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Functional genomics in plant virus research

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Abstract

Virus infections cause physiological changes, symptoms and eventual yield losses in plants. The complex networks of signalling pathways and biosynthetic reaction chains associated with disease development are challenging to elucidate. Similarly, plants respond to virus infection with general and virus-specific defence, which also involve a multitude of physiological changes. The studies on modulation of gene expression in the host plant following infection are pursued to elucidate the basis of the disease or defence. Various methods have been utilized for this purpose. In this report, we briefly review how differential display, Suppression Subtractive Hybridization (SSH), cDNA Amplified Fragment Length Polymorphism (cDNA-AFLP), Macro- and Microarrays, and Serial Analysis of Gene Expression (SAGE) have been employed for the study on virus-induced changes in plant gene expression and summarize their main results from the studies.

Introduction

The impact of viral infections on physiology and metabolism of the host plant is one of the leading research topics in molecular plant virology. The plant responses to virus infections are complex, as indicated by the modulation of transcriptional activity. Since first described (Wang & Maule, 1995), a wealth of data has accumulated on the impact of invading plant viruses on the expression of host genes.

Experimental approaches for investigating the changes in the transcriptional profiles induced by viral infection have evolved with the general, rapid technical improvement of techniques for the selection and analysis of differentially expressed transcripts. The initial differential screening technique (Hedrick et al., 1984) has been modified in various ways to make them less laborious and time-consuming. Many laboratories have switched to use the more sophisticated differential display of cDNA Amplified Fragment Length Polymorphism (cDNA-AFLP) (Bachem et al., 1996) and Suppression Subtractive Hybridization (SSH) (Diatchenko et al., 1996). Pre-existing sequence information on target genes is not required, which makes cDNA-AFLP and SSH applicable to any host species. In many cases, the differentially expressed sequences detected with the aforementioned techniques have been found to correspond to previously unknown genes, evidencing the potential of these techniques for gene discovery studies (Butterfield et al., 2004). These methods contributed to the first bulk of data on rearrangements of plant transcriptome in response to viral infections. Subsequently, the completion of the plant genome projects made fully representative microarrays and gene chips available for genome-wide expression studies (Schaffer et al., 2000; Wu et al., 2001). The full genomic sequences are also an important basis for the Serial Analysis of Gene Expression (SAGE) (Velculescu et al., 1995).

Differential display

One of the most popular differential display approaches is based on amplification of the mRNA 3'-ends. Single-stranded cDNA is synthesized and amplified by Polymerase Chain Reaction (PCR) using a selective oligo-dT primer and a random primer. The products are resolved on a polyacrylamide (sequencing) gel by electrophoresis and the band patterns between samples are compared (Liang and Pardee, 1992).

Many studies on virus-host interactions using differential display have aimed to isolate and characterize virus resistance related genes. Horvath et al. (1998) compared gene expression in suspension cells of tobacco (BY-2) challenged with SA to an untreated culture. The 15 isolated cDNA clones were classified to four groups of SA responsive genes according to the transcription kinetics and the elicitor. The expression kinetics of one gene per class was analysed following TMV infection. The kinetics of transcription was similar in TMV-treated and SA-treated plants.

Vallejos et al. (2000) combined differential display and Bulk Segregant Analysis (BSA) to detect differentially expressed genes associated with resistance to *Bean common mosaic virus* (BCMV, genus *Potyvirus*) conferred by the resistance (*R*) gene *I* in bean (*Phaseolus vulgaris*). A resistant (cv. Jamapa) and a susceptible (cv. Calima) bean genotype were crossed and gene expression compared between the bulked resistant and susceptible progeny. Thirty-one cDNAs were detected only in susceptible BCMV-infected plants and 17 only in the resistant plants and used as probes for segregation and linkage analysis. The genes for 10 cDNAs were linked to the *I* locus.

Yoda et al. (2002) used differential display to compare gene expression in leaves of a TMV-resistant tobacco cultivar before and after TMV infection. Fourteen genes potentially involved in viral defence were identified and their expression patterns examined by northern blot analysis. The *HRR3* transcript, containing a WRKY domain characteristic of DNA-binding proteins, was further studied. Activation of *HRR3* was independent of SA and not affected by mechanical wounding. Localisation of the HRR3 protein in the nucleus was observed using a HRR3-GFP fusion protein. Binding of the GST-HRR3 fusion protein to synthetic oligonucleotides containing part of the W-Box sequence was observed in gel mobility shift assays. HRR3 was unable to induce gene transcription by itself, as shown by transformation of tobacco with the pGAL4-HRR3 fusion protein. The authors suggested that this gene participate specifically in HR and that members of the WRKY family genes are differentially regulated. The results also confirmed that HR can occur through multiple signalling pathways, with or without involvement of SA.

Two further studies on the differentially expressed genes identified by Yoda et al (2002) are briefly described as examples of the typical down-stream investigations. The *HRR5* (Nt7TM1) transcript isolated by Yoda et al (2002) encodes a putative protein with seven transmembrane domains, suggesting involvement in transmission of extracellular signals through mechanisms independent of G-proteins (Yamaguchi et al., 2003). However, wounding of plants or treatment with SA, methyl jasmonate, ethylene and hydrogen peroxyde did not induce the *Nt7TM1* transcript. Use of GFP fusion proteins indicated that the Nt7TM1-GFP fusion protein was located around the nucleus and at the plasma membrane. The authors concluded that the protein may be involved in a unique signalling pathway in pathogen response. Sugimoto et al. (2004), in turn, characterized *HRR11* (Yoda et al., 2002) that was induced only in resistant tobacco genotypes and the expression declined when expression of PR proteins was initiated. Moreover, treatment with jasmonate and ethylene but not SA induced the gene. The deduced protein contains three conserved motifs characteristics of the AAA-type ATPases and was designed NtAAA1. Indeed, NtAAA1 could hydrolyse ATP and the activity was greatly enhanced by Mg²⁺. The NtAAA1-GFP fusion protein was localized in the cytoplasm. Silencing of *HRR11* provoked a higher accumulation of three HR-related proteins. This implies that NtAAA1 functions in mitigation of the HR.

Wada et al. (2004) used differential display to isolate transcripts that specifically accumulate in transgenic tobacco plants expressing an anti-sense construct of a DNA methyltransferase gene. Among the 31 differentially expressed genes, analysis of transcription and methylation of *ntALix1* provided evidence that the methylation pattern changed and that transcription was induced after TMV infection. The observations suggested that changes in methylation status due to virus infection trigger the activation of many resistance-related genes.

A few studies have sought for information about genes that a differentially expressed in susceptible plants infected with viruses. Differential Display PCR (DD-PCR) and amplified cDNA screening were used for the study on gene expression in transgenic *Arabidopsis thaliana* (Baji-A15) expressing the VI protein, an important virulence determinant of *Cauliflower mosaic virus* (CaMV, genus *Caulimovirus*), that alone confers a virus symptom-like phenotype in the transgenic plants (Geri et al., 1999). In this approach, first-strand cDNA synthesis was carried out with three special, 1-base anchored primers (H-T₁₁). For PCR amplification of cDNA, one of eight 13-mers (H-AP primers; GenHunter) and one of the three H-T₁₁ primers were used. Abundances of the transcripts (cDNAs) were estimated and compared using dot-blot hybridization and measurement of signal intensities. Ten genes were up-regulated and eight genes were down-regulated in both the transgenic and CaMV-infected non-transgenic (wt) plants, as compared the healthy wt plants. Only two genes showed an opposite change in the transgenic and CaMV-infected wt plants. The up-regulated genes included one for a putative protein homologous to myb proteins. This result was consistent with induction of a *myb* gene in TMV-resistant tobacco plants following TMV infection (Yang and Klessig,

1996). One of the down-regulated genes encoded a putative sulfotransferase protein. A sulfotransferase gene had previously been studied in *Arabidopsis* and found to be induced during HR or in response to SA and methyl jasmonate (Lacomme and Roby, 1996).

Anaya-Lopez et al. (2005) used differential display to understand the interaction between pepper (*Capsicum chinense*) and *Pepper huasteco yellow vein virus* (or Pepper huasteco virus; BHYVV, genus *Begomovirus*). They compared gene expression between mock-inoculated and BHYVV-inoculated, resistant plants, and sequenced three of the 45 differentially expressed transcripts. The deduced amino acid sequences of two transcripts had some identity to a methyltransferase protein and a NADP malic enzyme. Methyltransferase mutants in *Arabidopsis* exhibit impaired transcriptional and post-transcriptional gene silencing (Vaucheret et al., 2001). A gene for NADP malic enzyme of *Cucurbita pepo* is induced during infection by *Cucumber mosaic virus* (CMV, genus *Cucumovirus*) (Havelda and Maule, 2000).

Selective differential display strategies

Differential display can be carried out in a more selective way, so to capture and analyse transcripts of a targeted functional or structural group of genes. Chen and Chen (2000) adopted a slightly modified differential display strategy for targeted isolation of tobacco genes encoding WRKY DNA-binding proteins. The targeted differential display strategy used a degenerate primer and an oligo-dT primer for PCR-based amplification of cDNA from TMV-resistant and susceptible tobacco plants following infection with TMV. Two cDNA fragments, tWRKY3 and tWRKY4, were identified. The corresponding proteins were expressed *in vitro*. They recognized the W-box (TTGAC) but had a zinc-finger structure significantly different from other WRKY proteins. tWRKY3 and tWRKY4 were induced by TMV infection, which preceded the increase of expression of TBDA12 and many other genes related to plant defence. SA treatment induced the expression of tWRKY3 and tWRKY4. The authors postulated that transcriptional activation of the tWRKY3 and tWRKY4 genes and the enhanced phosphorylation of the corresponding proteins may be involved in the induction of TBDA12 complex during pathogen infection or SA treatment.

Sequence-Specific DNA Binding (SSDB) factors involved in the transcriptional activation of genes associated with HR to infection with *Tobacco mosaic virus* (TMV, genus *Tobamovirus*) were isolated using an oligo-based selection procedure by Wang et al. (1998). Briefly, these authors first selected oligonucleotides containing binding sequences for SSDB factors. The oligonucleotides were labelled and mixed with nuclear extracts from TMV-infected resistant or susceptible plants. By gel retardation assays, they identified two SSDB activities substantially enhanced and five SSDB activities suppressed during the TMV-induced HR. In order to identify the cDNAs encoding the corresponding SSDB factors, the oligonucleotides were labelled and used as probes in South-Western analysis to screen a cDNA library prepared from TMV-infected tobacco plants. Two clones corresponding to a unique SSDB factor (TDBA12) containing a highly conserved WRKY sequence were further studied. The TDBA12 protein always recognized a conserved 5-bp sequence (TTGAC). Transcription of TDBA12 was induced by salicylic acid (SA) or its biologically active analogues that induce pathogenicity-related (PR) genes. Enhanced binding activity of TDBA12 required protein-protein interactions and protein phosphorylation (Yang et al., 1999). Furthermore, DNA-binding activity similar to TDBA12 recognized specifically a W-box (TGACNNNNNNGTCA) in the promoter sequence of a class I chitinase gene (*CHN50*) involved in plant defence, which occurred after activation of TDBA12 (Yang et al., 1999).

To identify downstream target genes of WRKY proteins, Du and Chen (2000) screened the *Arabidopsis* genome to identify genes containing the [(T)GAC(C/T)] W-box sequence in the promoter region. Four genes coding for receptor-like protein kinases were identified. These genes were induced by salicylic acid treatment. The W-box elements of the promoter sequence of at least one of these genes were recognised by a WRKY protein. Their results emphasized that WRKY proteins regulate transcription of genes potentially involved in plant defence but also genes encoding proteins with regulatory functions.

cDNA-AFLP and SSH

Although cDNA-AFLP and SSH both utilize Polymerase Chain Reaction (PCR), they differ in many characteristics that can affect the choice of the researcher towards either method. cDNA-AFLP is sensitive and able to identify low-abundance targets, but it is expensive, technically difficult and rather prone to providing false positive results. On the other hand, SSH generates subtracted libraries containing only differentially expressed transcripts, normalizes the high- and low- expressed genes and makes detection of lower albeit differently expressed genes more likely. The SSH is relatively less expensive and easy to carry out, although many clones obtained may contain the same transcript.

The actual modulation of expression levels of the selected transcripts always needs to be reconfirmed using independent methods, such as northern blot analysis or real-time reverse transcription PCR (rtRT-PCR).

In tobacco plants (*Nicotiana tabacum* L.) infected with *Tobacco mosaic virus* (TMV, genus *Tobamovirus*), differential gene expression was analyzed in an incompatible TMV-host interaction expressed as a hypersensitive response (HR) using the standard methods of differential screening (Guo et al., 1998; 2000). Six groups of tobacco genes were isolated and cloned, whose expression was associated with local (LAR) and systemic acquired resistance (SAR). These genes were induced via a salicylic acid (SA) independent and in some cases also ethylene-independent pathways (Guo et al., 2000). In pepper plants (*Capsicum annuum* L.) responding with HR to TMV infection, differential screening revealed 22 unique genes that were up-regulated in the course of TMV infection, 16 of which were specifically associated with HR. They included genes involved in carbon metabolism and general metabolic processes, pathogenesis-related (PR) protein production and other plant defence mechanisms (Shin et al., 2001). This work was followed up by a series of successive studies, in which the expression and activity of eight of the HR-induced genes were further characterised.

The HR-specific gene expression profile in *Chenopodium* spp. was analyzed by Cooper (2001) using cDNA-AFLP and rtRT-PCR. *Chenopodium* spp. can limit the infection of many different viruses to inoculated leaves, which is visualized by development of local chlorotic or necrotic lesions. The author described 13 HR-related genes responsive to infections with two unrelated viruses, TMV and *Tobacco rattle virus* (TRV). Two genes contained typical domains of resistance (*R*) genes, whereas other genes were assigned specific roles in SA-dependent and SA-independent signalling pathways and cell detoxification.

cDNA-AFLP has also been used to provide insights on gene expression alterations associated with compatible plant-virus interactions. In apricot (*Prunus armeniaca*), the gene expression pattern was analyzed upon infection with *Plum pox virus* (PPV, genus *Potyvirus*) of a PPV-tolerant cultivar. Twenty-one genes belonging to different functional groups, such as metabolism, signal transduction, cytoskeletal elements, defence and stress, were found to be over-expressed (Schurdi-Levraud

Escalettes et al., 2006). Expression of five of these transcripts was assessed to be variable between a tolerant and a susceptible cultivar and possibly involved in the onset of virus tolerance. Responsiveness of gene expression to infection with *Bean common mosaic virus* (BCMV, genus *Potyvirus*) and higher temperatures was compared by cDNA-AFLP between a BCMV-susceptible and resistant genotype of bean (*Phaseolus vulgaris*) (Cadle-Davidson & Jahn, 2006). Fifty-nine interesting bands were observed and analyzed by northern blot hybridization, and DNA isolation and sequencing. Consequently, 17 unique transcripts were found.

SSH, coupled with different formats of RNA hybridization, has been used in a number of studies focused on the transcriptome of plant defense response to virus infection. Using a combination of SSH and macroarray, a library of over 1100 subtracted tomato (*Lycopersicon esculentum*) cDNA clones was built and analyzed for gene expression patterns following infections with a mild and severe strain of *Potato spindle tuber viroid* (PSTVd) or TMV (Itaya et al., 2002). This is the first and so far the only study on the modulation of host gene expression caused by a viroid. It was found that 38 (58 %) of the 65 differentially expressed genes were specifically induced or repressed by PSTVd but not TMV, whereas 15 % of the genes were specifically responding to TMV infection. The significant proportion of commonly modulated genes suggested that plants respond to the attacks of by viroids and viruses through certain common mechanisms.

SSH was used to study gene expression of *Nicotiana megalosiphon* modulated by infection with *Tomato mottle Taino virus* (ToMoTV, genus *Begomovirus*) (Collazo et al., 2006). A total of 67 up-regulated genes were detected, of which 25 % were unknown. Induction of 14 genes following ToMoV infection was verified using an RNA dot-blot assay. Many genes were pathogen defence-related.

SSH supplemented with analysis in an array format

SSH is a sensitive method to detect differentially expressed genes but other methods are required for comparing samples from many repeated experiments and, especially, for quantitative comparison of differences in gene expression levels. A useful strategy employed in some studies is to make a library of differentially expressed genes using SSH and transfer it to a solid support (array) for analysis of replicated experiments (Vuorinen et al., 2003; Ros et al., 2004). In a macroarray, transcripts are selected and isolated using SSH, cloned and immobilized on a membrane. Sample RNA is labelled by reverse transcription and the labelled cDNA hybridized on the membrane. The procedure largely resembles dot-blot hybridization. In a microarray, the spot size of the immobilized DNA is considerably smaller and the number of spots much larger than in a macroarray. Glass slides are mostly used as the solid support. The samples to be compared are labelled with different dyes, which allows direct comparison of their hybridization on the same spot (*i.e.*, with the same cDNA).

SSH followed by analyses with a macroarray was employed to compare gene expression in a susceptible and a resistant, near-isogenic maize line (*Zea mays*) following infection with *Sugarcane mosaic virus* (SCMV, genus *Potyvirus*) (Shi et al., 2005). Five cDNA libraries were generated. They were composed of 2688 clones, among which 302 differentially expressed genes were identified. Two-thirds of these genes were assigned to three functional classes, including *i*) cell rescue, defence, cell death and ageing, *ii*) signal transduction, and *iii*) transcription. Mapping of some of the differentially expressed transcripts on maize chromosomes indicted an association of a few of them with SCMV resistance loci (Shi et al., 2005).

SSH and subsequent, repeated tests on selected genes using a microarray was used to investigate the molecular interactions between potato (*Solanum tuberosum*) and *Potato virus A* (PVA, genus *Potyvirus*) (Vuorinen et al., 2003) and *Potato virus Y* (PVY, genus *Potyvirus*) (Pompe-Novak et al., 2006).

In the study on PVA-potato interactions, the goal was to test whether potato genotypes expressing 'non-necrotic resistance' (nnr) fail to support translocation of PVA from the inoculated leaves due to virus-induced defence responses. In the nnr-type of resistance, PVA accumulates in inoculated leaves to high titers similar to PVA-susceptible genotypes but causes no visible HR or other symptoms. BSA of the nnr and susceptible genotypes selected from a segregating F1 population was used to obtain samples from inoculated leaves. Genes differentially expressed in the two pools were isolated by SSH, sequenced and used for preparation of a microarray. However, analysis of samples from repeated experiments using the microarray and rtRT-PCR indicated that gene expression was not significantly different between the nnr and susceptible genotypes. It was concluded that translocation of PVA from the inoculated leaves fails due to a failure of the host factors to support movement of PVA in the nnr genotypes (Vuorinen et al., 2003; Gammelgård et al., 2006).

Pompe-Novak et al. (2006) studied the changes in gene expression of a potato cultivar following infection with the NTN strain of PVY that causes tuber necrosis. However, the analyses were carried out on leaves. SSH between healthy and virus-infected leaves resulted in 258 characterized transcripts of which 175 could be identified. A total of 185 of these genes were included in a microarray and used to detect genes induced in the leaves that reacted with necrotic symptoms to infection with PVY^{NTN}. Few significantly induced genes were detected in inoculated leaves, whereas 14 genes were upregulated in the systemically infected leaves (Pompe-Novak et al., 2006).

Microarrays for genome-wide analysis of gene expression

The first study monitoring simultaneously a large proportion of host genes for changes upon virus infection was carried out by Whitham and co-workers (2003). They utilized Arabidopsis GeneChip microarrays (Affymetrix) containing 8734 probe sets (genes). Furthermore, they included as many as five different positive-stranded RNA viruses in the study: *Turnip vein clearing virus* (TVCV, genus *Tobamovirus*), *Oilseed rape mosaic virus* (ORMV, tentative *Tobamovirus* member), *Potato virus X* (PVX, genus *Potexvirus*), CMV, and *Turnip mosaic virus* (TuMV, genus *Potyvirus*). The observed changes in gene expression were general for different viruses or virus-specific and putatively associated with defence or stress responses. Induction of 114 genes was common for all of the five viruses. They could be classified as genes involved in cell rescue, defense, cell death and ageing (35 genes), or cellular oxidative state (7 genes). Six heat shock protein (HSP)/heat shock cognate (HSC) genes and a few genes possibly involved in signal transduction (protein kinases) and transcription (DNA binding proteins) were also detected. Regarding virus or virus group specific responses, the tobamoviruses were found to elicit accumulation of HSPs more effectively than other viruses. Promoter analysis provided further evidence that different RNA viruses elicit common responses in susceptible hosts and some of the signalling pathways for these responses were novel.

Golem and Culver (2003) studied TMV-induced modulation of gene expression in *A. thaliana* ecotype Shahdara using cDNA microarrays that contained 8000 or

10000 *Arabidopsis* genes. This ecotype is susceptible to TMV and develops various types of severe symptoms following infection. A total of 68 genes showed either up- or down-regulated expression in inoculated or systemically infected leaves, or both. These genes could be classified according to the deduced products to transcription factors, antioxidants, metabolic enzymes, and transporters.

Microarrays can be reciprocally used between species or genera of the same plant family. Senthil et al. (2005) applied a microarray prepared from 10000 ESTs of potato for analysis of gene expression in *Nicotiana benthamiana*, both which belong to the family Solanaceae. The goal of the study was to test whether enveloped plant viruses that replicate in different subcellular compartments elicit unique changes in the gene expression of a susceptible host. The responses to infection with *Sonchus yellow net virus* (SYNV, genus *Nucleorhabdovirus*) that replicates in the nucleus were compared to those induced by *Impatiens necrotic spot virus* (INSV, genus *Tospovirus*) that replicates in the cytoplasm. The expression of 275, 2646 and 4165 genes was altered following infection with INSV at 2, 4 and 5 days post-inoculation (dpi), whereas SYNV affected the expression of 35, 665 and 1458 genes at 5, 11 and 14 dpi, respectively. Furthermore, infection with INSV resulted in larger fold changes in gene expression than infection with SYNV. The virus-specific difference included induction of small HSP genes and down-regulation of histone genes by INSV, and temporal induction of a gene for putative WRKY transcription factor by SYNV.

The first genome-wide study on host gene expression following a virus-specific defence response was done by Marethe et al. (2004). A genotype of *Arabidopsis* carrying the gene *RCY1* for HR to CMV was inoculated with strain Y of CMV. The 'resistome' determined based on stringent criteria consisted of 444 genes, of which most were unknown and many encoded putative small peptides with less than 100 amino acids. The known genes encoded, e.g., putative kinases and phosphatases, protein degradation machinery/proteases, transcriptional regulators, and included 80 defence-responsive genes. Analysis of promoter regions of the 444 genes indicated that nine *cis*-binding elements mapped to pathogen-responsive clusters of genes. The study also revealed that genes responding to bacterial and viral pathogens are closely linked in small gene clusters and their transcription may be co-regulated.

Serial Analysis of Gene Expression

The Serial Analysis of Gene Expression (SAGE) allows the quantitative analysis of a large number of gene transcripts (Velculescu et al., 1995; Matsumura et al., 1999). The SAGE approach is based on release and analysis of short fragments (tags) adjacent to the *Nla*III restriction site closest to the 3'-end of a cDNA. In brief, the mRNA pool is isolated, reverse-transcribed, digested with *Nla*III endonuclease and captured using magnetic streptavidin-coated or oligo-dT beads. Linkers ('docking molecules') are ligated to the captured cDNA fragments. The linker contains a restriction enzyme (*Bsm*FI) recognition site, which is used to release the short SAGE tags (9-15 bp) from beads. The tags are purified, blunt-ended and ligated to obtain ditags, which are subsequently PCR-amplified and digested with *Nla*III to remove the docking molecules. The ditags are purified and ligated to each other to obtain long concatemers which are cloned and sequenced. The relative numbers of copies of the different tags detected by this approach reflect the relative differences in the transcript expression levels. As the tag sequences can be used to identify the corresponding genes, especially in species of which the whole genome has been sequenced, the method can provide information about the relative expression levels of genes with known or predicted functions.

Fregene et al. (2004) applied SAGE on cassava (*Manihot esculenta*) to identify genes induced in genotypes resistant to the cassava mosaic disease (CMD) caused by *African cassava mosaic virus* (ACMV, genus *Begomovirus*). The CMD-resistant parental genotype contained a previously published CMD resistance gene (Akano et al., 2002). The SAGE tags were isolated from bulked CMD-resistant F1 progeny. For annotation of the tags, two approaches were used. An Expressed Sequence Tag (EST) library of 4000 clones was obtained by 3'-end cDNA amplification, sequenced and annotated by comparison to sequences in databanks. Subsequently, annotation of the SAGE tags was attempted based on the EST information. Alternatively, the SAGE tag sequences were used as sense primers and the reverse primer was designed according to the 3'-end cloning site of the vector plasmid to amplify the 3'-parts of the cDNAs. A total of 30 tags that showed a 4-fold increase in the expression level in the bulk of resistant genotypes upon ACMV-infection were reported. The genes identified belonged to two main functional groups: systemic acquired resistance (SAR), and genes involved in cell cycle activities and intra- and inter-cellular virus trafficking. The chromosomal positions of 24 SAGE tags were mapped. Only one tag derived from the transcript of a WRKY transcription factor showed polymorphism between the bulks of resistant and susceptible genotypes. The genetic linkage of the WRKY with the CMD resistance gene together with the induced expression of WRKY specifically in CMD-resistant genotypes provided provisional evidence of its involvement in the resistance mechanism.

Taken the aforementioned studies to consideration, it seems apparent that each plant-virus combination results in activation of both unique and common host responses, including those that are stress-related.

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