Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Analysis of gene expression profiles in response to *Sporisorium reilianum* f. sp. zeae in maize (*Zea mays* L.)

Tao Yu, Zhenhua Wang *, Xiaochun Jin, Xianjun Liu, Shuaishuai Kan

Maize Research Institute, Northeast Agricultural University, Mucai Street 59, Xiangfang District, Harbin, Heilongjiang 150030, China

ARTICLE INFO

ABSTRACT

Background: Head smut of maize, which is caused by *Sporisorium reilianum* f. sp. *zeae* (Kühn), is a serious disease in maize. In order to reveal the molecular mechanism of the resistance to head smut in maize, a microarray containing ~14,850 probes was used to monitor the gene expression profiles between a disease resistant near isogenic line (NIL) and a highly susceptible inbred line after *S. reilianum* was injected with an artificial inoculation method.
Results: Levels of expression for 3,532 genes accounting for 23.8% of the total probes changed after inoculation. Gene Ontology analysis revealed that the differentially expressed genes participated in physiological and biochemical pathways. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that plant–pathogen interaction, natural killer cell mediated cytotoxicity and benzoxazinoid biosynthesis pathways play important roles in resistance to head smut. Three head smut resistance-related candidate genes, CLAVATA1, bassinosteroid insensitive 1-associated receptor kinase 1 and LOC100217307 with leucine-rich repeat (LRR) conserved domains were identified, each of which is in maize mapping bin 2.09, a region previously shown to include a major QTL for head smut resistance. Furthermore, LOC100217307 was validated by quantitative real-time (qRT)-PCR inferring that this gene may be involved in the resistance to head smut of maize.
Conclusions: This study provided valuable information for cloning, functional analysis and marker assisted breeding of head smut resistance genes.

1. Introduction

Head smut of maize (*Zea mays* L.), caused by *Sporisorium reilianum* f. sp. *zeae* (Kühn), is a disease that occurs in many regions of North America, Australia, Asia, and southern Europe [1,2]. Since the 1970s, it has also become an important constraint in both seed and commercial maize production in temperate maize-growing areas of China [3]. Yield losses attributed to *S. reilianum* are estimated to be as high as 80% [3,4].

Head smut has become a major topic of study because of the increasing spread of the disease in recent years. To date, much research has studied the symptoms of the head smut [5,6,7], the pathogenic fungus that causes it [8,9,10,11], infection conditions [12,13,14,15], and the epidemic model [8,16,17]. Genetic analyses have revealed that a form of maize resistance to *S. reilianum* is inherited as a quantitative

trait [18]. Ali and Baggett [19] reported that maize resistance to *S. reilianum* was under polygenic control and the model of gene action was predominantly additive or dominant. Recently, several studies have adopted molecular markers to identify chromosomal regions conferring head smut resistance in maize. Ji et al. [20] identified two "consensus" quantitative trait loci (QTL), one located at chromosome bin 2.09, and the other at bin 3.04. Chen et al. [21] detected 4 QTLs with 94 individuals of BC1 derived from the cross between Ji 1037 (donor parent) and Huangzao 4 (recurrent parent). The major QTL (qHSR1) was also mapped to bin 2.09 and fine mapped into an interval of ~2 kb. At present, little information is available about the disease-resistance mechanism against *S. reilianum* in maize.

DNA microarray technology [22,23] has already become a routine tool in the search for the control of complex pathway and pathway interactions in animal, plant, and microbial development [24,25,26]. In recent years, the technique is increasingly being used in maize [27,28,29,30]. Hassan et al. [31] reported the expression profiles of healthy and *S. reilianum*-infected ears using a cDNA microarray to understand the basic molecular mechanisms of *S. reilianum*-induced changes in the floral architecture of maize. However, there has still been no report on a large-scale gene expression analysis of the developmental process of *S. reilianum*-infected maize. Therefore, more information
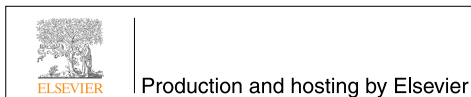
about the basic molecular mechanisms of *S. reilianum*-induced changes in maize is urgently needed.

The objectives of this study were to analyze differentially expressed genes involved in resistance against head smut in maize, to provide clues for further study on the disease-resistant reaction mechanism and the disease-resistant response, and to find head smut resistance-related candidate genes for use in genetic improvement.

2. Materials and methods

2.1. Biological materials and culture

Teliospores of *S. reilianum* were collected from smutted ears in a maize field at Harbin (Heilongjiang, China). They were rinsed twice for 10 min in sterile distilled water, treated for 12 min in 2% (w/v) chloramine T (Sigma, USA) and then washed in sterile distilled water. Teliospores were counted under the microscope with a Thoma cell and diluted with sterile water to obtain 10^6 spores ml^{-1} [15].

Two lines of maize were used in our experiments: L282 and Huangzao4. Near isogenic line (NIL) BC$_4$F$_4$ progeny L282 was derived from the BC$_4$F$_2$ with the susceptible background (Huangzao4) carrying the major resistance QTL from the resistant donor line (Qi319) in bin 2.09 using simple sequence repeat (SSR) marker-assisted selection. One hundred and five polymorphic SSR markers evenly distributed on 10 chromosomes were used for marker-assisted selection of the genetic background in subpopulations. The recovery rate of the recurrent parent's genetic background was 96.7%. Resistance responses of the maize lines to *S. reilianum* infection were recorded after artificial inoculation in the field from August to September 2009 and 2010 at Harbin, Heilongjiang Province, China (45.8°N, 126.5°E). Qi319 and L282, which both have major resistance loci to head smut in bin 2.09 region, are fully resistant to head smut and no susceptible individual has ever been observed in the field. Huangzao4, an elite Chinese inbred line, is highly susceptible to head smut with ~75% susceptible individuals in the field. In all the experiments, the seeds were sterilized with 2% (w/v) chloramine T for 15 min, and then washed with sterile distilled water. Seeds were germinated on sterile potato dextrose agar (PDA) for 3 d at 27°C. Seedlings free of microbial contamination were transferred into Erlenmeyer flasks, each containing 50 ml of m medium [32] enriched with glucose (1 g/L) instead of sucrose. The medium was solidified with 0.4% (w/v) Phytagel (Sigma, USA), and the pH was adjusted to 5.5. The seedlings were grown under 15-h-d conditions at 28°C, 50% relative humidity, and illumination at a minimum of 10,000 lx, and 9-h-night conditions at 20°C and 60% relative humidity [15].

2.2. Infection of maize plantlets

Each 5-day-old seedling was inoculated with 1 mL of sterile teliospores. These teliospores were rubbed on the mesocotyls, coleoptiles and radicles. Mock-inoculated seedlings with distilled and sterile water were set as the control group (CK). After inoculation, the seedlings were incubated at 28°C d/20°C night, 80% relative humidity, >10,000 lx light intensity, and a 16-h photoperiod.

2.3. RNA extraction and purification

The leaves of L282 and Huangzao4 at 1, 2, 4 and 7 d post-inoculation were collected. Ten leaves from mock-inoculated plants and *S. reilianum*-infected plants were selected and pooled for RNA extraction. RNA was extracted with Trizol (Invitrogen, USA). DNase treatment was performed using the RNase-Free DNase kit (Qiagen, USA), and RNA was purified with the RNeasy Plant Mini Kit (Qiagen, USA). The quality of the isolated RNA was determined with a spectrophotometer. Only samples with a 260-nm: 280-nm ratio between 1.8 and 2.1 and a 28S:18S ratio within 1.5 to 2 were processed further.

2.4. Microarray hybridization

RNA targets were prepared according to the manufacturer's protocol using GeneChip 3′ IVT Express Kit (Affymetrix, USA). Total RNA samples (100 ng) were reverse transcribed into double-stranded cDNA and then in vitro transcribed in the presence of biotin-labeled nucleotides using the GeneChip 3′ IVT Express kit (Affymetrix, USA), including poly(A) controls as recommended by the manufacturer. The quality and quantity of the biotinylated cRNA were determined using NanoDrop ND 1000 and Bioanalyzer 2100. Biotin-labeled cRNA samples (15 mg) were quantified by the RNA Nano Lab chip and fragmented randomly to 35 to 200 bp at 94°C in fragmentation buffer (Affymetrix, USA). The purified and fragmented cRNA (12.5 μg) was hybridized to GeneChip MaizeGenome Arrays (Affymetrix, USA). This system covers over 14,850 transcripts from the GenBank, dbEST, and RefSeq databases. Arrays were incubated for 16 h at 45°C, then automatically washed and stained with GeneChip Hybridization, Wash and Stain Kit (Affymetrix, USA). The probe arrays were scanned by a GeneChip Scanner 3000 (Affymetrix, USA) and Command Console Software 3.1 with default settings.

2.5. Data analysis

Raw data were normalized with a Microarray Suite 5.0 (MAS 5.0) algorithm, Gene Spring Software 11.0 (Agilent Technologies Santa Clara, USA). Calculation of fold change was conducted between L282 and Huangzao4 using GeneSpring GX software version 10.0 (Agilent Technologies, Santa Clara, USA). Only the genes with fold change > 3 and $P < 0.01$ were further analyzed. Differentially expressed genes were annotated using a BLAST search of GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). Cluster analysis was performed with the help of the Gene Cluster Program (http://rana.stanford.edu/software) to study the expression pattern of differentially expressed genes. The Gene Ontology database (GO, http://www.geneontology.org/index.shtml) for comparative Gene Ontology categories, including molecular function, biological process and cellular component. A more detailed analysis of the genes' association with physiological pathways was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/pathway.html). Each identified process was confirmed through PubMed/Medline.

2.6. Disease resistance candidate gene mining

The maize databases (http://www.maizesequence.org/) were used in a BLAST-based search of the entire maize genomic sequence to confirm the physical locations of all differentially expressed genes. Only the genes located on chromosome bin 2.09, which has a major QTL conferring resistance to maize head smut, were further analyzed. Then, BLASTN and BLASTX programs were run to search the genes for recognizable structural domains of most known plant disease resistance (*R*) genes.

2.7. qRT-PCR

To validate differential expression of the disease resistance candidate genes, we selected LOC100217307 using reverse transcription real-time RT PCR. The constitutively expressed β-*actin* was used for normalization. First a stand of cDNA was synthesized with the SuperScript II Reverse Transcriptase for RT-PCR (Invitrogen, USA). Primers for the PCR reactions were designed to have a melting temperature of about 60°C and to generate a PCR product between 100 and 200 bp. Primers are listed in Table 1. One microliter of cDNA was used as a template in real-time qPCR reactions, containing 10 μL 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 1 μL each of forward and reverse primer and 7 μL water. Reactions were performed using the 7900HT Real-Time PCR System (Applied

Table 1
Real-time PCR primers for the amplification of selected genes.

Gene Bank accession number	Target	Forward primer (5′–3′)/reverse primer (5′–3′)
NM_001143654	LOC100217307	TTCGCCCTCCTCCCACT CCCTTCCTCCTCAC GCTCT
	β-Actin	AAGCCGAGAGGAGCCATTATC AATGAA GAAACAGCAACAAAAGGA

Biosystems, USA) with the following cycling parameters: 50°C for 2 min, 95°C for 10 s, 40 cycles of 95°C for 15 s, 60°C for 1 min, followed by a disassociation stage (melting curve analysis). Each gene was analyzed in triplicate, after which the average threshold cycle (Ct) was calculated per sample. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

3. Results

3.1. Differentially expressed genes following inoculation

In all of the chips, the signal of microarray hybridization was clear, and the images had a relatively low background but high signal-noise ratio. In total, 3532 differentially expressed genes ($P < 0.01$) between L282 and Huangzao4 were detected under the various inoculation times using approximately 14,850 transcripts. Of them, 45.5% of the genes were up-regulated and 54.5% were down-regulated. Most differentially expressed genes were observed on the fourth day (Fig. 1).

3.2. Gene Ontology category analysis

These differentially expressed genes were classified into different functional categories according to the Gene Ontology (GO) project for biological processes, molecular function and cellular component. Based on GO terms, there were 1210 up-regulated and annotated genes in the head smut-resistant NIL following *S. reilianum* inoculation. The genes involved in receptor binding activity (38.3%) and catalytic activity (32.5%) were the two major groups, followed by genes involved in the transcription regulator activity (10.1%), transporter activity (6.2%), molecular transducer activity (4.3%), structural molecular activity (3.6%), electron carrier activity (3.2%), translation regulator activity (1.3%), and antioxidant activity (0.9%; Fig. 2a). Among the 1378 down-regulated and annotated genes, the genes were classified as follows: 427 genes mapped to binding, 353 genes mapped to catalytic activity, 111 genes mapped to transcription regulator activity, 63 genes mapped to transporter activity, 44 genes mapped to molecular transducer activity, 37 genes mapped to
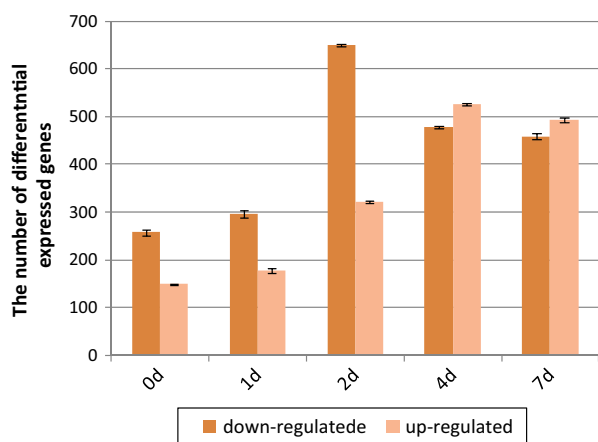

Fig. 1. Genes differentially expressed during different inoculation times were separated into two groups according to whether they were up-regulated or down-regulated.

structural molecular activity, 34 genes mapped to electron carrier activity, 13 genes mapped to antioxidant activity, and 9 genes mapped to translation regulator activity (Fig. 2b).

3.3. Pathway analysis

The KEGG pathway analysis for these differential expressed genes showed that the genes involved have been implicated in a plant–pathogen interactions (Fig. 3a), natural killer cell mediated cytotoxicity (Fig. 3b) and a benzoxazinoid biosynthesis pathway (Fig. 3c). The results showed changes in the expression of some genes among these three pathways. In the plant–pathogen interaction pathway, the expression of LOC542465, LOC542227, LOC100273405, TIDP2949 and pco145926 changed, and these genes are involved in Calcium/Calmodulin (CaM/CML), Calcium-Dependent Protein Kinase/Calmodulin-like Domain Protein Kinase (CDPK), and WRKY transcription factor 2033 (WRKY 2033). In the natural killer cell mediated cytotoxicity pathway, rop3 changed differently. It was up-regulated in L282, but down-regulated in Huangzao4. In the benzoxazinoid biosynthesis pathway, Bx7 and Bx6 are known to play an important role in DIMBOA biosynthesis which is a secondary metabolite with antibiotic properties.

3.4. Disease resistance candidate gene mining

Chromosome localization of differentially expressed genes responding to stress was carried out with maize sequence databases. 60 genes are located in bin 2.09, 42 of which are involved in biological processes (pco080442, pco097999, LOC100192560, pco132830, si605047e03, LOC100280966, LOC100281090, LOC100281811, LOC100282140, pco061870a, LOC100283036, LOC100283506, pco073973a, pco124486, LOC100285313, LOC100304375, RRB1, bx6, p1, ABCF1), molecular function (pco080442, pco097999, LOC100192560, pco132830, si605047e03, LOC100281090, LOC100281811, LOC100282140, pco061870a, LOC100283036, LOC100283506, pco073973a, pco124486, LOC100285313, LOC100304375, RRB1, bx6, p1, ABCF1) and cellular component (pco097999, pco132830, si605047e03, LOC100280966, LOC100281090, LOC100281811, LOC100282140, pco061870a, LOC100283506, pco073973a, pco124486, LOC100285313, LOC100304375, RRB1, bx6, p1, ABCF1). The rest were unknown (Zm.11637, Zm.12524, Zm.66924, Zm.23493, Zm.17226, 100191926, 100279445, 100193977, 100272381, Zm.3798, 100279927, 100273293, 100272843, Zm.6511, 100383635, Zm.7176,, Zm.8818, Zm.2306,). CLAVATA1, bassinosteroid insensitive 1-associated receptor kinase 1 and LOC100217307 have LRR conserved domain, which could possibly implicate them as head smut resistance candidate genes. CLAVATA1 was also closely linked to SSR148152 which was the newly developed SSR marker in head smut resistance by Chen et al. [21] (Table 2).

3.5. qPCR

To further confirm the results of the disease resistance candidate genes, LOC100217307 was tested by real-time PCR between L282 and Huangzao4 after *S. reilianum* inoculation. The qPCR results showed that the relative mRNA expression level of LOC100217307 was significantly different between L282 and Huangzao4 across different time points. LOC100217307 may be involved in maize defense response to head smut infection. In summary, although there were some differences between qPCR and the chip, the change trend of qPCR was basically consistent with the results of that from the microarray analysis (Fig. 4). Further studies should focus on cloning and characterization of these disease resistance candidate genes.
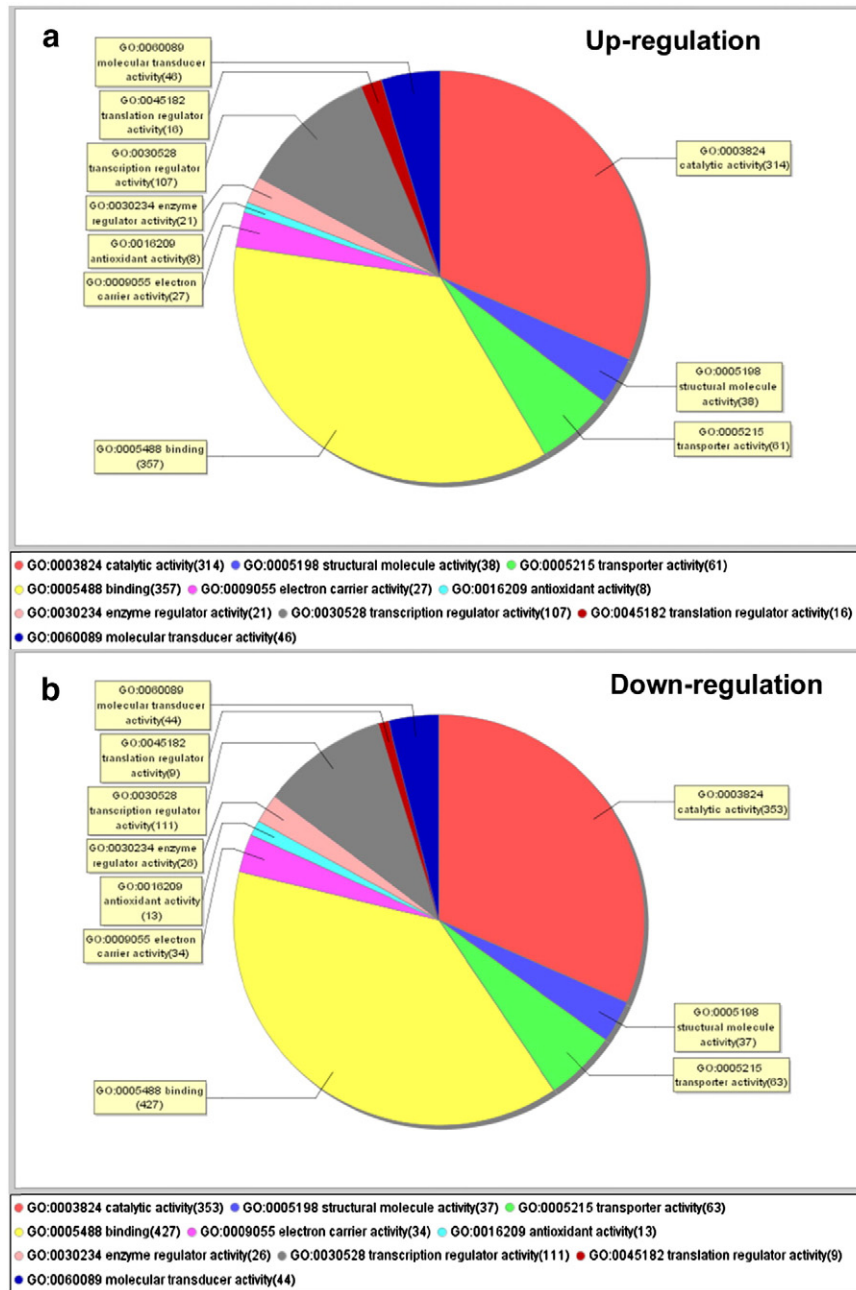
Fig. 2. Broad functional classifications for genes and gene products representing their corresponding biological processes, molecular function and cellular localization.

4. Discussion

4.1. Plant–pathogen interaction pathway

Plants are able to recognize potential microbial pathogens through pathogen-associated molecular patterns (PAMPs) by host sensors, which are known as pattern-recognition receptors (PRRs) that initiate a series of defense responses called PAMP-triggered immunity (PTI) [33]. PAMPs induce rapid and transient production of ROS in an oxidative burst within a few minutes after treatment. ROS are known to play important roles in cell signaling and plant development [34,35]. We found that the expression of CDPK, which is known to be essential in mediating tolerance to abiotic stress through involvement in ABA signaling and ROS detoxification, was altered dramatically under head smut stress [36]. CDPK resulted in increased accumulation of ROS through PTI pathway, and excess

ROS was responsible for cell death [37,38]. As a result, the pathogen was confined at and around the initial infection sites. There are ROS scavenge systems in plants, both enzymatic and non-enzymatic, which control the balance of ROS to prevent damage from excess ROS. Higher levels of detoxification of ROS and ROS were observed in *S. reilianum*-colonized inflorescence tissue than in the healthy inflorescences [31].

The concept of programmed cell death (PCD) and its importance for normal plant physiology was first proposed by Carl Leopold in 1961. In the case of a resistant interaction, PCD is induced by the plant, which is known as the hypersensitive response (HR), and acts to limit the spread of the pathogen. In this study, rop3 and other genes involved in the natural killer cell mediated cytotoxicity pathway were induced differentially in L282 and Huangzao4. Higher expression of these defense genes in resistant maize plants might be responsible for resistance against *S. reilianum*.

4.2. Benzoxazinoid biosynthesis pathway

The 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and its glucoside (glc) were originally reported more than 50 years ago [39]. DIMBOA, which exists in most Poaceaeous plants, has many bioactivities, including pesticidal, insecticidal, and allelopathic effects [40,41,42,43]. DIMBOA concentration in plumula of 8 corn inbreds and percent infection of head smut show significant correlation [44].

The results of this study showed that the expression of Bx7, a key gene in the biosynthesis of DIMBOA, was induced in L282 at the fourth day after inoculation. The expression of Bx6 in L282 was higher

than Huangzao4, which agrees with a previous study [45]. In this period, representative genes of benzoxazinoid biosynthesis pathway were up-regulated to a certain extent, which indicated that the infection of *S. reilianum* triggered host defense responses by this time in the resistant cultivar, and the maize resisted the infection through synthesis of secondary metabolites.

4.3. Cell wall synthesis pathway

Cell wall, especially the primary cell wall participates in cellular metabolic activities, i.e. regulation of cell growth, strengthening of tissues, or protection against attack by microorganisms [46].
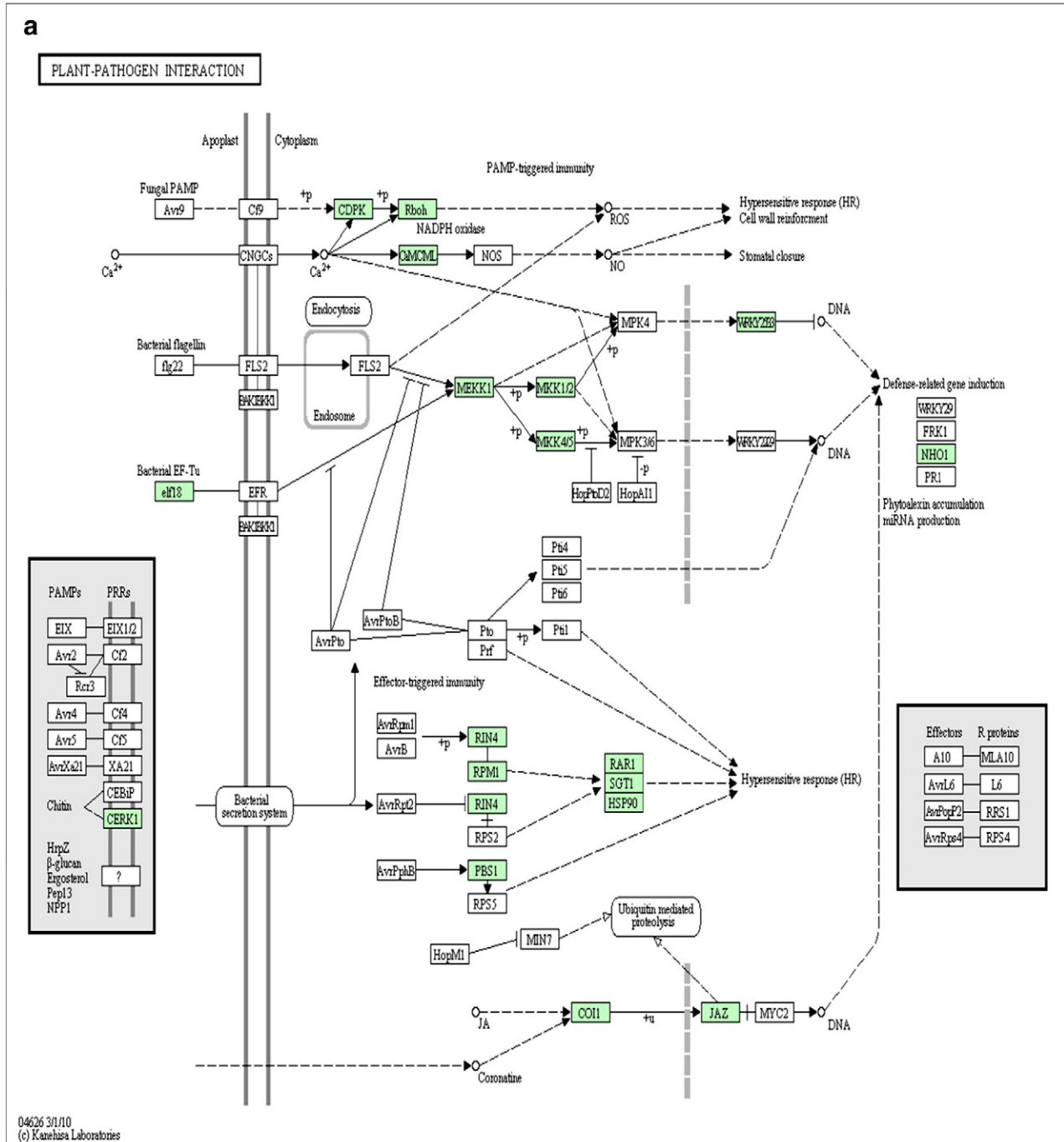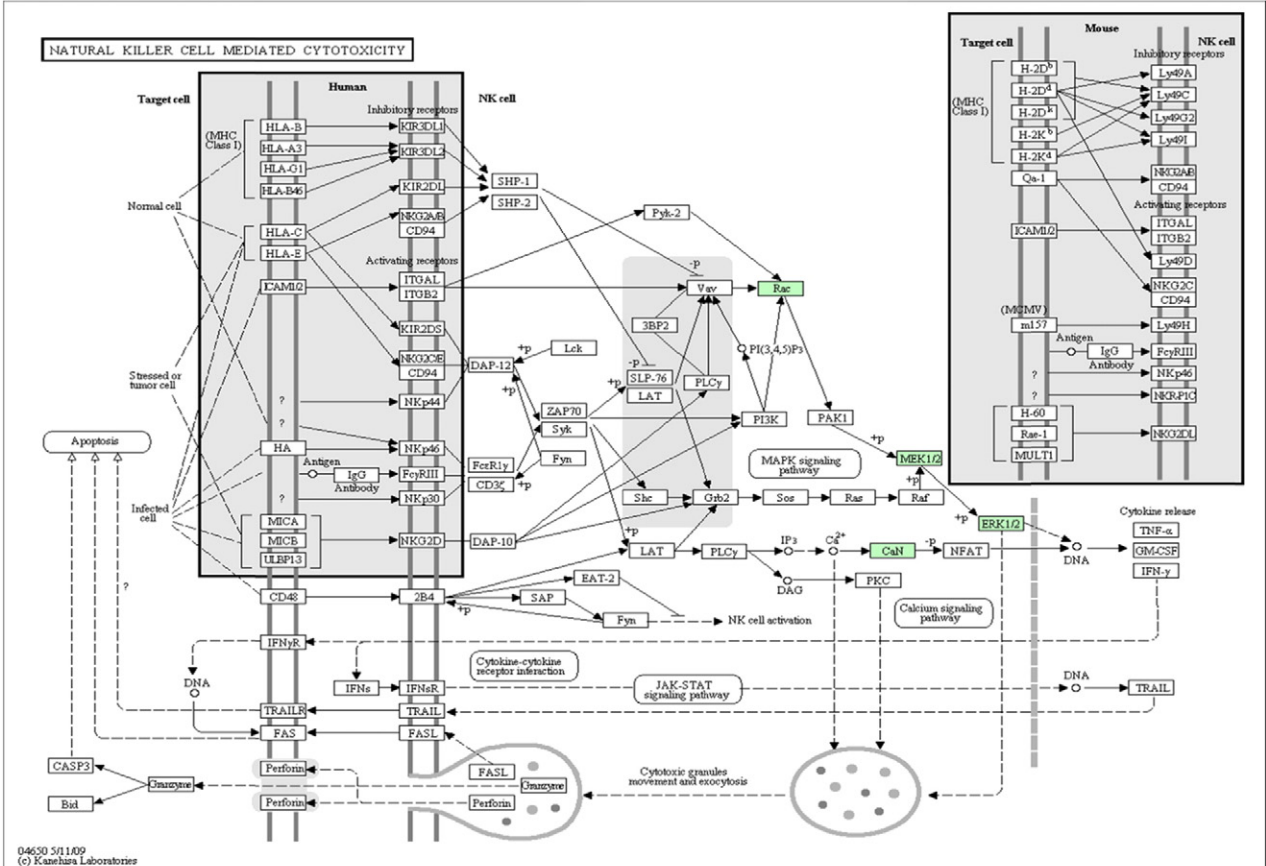

Fig. 3. KEGG pathway diagram showing genes involved in the (a) plant–pathogen interaction, (b) natural killer cell mediated cytotoxicity, and (c) benzoxazinoid biosynthesis pathway. The alterative genes were highlighted in green.
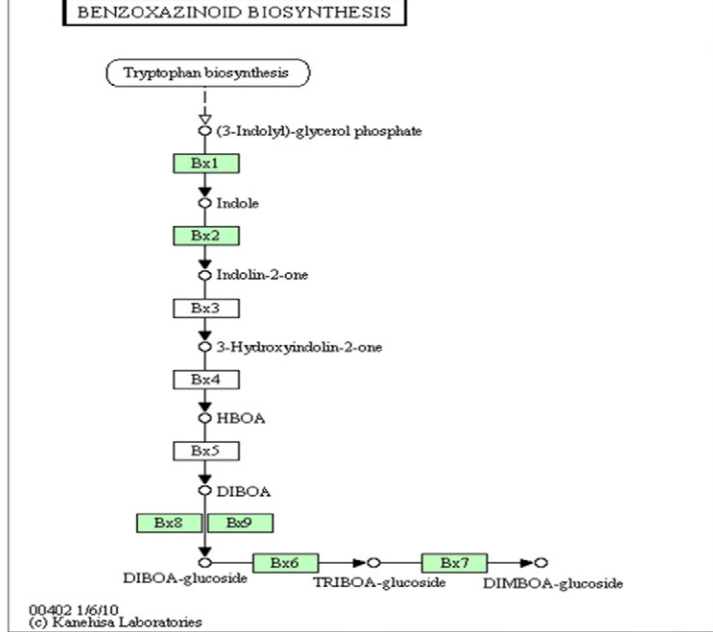
b

c

Fig. 3 (*continued*).

S. reilianum locally dissolves the epidermal cell wall to penetrate the maize root and never develops an appressorium at the root surface [15]. The results of this study demonstrate that the expression of cell wall metabolism related genes did not change obviously 7 d after inoculation. Therefore, thicker cell walls were not involved in the ability to resist head smut.

Many genes involved in other metabolic pathways, such as the biosynthesis of alkaloids derived from histidine and purine pathways; the biosynthesis of alkaloids derived from ornithine, lysine and nicotinate pathway; and the biosynthesis of alkaloids derived from shikimate pathway, changed. These metabolite pathways may play a role in resistance to pathogens.

Table 2
Candidate resistance gene in bin 2.09.

Gene ID	Probe ID	Description	Chr. location
100281253	Zm.10534.1.A1_x_at	Receptor protein kinase CLAVATA1	chr = 2:230583533..230585418
100280966	Zm.10830.1.S1_at	Bassinosteroid insensitive 1-associated receptor kinase 1	chr = 2:219629313..219636025
100217307	Zm.10111.1.A1_at	LOC100217307	chr = 2:222235488..222241159

4.4. Candidate disease resistance genes mining

Transposon tagging was used in Hm1 in maize to create the first cloned disease resistance gene (R gene) in plants [47]. To date, about 70 plant disease resistance (R) genes have been cloned. According to the conserved domain, Martin et al. [48] divided these known genes into five main categories: serine–threonine protein kinase (STK), leucine-rich repeats/trans-membrane (LRR/TM), nucleotide-binding site/leucine-rich repeats (NBS/LRR), leucine-rich repeats/trans-membrane/serine-threonine protein kinase (LRR/TM/STK) and Hm1, with the majority encoding NBS-LRR protein [49].

The NBS-LRR proteins are thought to recognize specific avirulence (Avr) proteins produced by pathogens and activate signal transduction cascades for complex defense responses [50]. Among the downstream cellular events that characterize the resistant state are rapid oxidative bursts, cell wall strengthening, the induction of defense gene expression, and rapid cell death at the site of infection [51].

Resistance to head smut is a complex quantitative inherited trait under polygenic control, and the molecular mechanism of the maize resistance to head smut is not clear. Up to now no one gene responsible to head smut resistance was cloned. We previously reported a major resistance QTL in bin 2.09 (qHS2.09) that explains up to 43.7% of the phenotypic variance and confers resistance to head smut in a population of 184 $F_{2:3}$ families derived from the cross of 'Mo17' and 'Huangzao4' [52]. Just as rhg1 of soybean cyst nematode (SCN), the qHS2.09 might be a complex locus with more than one functional gene [53]. These genes could interact together to initiate a series of signaling cascades leading to maize head smut resistance in bin 2.09. In this study, three candidate resistance genes with conserved domains were selected in bin 2.09, these three differential expression genes (CLAVATA1, bassinosteroid insensitive 1-associated receptor kinase 1 and LOC100217307) have potential applications in maize production and might be used to engineer a head smut resistance candidate gene. Future investigations will focus on identification of the function of candidate genes in maize by genetically modified (GM) technology. These will greatly enhance our understanding of the mechanisms of maize against head smut at the molecular level 9.

Accession numbers

Financial support

Author contributions

Proposed theoretical frame: ZW; Conceived and designed the experiments: ZW, TY; Contributed reagents/materials/analysis tools: ZW, XJ, XL; Wrote the paper: TY; Performed the experiments: TY, XJ, XL, SK; Analyzed the data: TY, XJ.

References

[1] Stromberg EL. Head smut of maize, a new disease in Minnesota. Phytopathology 1981;71:906.
[2] Bernardo R, Bourrier M, Olivier JL. Generation means analysis of resistance to head smut in maize. Agronomie 1992;12:303–6. http://dx.doi.org/10.1051/agro:19920403.
[3] Jin QM, Li JP, Zhang XW, Wang GX, Song SY, Liu YC, et al. Establishment IPM of system of corn diseases and pest insects in the spring corn belt. J Maize Sci 2000;8:84–8.
[4] Frederiksen RA. Head smuts of corn and sorghum. Proceedings of 32nd corn and sorghum research conference, 32. ; 1977. p. 89–104.
[5] Stromberg EL, Stienstra WC, Kommedahl T, Matyac CA, Windels CE, Geadelmann JL. Smut expression and resistance of corn to Sphacelotheca reiliana in Minnesota. Plant Dis 1984;68:880–4. http://dx.doi.org/10.1094/PD-68-880.
[6] Matyac CA, Kommedahl T. Occurrence of chlorotic spots on corn seedlings infected with Sphacelotheca reiliana and their use in evaluation of head smut resistance. Plant Dis 1985;69:251–4. http://dx.doi.org/10.1094/PD-69-251.
[7] Njuguna JGM. Combating head smut of maize caused by Sphacelotheca reiliana through resistance breeding. Proceedings of Eastern and Southern Africa Regional Maize Conference; 2002. p. 110–2.
[8] Halisky PM. Head smut of sorghum, sudangrass, and corn, caused by Sphacelotheca reiliana Kühn Clint. Hilgardia 1963;34:287–304.
[9] Al-Sohailv IA, Mankin CJ, Semeniuk G. Physiologic specialization of Sphacelotheca reiliana to sorghum and corn. Phytopathology 1963;53:723–6.
[10] Mehta BK, Frederiksen RA, Collier J. Evaluation of physiological specialization in Sphacelotheca reiliana. Phytopathology 1967;57:925–8.
[11] Schirawski J, Heinze B, Wagenknecht M, Kahmann R. Mating type loci of Sporisorium reilianum: Novel pattern with three a and multiple b specificities. Eukaryot Cell 2005;4:1317–27. http://dx.doi.org/10.1128/EC.4.8.1317-1327.2005.
[12] Téferi A, Petitprez M, Valles V, Albertini L. Influence of soil water potential and soil texture on infection of maize by head smut. Agronomie 1989;9:677–82.
[13] Ingold CT. Products of teliospore germination in Sporisorium spp. Mycol Res 1994;98:467–73. http://dx.doi.org/10.1016/S0953-7562(09)81206-9.
[14] Martinez C, Roux C, Dargent R. Biotrophic Development of Sporisorium reilianum f sp. Zeae in vegetative shoot apex of maize. Biochem Cell Biol 1999;89:247–53. http://dx.doi.org/10.1094/PHYTO.1999.89.3.247.
[15] Martinez C, Jauneau A, Roux C, Savy C, Dargent R. Early infection of maize roots by Sporisorium reilianum f sp. Zeae. Protoplasma 2000;213:83–92.
[16] Krueger W. Sphacelotheca reiliana on maize I. Infection and control studies. S Afr J Agric Sci 1962;5:43–56.
[17] Simpson WR. Head smut of corn in Idaho. Plant Dis Rep 1966;50:215–7.
[18] Mei ZB, Xu GY, Wang HC, Hou GD, Li JY. Pattern of inheritance of resistance to head smut in maize. Shanxi Nongye Kexue 1982;11:10–3.
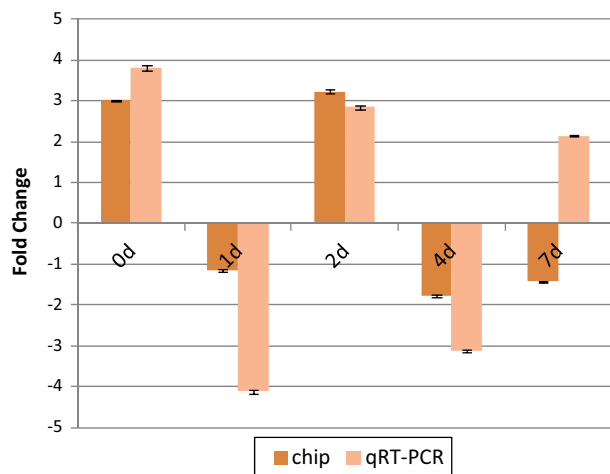
Fig. 4. Comparative results of qRT–PCR and Maize array. Column diagrams represent the average fold change of LOC100217307 expression across different time points after *S. reilianum* injection. Different color means different detection methods of gene chip and quantitative RT-PCR, respectively.

[19] Ali A, Baggett JR. Inheritance of resistance to head smut disease in corn. J Am Soc Hortic Sci 1990;115:668–72.

[20] Ji HL, Li XH, Xie CX, Hao ZF, Lü XL, Shi LY, et al. Comparative QTL mapping of resistance to *Sporisorium reiliana* in maize based on meta-analysis of QTL locations. J Plant Genet Resour 2007;8:132–9.

[21] Chen YS, Chao Q, Tan GQ, Zhao J, Zhang MJ, Ji Q, et al. Identification and fine-mapping of a major QTL conferring resistance against head smut in maize. Theor Appl Genet 2008;117:1241–52. http://dx.doi.org/10.1007/S00122-008-0858-4.

[22] Brown P, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat Genet 1999;21:33–7. http://dx.doi.org/10.1038/4462.

[23] Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995; 270:467–70. http://dx.doi.org/10.1126/science.270.5235.467.

[24] Zheng M, Wang X, Templeton LJ, Smulski DR, La Rossa RA, Storz G. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J Bacteriol 2001;183:4562–70. http://dx.doi.org/10.1128/JB.183.15.4562-4570.2001.

[25] Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI. Microarray expression analyses of *Arabidopsis* guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell 2004;16:596–615. http://dx.doi.org/10.1105/tpc.019000.

[26] Yamazaki K, Kuromitsu J, Tanaka I. Microarray analysis of gene expression changes in mouse liver induced by peroxisome proliferator-activated receptor α agonists. Biochem Biophys Res Commun 2002;290:1114–22. http://dx.doi.org/10.1006/bbrc.2001.6319.

[27] Lee JM, Williams ME, Tingey SV, Rafalski AJ. DNA array profiling of gene expression changes during maize embryo development. Funct Integr Genomics 2002;2:13–27. http://dx.doi.org/10.1007/s10142-002-0046-6.

[28] Liu XH, Fu J, Gu D, Liu W, Liu T, Peng Y. Genome-wide analysis of gene expression profiles during the kernel development of maize (*Zea mays L.*). Genomics 2008; 91:378–87. http://dx.doi.org/10.1016/j.ygeno.2007.12.002.

[29] Zhu Y, Fu J, Zhang J, Liu T, Jia Z, Wang J. Genome-wide analysis of gene expression profiles during ear development of maize. Plant Mol Biol 2009;70:63–77. http://dx.doi.org/10.1007/s11103-009-9457-2.

[30] Jia MA, Li Y, Lei L, Di D, Miao H, Fan Z. Alteration of gene expression pro- file in maize infected with a double-stranded RNA fijivirus associated with symptom development. Mol Plant Pathol 2012;13:251–62. http://dx.doi.org/10.1111/j.1364-3703.2011.00743.x.

[31] Hassan H, Becker A, Iven T, Feussner I, Schirawski J. *Sporisorium reilianum* infection changes inflorescence and branching architectures of maize. Plant Physiol 2011; 156:2037–52. http://dx.doi.org/10.1104/pp.111.179499.

[32] Bécard G, Fortin JA. Early events of vesicular–arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytol 1988;108:211–8. http://dx.doi.org/10.1111/j.1469-8137.1988.tb03698.x.

[33] Chen XW, Ronald PC. Innate immunity in rice. Trends Plant Sci 2011;16:451–9. http://dx.doi.org/10.1016/j.tplants.2011.04.003.

[34] Gapper C, Dolan L. Control of plant development by reactive oxygen species. Plant Physiol 2006;141:341–5. http://dx.doi.org/10.1104/pp.106.079079.

[35] Møller IM, Sweetlove LJ. ROS signaling — specificity is required. Trends Plant Sci 2010;15:370–4. http://dx.doi.org/10.1016/j.tplants.2010.04.008.

[36] Asano T, Hayashi N, Kikuchi S, Ohsugi R. CDPK-mediated abiotic stress signaling. Plant Signal Behav 2012;7:817–21. http://dx.doi.org/10.4161/psb.20351.

[37] Gao X, Cox Jr KL, He P. Functions of calcium-dependent protein kinases in plant innate immunity. Plants 2014;3:160–76. http://dx.doi.org/10.3390/plants3010160.

[38] Rodríguez-Serrano M, Bárány I, Prem D, Coronado MJ, Risueño MC, Testillano PS. NO, ROS, and cell death associated with caspase-like activity increase in stress-induced microspore embryogenesis of barley. J Exp Bot 2012;63:2007–24. http://dx.doi.org/10.1093/jxb/err400.

[39] Hietala PK, Virtanen AI. Precursors of benzoxazolinone in rye plants. II. Precursor I, the glucoside. Acta Chem Scand 1960;14:502–4.

[40] Barnes JP, Putnam AR. Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.). J Chem Ecol 1987;13:889–990. http://dx.doi.org/10.1007/BF01020168.

[41] Grombacher AW, Russell WA, Guthrie WD. Resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. J Kansas Entomol Soc 1989;62:103–7.

[42] Bravo HR, Lazo W. Antimicrobial activity of cereal hydroxamic acids and related compounds. Phytochemistry 1993;33:569–71.

[43] Sicker D, Frey M, Schulz M, Gierl A. Role of natural benzoxazinones in the survival strategy of plants. Int Rev Cytol 2000;198:319–46. http://dx.doi.org/10.1016/S0074-7696(00)98008-2.

[44] Wang JH, Wang QM. Study on the relation between DIMBOA and resistance to *Sphacelotheca reiliana* in maize. Acta Phytopathol Sin 1989;10:187–91.

[45] Ni Ch, Xiao YN, Wang FG, Zheng YL. The research of *Sporisorium reiliana*'s infection efficiency and spread course in maize based on PCR assays. Sci Agric Sin 2006; 39:1804–9.

[46] Wojtaszek P. Genes and plant cell walls: A difficult relationship. Biol Rev 2000; 75:437–75. http://dx.doi.org/10.1111/j.1469-185X.2000.tb00051.x.

[47] Johal GS, Briggs SP. Reductase activity encoded by the HM1 disease resistance gene in maize. Science 1992;258:985–7. http://dx.doi.org/10.1126/science.1359642.

[48] Martin GB, Bogdanove AJ, Sessa G. Understanding the functions of plant disease resistance proteins. Annu Rev Plant Biol 2003;54:23–61. http://dx.doi.org/10.1146/annurev.arplant.54.031902.135035.

[49] Dangl JL, Jones JDG. Plant pathogens and integrated defence responses to infection. Nature 2001;411:826–33. http://dx.doi.org/10.1038/35081161.

[50] Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J. The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. Plant J 2000; 23:441–50. http://dx.doi.org/10.1046/j.1365-313x.2000.00804.x.

[51] Morel JB, Dangl JL. The hypersensitive response and the induction of cell death in plants. Cell Death Differ 1997;4:671–83.

[52] Li XH, Wang ZH, Gao SR, Shi HL, Zhang SH. Analysis of QTL for resistance to head smut (*Sporisorium reiliana*) in maize. Field Crop Res 2008;106:148–55. http://dx.doi.org/10.1016/j.fcr.2007.11.008.

[53] Ruben E, Jamai A, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, et al. Geno- mic analysis of the *rhg1* locus: Candidate genes that underlie soybean re- sistance to the cyst nematode. Mol Genet Genomics 2006;276:503–16. http://dx.doi.org/10.1007/s00438-006-0150-8.