Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2012) 13(3), 251–262 DOI: 10.1111/J.1364-3703.2011.00743.X

Alteration of gene expression profile in maize infected with a double-stranded RNA fijivirus associated with symptom development

MENG-AO JIA[1], YONGQIANG LI[1], LEI LEI[1], DIANPING DI[2], HONGQIN MIAO[2] AND ZAIFENG FAN[1,*]

[1]State Key Laboratory of Agrobiotechnology and Department of Plant Pathology, China Agricultural University, Beijing 100193, China
[2]Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, Baoding, China

SUMMARY

Maize rough dwarf disease caused by *Rice black-streaked dwarf virus* (RBSDV) is a major viral disease in China. It has been suggested that the viral infection of plants might cause distinct disease symptoms through the inhibition or activation of host gene transcription. We scanned the gene expression profile of RBSDV-infected maize through oligomer-based microarrays to reveal possible expression changes associated with symptom development. Our results demonstrate that various resistance-related maize genes and cell wall- and development-related genes, such as those for cellulose synthesis, are among the genes whose expression is dramatically altered. These results could aid in research into new strategies to protect cereal crops against viruses, and reveal the molecular mechanisms of development of specific symptoms in rough dwarf-related diseases.

INTRODUCTION

It is known that viruses can alter the transcription network of their hosts. These alterations, which can be caused by direct and indirect effects of viral replication, may eventually result in the development of disease. The innovation in detection technology and the enrichment of genome sequences and annotation in diverse organisms, especially in maize (Alexandrov *et al*., 2009; Gardiner *et al*., 2004; Liang *et al*., 2008), have made it possible to observe global gene expression patterns based on microarrays for any developmental stage, tissue type or environmental factor (Casati and Walbot, 2003; Dowd *et al*., 2004; Moy *et al*., 2004; Rensink and Buell, 2005., Schenk *et al*., 2000; Zou *et al*., 2005).

Rough dwarf disease has been devastating maize (*Zea mays*) production in China for decades and the causal agent of this disease has been identified to be *Rice black-streaked dwarf virus* (RBSDV) (Zhang *et al*., 2001). RBSDV belongs to the genus *Fijivirus* within the family *Reoviridae*, whose genome consists of 10 double-stranded RNA segments (S1–S10) (Milne *et al*., 2005), and can be transmitted by small brown planthopper (*Laodelphax striatellus*) in a persistent manner. The major infected cereals (such as rice, maize and wheat) show severe growth abnormalities, such as plant dwarfing, leaf dark greening and vein clearing to a white streak with small white enations on the lower surfaces of leaves and sheaths (Kuribayashi and Shinkai, 1952). Protein P1 (encoded by S1) is a putative RNA-dependent RNA polymerase, and P2 and P4 are the major core structural protein and outer shell B-like protein, respectively (Zhang *et al*., 2001). Previous research results have indicated that S8 and S10 encode the major inner caspid and outer caspid proteins, respectively, and S6, S7 and S9 encode nonstructural proteins (Isogai *et al*., 1998). Among the nonstructural proteins, P6 is likely to be a silencing suppressor (Zhang *et al*., 2005). Both P7-1 and P9-1, encoded by S7 and S9 (each of which contains two open reading frames), have been suggested to be involved in the formation of the tubular structure and viroplasm during infection, respectively (Isogai *et al*., 1998; Zhang *et al*., 2009). As well as serving as the inner caspid protein, P8, with the nuclear localization signal peptide, can enter into the nucleus and play a role in the inhibition of transcription (Liu *et al*., 2007). Until recently, because of the paucity of data on the interactions between RBSDV and its host plants, especially for maize, there have been no valid strategies to control this disease, and research has been confined to the exploration of possible functions of virus components *in vitro* or in nonhost plants.

Transcriptome analyses via microarray for some model plants, such as *Arabidopsis thaliana*, *Nicotiana benthamiana* and rice (*Oryza sativa*), infected with viruses, have provided references to identify the gene expression networks underlying disease symptom expression (Dardick, 2007; Espinoza *et al*., 2007; Golem and Culver, 2003; Marathe *et al*., 2004; Satoh *et al*., 2010; Senthil *et al*., 2005; Shimizu *et al*., 2007; Whitham *et al*., 2006; Yang *et al*., 2007). *Sugarcane mosaic virus* (SCMV) resistance candidate genes in maize have also been identified successfully by the same method (Uzarowska *et al*., 2009). It is now feasible to characterize the differential expression of host genes responsive to RBSDV infection with the help of microarray analysis and the increasing maize gene annotation data (Alexandrov *et al*., 2009; Gardiner *et al*., 2004). Thus, it is expected that some genes playing key roles

*Correspondence: Email: fanzf@cau.edu.cn

in symptom development may be found through microarray analysis, leading to the understanding of the processes of RBSDV infection.

In this study, we investigated and analysed the alterations in gene expression in maize infected by RBSDV with 43 803 probes, and found about 4000 genes whose expression changed significantly. The gene expression profile changes may aid in the understanding of the mechanisms of symptom expression and in the search for genes with resistance against RBSDV.

RESULTS

Development of symptoms and virus accumulation

Previous detailed electron micrographs have indicated that the RBSDV particle population in viroplasm is small when the leaves start to turn dark green and when discontinuous vein clearing (DVC) starts to appear (Chen *et al*., 2004), but increases dramatically when DVC turns to white streaks (Fig. 1A) and dwarfing symptoms start to appear (Fig. 1B). Our results from double-stranded RNA extraction agree with this report, and show that viral genomic RNA accumulation in systemic leaves is similar to that in inoculated leaves during this infection phase (Fig. 1C).

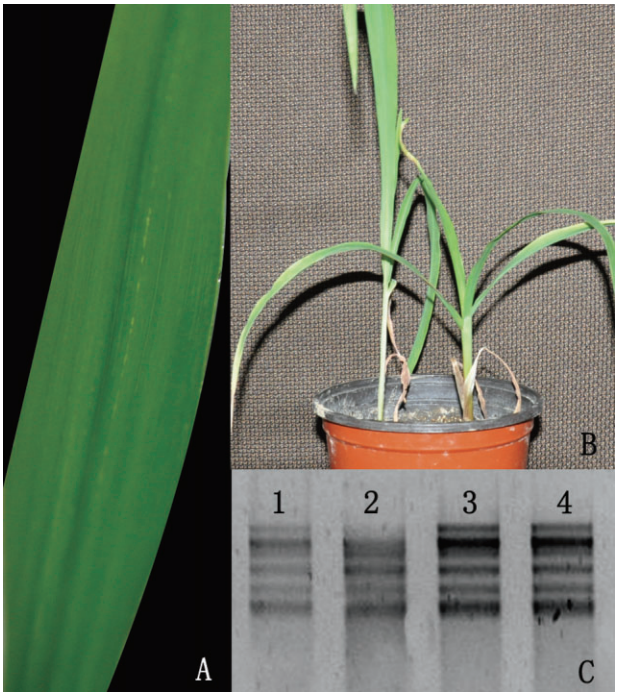


Fig. 1 The phenotype of *Rice black-streaked dwarf virus* (RBSDV)-infected maize and the comparative abundance of virus genome accumulation. (A) The early symptom of white streaks. (B) Comparison between healthy (left) and RBSDV-infected (right) maize at the time of the early symptom of white streaks. (C) Abundance of RBSDV virus genome in different infection phases. Double-stranded RNAs of the viral genome were extracted from the same amount of maize leaves infected by RBSDV in different phases, and analysed by 1.5% agarose gel. Lane 1, an abundance of the virus genome in an inoculated leaf when the colour is turning to dark green. Lane 2, an abundance of virus genome at the time of appearance of discontinuous vein clearing (DVC). Lane 3, an abundance of virus genome in the inoculated leaf during the transition from DVC to white streaks. Lane 4, an abundance of virus genome in a newly developed leaf during the transition from DVC to white streaks. All measurements were repeated three times.

Microarray analysis of the maize transcript network influenced by RBSDV

During the transition of DVC to white streaks, large amounts of virus particles accumulate, and the dwarfing symptoms of maize seedlings appear. In this phase, virus replication interferes with physiological activities, most of which are regulated by the host transcription network. Thus, we chose maize materials infected by RBSDV in this phase to analyse transcription network alterations via microarray analysis.

To reduce the biological errors caused by natural variation, every 10 inoculated seedlings exhibiting similar symptoms were harvested as one sample, and three more samples were prepared by the same method as replicates; the mock-inoculated sample was prepared in the same way, as reported by Shimizu *et al*. (2007). Transcriptome analysis was performed using the Agilent maize microarray chip (G2519F), containing 43 803 probes covering several different varieties (sources from RefSeq and GenBank). The identification criteria of gene expression alteration induced by RBSDV infection were as follows: (i) gene expression should be detected at a statistically significant level in all samples; (ii) gene expression should occur in the same direction in at least three replicates; and (iii) the $\log_2$-based ratio of (RBSDV-inoculated sample/mock-inoculated sample) $\geq$0.58 or $\leq$–0.58 (this ratio was defined as the fold change, FC).

Derived from more than 43 000 probe signals detected in the chip, the expression of 4099 genes was altered significantly according to the identification standards influenced by RBSDV infection (Fig. 2A, B; Fig. S1, see Supporting Information). Of these, 1840 genes had been annotated, with approximately one-third possessing known or hypothetical functions. Analysis of the annotated data suggested that more than 60% of these genes were involved in amino acid/protein metabolism and energy metabolism; other genes were related to carbohydrate biosynthesis, fatty acid and lipid metabolism, nucleotide metabolism, secondary metabolites and hormone metabolism (Fig. 2C). This large amount of data contains very useful information on the relationships between this virus and its maize host. Among the alterations in expression, we focused on functional categories related to resistance and disease symptoms in order to search for the potential pathogenic mechanism and resistance resources.

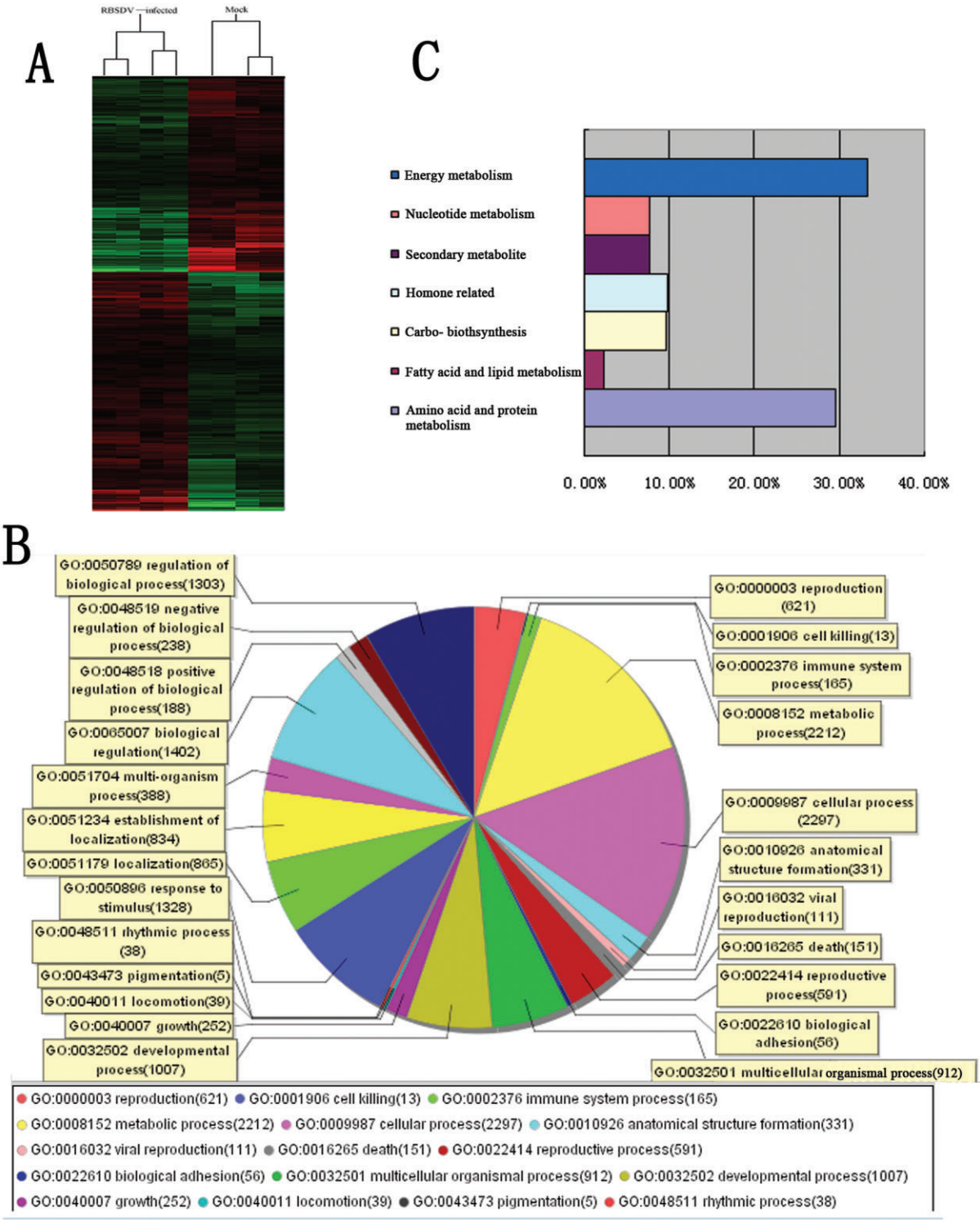


Fig. 2 Overview of the microarray results. (A) Hierarchical clustering by *Rice black-streaked dwarf virus* (RBSDV)-infected and mock-inoculated samples. The expression data of 4099 genes which were altered significantly in four replicates were clustered through Cluster and Treeview. Gene expression is shown in the heat map as up-regulated (red), down-regulated (green) and no change (black). (B) Pie chart of gene ontology at the biological process level. (C) The functional categories of annotated genes, and references from KEGG and Gramene (Kanehissa *et al*., 2008; Liang *et al*., 2008).

Defence and stress response-related genes

When a plant suffers biotic or abiotic stresses, the expression of groups of defence response-related genes changes significantly following signal burst (Marathe *et al*., 2004; Satoh *et al*., 2010; Senthil *et al*., 2005; Shimizu *et al*., 2007; Uzarowska *et al*., 2009; Whitham *et al*., 2006). By comparing our microarray analysis with previous reports, we found that many genes showed a similar performance under different pathogen stresses (Table 1), such as chitinase chem5 [Gene ID: 542525], pathogenesis-related 1 (*PR-1*) [Gene ID: 542352], glutathione-*S*-transferase [Gene ID: 100285806], heat shock protein [Gene ID: 100283530], MYB transcription factor family (e.g. [Gene ID: 100283510]) and WRKY family (e.g. [Gene ID: 100281682]). At the same time, our probes also detected many defence or stress-related genes which have rarely been reported in maize, such as ferredoxin 1 [Gene ID: 542275], barley *Mlo* defence gene homologue 8 [Gene ID: 542065], autophagy-related gene 7 (*ATG7*) [Gene ID: 100233231] and *ATG4* [Gene ID: 100240690]. We found that some of these genes were altered dramatically [e.g. FC of the *Mlo* homologue 8 gene was up to 3.9 times, i.e. $\log_2$(FC) was 1.98]. Although it is not known how these maize genes are involved in the resistance response, they have been confirmed to play important roles in defence in other plant hosts (Buschges *et al*., 1997; Dayakar *et al*., 2003; Matsumura *et al*., 1999).

Table 1 Alterations in expression of defence- and stress-related genes in maize infected by *Rice black-streaked dwarf virus* (RBSDV).

Gene ID	Annotation	$\log_2(FC_{ave})$
Signal transduction		
100282408	Serine/threonine protein kinase Eg2-like	0.7690
100285649	Serine/threonine protein kinase NEK4	−1.4860
542671	Serine/threonine protein kinase 1	1.1539
100280516	Serine/threonine protein kinase RIO2	0.8344
100286383	Receptor-like protein kinase RK20-1	0.6107
100281569	Receptor-like kinase	0.7255
541867	AKIN protein kinase	0.9058
100282637	Protein kinase APK1B	1.2207
100284960	Nodulation receptor kinase	−1.2314
100281445	Lectin-like receptor kinase 7	1.7102
100281768	MPK17-2, putative MPK	−2.1617
100285625	Brassinosteroid LRR receptor kinase	1.1784
100281682	WAK1-OsWAK receptor-like cytoplasmic kinase	0.6203
100281741	WAK53-OsWAK receptor-like cytoplasmic kinase	1.5851
Transcription factor		
541969	R2R3 Myb transcription factor MYB-IF35	1.1157
100284980	SANT/MYB protein	−4.2056
100283600	Myb-related transcription activator	−1.3317
100286254	Myb-CC type transcription factor	−1.4399
100283510	MYB59	1.4364
100281741	WAK53-OsWAK receptor-like cytoplasmic kinase	1.5851
100281370	WRKY DNA-binding domain-containing protein	−1.8242
100281820	WRKY23	−1.7370
100284723	DNA-binding WRKY	0.6466
100280991	PHD finger protein	1.0375
Resistance related		
542352	Pathogenesis-related 1	2.0635
542525	Chitinase chem5	2.0772
100285806	Glutathione-*S*-transferase	1.0135
100181280	Peroxidase 1	0.9777
100285457	Peroxidase 16	2.1215
100183530	Heat shock factor protein 7	−2.5179
100126543	Heat shock complementing factor 1	0.7161
100285707	Phloem-specific lectin	1.4656
542275	Ferredoxin 1	2.3537
542065	Barley Mlo defence gene homologue 8	1.9881
100240690	Autophagy-related 4	0.9828
100233231	Autophagy-related 7	−1.4560

Fold change (FC) = (normalized signal of RBSDV-inoculated sample)/(normalized signal of mock-inoculated sample). FC_{ave} is the mean of four replicates in our analyses.

Genes related to dwarf symptoms

Dwarfing is the major symptom of maize infected by RBSDV. Several pathways are involved in plant height determination, but cell wall-related genes play pivotal roles, such as cellulose synthase (*CESA*) genes and the *CESA*-like gene family (Kalluri and Joshi, 2004; Li *et al*., 2009; Pagant *et al*., 2002; Xiong *et al*., 2010). In the microarray analysis, most probes of cell wall-related genes showed significant alterations. Among these alterations, we observed that most of the cellulose synthesis-related genes (Table 2), for example, *CESA 1* [Gene ID: 541802], *CESA 9* [Gene ID: 541807] and dynamin-related protein (DRP) C [Gene ID: 100280709]), were suppressed in this infection phase, but the genes involved in cell wall composition and structural proteins (e.g. glycosyl transferase family [Gene ID: 100281324], β-galactosidase [Gene ID: 100185111] and glycine-rich protein [Gene ID: 542725]) were altered in the opposite direction (Table 2). These results show that most cell wall-related genes are influenced by RBSDV infection, and that the dwarfing symptoms may be caused by a significant decrease in *CESA* expression.

Genes related to developmental abnormalities

During RBSDV infection, in addition to severe dwarfing symptoms, other serious damage includes developmental abnormalities, such as the loss of fertility. A huge network controls plant vegetative and reproductive development, involving numerous pathways and chemical compounds. Among these complex factors, plant hormones play special roles as they are widely involved in developmental regulation (Bortiri and Hake, 2007; Cecchetti *et al*., 2008; He *et al*., 2003; Sakata *et al*., 2010; Tanurdzic and Banks, 2004; Teale *et al*., 2006). Through microarray analysis, it was observed that many plant hormone biosynthesis-related genes and plant hormone response-related genes were altered significantly (Table 3), including intermediate product biosynthesis-related

Table 2 Expression alterations of cell wall-related genes in maize infected by *Rice black-streaked dwarf virus* (RBSDV).

Gene ID	Annotation	$Log_2(FC_{ave})$
Polysaccharide synthesis		
541802	Cellulose synthase 1 (CESA 1)	−1.7690
542624	Cellulose synthase 4 (CESA 4)	−0.8558*
541804	Cellulose synthase 6 (CESA 6)	−1.0497
541805	Cellulose synthase 7 (CESA 7)	−1.0397
541806	Cellulose synthase 8 (CESA 8)	−0.6831*
541807	Cellulose synthase 9 (CESA 9)	−2.2089
542685	Cellulose synthase 10 (CESA 10)	−1.1643
100281536	Cellulose synthase-like family F (CSLF3)	−0.8388*
100280709	Dynamin-related protein C (DRP)	−1.8552
100285082	KOBITO1 (KOB1)	0.7004
Reassembly and structural proteins		
100281146	Endo-1,4-β-glucanase	1.1302
541706	Endo-1,3–1,4-β-D-glucanase	−0.6380*
100285111	β-Galactosidase	1.4255
100285007	Ploygalacturonase	−1.4066
100285881	Polygalacturonase	1.0819
100281324	Glycosyl transferase (GT47)	1.3259
100283642	Glycosyl transferase (GT1)	0.6986
100284356	Pectate lyase	5.1699
100282661	Pectinesterase inhibitor	−1.0353
542725	Glycine-rich protein 1	0.9552
100282674	Fasciclin-like arabinogalactan protein 8	1.3574

*To conform with the criterion for data filtering, these data were not used for cluster analysis, and are listed here only for comparison with other microarray results.

Fold change (FC) = (normalized signal of RBSDV-inoculated sample)/(normalized signal of mock-inoculated sample). FC_{ave} is the mean of four replicates in our analyses.

enzymes (e.g. 6-phosphogluconolactonase [Gene ID: 100304292]) and auxin (indole-3-acetic acid, IAA) response genes (e.g. Aux/IAA family [Gene ID: 100283352], SAUR (small auxin up RNA, mRNA increase in response to auxin) family [Gene ID: 100184202], auxin response factor (ARF) [Gene ID: 100283409], [Gene ID: 100185200]). Moreover, we also detected that sex determination-related genes, such as *Anther ear* [Gene ID: 542253] (Bensen *et al*., 1995; Bortiri and Hake, 2007; Tanurdzic and Banks, 2004), flowering time-related genes, such as *Flowering locus D* [Gene ID: 100281678] (He *et al*., 2003), anther structure-related genes, such as anther-specific proline-rich protein gene [Gene ID: 100284023] (Evrard *et al*., 1991), and pollen development-related genes, such as UDP-sugar pyrophosphorylase gene [Gene ID: 100285949] (Schnurr *et al*., 2006), were altered dramatically. Expression alterations of the above genes could affect maize development directly.

Validation by Northern blot analyses

The microarray revealed the expression profiles of thousands of genes. In order to assess the validity of these microarray data, eight genes with varying degrees of alteration were selected to verify some of the results by Northern blotting. The probes for Northern blotting were synthesised by reverse transcription and touch-down polymerase chain reaction (PCR) with degenerate primers (Table S1, see Supporting Information). The results of Northern blotting were consistent with the microarray data (Fig. 3), suggesting that the alterations in gene expression detected by microarray analysis reflect the actual transcription differences in mock-inoculated and RBSDV-infected maize plants.

DISCUSSION

Dwarfing symptoms and their relation to CESA

Plant height, especially that of maize, is an important agronomic trait, and is often correlated with the establishment of cell size and cell shape, which are associated with metabolism and new cell plate biosynthesis (Reiter *et al*., 1993; Somerville *et al*., 2004). The cell wall is composed mostly of polysaccharides (Carpita and Gibeaut, 1993; McCann *et al*., 2001; Ridley *et al*., 2001; Somerville, 2006), a major class of which is cellulose. Previous studies have shown that some important membrane proteins are involved in cellulose synthesis, such as KOBITO1 (KOB1), KORRIGAN1 (KOR1) and DRP (Nicol *et al*., 1998; Pagant *et al*., 2002; Xiong *et al*., 2010). Of the numerous genes involved in cell wall biosynthesis, those encoding the CESA superfamily, which contain CESA active subunits and CESA-like (CLS) proteins, are very important. Recent research has shown that the CSL D4 rice mutant, *nd1*, has an obvious dwarf phenotype (Li *et al*., 2009), similar to the symptom of rice infected by either RBSDV or *Rice dwarf virus* (RDV).

Plant height can be influenced by infections with different viruses. Compared with the stunting symptom of rice infected by

Table 3 Expression alterations of development-related genes in maize infected by *Rice black-streaked dwarf virus* (RBSDV).

Gene ID	Annotation	$\log_2(FC_{ave})$
Plant hormone biosynthesis		
541781	Dihydrolipoamide *S*-acetyltransferase	0.9231
100304292	6-Phosphogluconolactonase	0.7668
100284499	Phospho-2-dehydro-3-deoxyheptonate aldolase 1	−1.6588
100282968	Phosphomevalonate kinase	0.6500
100281237	Phosphoglycerate kinase	−1.2948
606484	Deoxy xylulose synthase 1	−1.4430
542316	Enolase 2	−1.8034
542598	Malate dehydrogenase 5	−1.8034
542578	2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	1.1068
100136883	Phytoene synthase 3	−1.8609
Plant hormone response		
100285214	Aux/IAA family IAA25-auxin responsive	1.0230
100283352	Aux/IAA family IAA7-auxin responsive	−0.8990
100284202	SAUR family member SAUR20-auxin responsive	−1.3317
100283200	SAUR family member SAUR55-auxin responsive	−1.0590
100286042	SAUR family member SAUR40-auxin responsive	−1.1178
541998	Auxin import carrier	−1.0000
542427	Auxin induced protein	−1.5862
100281451	Auxin efflux carrier	−2.2178
542294	Auxin-binding protein homologue 4	0.7588
100285200	Auxin transporter-like protein 1	−1.5547
100283409	ARF, auxin response factor 75	−2.4983
100285200	ARF, auxin response factor 1	−1.5547
100281703	Auxin-independent growth promoter	0.6148
100285167	Auxin-independent growth promoter-like protein	−2.4127
100101521	Zein	0.8011
100285966	Ethylene response factor 1	2.3903
100284152	Jasmonate-induced protein	−1.6915
100281173	Cytokinin-*O*-glucosyltransferase 3	0.9601
Flowering related		
542253	Anther ear 1	−1.0538
100281678	Flowering locus D	0.6616
100284023	Anther-specific proline-rich protein	−1.3339
100285949	UDP-sugar pyrophospharylase	0.8822

Fold change (FC) = (normalized signal of RBSDV-inoculated sample)/(normalized signal of mock-inoculated sample). FC_{ave} is the mean of four replicates in our analyses.

Rice stripe virus (RSV), dwarfism is more characteristic of rice infected by RDV and of maize infected by RBSDV. According to the microarray data published (Satoh *et al*., 2010; Shimizu *et al*., 2007), we compared the alterations in cell wall-related genes caused by the three viruses. The results showed that *CESA* genes were altered in the same direction (decreased significantly) by RSV, RDV and RBSDV infection (Fig. 4A), but the alterations in structural proteins and cell wall assembly-related genes differed (Fig. 4B, C). In combination with the phenotype of the CSL D4 mutant *nd1* (Li *et al*., 2009), these comparative analyses suggest that, in rice infected with these viruses, both *CESA* genes and structure assembly-related genes contribute to dwarfism formation; however, for RBSDV-infected maize, the decrease in expression of *CESA* genes likely contributes more to dwarfing symptoms.

Possible reasons for infertility in diseased plants

The major damage caused by maize rough dwarf disease is the decrease, or even loss, of fertility. It is true that insufficient accumulation of nutrients caused by the inhibition of vegetative development is one reason, but other factors (including hormone regulation, sex determination, flowering time and anther structure) are more relevant.

Auxin, which is an important plant hormone, can have a significant effect on plant development at low concentrations (Teale *et al*., 2006). In addition to being associated with cell expansion and division, auxin can affect anther dehiscence, pollen maturation and filament elongation (Cecchetti *et al*., 2008). It can also reverse the male sterility caused by environmental stresses, e.g. high temperature, and associated with pathogen infection (Padamanabban *et al*., 2005; Sakata *et al*., 2010). Some types of F-box protein, such as TIR1, are auxin receptors; therefore, auxin can regulate the expression of certain genes in combination with ARF and auxin response element (ARE) via the ubiquitin pathway (Teale *et al*., 2006; Ulmasov *et al*., 1999). In our microarray results, we did not obtain auxin biosynthesis patterns directly through hormone biosynthesis-related gene alterations, but observed that auxin carrier genes (e.g. Table 3 [Gene ID: 100281451]) and most

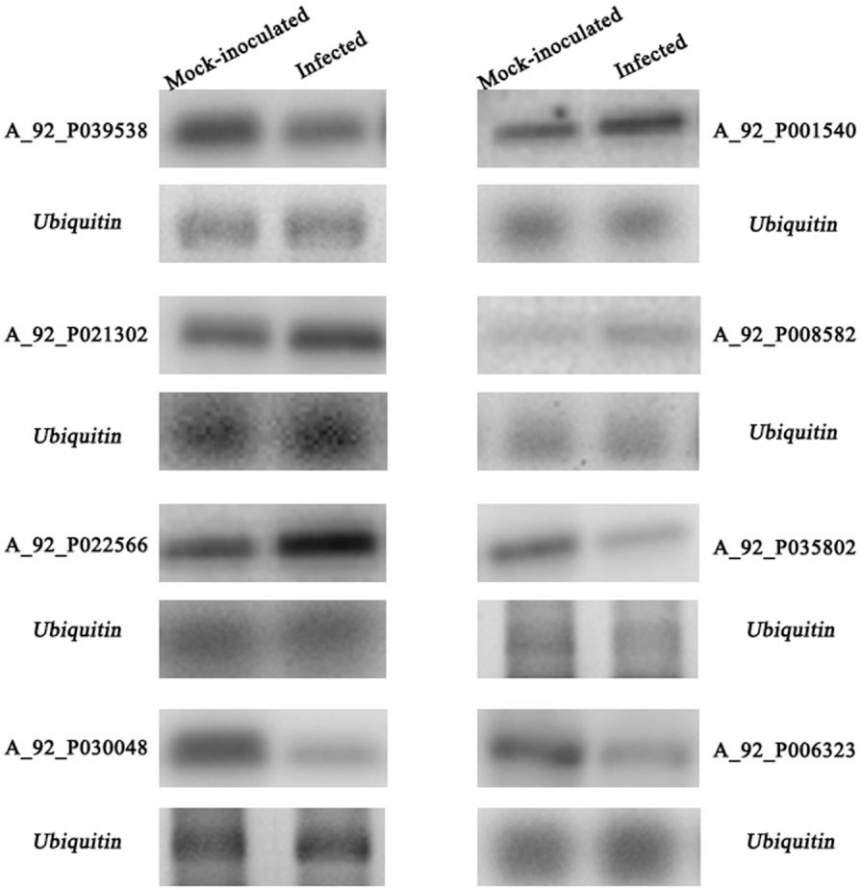


Fig. 3 RNA gel blot analyses of the expression alterations of eight representative genes after infection of maize by *Rice black-streaked dwarf virus* (RBSDV). Approximately 1 μg of poly(A)-rich RNA was prepared for each hybridization reaction. The reverse transcription-polymerase chain reaction (RT-PCR) products of eight genes expressing differentially in microarray analyses were used to prepare the probes. The labels represent the source information of the probes: A_92_P039538, RNA-binding S; A_92_P021302, *Flowering locus D*; A_92_P022566, *Mlo*; A_92_P030048, autophagy-related 7; A_92_P001540, cellulose synthesis-related gene; A_92_P008582, lipoxygenase; A_92_P035802, cellulose synthase; A_92_P006323, *Anther ear 1*. (Details of the related information are included in Table S1.) Rehybridization with the *ubiquitin* probe was performed after nylon membrane stripping with boiling sodium dodecylsulphate (SDS) solution. 'Mock-inoculated' indicates the mock-inoculated maize samples, and 'Infected' indicates maize samples infected with RBSDV after inoculation.

of the SAUR family genes (e.g. Table 3 [Gene ID: 100284202]) decreased significantly; this suggests that the level of auxin can decrease. The decrease in ARF means that genes regulated by auxin may also be down-regulated. Under these conditions, auxin-related anther and pollen development will be oppressed.

Maize is an example of a monoecious species that produces only unisexual flowers in male and female inflorescences, referred to as the tassel and ear, respectively (Tanurdzic and Banks, 2004). Unisexuality in maize occurs through the selective elimination of pistils in tassel florets (Irish and Nelson, 1989). The maize mutant (*an1*) of the anther ear gene *An1*, which has been confirmed to be involved in the synthesis of ent-kaurene, the first tetracyclic intermediate in the gibberellin (GA) biosynthetic pathway (Bensen *et al*., 1995), exhibits recessive and masculinized ears by preventing stamen abortion in female florets (Wu and Cheung, 2000). In our results, the expression of the *Anther ear* gene was decreased significantly (Table 3). Hence, it may be a factor underlying the dwarfing symptom and fertility anomaly.

We also observed that the expression of the *Flowering locus D* gene increased and the anther-specific proline-rich protein gene decreased. By histone acetylation, the *Flowering locus D* gene can down-regulate *Flowering locus C*, a MADS-box transcription factor that blocks transition from vegetative to reproductive development, for normal developmental regulation (Michaels and Amasino, 1999; He *et al*., 2003). In addition, anther-specific proline-rich protein is involved in the maintenance of anther structure. Our results show that the flowering programme is switched on ahead of time, and that the anther structure may be abolished to some extent.

Taken together, the infertility caused by RBSDV infection may be a result of an imbalance of auxin biosynthesis and auxin-mediated pathways, disturbance of sex determination and interference with the flowering schedule and anther structure.

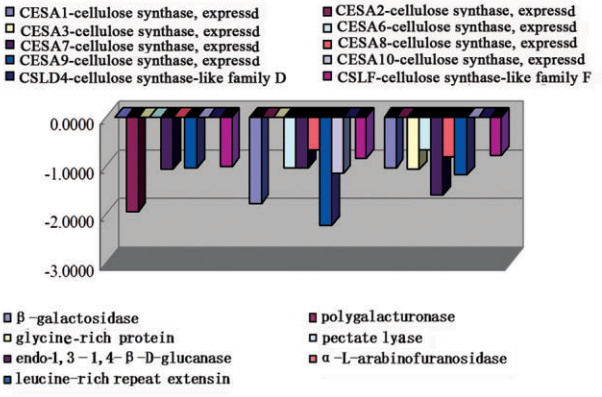
Plant immunity-related genes

Many resistance genes have been identified against fungi, bacteria and viruses, and most elicit a resistance signal and switch on the death programme in infected cells on recognition of the pathogen (Buckner *et al*., 1998; Maule *et al*., 2002; Staskawicz *et al*., 1995; Whitham *et al*., 2006). The *Mlo* gene, found in barley and in most plants, works differently. The resistance against the barley powdery mildew pathogen originates from the *Mlo* mutant gene *mlo*, and confers a broad-spectrum resistance to almost all known isolates of the fungal pathogen in barley (Buschges *et al*., 1997); the resistance remains after knocking down *Mlo* expression by

A

Cellulose synthesis related gene	Alteration caused by RDV (1)	by RBSDV	by RSV(2)
CESA1 - cellulose synthase, expressed	ns	-1.7691	-1.03
CESA2 - cellulose synthase, expressed	-1.9499	nd	ns
CESA3 - cellulose synthase, expressed	ns	nd	-1.08
CESA6 - cellulose synthase, expressed	ns	-1.0497	-0.69
CESA7 - cellulose synthase, expressed	-1.0635	-1.0397	-1.6
CESA8 - cellulose synthase, expressed	ns	-0.6831	-0.82
CESA9 - cellulose synthase, expressed	-1.0426	-2.2089	-1.16
CESA10 - cellulose synthase, expressed	ns	-1.1643	ns
CSLD4 - cellulose synthase-like family D	ns	nd	ns
CSLF - cellulose synthase-like family F;	-1.0144	-0.8388	-0.7782
Reassembly & Structural protein			
β-galactosidase	-1.8427	1.4255	-0.5142
Polygalacturonase	-1.7528	1.0819	ns
Glycine–rich protein	-4.00185	0.9552	0.4667
Pectate lyase	-2.3730	0.9415	ns
Endo-1,3-1,4-β-D-glucanase	-1.6651	-0.638	ns
α-L-arabinofuranosidase	-3.8620	nd	ns
Leucine-rich repeat extensin	-3.9561	ns	-0.1567

B



C

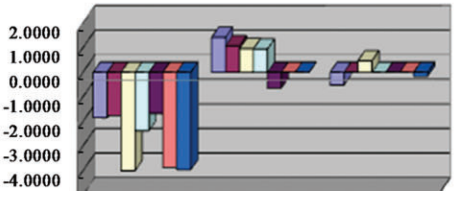


Fig. 4 Comparison of microarray data of the differential expression of cell wall-related genes caused by infection with each of three viruses [*Rice dwarf virus* (RDV), *Rice black-streaked dwarf virus* (RBSDV) and *Rice stripe virus* (RSV)]. (A) Table showing data collected from: (1) Shimizu *et al*. (2007); the fold change (FC) data in this report were calculated from the normalized signal of mock/normalized signal of RDV infection. Thus, we modified the data according to $\log_2$(normalized signal of inoculated/normalized signal of mock) in our comparison; (2) Satoh *et al*. (2010); we chose the data reflecting the gene expression patterns 9–12 days post-inoculation (the virus population accumulated dramatically at this stage). ns, not significant. nd, not detected. (B) Comparison of microarray data of differentially expressed cellulose synthesis-related genes caused by infection with RDV, RBSDV or RSV. The expression alterations of the cellulose synthesis gene family caused by infection with the three viruses were in the same direction. (C) Comparison of the microarray data of differentially expressed structure- or reassembly-related genes caused by infection with RDV, RBSDV or RSV. The expression alterations on infection with each of the three viruses differed to varying degrees.

RNA interference (RNAi) (Schweizer *et al*., 2000). It has been suggested that *Mlo* genes can block multiple defence-related functions. In our analysis, we observed that *Mlo* homologue gene 8 in maize increased almost four-fold post-RBSDV infection [Table 1, $\log_2$(FC) = 1.9]. No previous study has focused on the *Mlo* gene during virus interaction; however, according to its action mechanism, we suggest that RBSDV may induce and take advantage of an increase in *Mlo* gene expression to repress certain defence-related genes for infection development. Thus, the *Mlo* gene could be a potential target candidate in breeding for virus resistance.

ATG and plant immunity

During the process of cell death, degradation is the first step. Autophagy, which comprises two patterns, microautophagy and macroautophagy, is one of the most important degradation pathways. During the autophagy process, cytoplasmic materials, such as long-term proteins and abnormal organelles, can be enclosed in double-membrane-bound vesicles that are targeted to the vacuole and lysosome for degradation (Levine and Klionsky, 2004). In *Arabidopsis*, research on autophagosome formation has provided detailed information that ATG4 and ATG7 are necessary for the ATG8–phosphatidylethanolamine complex to enter into autophagosomes (Abeliovich *et al*., 2000). In *Nicotiana benthamiana*, autophagosome formation was blocked and virus expansion was not confined by the hypersensitive response when the ATG *Beclin1* was silenced (Liu *et al*., 2005). This means autophagy is not dispensable for plant immunity, especially for virus resistance. In our microarray results, we found that *ATG7* expression decreased by about 70% and *ATG4* expression increased by 1.9-fold [Table 1, $\log_2$(FC) of −1.4560 and 0.9828, respectively]. This implies that RBSDV infection influences the autophagy pathway. The relationship between this influence and maize resistance could be an interesting project to explore.

Possible mechanisms of the alteration in gene expression

There have been many reports on virus-induced gene expression alteration, but the mechanisms remain unknown. Modification taking place at the transcript level (changing the amount of mRNA by an effect on a promoter, transcription factor, enhancer or inhibitor) is one mechanism. Run-on analysis has shown that alterations in the expression of many host genes caused by virus take place at the transcriptional level in *N. benthamiana* and tomato (*Solanum lycopersicum*) (Havelda *et al*., 2008). Previous results have indicated that certain proteins, which are either encoded or induced by virus infection, can serve as transcription inducers or suppressors in human macrophages (Eisen-Vandervelde *et al*., 2004). Modifications taking place at the post-transcriptional level (degrading target mRNA by endogenous small RNA-directed RNA interference, such as microRNA and ta-siRNA) represent another mechanism. As with most other plant viruses, RBSDV encodes a protein (P6) that functions as an RNA silencing suppressor, and this suppressor can interfere with host DNA methylation (Zhang *et al*., 2005). Hence, it is possible that the P6 protein may play a role in the transcriptome alteration caused by RBSDV infection.

EXPERIMENTAL PROCEDURES

Plant growth conditions and inoculation with RBSDV

Wheat plants infected with RBSDV were collected as a virus source from Hebei Province, China. Seeds of the maize inbred line Zheng 58 were soaked for 24 h at room temperature, and kept wet at 37 °C for 24 h in the dark after the water had been discarded. Germinated seeds were sown in a pot filled with 300 mL of commercial nutrient soil and submerged in water inside a container. These pots were kept in a glasshouse at 25 °C under natural sunlight. Fifteen-day-old (three leaves) seedlings were exposed to small brown planthoppers carrying RBSDV (five insects on each seedling) for 3 days in specific inoculation chambers. The inoculated plants were taken after removing the insects completely, and grown in a glasshouse at 25 °C under natural sunlight until white streaks emerged on the first upper newly expanded leaf. The aerial parts of whole plants were harvested.

RNA extraction

Total RNA was extracted from RBSDV-infected and mock-inoculated plants. To reduce the experimental variation, four sets of seedlings, each including 10 virus-infected and 10 mock-inoculated seedlings, were harvested. Each set of 10 seedlings was homogenized with a mortar and pestle in liquid nitrogen as one sample, and RNA was extracted with Trizol agent (TianGen, Beijing, China). The total RNA concentration was examined with a spectrophotometer (Nanodrop ND-2000, ThermoFisher Scientific, Wilmington, DE, USA), and the RNA sample integrity was verified by a Bio-Analyzer 2100 (Agilent Technologies, Waldbronn, Germany).

Microarray hybridization and data analysis

A 2-μg aliquot of total RNA from RBSDV-inoculated and mock-inoculated plants was used to synthesize fluorophore-labelled cRNA using cyanine 3-CTP (Cy3C) as described previously ('One-color microarray-based gene expression analysis protocol', Version 5.5; Agilent Technologies). Samples were purified using a Qiagen RNeasy Kit (Qiagen, Hilden, Germany). The quality of cyanine-labelled cRNA samples, including yield, concentration, amplification efficiency and abundance of cyanine fluorophore, was determined by an ND-1000 spectrophotometer at A_{260} and A_{550}. Once the concentration had been determined, cyanine-labelled cRNA fragmentation and microarray slide hybridization followed (Agilent Technologies). After hybridization, the microarray slides were washed according to the standard Agilent protocol and scanned on a microarray at 550 nm using an Agilent microarray scanner (G2505B). The information produced by the scanner was loaded into the image analysis program 'Feature Extraction

version 9.5' to establish standard data for statistical analysis, and all microarray slides were checked for background evenness.

Datasets were further analysed according to published procedures that consisted of one-colour experimental methods and utilized gProcessedSignal values determined using Agilent Feature Extraction software, including aspects of the signal-to-noise ratio, spot morphology and homogeneity (Gobert *et al*., 2009; Patterson *et al*., 2006). If the processed signal intensity was less than two-fold the value of the processed signal error, the features were deemed 'Absent'. If the measured intensity was at a saturated value or if there was a substantial amount of variation in the signal intensity within pixels of a particular feature, the features were deemed 'Marginal'. Otherwise, the features were deemed 'Present'. Data points were included only if 'Present' or 'Marginal' and probes were retained if all data points were 'Present'. Experimental data were analysed using GeneSpring software, version 7.3.1 (Agilent Technologies/Silicon Genetics, Redwood City, CA, USA). The FC analysis data were filtered by *t*-test ($P \leq 0.05$), and hierarchical clustering analysis was supported by the Cluster program. The gene ontology analysis was performed at the SBS server of Ebioservice (http://www.ebioservice.com), which was connected to the Gene Ontology website (http://www.geneotology.org). The annotation information was obtained from GenBank and KEGG (http://www.genome.jp/kegg). All the expression profiles are available as Gene Series (GSE) 27021 in the Gene Expression Omnibus (GEO) at the website of the National Center for Biotechnology Information (NCBI).

Reverse transcription and touch-down PCR

After removal of DNA by RNase-free DNase (Takara, Dalian, China), 5 μg of RNA was reverse transcribed in a 20-μL reaction mixture containing M-MLV reverse transcriptase (Promega, Madison, WI, USA) with oligo $(dT)_{18}$ and random six-mers (Takara) as primers at 37 °C for 60 min. PCR was performed with 1 μL of cDNA reaction mixture in a final volume of 25 μL using *Taq* DNA polymerase (FunGenome, Beijing, China). The primers, which were designed to generate selected PCR products of 500–1500 bp in length, are listed in Table S1. The touch-down PCR programme was as follows: initial denaturation for 5 min at 95 °C; 10 touch-down cycles with the annealing temperature decreasing by 1 °C per cycle; 25 cycles at the final touch-down temperature; followed by the final extension for 10 min at 72 °C.

Northern blotting

The protocols and sources of maize samples for total RNA extraction were the same as those used for microarray analysis. Poly(A)-rich mRNA was enriched using the PolyATract Kit (Promega) according to the manufacturer's instructions.

Poly(A)-enriched RNA, which was dissolved in formamide, was electrophoresed on 1.2% agarose gel containing formaldehyde. DNA probes, synthesized by reverse transcription and touch-down PCR, were labelled with [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) using a Random Primer Labelling Kit (Takara). RNA hybridization was performed in 50% formamide, 6 × standard saline citrate (SSC), 0.5% (w/v) sodium dodecylsulphate (SDS), 5 × Denhardt's reagent and denatured salmon sperm DNA (100 μg/mL) at 42 °C. Northern blots on nylon membranes were washed in 2 × SSC/0.2% SDS solution at 65 °C (low stringency) or 68 °C (high stringency) for 30 min. Stripping was performed with a boiling solution of 0.1% (w/v) SDS. Typhoon Trio Variable Imager (GE Healthcare, Uppsala, Sweden) was used for signal detection and analysis.

ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (#2012CB114004), a grant (IRT1042) from the Ministry of Education and a grant (2009ZX08003-011B) from the Ministry of Agriculture, China.

REFERENCES

Abeliovich, H., Dunn, W.A., Kim, J. and Klionsky, D.J. (2000) Dissection of autophagosome biogenesis into distinct nucleation and expansion steps. *J. Cell Biol*. **151**, 1025–1034.

Alexandrov, N.N., Brover, V.V., Freidin, S., Troukhan, M.E., Tatarinova, T.V., Zhang, H., Swaller, T.J., Lu, Y.P., Bouck, J., Flavell, R.B. and Feldmann, K.A. (2009) Insights into corn genes derived from large-scale sequencing. *Plant Mol. Biol*. **69**, 179–194.

Bensen, R.J., Johal, G.S., Crane, V.C., Tossberg, J.T., Schenable, P.S., Meelay, R.B. and Briggs, S.P. (1995) Cloning and characterization of the maize *An1* gene. *Plant Cell*, **7**, 75–84.

Bortiri, E. and Hake, S. (2007) Flowering and determinacy in maize. *J. Exp. Bot*. **58**, 899–907.

Buckner, B., Bucker-Janick, D., Gray, J. and Johal, G.S. (1998) Cell-death mechanism in maize. *Trends Plant Sci*. **3**, 218–223.

Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., van Daelen, R., van der Lee, T., Diergaarde, P., Groenenddijk, J., Topsch, S., Vos, P., Salamini, F. and Schulze-Lefer, P. (1997) The barley *MLO* gene: a novel control element of plant pathogen resistance. *Cell*, **88**, 695–705.

Carpita, N.C. and Gibeaut, D.M. (1993) Structure models of primary cell wall in flowering plants: consistency of molecular structure with the physical properties of the wall during growth. *Plant J*. **3**, 1–30.

Casati, P. and Walbot, V. (2003) Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. *Plant Physiol*. **132**, 1739–1754.

Cecchetti, V., Altamura, M.M., Falasca, G., Costantino, P. and Cardarelli, M. (2008) Auxin regulates Arabidopsis anther dehiscence, pollen maturation, and filament elongation. *Plant Cell*, **20**, 1760–1774.

Chen, S.X., Hong, J., Lu, Y.P. and Lei, J.L. (2004) Study on relation between RBSDV infecting stages in maize vein cell and active transmission of *Laodelphax striatellus* Fallen. *Virol. Sin*. **19**, 153–157.

Dardick, C. (2007) Comparative expression profiling of *Nicotiana benthamiana* leaves systemically infected with three fruit tree viruses. *Mol. Plant–Microbe Interact*. **20**, 1004–1017.

Dayakar, B.V., Lin, H., Chen, C., Ger, M., Lee, B., Pai, C., Chow, D., Huang, H., Hwang, S., Chung, M. and Feng, T. (2003) Ferredoxin from sweet pepper (*Capsicum annuum* L.) intensifying harpin(pss)-mediated hypersensitive response shows an enhanced production of active oxygen species (AOS). *Plant Mol. Biol*. **51**, 913–924.

Dowd, C., Wilson, I.W. and McFadden, H. (2004) Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp. *vasinfectum*. *Mol. Plant–Microbe Interact*. **17**, 654–667.

Eisen-Vandervelde, A.L., Waggoner, S.N., Yao, Z.Q., Cale, E.M., Hahn, C.S. and Hahn, Y.S. (2004) Hepatitis C virus core selectively suppresses interleukin-12 synthesis in human macrophage by interfering with AP-1 activation. *J. Biol. Chem*. **279**, 43 479–43 486.

Espinoza, C., Medina, C., Somerville, S. and Arce-Johnson, P. (2007) Senescence-associated genes induced during compatible viral interactions with grapevine and Arabidopsis. *J. Exp. Bot*. **58**, 3197–3212.

Evrard, J., Jako, C., Saint-Guily, A., Weil, J. and Kuntz, M. (1991) Anther-specific, developmentally regulated expression of genes encoding a new class of proline-rich protein in sunflower. *Plant Mol. Biol*. **16**, 271–281.

Gardiner, J., Schroeder, S., Polacoo, M.L., Sanchez-villeda, H., Feng, Z., Morgante, M., Landewe, T., Fengler, K., Useche, F., Hanafe, M., Tingey, S., Chou, H., Wing, R., Soderlund, C. and Core, E. (2004) Anchoring 9371 maize expression

sequence tagged unigenes to the bacterial artificial chromosome contig map by two dimensional overgo hybridization. *Plant Physiol*. **134**, 1317–1326.

Gobert, G.N., Moertel, L., Brindley, P.J. and McManus, D.P. (2009) Developmental gene expression profiles of the human pathogen *Schistosoma japonicum*. *BMC Genomics*, **10**, 128–146.

Golem, S. and Culver, J.N. (2003) Tobacco mosaic virus induced alterations in the gene expression profile of *Arabidopsis thaliana*. *Mol*. *Plant–Microbe Interact*. **16**, 681–688.

Havelda, Z., Varallyay, E., Valoczi, A. and Burgyan, J. (2008) Plant virus infection-induced persistent gene down-regulation in systemically infected leaves. *Plant J*. **55**, 278–288.

He, Y., Michael, S.D. and Amasino, R.M. (2003) Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science*, **302**, 1751–1754.

Irish, E.E. and Nelson, T. (1989) Sex determination in monoecious and dioecious plants. *Plant Cell*, **1**, 737–744.

Isogai, M., Uyeda, I. and Lee, B. (1998) Detection and assignment of proteins encoded by rice black-streaked dwarf fijivirus S7, S8, S9 and S10. *J. Gen. Virol*. **79**, 1487–1494.

Kalluri, U.C. and Joshi, C.P. (2004) Differential expression patterns of two cellulose genes are associated with primary and secondary cell wall development in aspen trees. *Planta*, **220**, 47–55.

Kanehissa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T. and Yamanishi, Y. (2008) KEGG for linking genomes to life and the environment. *Nucleic Acids Res*. **36**, D480–D484.

Kuribayashi, K. and Shinkai, A. (1952) On the rice black-streaked dwarf disease, a new disease of rice in Japan. *Ann. Phytopathol. Soc. Jpn*. **16**, 41.

Levine, B. and Klionsky, D.J. (2004) Development by self-digestion: molecular mechanism and function of autophagy. *Dev. Cell*, **6**, 463–477.

Li, M., Xiong, G., Li, R., Cui, J., Tang, D., Zhang, B., Pauly, M., Cheng, Z. and Zhou, Y. (2009) Rice cellulose synthase-like D4 is essential for normal cell-wall biosynthesis and plant growth. *Plant J*. **60**, 1055–1069.

Liang, C., Jaiswal, P., Hebbard, C., Avraham, S., Buckler, E.S., Casstevens, T., Hurwitz, B., McCouch, S., Ni, J., Pujar, A., Ravenscroft, D., Ren, L., Spooner, W., Tecle, I., Thomason, J., Tung, C., Wei, X., Yap, I., Youens-Clark, K., Ware, D. and Stein, L. (2008) Gramene: a growing plant comparative genomics resource. *Nucleic Acids Res*. **36**, D947–D953.

Liu, H., Wei, C., Zhong, Y. and Li, Y. (2007) Rice black-streaked dwarf virus minor core protein P8 is a nuclear dimeric protein and represses transcription in tobacco protoplasts. *FEBS Lett*. **581**, 2534–2540.

Liu, Y., Schiff, M., Czymmek, K., Tallóczy, Z., Levine, B. and Dinesh-Kumar, S.P. (2005) Autophagy regulates programmed cell death during the plant innate immune response. *Cell*, **121**, 567–577.

Marathe, R., Guan, Z., Anandalakshmi, R., Zhao, H. and Dinesh-Kumar, S.P. (2004) Study of *Arabidopsis thaliana* resistome in response to cucumber mosaic virus infection using whole genome microarray. *Plant Mol. Biol*. **55**, 501–520.

Matsumura, T., Kimata-Ariga, Y., Sakakibara, H., Sugiyama, T., Murata, H., Takao, T., Shimonishi, Y. and Hase, T. (1999) Complementary DNA cloning and characterization of ferredoxin localized in bundle-sheath cell of maize leaves. *Plant Physiol*. **119**, 481–488.

Maule, A., Leh, V. and Lederer, C. (2002) The dialogue between virus and hosts in compatible interactions. *Curr. Opin. Plant Biol*. **5**, 279–284.

McCann, M.C., Bush, M., Milioni, D., Sado, P., Stacey, N.J., Catchpole, G., Defernez, M., Carpita, N.C., Hofte, H., Ulvskov, P., Wilson, R.H. and Roberts, K. (2001) Approaches to understanding the functional architecture of the plant cell wall. *Phytochemistry*, **57**, 811–821.

Michaels, S.D. and Amasino, R.M. (1999) Flowering locus C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*, **11**, 949–956.

Milne, R.G., del Vas, M., Harding, R.M., Marzachi, R. and Mertens, P.P.C. (2005) Genus *Fijivirus*. In: *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses* (Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A., eds), pp. 534–542. London: Elsevier/Academic Press.

Moy, P., Qutob, D., Chapman, B.P., Atkinson, I. and Gijzen, M. (2004) Patterns of gene expression upon infection of soybean plants by *Phytophthora sojae*. *Mol. Plant–Microbe Interact*. **17**, 1051–1062.

Nicol, F., His, I., Jauneau, A., Vernhettes, S., Canut, H. and Höfte, H. (1998) A plasma membrane-bound putative endo-1,4-β-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. *EMBO J*. **17**, 5563–5576.

Padamanabban, M.S., Goregaoker, S.P., Golem, S., Shiferaw, H. and Culver, J.N. (2005) Interaction of the tobacco mosaic virus replicase protein with the Aux/IAA protein PAP1/IAA26 is associated with disease development. *J. Virol*. **79**, 2549–2558.

Pagant, S., Bichet, A., Sugimoto, K., Lerouxel, O., Desprez, T., McCann, M., Lerouge, P., Vernhettes, S. and Höfte, H. (2002) *KOBITO1* encodes a novel plasma membrane protein necessary for normal synthesis of cellulose during cell expansion in Arabidopsis. *Plant Cell*, **14**, 2001–2013.

Patterson, T.A., Lobernhore, E.K., Fulmer-Smentek, S.B., Collins, P.J., Chu, T.M., Bao, W., Fang, H., Kawasaki, E.S., Hager, J., Tikhonova, I.R., Walker, S.J., Zhang, L., Hurban, P., de Longueville, F., Fuscoe, J.C., Tong, W., Shi, L. and Wolfinger, R.D. (2006) Performance comparison of one-color and two-color platforms in the MicroArray Quality Control (MAQC) project. *Nat. Biotechnol*. **24**, 1140–1150.

Reiter, W.D., Chapple, C.C. and Somerville, C.R. (1993) Altered growth and cell walls in a fucose-deficient mutant of *Arabidopsis*. *Science*, **261**, 1032–1035.

Rensink, W.A. and Buell, C.R. (2005) Microarray expression profiling resources for plant genomics. *Trends Plant Sci*. **10**, 603–609.

Ridley, B.L., O'Neil, M.A. and Mohnen, D. (2001) Pectin: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, **57**, 929–967.

Sakata, T., Oshino, T., Miura, S., Tomabechi, M., Tsunaga, Y., Higashitani, N., Miyazawa, Y., Takahashi, H., Watanabe, M. and Higashitani, A. (2010) Auxin reverses plant sterility caused by high temperature. *Proc. Natl. Acad. Sci. USA*, **107**, 8569–8574.

Satoh, K., Kondoh, H., Sasaya, T., Shimizu, T., Choi, I.R., Omura, T. and Kikuchi, S. (2010) Selective modification of rice (*Oryza sativa*) genes expression by rice stripe virus infection. *J. Gen. Virol*. **91**, 294–305.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, J.M. (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. *Proc. Natl. Acad. Sci. USA*, **97**, 11 655–11 660.

Schnurr, J.A., Storey, K.K., Jung, H.G., Somers, D.A. and Gronwald, J.W. (2006) UDP-sugar pyrophosphorylase is essential for pollen development in Arabidopsis. *Planta*, **24**, 520–532.

Schweizer, P., Pokorny, J., Schulze-lefert, P. and Dudler, R. (2000) Double strand RNA interferes with cell function at single-cell level in cereals. *Plant J*. **24**, 895–903.

Senthil, G., Liu, H., Puram, V.G., Clark, A., Stromberg, A. and Goodin, M.M. (2005) Specific and common changes in *Nicotiana benthamiana* gene expression in response to infection by enveloped viruses. *J. Gen. Virol*. **86**, 2615–2625.

Shimizu, T., Satoh, K., Kikuchi, S. and Omura, T. (2007) The repression of cell wall- and plastid-related genes and the induction of defense-related genes in rice plant infected with rice dwarf virus. *Mol. Plant–Microbe Interact*. **20**, 247–254.

Somerville, C. (2006) Cellulose synthesis in higher plants. *Annu. Rev. Cell Dev. Biol*. **22**, 53–78.

Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., Osborne, E., Paredez, A., Persson, S., Raab, T., Vorwerk, S. and Youngs, H. (2004) Towards a systems approach to understanding the plant cell wall. *Science*, **306**, 2206–2210.

Staskawicz, B.J., Ausubel, F.M., Ellis, J.G. and Jones, J.D. (1995) Molecular genetics of plant disease resistance. *Science*, **268**, 661–667.

Tanurdzic, M. and Banks, J.A. (2004) Sex-determining mechanisms in land plants. *Plant Cell*, **16**, S61–S71.

Teale, W.D., Paponov, I.A. and Palme, K. (2006) Auxin in action: signaling, transport and the control of the plant growth and development. *Nat. Rev. Mol. Cell Biol*. **7**, 847–859.

Ulmasov, T., Hagen, G. and Guilfoyle, T.J. (1999) Activation and repression of transcription by auxin-response factors. *Proc. Natl. Acad. Sci. USA*, **96**, 5844–5849.

Uzarowska, A., Dionisio, G., Sarholz, B., Piepho, H.P., Xu, M., Ingvardsen, C.R., Wenzel, G. and Lübberstedt, T. (2009) Validation of candidate genes putatively associated with resistance to SCMV and MDMV in maize (*Zea mays* L.) by expression profiling. *BMC Plant Biol*. **5**, 279–284.

Whitham, S.A., Yang, C. and Goodin, M.M. (2006) Global impact: elucidating plant responses to viral infection. *Mol. Plant–Microbe Interact*. **19**, 1207–1215.

Wu, H. and Cheung, A.Y. (2000) Programmed cell death in plant reproduction. *Plant Mol. Biol*. **44**, 267–281.

Xiong, G., Li, R., Qian, Q., Song, X., Liu, X., Yu, Y., Zeng, D., Wan, J., Li, J. and Zhou, Y. (2010) The rice dynamin-related protein DRP2B mediates membrane trafficking and thereby plays a critical role in secondary cell wall cellulose biosynthesis. *Plant J*. **64**, 56–70.

Yang, C., Guo, R., Jie, F., Nettleton, D., Peng, J., Carr, T., Yeakley, J.M., Fan, J.B. and Whitham, S.A. (2007) Spatial analysis of *Arabidopsis thaliana* gene expression in response to turnip mosaic virus infection. *Mol. Plant–Microbe Interact*. **20**, 358–370.

Zhang, C., Liu, Y., Liu, L., Lou, Z., Zhang, H., Miao, H., Hu, X., Pang, Y. and Qiu, B. (2009) Rice black streaked dwarf virus P9-1, an α-helical protein, self-interacts and forms viroplasms *in vivo*. *J. Gen. Virol*. **89**, 1770–1776.

Zhang, H., Chen, J. and Adams, M. (2001) Molecular characterization of segments from 1 to 6 of Rice black-streaked dwarf virus from China provides the complete genome. *Arch. Virol.* **146**, 2331–2339.

Zhang, L., Wang, Z., Wang, X., Zhang, W., Li, D., Han, C., Qu, Y. and Yu, J. (2005) Two virus-encoded RNA silencing suppressors, P14 of Beet necrotic yellow vein virus and S6 of Rice black-streaked dwarf virus. *Chin. Sci. Bull.* **50**, 305–310.

Zou, J., Rodriguez-Zas, S., Aldea, M., Li, M., Zhu, J., Gonzalez, D.O., Vodkin, L.O., DeLucia, E. and Clough, S.J. (2005) Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific down regulation of photosynthesis. *Mol. Plant–Microbe Interact.* **18**, 1161–1174.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Pie chart of gene ontology at the levels of molecular functions and cellular components.

Table S1 Primers used for the synthesis of probes in Northern blotting.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.