), the peptides were eluted onto the target plate with an equal volume of a freshly prepared 5 mg/ml solution of 4-hydroxy-α-cyano-cinnamic acid in 50 % (v/v) ACN containing 0.1 % TFA. The samples were analyzed on a 4800 Plus MALDI TOF/TOF TM Analyzer (Applied Biosystems, ABI, USA).

The data were searched with GPS Explorer (GPS Explorer TM software, ABI, USA) using MASCOT (Matrix Science, London, UK) as a search engine. The following parameters were set: the database was NCBI (nr); the taxonomy was set as Viridiplantae (Green Plant) and Rosaceaea; and the maximum number of missed cleavages was set as 1. The quality error

scope setting was 0.4 Da. The unmentioned parameters were set according to the default values in the software. To determine the identification confidence, the results with protein scores above 60 were chosen as positive. Only the best matches with high confidence levels were selected.

Results

Comparison of proteome expressions induced by the *B. berengeriana* f. Sp. *piricola* pathogen in susceptible and resistant branches

Using two-dimensional gel electrophoresis, we explored the alterations of the total proteins induced by the *B. berengeriana* f. sp. *piricola* pathogen in the bark of susceptible and resistant branches. According to the software analysis, more than 800 protein spots were detected from both the susceptible and resistant samples (Fig. 1). Compared with each respective control, and with at least a 1.5-fold quantitative change as the criteria, 27 protein spots were found to be differentially expressed and were successfully identified in two different inoculation assays (Fig. 1). Of these proteins, three (spots 13, 14, and 26) were induced in the bark of infected susceptible branches and two (spots 14 and 26) were induced in the bark of challenged resistant branches. The induced proteins may be related to the defense responses in the bark of susceptible and resistant branches.

Protein identification and functional categorization

Based on the criteria described in the "Materials and Methods" section, 27 spots were successfully identified using two inoculation assays. Among the spots, eight proteins were highly similar to proteins from the *Malus × domestica* species, and the remaining proteins (19) were identified in other databases. For these differentially expressed proteins, 17 and 13 proteins were up-regulated in the bark of susceptible and resistant branches, respectively, whereas 10 and 14 proteins were down-regulated in the bark of susceptible and resistant branches, respectively (Fig. 2). All of the identified proteins induced by the pathogen are listed in Table 1.

Notably, there were no pathogen proteins identified in our assays, which may be due to the low amount of fungal biomass compared with that of the host plant.

Based on the previous categorizations of protein functions (Bevan et al. 1998), the identified proteins could be grouped into five classes. These functional classes include metabolism and energy production (29.7 %; Class I), protein synthesis (7.4 %; Class II), defense response (22.2 %; Class III), cell structure (7.4 %; Class IV), and unclear classification (33.3 %; Class V) (Table 1; Fig. 3).

Differentially expressed proteins in the bark of susceptible and resistant branches after being challenged by the *B. berengeriana* f. Sp. *piricola* pathogen

After inoculation with the *B. berengeriana* f. sp. *piricola* pathogen, a total of 27 proteins were differentially expressed in the bark of susceptible and resistant branches, respectively (Fig. 1). Of these proteins, eight were related to Class I (spots 1, 5, 7, 8, 10, 12, 18, and 27), two were related to protein synthesis (Class II) (spots 13 and 17), six were related to defense response (Class III) (spots 6, 15, 16, 20, 24, and 26), two were related to cell structure (Class IV) (spots 4 and 22), and nine had unclear classifications (Class V) (spots 2, 3, 9, 11, 14, 19, 21, 23, and 25) (Table 1). According to our results, the proteins having unclear classifications constituted the largest group (Fig. 3), and, when compared with the control samples, they all accumulated in the susceptible sample, including an induced protein (spot 14) (Fig. 2), while, four of them were down-regulated in the resistant sample (spots 9, 11, 23, and 25).

In addition to the proteins with unclear classifications, there were five proteins that were differentially expressed between the bark of susceptible and resistant branches, including three metabolism and energy production proteins (spots 8, 10, and 18), one actin (spot 4) and one defense response protein (spot 16). Three of these proteins (spots 4, 10, and 18) were up-regulated in a susceptible host, but down-regulated in a resistant host. The other two proteins (spots 8 and 16) were down-regulated in a susceptible host, but up-regulated in a resistant host. The differentially expressed proteins may be related to the different resistance levels of each host.

There were six proteins were categorized as defense response proteins, and except for one induced protein (spot 26), they were all down-regulated in the bark of the susceptible sample after infection by the pathogen.
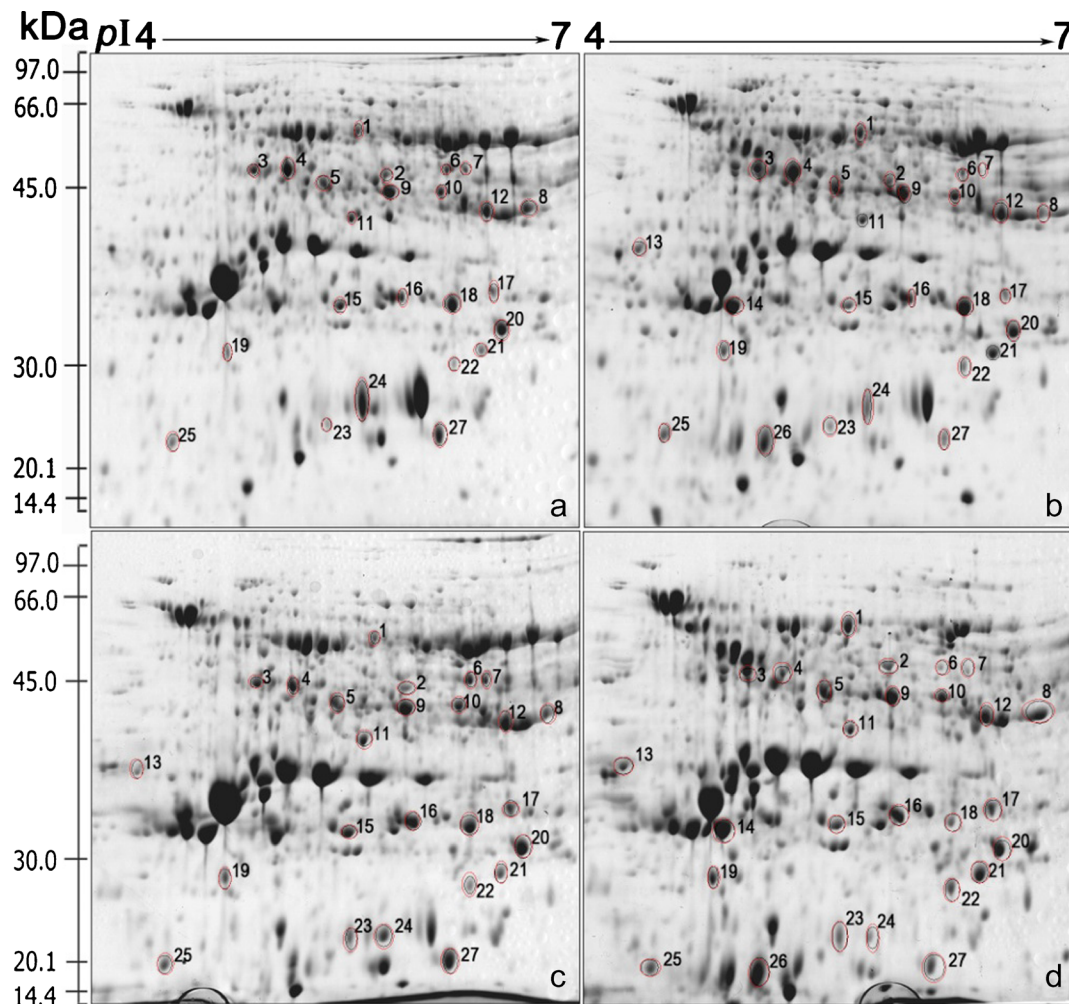
Fig. 1 The 2-DE analysis of protein spots induced by the *B. berengeriana* f. sp. *piricola* pathogen in the bark of susceptible and resistant branches. The 2-D gels (pH = 4–7) were stained with CBB R-350. The approximate molecular masses and *p*Is are indicated in the margins. Circles indicate the 27 proteins identified by MALDI-TOF-TOF/MS that had an at least 1.5-fold change in abundance between the control and treated samples. The proteins that changed were numbered, and the numbers correspond to the numbers in Table 1. This figure represents three biological replicates. **a** Control susceptible branches; **b** Infected susceptible branches; **c** Control resistant branches; and **d** Infected resistant branches

However, one protein was up-regulated in the resistant sample (spot 16). Of the proteins, two spots (spots 24 and 26) were identified as the same major allergen mal d1. However, the two proteins exhibited different expression patterns. Spot 24 was down-regulated in each host after the pathogen challenge, while spot 26's expression was induced dramatically in each host. Combined with the other four differentially expressed defense-response proteins, SAMS (spot 6), APX (spot 15), abscisic stress ripening-like protein (spot 16), and oxygen-evolving enhancer protein (spot 20), the results indicate that when confronted with a pathogen challenge, the susceptible and resistant hosts exhibited different self-defense response mechanisms.

Discussion

For ring rot disease, few proteomic analyses have been used to analyze the apple host–pathogen interactions. To elucidate the changes in protein abundances that occurs in susceptible and resistant apple branches responding to the *B. berengeriana* f. sp. *piricola* pathogen, we conducted two different
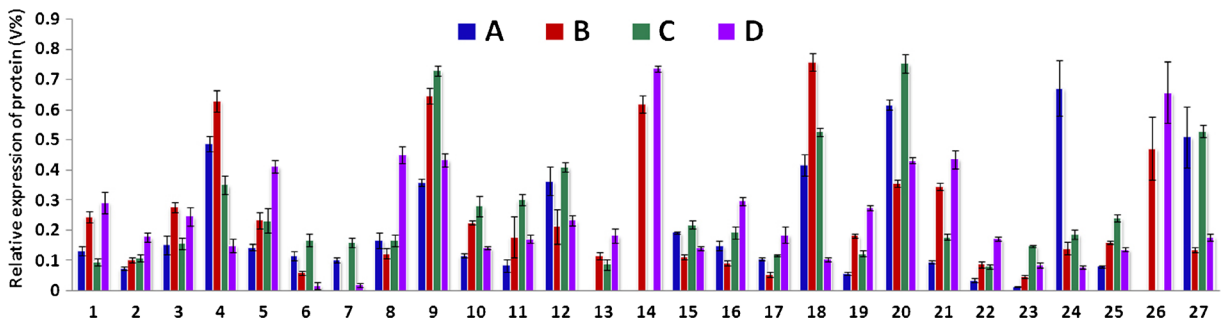
Fig. 2 Proteins whose abundances were quantitatively changed by the *B. berengeriana* f. sp. *piricola* pathogen in the bark of susceptible and resistant branches. **A** Control susceptible branches; **B** Infected susceptible branches; **C** Control resistant branches; and **D** Challenged resistant branches. The protein spots are numbered according to those in Fig. 1 and Table 1. Data are representative of three independent experimental replicates and given as intensity means ± S.D. Y axis: Relative expression of the spot (V%)

inoculation assays. Here, we discuss the possible defense mechanisms that occurred in the two different hosts when faced with pathogen infection.

The metabolism and energy production-related proteins

In our study, almost a third of the proteins identified were involved in metabolism and energy production. Of these proteins, four and three proteins were up-regulated in susceptible and resistant hosts, respectively, and four and five proteins were down-regulated in susceptible and resistant hosts, respectively. In addition to one photosynthesis-related protein (spot 7), the others are all related to energy production. The proteins identified in the bark of the branches were quite different than those found in apple leaves induced by *Alternaria alternata* (Zhang et al. 2015). In the latter, most of the metabolism and energy production-related proteins were related to photosynthesis, and these different results may be owing to the differences in photosynthetic capacities of the two tissues. The up- and down-regulated proteins identified in each host also indicated that during the interaction process, the hosts may use different metabolic pathways in response to the pathogen infection or challenge to provide an energy base to complete the resistance reaction (Fang et al. 2012).

Defense-related proteins

There were six differentially expressed defense-related proteins identified in the susceptible and resistant hosts, and two of them were PR proteins, including the Mal d1 (spots 24 and 26) protein.

Usually, PR proteins coded by the host plant play important roles in host disease resistance. Numerous PR proteins have been detected in many model plants (van Loon et al. 2006; Nandi 2016). PR proteins can be categorized into 17 groups based on their biological activities, including the major allergen mal d1 (PR-10) (Loon et al. 2006). In our studies, there were two proteins annotated to PR-10, and they were differentially expressed after pathogen exposure in the susceptible and resistant hosts. Mal d1, as an allergen, has been identified as having 12 members in its family and has a high homology to PR-10 proteins (Beuning et al. 2004). Mal d1 was also related to pathogen infection in previous reports (Pühringe et al. 2000; Mayer et al. 2011). In our experiment, two Mal d1 allergens (spots 24 and 26) were differentially expressed, with one spot (spot 24) being down-regulated in the two hosts, while the other (spot 26) was highly induced in the two hosts after infection or challenged with the pathogen. This phenomenon may be related to the post-translational modification of proteins.

Reactive oxygen species can be scavenged by some anti-oxidative enzymes. APX is an important enzyme involved in scavenging H_2O_2, and it acts as a signal to activate defense responses (Faize et al. 2012). In our studies, APX was down-regulated in susceptible and resistant host plants, consistent with a previous report (Palanisamy and Mandal 2014). Because the APX levels were not dramatically different in each host, we speculated that the expression of APX may not contribute to the different resistance levels of the two hosts.

The abscisic stress ripening-like (ASR) protein (spot 16), which localized in both cytoplasmic and nuclear chromatin, may play essential protective roles in signal pathways under stress conditions (Li et al. 2012). In our

Table 1 Differentially expressed proteins identified from the bark of susceptible and resistant branches
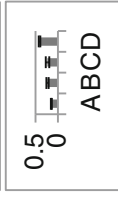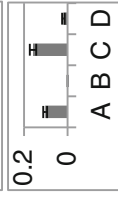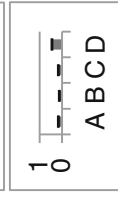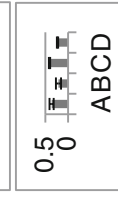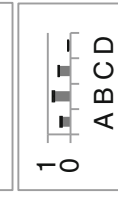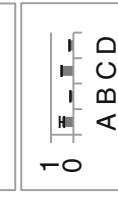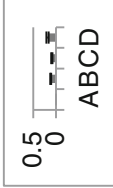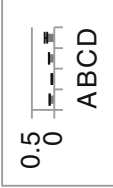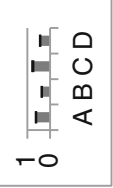
Spot No.	Spot % volume variation	Description	Accession No.	Theo. Mr.(kDa)/pI	Exp. Mr.(kDa)/pI	Functional categories	Protein Score	S. C(%)	Pep Count.	Species
Metabolism and energy production										
1		ATP synthase beta subunit	gi|7708676	52.10/5.02	58.21/5.58	I	262	21	2	*Thunbergia coccinea*
5		beta-cyanoalanine synthase 1	gi|93359259	41.08/7.6	45.18/5.32	I	291	29	12	*Malus x domestica*
7		glutamate-1-semialdehyde 2,1-aminomutase 2	gi|297816078	50.75/6.36	49.31/6.3	I	121	35	5	*Arabidopsis lyrata subsp.*
8		cytosolic malate dehydrogenase	gi|78216493	35.97/6.01	44.56/6.67	I	463	46	12	*Malus x domestica*
10		glutamine synthetase nodule isozyme-like	gi|470104403	39.20/5.64	45.20/6.01	I	277	24	6	*Fragaria vesca subsp. Vesca*
12		cytosolic malate dehydrogenase	gi|78216493	35.97/6.01	43.62/6.30	I	334	38	9	*Malus x domestica*
18		cytosolic triose phosphate isomerase	gi|414550	27.37/5.24	35.21/63.20	I	150	23	4	*Arabidopsis thaliana*
27		carboxylase/oxygenase large subunit	gi|75758075	51.99/5.91	21.1/6.1	I	406	48	20	*Eryngium giganteum*

Table 1 (continued)

Spot No.	Spot % volume variation	Description	Accession No.	Theo. Mr(kDa)/pI	Exp. Mr(kDa)/pI	Functional categories	Protein Score	S. C(%)	Pep Count.	Species
Protein synthesis										
13		ran-binding protein 1 homolog c-like isoform 2	gi|470116263	24.41/4.66	42.1/4.1	II	195	29	6	*Fragaria vesca*
17		ribosomal protein 30S subunit	gi|170131	33.97/6.69	37.2/6.51	II	114	30	6	*Spinacia oleracea*
Defense response										
6		s-adenosylmethionine synthetase 1	gi|224101473	43.61/5.5	49.2/6.11	III	487	48	13	*Populus trichocarpa*
15		ascorbate peroxidase	gi|288188862	16.51/4.66	35.26/5.43	III	123	34	5	*Malus pumila*
16		abscisic stress ripening-like protein	gi|16588758	20.74/5.68	36.8/5.9	III	72	29	3	*Prunus persica*
20		oxygen-evolving enhancer protein 2	gi|470136236	28.52/8.3	33.67/6.72	III	68	23	2	*Fragaria vesca*
24		Mal d 1	gi|332271283	17.67/5.67	25.4/5.49	III	185	21	4	*Malus x domestica*
26		major allergen mal d 1	gi|4590376	17.69/5.67	23.1/4.82	III	163	28	6	*Malus x domestica*

Table 1 (continued)
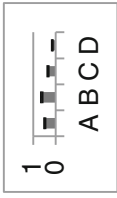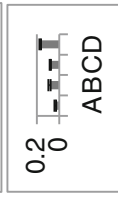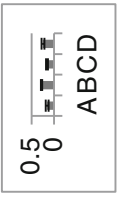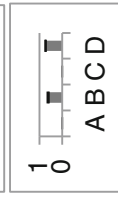
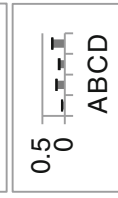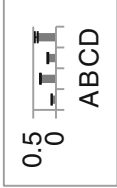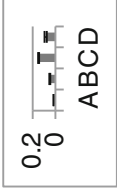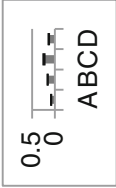| Spot No.	Spot % volume variation	Description	Accession No.	Theo. Mr(kDa)/pI	Exp. Mr(kDa)/pI	Functional categories	Protein Score	S. C(%)	Pep Count.	Species
Cell structure										
4		Actin, partial	gi|355329944	40.37/5.67	49.3/5.19	IV	587	51	14	Malus x domestica
22		actin-depolymerizing factor	gi|33772153	11.19/8.76	31.1/6.31	IV	268	23	3	Malus x domestica
Unclear classification										
2		Hypothetical PRUPE _ppa006513	gi|462411618	42.18/5.82	47.2/5.75	V	289	28	7	Prunus persica
3		hypothetical PRUPE _ppa006628	gi|462414559	43.50/5.46	47.36/4.9	V	259	34	8	Prunus persica
9		hypothetical PRUPE _ppa007772	gi|462411755	39.12/5.88	43.25/5.91	V	222	26	5	Prunus persica
11		hypothetical PRUPE _ppa009534	gi|462420277	28.67/8.43	41.6/5.38	V	366	31	8	Prunus persica
14		uncharacterized protein	gi|356545090	38.07/6.51	34.9/4.75	V	107	23	1	Glycine max
19		hypothetical PRUPE _ppa010791	gi|462420794	25.50/5.05	32.1/4.67	V	155	39	10	Prunus persica

Table 1 (continued)

Spot No.	Spot % volume variation	Description	Accession No.	Theo. Mr.(kDa)/pI	Exp. Mr:(kDa)/pI	Functional categories	Protein Score	S. C(%)	Pep Count.	Species
21		hypothetical PRUPE _ppa012487	gi|462405445	18.04/6.17	31.1/6.31	V	195	34	8	*Prunus persica*
23		hypothetical PRUPE _ppa012643	gi|462398223	17.72/5.47	23.21/5.3	V	292	37	9	*Prunus persica*
25		hypothetical PRUPE _ppa010963	gi|462398057	24.23/8.96	22.1/4.47	V	132	39	4	*Prunus persica*

*Y axis: Relative expression of the spot (V%)

*X axis: column: A: Control susceptible branches; B: Infected susceptible branches; C: Control resistant branches; and D: Infected resistant branches

The "protein score" obtained from Mascot for each match and Mascot scores greater than threshold (60) are statistically significant ($P < 0.05$)

Theo. Mr. (kDa)/pI, theoretical molecular mass and isoelectric point based on amino acid sequence of the identified protein

Expt. Mr. (kDa)/pI, experimental molecular mass and isoelectric point estimated from the 2-D gels

Data are representative of three independent biological replicates and given as intensity means ± S.D
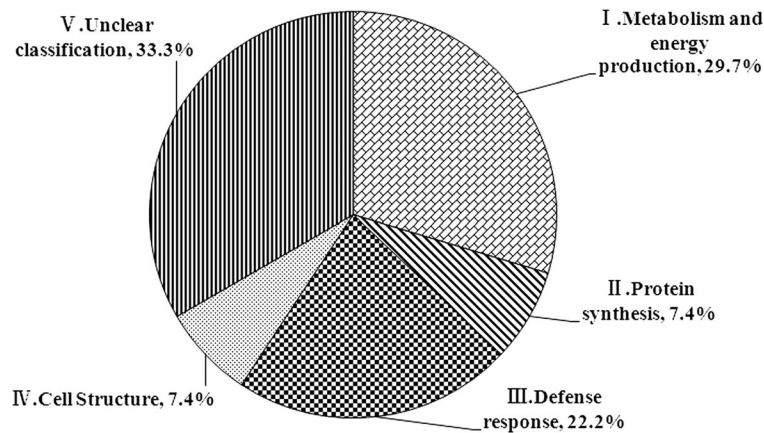
Fig. 3 Functional categorization of the identified proteins that were differentially regulated in the bark of susceptible and resistant branches infected by the *B. berengeriana* f. sp. *piricola* pathogen. A total of 27 identified proteins were assigned to the functional categories. The Roman numerals of the categories correspond to the functional categories described in Table 1. The percentages represent the proportions of proteins in each category

study, the ASR protein exhibited a differential expression pattern between the susceptible and resistant hosts, being up-regulated in the resistant sample and down-regulated in the susceptible one, which was consistent with other previous data (Salekdeh et al. 2002). We speculated that only the up-regulation of the ASR protein in the resistant host may be involved in defense responses.

In addition, SAMS, as a key factor involved in polyamine synthesis, also plays a crucial role in the host plant response to pathogen challenge (Izhaki et al. 1995). Previous study showed that transcript level of *SAMS1* and *SAMS2* were all sharp decreased after 24 h post-inoculation, and the resistant line showed more dramatic reduction than susceptible line (Nazmul et al. 2007). In our study, compared with the control, the SAMS (spot 6) decreased more than 10-fold in the resistant host, while the level was much higher in the susceptible host (Fig. 2), which may be owing to their different disease resistance properties.

Other proteins

In our results, 10 of the 27 proteins had reciprocal expression patterns in susceptible and resistant branches (Fig. 2), including some basic physiologically regulated proteins and some proteins with unclear functions. The results indicated that some metabolic and protein synthesis pathways may also be involved in the disease resistance of susceptible and resistant branches. Further studies will be performed to determine the specific functions of the proteins categorized in Class V.

Conclusions

In summary, our present work, using a comparative proteomic approach, was an attempt to elucidate the different responses of susceptible and resistant apple branches against the *B. berengeriana* f. sp. *piricola* pathogen and identify defense-related proteins. The present study highlights several defense-related proteins, including Mal d1, ASR, and SAMS, which may play crucial roles in the defense functions of susceptible and resistant apple branches in response to pathogen infection or challenge. In addition to defense-related proteins, other primary metabolic proteins and proteins with unclear functions may also be involved in the defense actions of susceptible and resistant hosts. Nevertheless, considering the complexity of the antifungal mechanisms of apple branch hosts against ring rot disease, determining the detailed molecular mechanisms involved in the interactions of apple hosts and the ring rot disease-causing pathogen will require further investigations.

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