Construction of a cDNA Library of *Vitis pseudoreticulata* Native to China Inoculated with *Uncinula necator* and the Analysis of Potential Defence-related Expressed Sequence Tags (ESTs)

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Expressed sequence tags (ESTs) constitute a rapid and informative strategy for studying the gene expression profiles of specific stages of annual and perennial plant species. In the study undertaken for this report, a cDNA library was constructed from the young leaves of Chinese wild *Vitis pseudoreticulata* inoculated with *Uncinula necator* (Schwein.) Burrill. The leaves were harvested at various times after inoculation for total RNA extraction, which was used to generate ESTs. In our study, 107 cDNA clones were sequenced from either the 5' or 3' end of the cDNAs. Of these, 60 unigenes (56.1%) were functionally characterised by the BLASTX matches to known-function proteins, and 20 unigenes (18.7%) matched significantly with proteins with unknown function in the public databases. The remaining 27 unigenes (25.2%) failed to show significant homology to any proteins in the public databases, suggesting that they represented novel sequences. Some functional genes identified from the cDNA library and their potential with plant defence system is discussed.

Powdery mildew of grapevines, caused by *Uncinula necator*, is a worldwide fungal disease. The pathogen is believed to have originated in North America and to have spread to Europe before 1850 (Pearson, 1988). Powdery mildew was observed in China for the first time in the 1950s (Wang, 1993). There have been reports of local powdery mildew disease outbreaks and potential for damage in the major grape production areas in China (He, 1993; Wang, 1993; Wang *et al.*, 1995; Zhang, 2001).

Grapevines (*Vitis* species) are cultivated commercially in more than 60 countries over a combined area of about 9 million ha (Anonymous, 1988). The leading cultivated species by far is *Vitis vinifera* L. It is supported by the multitude of uses of its fruit for producing table grapes, wine, juice and raisins. However, most of the European grape cultivars with a fine quality and high yield are susceptible to fungal diseases, which cause extensive losses in yield and quality. This has been the one of the most serious problems for grapevine cultivation. The most threatening among these fungi are powdery mildew, anthracnose, downy mildew, and grey mould rot. Powdery mildew is the most devastating fungal disease of grapevines worldwide, reducing yield, vine growth and vigour, and fruit quality (Pool *et al*., 1984; Gadoury *et al*., 2001).

Wild species are often valuable sources of resistance to crop pathogens. This is obviously the case for grapevine, where *V. vinifera* is susceptible to most pathogens whereas resistance to the same pathogens can be found in wild grapevine species (Boubals, 1959; 1961; 1966; Eibach *et al*., 1989). Among these wild species, *Muscadinia rotundifolia* offers the highest level of resistance against the widest range of pathogens. Small (1913) proposed reclassification of *Muscadinia* as a distinct genus. The discovery that the chromosome numbers were different in *Vitis* (2n = 38) and *Muscadinia* (2n = 40) gave new support to this proposal (Branas, 1932). Programmes aimed at the introgression of resistance genes from *M. rotundifolia* into *V. vinifera* were developed (Wylie, 1871; Detjen, 1919a; 1919b), but have been limited by the high sterility of the hybrids (Patel & Olmo, 1955; Nesbitt, 1966; Olmo, 1971; Bouquet, 1986). So, many international scientists undertake research on the resistance to *Uncinula necator* of grapevine using *V. vinifera*. Although a number of pathogenesis-related (PR) cDNA clones from *V. vinifera* cultivars Sultana and Cabernet Sauvignon infected with powdery mildew, such as chitinases (PR-2), beta-1,3-glucanases (PR-3), and thaumatin-like (TL) proteins (PR-5), have been reported (Jacobs & Robinson, 1999), the expression of chitinase genes in *V. vinifera* cultivar *Ugni blanc* induced by *Uncinula necator* has also been reported (Robert *et al*., 2002). In addition, a number of resistance gene analogs linked to a powdery mildew resistance locus in *V. vinifera* have been isolated (Donald *et al*., 2002). *V. vinifera* is not a desirable natural source of resistance to *U. necator*.

Consequently, it is desirable to identify natural sources of resistance to *U. necator* that might be employed to increase the resistance of cultivated vines. China is one of the major centres of origin of *Vitis* species (He *et al*., 1991). Chinese wild *Vitis* species

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are an important source of powdery mildew resistance, and these grapevine species originate from all parts of China. Chinese wild *Vitis* species are resistant to a number of pathogens known to affect cultivated grapevines, including powdery mildew, anthracnose, downy mildew, white rot and ripe rot (Wang *et al*., 1995; Wang & He, 1997; Wang *et al*., 1998; Luo *et al*., 2002; Wang *et al*., 2002; Wang *et al*., 2003; Xu *et al*., 2003). In addition to their potential as sources of disease resistance, Chinese wild *Vitis* species do not have the foxy flavour that limits the use of some of the American native grapes in breeding programmes (Alleweldt & Possingham, 1988). Therefore, they could provide an important source of resistance to a number of economically significant diseases. Understanding the host defence mechanism and identifying key genes in the resistant germplasm should provide valuable information and foundational resources for the timely and efficient molecular breeding of highly resistant cultivars.

In this study, in order to obtain more information about the interaction between host plant and pathogen at the molecular level of Chinese wild *Vitis* species in particular, and a cDNA library for study purposes was established using pilot-expressed sequence tags (ESTs). We undertook a moderate gene survey by means of the generation, sequencing and analysis of *Vitis pseudoreticulata* clone Baihe-35-1 ESTs, aiming to unveil new features and shed more light on lingering questions related to resistance, host interaction and molecular systematics of this species.

MATERIALS AND METHODS

Plant materials and treatments

V. pseudoreticulata clone Baihe-35-1 of the Chinese wild *Vitis* specie, which is highly resistant to powdery mildew and which is kept in the grape germplasm resources orchard, Northwest A&F University, Yangling Shaanxi, People's Republic of China, was used in this study. The powdery mildew inoculation was carried out under natural field conditions. Before inoculation, the upper sides of the infected young leaves of Baihe-35-1 were pre-sprayed with sterile water. The upper sides of the leaves were then inoculated with spores by the dry-pressing method (Zhang *et al*., 2001). Spores were harvested from *V. pseudoreticulata* clone Baihe-35-1 leaves infected with *Uncinula necator* from 08:00 am to 10:00 am on 8 July 2003. The inoculated leaves were immediately covered with paper bags to prevent infection with other pathogens.

Extraction of total RNA from *V. pseudoreticulata* clone Baihe-35-1

At one, two, three, four, five, six and seven days post-inoculation, leaves were collected from *V. pseudoreticulata* clone Baihe-35-1 and snap frozen in liquid nitrogen. Total RNA was isolated from the above leaf samples with Zhang's SDS/phenol method (Zhang *et al*., 2003). The concentration of total RNA was estimated by measuring the O.D. in a spectrophotometer at 260_{nm}.

Construction of cDNA library

The pool of total RNA from *V. pseudoreticulata* clone Baihe-35-1 at various times (1 µg) was used to construct the cDNA library. According to the manufacturer's instructions, the synthesis of cDNA and an adaptor ligation were performed using the SMART™ cDNA Library Construction Kit (CLONTECH, USA). The resulting cDNA was directly sub-cloned into the λTriplEx2 vector and packaged using MaxPlax™ lambda packaging extracts (Epicentre Technologies) according to manufacturer's guidelines. The resulting primary library contained 3.0×10^5 pfu/ml.

Converting λTriplEx2 to pTriplEx2

The conversion of a λTriplEx2 phagemid to a pTriplEx2 plasmid involved in vivo excision and circularisation of a complete plasmid from the recombinant phage. The λTriplEx2 multiple cloning site (MCS) is located within an embedded plasmid (pTriplEx2), which is flanked by loxP sites at the λ junctions. When the recombinant phage is transduced into *Escherichia coli* strain BM25.8, Cre recombinase is expressed in the *E. coli* BM25.8. In this system, the plasmid is released automatically as a result of Cre recombinase-mediated site-specific recombination at the loxP sites. The excised plasmid is propagated stably in *E. coli*.

Plasmid preparation

A total of 2 µl λphage (3.0×10^5pfu/ml) was combined overnight with 200 µl of BM25.8 host cell culture and the mixture was incubated at 31°C for 30 min without shaking. LB broth (400 µl) was added to the mixture, which was incubated at 31°C for an additional 1 h with shaking (225 rpm). Finally, the infected cell suspension was spread on a Luria-Bertani (LB)/ampicillin plate to obtain isolated colonies. Well-isolated colonies were randomly picked from each clone and plasmid DNA samples were prepared separately. The plasmid was prepared by using the Wizard® Plus SV Minipreps DNA purification system (Promega, USA). The plasmid DNA was stored at -20°C.

Sequencing

Purified plasmid DNA was sequenced to obtain the 5' end or 3' end sequence of the insert with a modified SMART primer (5'-CTCGGGAAGCGCGCCATTGTGTTGGT-3') or SP6 primer and Applied Bio-systems Big Dye Sequencing Mix. The sequencing reactions were separated on an Applied Bio-systems 3700 DNA analyser.

Sequence editing

Each sequence obtained was edited using Vecscreen (http://www.ncbi.nlm.nih.gov) to remove flanking vector sequences and assessed manually to determine sequence quality. The author edited anomalous clone sequences manually after examination of their corresponding chromatogram files. Three classes of anomalous sequences were also excluded: (1) sequences without inserts at all; (2) sequences with reverse inserts; and (3) sequences with incorrect adapters.

Homology comparisons

Each edited EST was translated in all six reading frames and compared with the non-redundant database at the National Centre for Biotechnology (NCBI) using the BLASTX program, which compares translated nucleotide sequences with protein sequences. Default BLAST parameter values were used, except for the following settings: Expect = 1, Alignments = 10, and Descriptions = 10. Sequences that returned no significant similarity were again compared using BLASTN, which compares nucleotide sequences with nucleotide sequences, with Expect = 1, Alignments = 10, and Descriptions = 10. Homologies to negative reading frames were disregarded, except in clones with inserts in the reverse orientation. Putative identifications for the ESTs were assigned based on the results of the BLAST searches and, in some cases, with information contained in related abstracts in PUBMED. The non-redundant set

of *V. pseudoreticulata* clone Baihe-35-1 ESTs was deposited in GenBank (GenBank accession nos. are *AY848693; DQ336280-336289; DQ339462-339464 DQ354157-354161; DT646287; DT661587-661592; DT661594-661601; DT661603; DT661605; DT661608; DT661610-661611; DT661613-661616; DT661618-661619; DV182076-182078; DV182080-182082; DV182084-182096; DV182098-182115; DV182117-182119; DV182121-182135; DV671632-671634; DV671636-671638; DY242191)*.

Analysis of ESTs

cDNA sequences generated from each cDNA clone were carefully edited to remove the vector sequence and the low-quality sequences. ESTs longer than 150 bp and containing no more than 4% ambiguity were considered useful for data analysis (Franco *et al*., 1995). Using the basic local alignment search tool (BLAST) service, at NCBI, sequences were subjected to search against the protein and nucleic acid databases. Sequence similarities identified by the BLAST programs were considered statistically significant at an E value of $\leq 10^{5}$.

Semi-quantitative RT-PCR

Using total RNA as template, one-step reverse transcription PCR was performed with M-MLV reverse transcriptase according to the manufacturer's instructions (Promega, USA). Primers of defence-related genes and a reference gene were designed for semi-quantitative RT-PCR (Table 3). PCR reactions were performed in a total volume of 25 µL comprising 170 ng DNA, 1.25 mM dNTPs, 10 µM of each primer, 37.5 mM $MgCl_2$ and 0.2 unit of Taq DNA polymerase (Henan Sino-American Biotechnology Co., Ltd.). The PCR cycling conditions were an initial 94°C for 5 min; 30 cycles of 94°C for 1 min, 56°C for 30 s and 72°C for 50 s; and a final cycle at 72°C for 10 min.

RESULTS

Construction of *V. pseudoreticulata* clone Baihe-35-1 cDNA library

The pool of total RNA from *V. pseudoreticulata* clone Baihe-35-1 at various times (1 µg) was used to construct the cDNA library. The primary titre of the library was 3.0×10^{5} pfu/ml. The size of insert fragments basically ranged from 0.5 kb to 2.0 kb, with an average of 0.9 kb, and the recombinant phage in the library was 96.9%.

Characterisation of EST sequence

The cDNA library generated from *V. pseudoreticulata* clone Baihe-35-1 was used as a source of ESTs. All ESTs from the cDNA library that were putatively identified from matches to database sequences of known functions were classified into general biochemical and metabolic functional categories (Table 1). The best matches for all the ESTs generated were to plant gene sequences, suggesting that none of the cDNA clones were copies of fungal mRNA. Of the 107 *V. pseudoreticulata* ESTs isolated, 56.1% (60) possessed high sequence similarity to existing sequence entries of known function in the databases. The remaining 43.9% (47) represented sequences of no currently known function. Twenty of the 47 unknown ESTs (18.7% of the entire sequenced cDNA library) were similar to sequences for genes coding for putative uncharacterised or hypothetical proteins. The remaining 17 (15.8% of the whole cDNA library) did not exhibit sequence similarity to any known DNA or protein sequence in the databases. The known ESTs in this report were classified according to function as predicted by BLASTX (Table 1).

TABLE 1

Classification of ESTs according to function prediction by BLASTX match.

Putative identification	No. of ESTs	Percentage of total (%)
Primary metabolism	15	14%
Protein synthesis and sorting	13	12.1%
Defence-related protein	6	5.6%
Signal transduction and hormone	5	4.7%
Secondary metabolism	1	0.9%
Stress-induced protein	2	1.9%
Cell wall structure and metabolism	2	1.9%
Gene expression and chromatin metabolism	4	3.7%
Transcriptional factor	2	1.9%
Reactive oxygen	3	2.8%
Membrane transport	2	1.8%
Others	5	4.8%
Unknown function	20	18.7%
No match	27	25.2%
Total	107	100%

Defence-related ESTs

Seventeen ESTs were identified from the cDNA library to be potentially associated with plant defence responses. The level of redundancy of the defence-related ESTs was 15.8% (Table 2), coding for specific antimicrobial proteins, signalling, receptors, protein kinase and defence-activating proteins. This search revealed sequence matches to cDNA clones/ESTs from related *Vitis* species or other organisms (Table 2), some of which were generated from stressed and disease-affected plant tissues.

Analysis of semi-quantitative RT-PCR

Semi-quantitative RT-PCR products were separated by electrophoresis in 1.0% agarose/EB gel with GeneSnap from SynGene (Fig. 1). As Fig. 1 shows, these expression patterns in mRNA levels of chitinase, PAL, PPO, PR10, OAO, 4-CcoAL, HSP90 and MT gene compared to GAPDH displayed significant changes after the first day of inoculation. It depicts that these genes were involved in the resistant mechanism of powdery mildew.

DISCUSSION

To increase the probability of cloning the *V. pseudoreticulata* clone Baihe-35-1 transcripts involved in disease response, leaf tissue taken from a resistant *V. pseudoreticulata* plant (clone Baihe-35-1) was used for cDNA library construction, and the analysis was performed over a time span of seven days while under challenge from *Uncinula necator*. A random cDNA sequencing strategy was better than an mRNA differential display approach (Wang, 2004; Xu & Wang, 2009). So, the use of an EST approach was a successful way of identifying genes in *V. pseudoreticulata* involved in a disease response to *Uncinula necator*. One hundred and seven ESTs from the cDNA library were identified. Of these, 56% were significantly similar to known gene sequences in entries in the databases searched. This value is higher than that observed in the analysis of cDNA libraries constructed from sugar

TABLE 2

Potential *V. pseudoreticulata* defence-related ESTs matched with known genes of other organism.

EST No.	Putative homologue	Organism	Accession No.	E value	Program
VP422-5	Class IV chitinase	*Vitis vinifera*	DT661595	2e-134	BLASTn
VP285-5	Gene for phenylalanine ammonia-lyase	*Vitis vinifera*	DV182110	5e-06	BLASTn
VP306-5	RUB1 conjugating enzyme	*Arabidopsis thaliana*	DQ336285	7e-90	BLASTx
VP6-5	Cysteine protease	*Daucus carota*	DV182129	1e-49	BLASTx
VP189-5	Polyphenol oxidase	*Vitis vinifera*	DV182119	2e-39	BLASTx
VP278-5	Oxalic acid oxidase	*Brassica napus*	DQ336283	5e-35	BLASTx
VP472-5	Pathogenesis-related protein 10	*Vitis vinifera*	DQ336289	8e-79	BLASTx
VP141-5	Cytochrome P450	*Panax ginseng*	DV182125	2e-24	BLASTx
VP68-5	Cyclase family protein	*Arabidopsis thaliana*	DQ336281	2e-84	BLASTx
VP336-5	Putative receptor kinase	*Solanum demissum*	DQ336286	2e-05	BLASTx
VP303-5	Similar to serine/ threonine kinase 9	*Arabidopsis thaliana*	DT661618	1e-07	BLASTx
VP73-5	4-coumarate: coenzyme A ligase	*Nicotiana tabacum*	DV182129	8e-25	BLASTx
VP272-5	Heat shock protein 90	*Nicotiana tabacum*	DV182112	5e-111	BLASTx
VP344-5	Putative galactosyltransferase	*Oryza sativa*	DV182106	2e-36	BLASTx
VP445-5	Apiose/xylose synthase	*Arabidopsis thaliana*	DQ339463	1e-120	BLASTx
VP7-3	Metallothionein-like protein 1 (MT-1)	*Cicer arietinum*	DV182091	2e-08	BLASTx
VP300-5	Type 2 metallothionein	*Arachis hypogaea*	DV182094	3e-15	BLASTx

TABLE 3

The pair of primers of defence-related genes and reference gene for semi-quantitative RT-PCR.

Defence-related genes/ reference gene	Upstream primer	Downstream primer
GAPDH	5'-AACATTGTGCCAACATCCA-3'	5'-ACCCCATTCATTGTCATAC-3'
Chitinase	5'-AGTCAGCTCGGGGTTTCA-3'	5'-TGGAATCACAAATTTGTGTCT-3'
PAL	5'-CGGTTAAATCATGATTATTTCC-3'	5'-TCCAGAAACCAAATATAACCT-3'
PPO	5'-GATACGGATTGGCTTGACG-3'	5'-GGCCTTGGAACTTCTACTCTT-3'
PR10	5'-ATGGGTGTTTTCACTTACGA-3'	5'- TTAATAGGCATCAGGGTGTG-3'
OAO	5'-ATGTTTCTCCCAATTCTCTG-3'	5'-TCATAATGTCACCCTTCTTAAG-3'
4-CcoAL	5'-GTTCCAGTTGCATTCATTGT-3'	5'-ACATGAAACATGTTATACATC-3'
HSP90	5'-ACATCGAAACCCTAAATCTC-3'	5'-ATGAGATCCTTGAGTCGG-3'
MT	5'-AGGTGACTGATCATTTTCTC-3'	5'-TACACTGACCAAATACTAAGC-3'

cane leaf roll (38%) (Carson & Botha, 2000), various tissues and growth stages in rice (25%) (Yamamoto & Sasaki, 1997), maize leaf (20%) (Keith *et al*., 1993) and *Arabidopsis* (32%) (Newman *et al*., 1994). The analysis of ESTs in this study identified a range of genes likely to be involved in defence against pathogen attack, and provided some insight into the complex phenotype of powdery mildew resistance in Chinese wild *Vitis* species. By isolating cDNA from the leaf tissue of *V. pseudoreticulata* plant clone Baihe-35-1, challenged with *Uncinula necator*, this study aimed to enrich the cDNA library for the presence of defence-related sequences so that a number of ESTs involved in defence responses could be identified. The enrichment process appeared to have been successful due to the relatively high number of defence-related ESTs identified (5.6%) and the high level of redundancy of defence-related ESTs observed in this library. The number of ESTs from the *V. pseudoreticulata* plant clone Baihe-35-1 cDNA library that were tentatively identified as defence related was higher than that reported in a small library consisting of 250 sugarcane sequences. These were not enriched for defence-related transcripts and it possessed only one EST (0.4%) that was identified as defence related (Yamamoto & Sasaki, 1997). However, in a cDNA library consisting of 430 clones constructed from fruits, peels and carpels from Fuji apple (*Malus domestica* Borkh.), 34 defence/stress-related ESTs were identified, which contributed to 7.9% of the library (Sung *et al*., 1998). Some of these ESTs were for genes that encoded proteins similar to

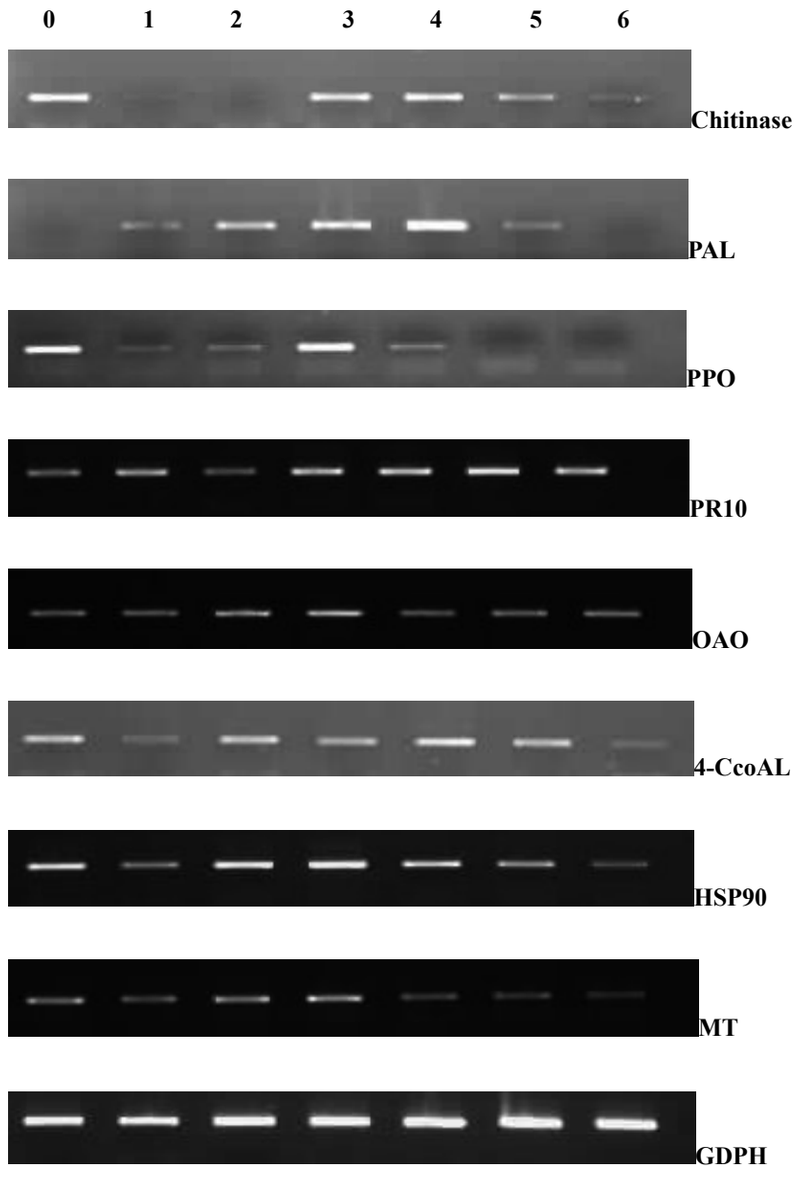

FIGURE 1

Expression patterns of chitinase, phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO), pathogenesis-related protein (PR10), oxalic acid oxidase (OAO), 4-Coumarate coenzyme ligase (4-CcoAL), heat shock protein (HSP90) and metallothionein (MT) in different periods of inoculation. glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a reference gene; 0: un-inoculated leaves; 1-6: leaves from one to six days after inoculation with *Uncicula necator*.

antioxidant enzymes, which Sung *et al*. (1998) suggested were needed to eliminate oxygen radicals to prevent the formation of the harmful lipid peroxide in the actively dividing cells and growing tissues of developing fruits. This indicated the importance of the type of tissue used to construct a cDNA library as a source of ESTs in order to identify ESTs related to disease response. In this study, the 17 unique potential defence-related ESTs represented genes coding for enzymes and proteins involved in different levels of defence, including recognition events between the plant and pathogen, signalling transduction and regulation, oxidative stress and the hypersensitive response, and specific defence-related/pathogenesis-related proteins. Further analysis of gene expression on mRNA level indicated that chitinase, PAL, PPO, PR10, OAO, 4-CcoAL, HSP90 and MT were involved in the resistant mechanism of powdery mildew (Fig. 1), and this result is consistent with previous studies on these genes in plant responses to abiotic and biotic stresses.

Two ESTs (VP422-5,VP472-5), representing pathogenesis-related protein genes, encoded respectively for class IV chitinase and PR10 from *Vitis vinifera,* as were the ESTs for disease resistance response protein. Chitinase (EC 3.2.1.14), which degrades chitin, a β-1,4-linked homopolymer of N-acetyl-D-glucosamine (GlcNAc), is widely distributed in many species of higher plants. It has been postulated that plant chitinases play an important role in the defence mechanism against pathogenic fungi that contain chitin in their cell walls (Nasser, 1990). EST

(VP472-5) shared similarity with sequences that encoded a PR10 from *Vitis vinifera*, The vast majority of proteins of the PR-10 family represent the products of genes that are expressed under influence of fungal and bacterial pathogens and show homology to pollen allergens from trees (Walter, 1996). There are a few reports that suggest that these proteins have ribonuclease function (Bantignies, 2000). It is supposed that PR10 proteins throughout the RNA degradation of pathogens might be involved in a defence mechanism in plants during pathogen attack.

Many end products of phenylpropanoid metabolism may also have direct antimicrobial effect, such as phytoalexins, resveratrol and flavonoids, or may serve to reinforce the cell wall, such as lignin. Three defence-related ESTs that encode for enzymes involved in the phenylpropanoid pathway (VP285-5 and VP73-5 separately showed strong similarity to PAL, 4-CcoAL) were identified from this cDNA library. The work of Langcake and Pryce (1977) on stilbenes showed that resveratrol was synthesised in grapevine in response to fungal attacks. These authors also considered as phytoalexins, all oxidation products of resveratrol as dimers, trimers and more highly polymerized oligomers of resveratrol, known as viniferins. It was shown that these phytoalexins were also produced in response to different biotic and abiotic stresses. Although these ESTs were expressed in resistant *V. pseudoreticulata* leaf tissue, further investigation is required to conclusively determine the function of this gene in Chinese wild *Vitis* plants (Jeandet *et al*., 2002).

Oxalic acid produced by this pathogenic fungus played an essential role in its pathogenic capabilities. During infection, the fungus produces high levels of a necrosis phytotoxin identified as oxalic acid. The role of oxalic acid in the pathogenicity process is still unclear. However, oxalic acid may have a number of functions in the infection process, including chelating calcium from the cell wall, thus making the pectic fraction more available to fungal hydrolases, and providing the acid pH needed for the maximum activity of the wall-degrading enzymes released by the pathogenic fungus (Keates *et al*., 1996). One part of the plant's defensive response to this may be the production of oxalate oxidase, which is an oxalic acid-degrading enzyme (ÇaliŞkan, 1998). A better understanding of oxalate biology might enable us to manipulate various aspects of these organisms.

Furthermore, the sequence of EST VP272-5 was very similar to genes coding for heat shock protein 90 (HSP90). Heat shock protein ESTs were also abundant in the Cgm-Malva interaction, where the mRNA was extracted at the transition between biotrophy and necrotrophy (Goodwina *et al*., 2004). Heat shock proteins in the hsp70 and hsp90 families have been found to be related to host resistance and are believed to be involved in signal transduction for plant defence responses (Kanzaki *et al*., 2003).

The sequences of VP7-3 and VP300-5 aligned strongly with the genes encoding metallothionein-like (MT) proteins from plants. Inducible responses to heavy metals have been found for some plant MT-like genes, including two isoforms of MT-like genes in *A. thaliana* (Zhou & Goldsborough, 1994) and an MT-like gene in *Triticum aestivum* L. (Snowden & Gardner, 1993). Whereas the sequestration of metal ions released due to macromolecular degradation during senescence and hypersensitive responses in incompatible reactions (with pathogens) is a viable function for MT-like genes in plants, it may be more likely that metallothioneins are synthesised as protectants against the effects of oxidative damage. It has been proposed that MTs in animal tissues have a role in protection against the effects of reactive oxygen species by acting as antioxidants, since MTs are potent scavengers of hydroxyl radicals (Muira *et al*., 1997).

CONCLUSIONS

Our results show that the sequencing of ESTs from the *V. pseudoreticulata* plant (clone Baihe-35-1) library gives a global view of gene activities in the plant. The analysis of ESTs in this study identified a range of genes likely to be involved in defence against pathogen attack, and provided some insight into the complex phenotype of powdery mildew resistance in Chinese wild *Vitis* species. A number of sequences were identified as strong candidates for expression in *V. pseudoreticulata* as a defence response to *Uncinula necator* infection. Perhaps the most important group of ESTs identified in this study are those of unknown function. The ESTs making up this category may include previously uncharacterised defence and stress-related genes, which may be identified as the sequence databases continue to grow and by future microarray expression studies using the *V. pseudoreticulata* ESTs. On the other hand, ESTs are also useful as molecular markers for the construction of high-density genetic linkage maps. This is the first report of the analysis of ESTs from *V. pseudoreticulata* in a cDNA library and may have significant application for future studies on gene expression, mapping and genetic manipulation in *V. pseudoreticulata*.

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