Front. Agric. China 2010, 4(1): 10–17
DOI 10.1007/s11703-010-0094-y

RESEARCH ARTICLE

Jinhua WU, Zhiying MA, Yingang HU, Hong ZHANG, Changyou WANG, Qiuying WANG, Wanquan JI

Expression of special genes inhibited by powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat germplasm N9436

Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most important fungal diseases in common wheat (*Triticum aestivum* L.) worldwide. Wheat germplasm N9436 is resistant to powdery mildew. In the present study, a backward subtracted cDNA library was constructed with cDNA from N9436 leaves inoculated by *Blumeria graminis* as the driver and cDNA from uninoculated N9436 leaves as the tester. A total of 120 positive clones were randomly chosen from the SSH-cDNA library and were amplified with sp6 and t7 primers to examine the insert size. After screening the repeated and redundant sequences, 59 expressed sequence tags (EST) were acquired. Nucleic acid and protein homology search were performed using the basic local alignment search tool (BLAST) program with the default settings at NCBI website (http://www.ncbi.nlm.nih.gov). BlastX results in nr-protein database revealed that 23 ESTs were highly homologous with known proteins involved in primary metabolism, energy metabolism, transport, signal transduction, and disease resistance and defenses. BlastNr results showed that 47 and 10 ESTs had high identities with known Unigene and function-unknown ESTs, respectively, and two ESTs matched none in the nr-database. Twenty-one ESTs were both in the nucleic acid and protein databases, including seven ESTs associated with powdery mildew resistance. Among them, one was responsible for signal transduction and six for systemic acquired resistance (SAR) system.

Keywords wheat, powdery mildew, suppression subtraction hybridization (SSH), expressed sequence tags (EST)

Received September 7, 2009; accepted October 9, 2009

Jinhua WU, Zhiying MA
College of Agronomy, Agricultural University of Hebei, Baoding 071001, China

Jinhua WU, Yingang HU, Hong ZHANG, Changyou WANG, Qiuying WANG, Wanquan JI (✉)
College of Agronomy, Northwest A&F University, Yangling 712100, China
E-mail: jiwanquan2003@126.com

1 Introduction

Wheat (*Triticum aestivum* L.) is the most widely cultivated and important food crop in the world. It is a stable food crop for about 35% of the human population (Huang and Röder, 2004). Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*syn. Erysiphe graminis* f. sp. *tritici*), is one of the important fungal diseases in many regions in the world and produces significant annual yield losses, especially in China (McIntosh and Brown, 1997). China is the largest epidemic region in the world, and severe losses in grain yield have been reported in any epidemic year. Research on the mechanism of disease resistance is the most effective, economical, and environmentally safe approach to control plant diseases (Liao et al., 2007). There are two methods to improve the disease resistance of the varieties. One is utilization of new resistant genes, while the other is inducing the expression of defense genes. The defense genes are the main factors for disease resistance in plants, although the resistance genes decide whether the plant resistance system is open or not (Luo et al., 2002a). It is valuable to study the mechanism of system acquired resistance (SAR) because SAR is a lasting and broad-spectrum resistance.

Luo et al. (2002b) obtained 65 expressed sequence tags (ESTs) in wheat by constructing a suppression subtraction hybridization (SSH) library at early inoculation. The signal transduction systems of salicylic acid (SA), mitogen-activating protein (MAP), and phenylpropanoid metabolism were observed to be involved in the powdery mildew resistance of resistant wheat. He and Wang (2005) cloned *WRP1* and *RPW2* genes found in the *T. aestivum* X *Agropyron elongatum* alien substitution line "Shannong 551" inoculated with *E. graminis* by using the techniques of cDNA representational difference analysis (RDA) and rapid amplification of cDNA ends (RACE). Niu et al. (2001) constructed two cDNA libraries, with or without inoculation with *E. graminis* for 48h in the 6VS/6AL translocation line between *T. aestivum* and *Haynaldia villosa* and primarily disclosed their differential

expression. SSH is an effective method to offer the theoretical foundation for separation and cloning candidate genes that express differently in the cDNA library (Diatchenko et al., 1996). Using SSH and other approaches, such as in silico cloning (Chen et al., 2006) and microarray (Wong et al., 2007), a full-length cDNA encoding wheat peroxisomal ascorbate peroxidase (pAPX) was cloned. There were 126 salinity tolerant cDNAs identified and isolated from the roots of *Bruguiera cylindrica* (L.) Blume (Ouyang et al., 2007). Genes associated with salt stress and albino (Lin et al., 2006) were identified in tomato (*Lycopersicon esculentum* Mill.) and bamboo (*Bambusa edulis* Murno), respectively.

A few studies have been carried out to isolate genes found in grain plants related to powdery mildew. Luo et al. (2002), using wheat, constructed the mixed cDNA library 24 h, 48 h, and 72 h after inoculation with *E. graminis* in order to isolate genes related to starting and sustainable effect of SAR, especially the genes involved in signal transduction. Using barley (*Hordeum vulgare*) and gene chip technology, Cao et al. (2006) selected resistant genes by mixing *H. villosa* RNA extracted after inoculation with *E. graminis*.

This research aims to study, by using the SSH method, the molecular mechanism of the resistance to powdery mildew as expressed in wheat resistant germplasm. We also seek to disclose the molecular mechanism of the interaction between wheat and powdery mildew.

2 Materials and methods

2.1 Materials

Wheat germplasm N9436 was provided by the College of Agronomy, Northwest A&F University. It had multiple spikelets and high resistance to powdery mildew. The pathogen race of powdery mildew was Guanzhong No. 4 of *Blumeria graminis* f. sp. *tritici*, which is the dominant pathogenic race in the Guanzhong region of Shaanxi Province, China.

The wheat seedlings were planted in a greenhouse for about three weeks. Then, the seedlings were divided into two groups. One group, the control group, was inoculated with spores of Guanzhong No. 4 and was identified for SSH analysis as the 'driver'. The other group was not inoculated and this group was known as the 'tester'. Wheat leaves were inoculated for 72 h.

2.2 Construction of SSH-cDNA and bioinformation analysis

Following the prescribed method, total RNA was extracted and then reversely transcribed into cDNA. The SSH was performed with inoculated-leaves as the driver and noninoculated leaves as the tester using the specification of PCR Select cDNA Subtraction Kit (Clontech, USA) (Wu et al., 2008; Yu et al., 2008).

ESTs obtained were analyzed using nonredundant protein (BLASTX) and nucleic acid (BLASTN) databases in the NCBI website (http://www.ncbi.nlm.nih.gov). The evaluation rule of significant sequences was E-Value less than 1×10^{-5} and identities more than 40% (Li et al., 2004). Then, the sequences qualified were classified according to the function (Bevans et al., 1998).

3 Results

3.1 Construction of SSH library

The total extracted RNA was detected by a nucleic acid and protein detector. It displayed values of A_{260}/A_{280} between 1.8 and 2.0 and that A_{260}/A_{230} was greater than or equal to 2.0. The double-strand cDNA from reverse transcription showed a disperse band, ranging from 200 bp to 2000 bp. The library was shown to be qualified for SSH.

3.2 Analysis of ESTs

There were 120 positive clones selected randomly and then sequenced, and 59 ESTs were obtained with nonrepeated sequences. They were submitted to GenBank (accession numbers from EX567298 to EX567356 and dbEST-Id from 50073262 to 50073320).

3.2.1 BLASTX

There were 23 ESTs that had homology to the known proteins with a percentage of 39% (Table 1). These proteins were involved in primary metabolism (9%), energy metabolism (44%), transcription (4%), transport (9%), signal transduction (4%), and disease and defense (26%). In addition, unknown ESTs occupied 4% (Fig. 1).

Energy metabolism had the largest proportion (44%) including chlorophyll a/b-binding protein (F189) and ribulose-1,5-bisphosphate carboxylase/oxygenase (F2, F50-2, F113, F125-1, F138). Rubisco is the key enzyme of the Calvin-Benson-Basham cycle, catalyzing the first step, in which CO_2 is reductively assimilated into organic carbon. Rubisco is also a bifunctional enzyme that controls the reduction of CO_2 and the oxygenolysis of ribulose-1,5-bisphophate. Since Rubisco is responsible for the overwhelming amount of carbon fixation by plants, nearly all primary production is linked to the function of this enzyme. Besides, Rubisco is a well-studied enzyme because of its extensive agricultural and environmental significance (Selesi et al., 2005). Ferredoxin-NADP(H) oxidoreductase (F84) can catalyze the electron transfer between NADP(H) and the proteins ferredoxin or flavodoxin (Thomas et al., 2006; Nascimento et al., 2007). Phosphoribulokinase (F105) is found in most

Table 1 Result of BlastX sequence homology analysis to nr-protein database

clone	GenBank Acc No.	*E*-value	identity/%	source	putative gene
energy					
F2	EX567299	2e-37	100%	*T. aestivum*	ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit
F9	EX567314	3e-11	100%	*T. aestivum*	putative oxygen-evolving complex precursor
F50-2	EX567302	3e-13	100%	*T. aestivum*	ribulose bisphosphate carboxylase small clone
F81-1	EX567343	9e-49	98%	*H. vulgare*	carbonic anhydrase, chloroplast precursor
F84	EX567328	1e-16	93%	*T. aestivum*	ferredoxin-NADP(H) oxidoreductase
F105	EX567308	5e-20	100%	*T. aestivum*	phosphoribulokinase (PRK)
F113	EX567316	6e-40	98%	*T. aestivum*	ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit
F125-1	EX567326	3e-13	100%	*T. aestivum*	ribulose bisphosphate carboxylase small chain
F138	EX567303	5e-04	100%	*T. aestivum*	ribulosebisphosphate carboxylase small subunit
F189	EX567345	1e-09	85%	*O. sativa*	chloroplast chlorophyll a-b binding protein
signal transduction					
F188	EX567310	5e-05	69%	*P. sativum*	thioredoxin
transporter					
F23	EX567311	2e-15	100%	*H. vulgare*	chloroplast inner envelope protein, putative
F136-1	EX567305	2e-32	98%	*T. monococcum*	serine hydroxymethyltransferase
metabolism					
F7	EX567317	6e-15	73%	*A. thaliana*	putative sterol C4- methyl oxidase
F136-2	EX567312	7e-20	100%	*A. thaliana*	serine-type endopeptidase/serine-type peptidase/ trypsin
disease and defense					
F76	EX567338	2e-17	100%	*T. aestivum*	stress responsive protein
F110-1	EX567331	1e-73	97%	*T. aestivum*	glyoxalase
F125-2	EX567342	5e-09	78%	*O. sativa*	beta-1, 3-glucanase
F135	EX567336	2e-17	100%	*T. aestivum*	non-specific lipid transfer protein 1 precursor (LTP1)
F146-2	EX567337	2e-22	60%	*H. vulgare*	lipoxygenase 2 (lox2:Hv:2 gene)
F167	EX567321	1e-19	94%	*A. thaliana*	hydroxypyruvate reductase
transcription					
F48	EX567353	1e-15	100%	*Z. officinale*	putative reverse transcriptase
unclear classification					
F87	EX567339	2e-15	60%	*O. sativa*	hypothetical protein

phototrophic organisms. Next to Rubisco, it is one of the key enzymes of the reductive pentose phosphate pathway as it catalyzes the only reaction by which the Calvin cycle intermediates can be provided for further CO_2 fixation (Michels et al., 2005; Runquist and Miziorko, 2006).

Of the total ESTs, disease and defense translated proteins accounted for a large proportion (26%). These proteins included stress protein (F76) and pathogenesis-related proteins, such as β-1,3 glucanases (F125-2), nonspecified transfer protein 1 precursor (F135), glyoxalase (F110-1), and lipoxygenase (F146-2). Large expression of nonspecific lipid-transfer protein was induced by the invasion of pathogen. The growth of the pathogen was inhibited at different degrees by lipid transfer protein. Van Loon and van Strien (1999) designated this lipid transfer protein, such as F135 (Table 1), as "pathogen-related protein 14."

Lipoxygenases were proposed to be a possible factor responsible for the pathology of certain diseases (Ichiro et al., 2006). The glyoxalase system was proposed to be involved in various functions that include regulation of cell division and proliferation, microtubule assembly, and protection against oxoaldehyde toxicity. Glyoxalase enzymes are important for the glutathione (GSH)-based detoxification of methylglyoxal. This reaction is catalyzed by glyoxalase I and glyoxalase II (Singla-Pareek, 2003).

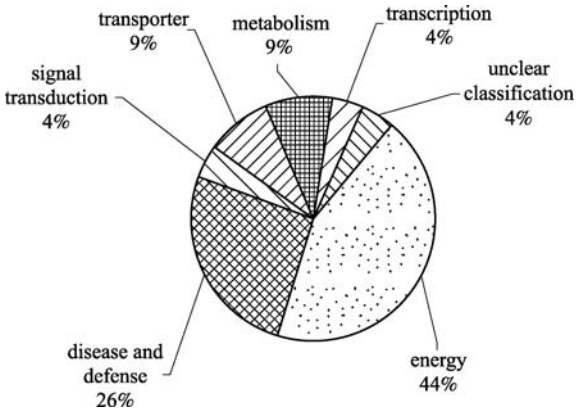

Fig. 1 The functional category and the ratio of ESTs based on the results of BlastX

3.2.2 BLASTNr

There were 47 ESTs with high homology to the Unigene and 12 ESTs had no homologous sequences in nonhuman and nonmouse EST database (Table 2). Of the total, unknown proteins accounted for 20%.

3.2.3 Comparison of BLASTX and BLASTNr

Among all ESTs, there were 21 ESTs with uniform function in different databases. Twenty-six ESTs were highly homologous with known nucleotides after BLASTNr, including glyoxysomal malate dehydrogenase (F71-1), putative thiamine biosynthesis protein (F12), triose phosphate translocator (F163-1), calmodulin binding (F75-1), putative ribosomal S1 protein (F15), and catalase (F134).

3.2.4 Expression of genes inhibited by powdery mildew

There were seven related proteins of powdery mildew including one protein of signal transduction, five SAR PR proteins, one SAR defense protein, and one protein of cellular defense (Table 3).

Among all ESTs, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit had the highest expression, followed by putative thiamine biosynthesis protein, ribulose-bisphosphate carboxylase, SMO1-2 (Sterol C4-Methyl Oxidase), phosphoribulokinase, plasma membrane H^+-ATPase (ha1), ferredoxin-NADP(H) oxidoreductase, electron carrier/electron transporter/iron ion binding, SHM1 (Serine hydroxymethyltransferase 1), transporter, lipid transfer protein precursor, serine-type endopeptidase, small subunit, carbonic anhydrase, etc. (Fig. 2).

One to twenty represent 1 ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit, 2 putative thiamine biosynthesis protein, 3 ribulose-bisphosphate carboxylase, 4 SMO1-2 (Sterol C4-Methyl Oxidase), 5 phospho-ribulokinase, 6 plasma membrane H^+-ATPase (ha1), 7 ferredoxin-NADP(H) oxidoreductase, 8 electron carrier/electron transporter/iron ion binding, 9 SHM1 (Serine hydroxymethyltransferase 1), 10 transporter, 11 lipid transfer protein precursor, 12 serine-type endopeptidase, 13 small subunit, 14 carbonic anhydrase, 15 catalytic protein, 16 caspase/cysteine-type endopeptidase, 17 putative phosphoenolpyruvate carboxylase kinase, 18 amino acid binding protein, 19 DNA binding, and 20 others.

4 Discussion

In the SSH-cDNA backward library, 72 h after inoculation, it was found that large quantities of proteins were expressed, such as ribulose-1,5-bisphosphate carboxylase/oxygenase, β-1,3-glucanase, non-specific lipid transfer protein 1 precursor, glyoxalase, lipoxygenase, hydroxypyruvate reductase, stress responsive protein, and thioredoxin.

Yu et al. (2007) reported the high expression of Rubico in wheat when induced by *Puccinia striiformis*. Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO; EC4.1.1.39) is a key enzyme in the Calvin-Benson-Bassham (CBB) cycle (Yoshizawa et al., 2004), which is important in the assimilation of CO_2 and modification of amino acids at the gene or protein level. Furthermore, I Rubisco is probably associated with oxygen stress.

The genes for disease resistance and defense accounted for a high percentage. Our results show that there was accumulation of several pathogenisis-related proteins (PRs) in the wheat resistant to the powdery mildew. The defense response was completely induced owing to the accumulation of several PRs, and accordingly, the pathogen was inhibited in growth and extension.

Beta-1, 3-glucosidase can catalyze the hydrolysis of β-1,3 and β-1,6-glucan, which is involved in cell division, seed germination, bud dormancy, flowers formation, and fruit maturation. One full length cDNA of β-1, 3-glucosidase was cloned from a cotton cultivar "Hai7124" (Gao et al., 2007). The coding region of β-1,3-glucosidase was cloned from wheat induced by stripe rust (Liu et al., 2007). Lipid transfer protein plays a role not only in lipid transfer and keratogenesis but also in the plant defense response (Gomes et al., 2003; Maldonado et al., 2003).

From the above, we conclude that the *B. graminis* f. sp. *tritici* inhibits the expression of several wheat leaf proteins after 72 h inoculation. Moreover, the degree of effect from B. graminis f. sp. *tritici* may be due to inoculation duration and concentration. It still needs further study.

There are similar mechanisms for biotic and abiotic stresses. It was found in our research that there is the same molecular mechanism operating between biotic and abiotic stresses. Wang et al. (2007) observed that lipid transfer protein was expressed in *Tamarix androssowii* under drought stress. Liu et al. (1999) revealed that lipid transfer

Table 2 Result for homological alignment with function genes in nr-nuclear database

clone	GenBank Accn No.	*E*-value	identity/%	source	putative gene
energy					
F2	EX567299	2e-108	99	*T. aestivum*	ribulose-1, 5-bisphosphate carboxylase/ oxygenase large subunit
F9	EX567314	3e-46	100	*T. aestivum*	ribulose-1, 5-bisphosphate carboxylase/ oxygenase large subunit
F12	EX567298	9e-91	100	*O. sativa*	putative thiamine biosynthesis protein
F50-2	EX567302	4e-114	98	*A. thaliana*	ribulose-bisphosphate carboxylase
F81-1	EX567343	5e-174	100	*A. thaliana*	carbonic anhydrase
F84	EX567328	1e-94	95	*T. aestivum*	ferredoxin-NADP(H) oxidoreductase
F105	EX567308	5e-113	100	*T. aestivum*	phosphoribulokinase
F111	EX567325	1e-45	100	*A. thaliana*	ribulose-bisphosphate carboxylase
F113	EX567316	2e-121	99	*T. aestivum*	ribulose-1, 5-bisphosphate carboxylase large subunit
F125-1	EX567326	3e-41	97	*A. thaliana*	ribulose-bisphosphate carboxylase
F138	EX567303	2e-97	100	*T. aestivum*	small subunit
F146-1	EX567327	4e-56	100	*T. aestivum*	ferredoxin-NADP(H) oxidoreductase
F85-2	EX567324	7e-48	100	*T. aestivum*	phosphoribulokinase
F167	EX567321	9e-74	98	*A. thaliana*	oxidoreductase, acting on the CH-OH group of donors, NAD or NADP as acceptor
F170-2	EX567346	1e-44	100	*A. thaliana*	CA2 (carbonic anhydrase 2) carbonate dehydratase/zinc ion binding
F189	EX567345	9e-74	100	*A. thaliana*	chlorophyll binding
metabolism					
F6	EX567335	5e-92	99	*A. thaliana*	catalytic
F7	EX567317	4e-114	99	*A. thaliana*	SMO1-2(sterol C4-methyl oxidase)
F71-1	EX567352	8e-85	99	*T. aestivum*	Glyoxysomal malate dehydrogenase
F120	EX567304	6e-138	100	*A. thaliana*	SMO1-2 (sterol C4-methyl oxidase)
F136-2	EX567312	3e-38	92	*A. thaliana*	serine-type endopeptidase/ serine-type peptidase/trypsin
F137	EX567322	3e-68	100	*A. thaliana*	LOL3 (LSD ONE LIKE 3); caspase/ cysteine-type endopeptidase
F153	EX567356	7e-80	100	*A. thaliana*	catalytic/ hydrolase
signal transduction					
F75-1	EX567330	4e-88	99	*A. thaliana*	calmodulin binding
F143	EX567341	4e-119	100	*A. thaliana*	electron carrier/electron transporter/ iron ion binding
F162	EX567320	1e-72	98	*O. sativa*	putative phosphoenolpyruvate carboxylase kinase
F188	EX567310	6e-65	99	*A. thaliana*	thiol-disulfide exchange intermediate
transporter					
F3-2	EX567301	7e-74	100	*T. aestivum*	plasma membrane H^+-ATPase (ha1)
F58	EX567313	6e-45	96	*A. thaliana*	transporter
F136-1	EX567305	4e-109	99	*A. thaliana*	SHM1 (Serine hydroxymethyltransferase 1)
F163-1	EX567344	4e-45	99	*T. aestivum*	triose phosphate translocator (tpt1)
transcription					
F45-1	EX567319	9e-100	100	*A. thaliana*	amino acid binding protein
F48	EX567353	3e-136	99	*A. thaliana*	ATPHB3 (PROHIBITIN 3)
F157-1	EX567300	3e-53	98	*A. thaliana*	DNA binding
protein synthesis, destination and storage					

(*Continued*)

clone	GenBank Accn No.	*E*-value	identity/%	source	putative gene
F15	EX567334	2e-44	98	*O. sativa*	putative ribosomal S1 protein (with alternative splicing)
disease and defense					
F71-2	EX567351	1e-72	100	*T. aestivum*	chalcone synthase (CHS)
F76	EX567338	7e-59	100	*T. aestivum*	stress responsive protein
F81-2	EX567340	2e-54	100	*T. aestivum*	biostress-resistance-related protein
F134	EX567329	8e-49	94	*T. aestivum*	catalase (wcat1)
F110-1	EX567331	0	99	*T. aestivum*	glyoxalase
F125-2	EX567342	1e-42	94	*A. thaliana*	glucan 1,3-beta-glucosidase
F126-2	EX567333	3e-47	100	*T. aestivum*	lipid transfer protein precursor (LTP2)
F135	EX567336	4e-93	100	*T. aestivum*	lipid transfer protein precursor (LTP1)
F140	EX567332	2e-58	100	*T. aestivum*	cold acclimation protein
F146-2	EX567337	2e-143	98	*A. thaliana*	lipoxygenase
F172-1	EX567354	7e-66	91	*A. thaliana*	lipid transfer protein
unclear classification					
F155-1	EX567315	6e-83	90	*O. sativa*	unnamed protein product
unknown proteins					
F69-1	EX567306				
F42	EX567307				
F4-1	EX567309				
F23	EX567311				
F119	EX567323				
F87	EX567339				
F110-2	EX567347				
F179	EX567348				
F175	EX567349				
F112	EX567355				
F178	EX567318				
F172-2	EX567350				

Table 3 Resistance-related genes to powdery mildew

the kind of gene	the name of gene	the number of gene
signal transduction	thioredoxin	1
pathogenesis-related protein of SAR	beta-1,3-glucanase	1
	non-specific lipid transfer protein 1 precursor	1
	glyoxalase	1
	lipoxygenase 2	1
	hydroxypyruvate reductase	1
defense protein of SAR	stress responsive protein	1

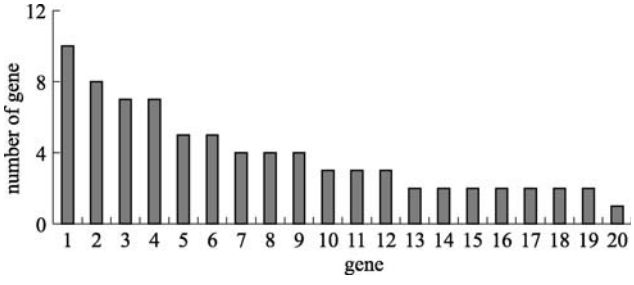

Fig. 2 Abundance of different genes induced by pathogen inoculation

protein from barley (*H. vulgare* L.) and maize (*Zea mays* L.) could inhibit the growth of several kinds of pathogens, and lipid transfer protein from barley and tomato (*Lycopersicon esculentum* Mill.) could be induced by controlling certain environmental factors, such as low or high temperature and drought. Plant resistance to powdery mildew is not due to a single entity but rather is a complex and systematic process, including physiological and

biochemical activities. It reflects the overall harmony of life's responses and reactions. The common mechanisms for both abiotic stresses and resistant reactions provide the basis for exploring and using a widely adaptable resistance system.

Acknowledgements This work was supported by the National S&T Support Plan "Wheat Breeding" (No. 2006BAD02A08), Renewal and Utilization of Germplasm Resources Propagation in Wheat (No. NB07-2130135-(25-30-0301)) and Key Special Project of Shaanxi Province "Research on the Molecular Breeding Technology of Wheat Resistance Genes" (No. 2006kz05-G3), China.

References

Bevans M, Bancroft I, Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W, Van Staveren M, Stiekema W, Drost L, Ridley P, Hudson S A, Patel K, Murphy G, Piffanelli P, Wedler H, Wedler E, Wambutt R, Weitzenegger T, Pohl T M, Terryn N, Gielen J, Villarroel R, De Clerck R, Van Montagu M, Lecharny A, Auborg S, Gy L, Kreis M, Lao N, Kavanagh T, Hempel S, Kotter P, Entian K D, Rieger M, Schaeffer M, Funk B, Mueller-Auer S, Silvey M, James R, Montfort A, Pons A, Puigdomenech P, Douka A, Voukelatou E, Milioni D, Hatzopoulos P, Piravandi, Obermaier B, Hilbert H, Düsterhöft, Moores T, Jones J D G, Eneva T, Palme K, Benes V, Rechman S, Ansorge W, Cooke R, Berger C, Delseny M, Voet M, Volckaert G, Mewes H W, Klosterman S, Schueller C, Chalwatzis N (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature, 391(66): 485–488

Cao A Z, Li Q, Chen Y P, Zou X W, Wang X E, Chen P D (2006). Screening resistance-related gene to powdery mildew in *Haynaldia villosa* using barly genechip and studying its mechanism of resistance. Acta Agron Sin, 32(10): 1444–1452 (in Chinese)

Chen Y P, Wang H Z, Wang X E, Cao A Z, Chen P D (2006). Cloning and expression of peroxisomal ascorbate peroxidase gene from wheat. Mol Biol Rep, 33: 207–213

Diatchenko L, Lauy F C, Campbell A P, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov E D, Siebert P D (1996). Suppression subtractive hybridization: A method for generating differentially regulated or tissue specific cDNA probes and libraries. Proceedings of National Academy Sciences of the USA, 93(12): 6025–6030

Gao Y L, Guo W Z, Wang L, Zhang T Z (2007). Cloning and characterization of one-1, 3-glucanase gene cDNA in cotton (*Gossypium barbadense* L.). Acta Agron Sin, 33(8): 1310–1315 (in Chinese)

Gomes E, Sagot E, Gaillard C, Laquitaine L, Poinssot B, Sane jouand Y H, Delrot S, Coutos-Thevenot P (2003). Nonspecific lipid-transfer protein genes expression in grape (*Vitis* sp.) cells in response to fungal elicitor treatments. Mol Plant Microbe Interact, 16(5): 456–464

He D Y, Wang H G (2005). Cloning of a new phosphoserine aminotransferase in the substitution resistant to powdery mildew of wheat-*Agropyron elongatum*. Chin Sci Bull, 50(6): 535–539 (in Chinese)

Huang X Q, Röder M S (2004). Molecular mapping of powdery resistance genes in wheat: a review. Euphytica, 137: 203–223

Ichiro K, Kazunori T, Tokuko T, Makiko K (2006). Radical scavenger can scavenge lipid allyl radicals complexed with lipoxygenase at lower oxygen content. Biochem J, 395: 303–309

Li R, Rimmer R, Buchwaldt L, Sharpe A G, Séguin-Swartz G, Coutu C, Hegedus D D (2004). Interaction of *Sclerotinia sclerotiorum* with a resistant *Brassica napus* cultivar: expressed sequence tag analysis identifies genes associated with fungal pathogenesis. Fungal Genet Biol, 41(8): 735–753

Liao Y, Zhang Z Y, Du L P, Xu H J, Yao W L, Shi J Y, Ren Z L, Xin Z Y (2007). Isolation of *RAR1*gene from *Thinopyrum intermedium* and analysis of its function in wheat background. Sci Agric Sin, 40(8): 1667–1674 (in Chinese)

Lin C S, Lai Y H, Sun C W, Liu N T, Tsay H S, Chang W C, Chen J J W (2006). Identification of ESTs differentially expressed in green and albino mutant bamboo (*Bambusa edulis*) by suppressive subtractive hybridization (SSH) and microarray analysis. Plant Cell Tiss Organ Cult, 86: 169–175

Liu B, Cui S P, Wang X J, Huang L L, Kang Z S (2007). Cloning and prodaryotic expression of a wheat β-1,3-glucanase gene induced by *Puccinia striiformis*Westend f. sp. *tritici* Eriks in *E. coli*. J Northwest A&F Univ (Nat Sci Edn), 35(6): 125–129 (in Chinese)

Liu X F, Xu J Z, Hou Y Q, Zhan S X, Ge X C, Chao K M (1999). Isolation and characterization of a lipid transfer protein gene from rice. Acta Bot Sin, 41(7): 736–740 (in Chinese)

Luo M, Kong X Y, Huo N X, Zhou R H, Jia J Z (2002a). ESTs analysis of resistance to powdery mildew in wheat at primary infected stage. Acta Genet Sin, 29(6): 525–530 (in Chinese)

Luo M, Kong X Y, Huo N X, Zhou R H, Jia J Z (2002b). Analysis of gene resistant to powdery mildew by suppression subtractive hybridization. Chin Sci Bull, 47(16): 1237–1241 (in Chinese)

Maldonado A M, Doerner P, Dixon R A, Lamb C J, Cameron R K (2002). A putative lipid transfer protein involved in systemic resistance signaling in *Arabidopsis*. Nature, 419(6905): 399–403

McIntosh R A, Brown G N (1997). Anticipatory breeding for resistance to rust diseases in wheat. Annu Rev Phytopathol, 35: 311–326

Michels A K, Wedel N, Kroth P G (2005). Diatom plastids possess a phosphoribulokinase with an altered regulation and no oxidative pentose phosphate pathway. Plant Physiology, 137: 911–920

Nascimento A S, Catalano-Dupuy D L, Bernardes A, Oliveira Neto M, Santos M A M, Ceccarelli E A, Polikarpov I (2007). Crystal structures of *Leptospira interrogans* FAD-containing ferredoxin-$NADP^+$ reductase and its complex with $NADP^+$. BMC Structural Biology, 7: 69

Niu J S, Yu L, Chen P D, Liu D J (2001). cDNA library construction and identification of *Triticum aestivum-Haynaldia villosa* 6VS/6AL translocation line. J Nanjing Agric Univ, 24(1): 5–8 (in Chinese)

Ouyang B, Yang T, Li H, Zhang L, Zhang Y, Zhang J, Fei J, Ye Z (2007). Identification of early salt stress response genes in tomato root by suppression subtractive hybridization and microarray analysis. J Exp Bot, 58: 507–520

Runquist J A, Miziorko H M (2006). Functional contribution of a conserved, mobile loop histidine of phosphoribulokinase. Protein Science, 15: 837–842

Selesi D, Schmid M, Hartmann A (2005). Diversity of Green-Like and Red-Like Ribulose-1, 5-bisphosphate carboxylase/oxygenase large-

subunit genes (*cbbL*) in differently managed agricultural soils. Applied and Environmental Microbiology, 71: 175–184

Singla-Pareek S L, Reddy M K, Sopory S K (2003). Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. PNAS, 100(25): 14672–14677

Thomas J C, Ughy B, Lagoutte B, Ajlani G (2006). A second isoform of the ferredoxin: NADP oxidoreductase generated by an in-frame initiation of translation. PANS, 103(48): 18368–18373

Van Loon L C, van Strien E A (1999). The families of pathogenesis-related proteins, their activities and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol, 55: 85–97

Wang Y C, Li H Y, Yang C P, Zhang G D (2007). Study on expression of genes in *Tamarix*and *rossowii* under drought stress using cDNA microarray technology. Bull Bot Res, 27(2): 186–194 (in Chinese)

Wong Y Y, Ho C L, Nguyen P D, Teo S S, Harikrishna J A, Rahim R A, Wong M C V L (2007). Isolation of salinity tolerant genes from the mangrove plant, *Bruguiera cylindrica* by using suppression subtractive hybridization (SSH) and bacterial functional screening. Aquatic Bot, 86(2): 117–122

Wu J H, Hu Y G, Zhang H, Wang C Y, Wang Q Y, Ji W Q (2008). Expression of Special Genes Resistant to Powdery Mildew (*Blumeria graminis* f. sp. *tritici*) in Wheat Germplasm N9436. Acta Agron Sin, 34(7): 1143–1152 (in Chinese)

Yoshizawa Y, Toyoda K, Arai H, Ishii M, Igarashi Y (2004). CO_2-responsive expression and gene organization of three ribulose-1, 5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in *Hydrogenovibrio marinus* Strain MH-110. Journal of Bacteriology, 186(17): 5685–5691.

Yu X D, Qu Z P, Guo J, Yu X M, Huang X L, Han Q M, Huang L L, Kang Z S (2008). Construction and preliminary analysis of the compatible SSH cDNA library between *Puccinia striiformis* and wheat. Sci Agric Sin, 41(5): 1267–1273

Yu X M, Yu X D, Qu Z P, Han Q M, Guo J, Huang L L, Kang Z S (2007). Construction of wheat SSH cDNA library induced by *Puccinia striiformis* and analysis of expressed sequence tags. Acta Phytopathol Sin, 37(1): 50–55 (in Chinese)