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# Sugarcane Transcript Profiling Assessed by cDNA-AFLP Analysis during the Interaction with *Sugarcane Mosaic Virus*

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## Abstract

**Sugarcane mosaic caused by *Sugarcane Mosaic Virus* (SCMV) is one of the most important virus diseases of sugarcane. In the present study, changes in the transcription profile obtained by cDNA-AFLP analysis were investigated in two sugarcane varieties contrasting to SCMV resistance, when challenged with a severe virus strain. Healthy plants derived from meristem tip tissue culture were mechanically inoculated under greenhouse controlled conditions and sampled at 24, 48 and 72 hours after inoculation. A total of 392 transcript-derived fragments (TDFs) were verified in the resistant variety against 380 in the susceptible one. The two sugarcane genotypes showed differential behavior in the number of induced and repressed TDFs along the time-course samplings. Ten out of 23 sequenced TDFs (unique from the resistance variety), showed identity with known plant sequences, mostly related to plant defense mechanisms against pathogens. The cDNA-AFLP technique was effective in revealing changes in the transcription profile within and between contrasting varieties when challenged by SCMV.**

## Keywords

**Plant Resistance, Plant Viruses, Molecular Markers, Candidate Genes, cDNA-AFLP, SCMV**

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## 1. Introduction

The mosaic disease in different poaceous species can be caused by a subgroup of seven distinct members of family *Potyviridae*: *Sugarcane Mosaic Virus* (SCMV) which names the subgroup, *Sorghum Mosaic Virus* (SrMV), *Maize Dwarf Mosaic Virus* (MDMV), *Johnson Grass Mosaic Virus* (JGMV), *Pennisetum Mosaic Virus* (PenMV), *Zea Mosaic Virus* (ZeMV), belonging to the genus *Potyvirus*, and *Sugarcane Streak Mosaic Virus* (SCSMV) belonging to the new genus *Poace Virus* [1]. The mosaic of sugarcane occurs in more than 70 countries and only SCMV, SrMV and SCSMV can naturally infect this crop [2] [3]. However, in Brazil, only SCMV is naturally found infecting sugarcane [3]. Initial symptoms of infection consist of chlorotic spots linearly distributed in the middle or at the base of the leaves evolving into elongated areas forming a typical mosaic, which can increase in severity with leaf aging. Plant growth can be markedly reduced, depending on the virus strain and the sugarcane variety, especially when infection occurs in the early stages of plant development [4]. The control of mosaic is achieved mainly by the use of resistant varieties, along with roughing in nurseries and first crop cycle, which makes screening for resistance to this disease a crucial step in sugarcane breeding programs worldwide.

Despite the importance of mosaic to the crop, little is known about the transcript profile of sugarcane infected by SCMV. In the last years several approaches using molecular tools have become available to identify genes of interest in the development of new varieties. The cDNA-AFLP approach [5] is based on the AFLP (Amplified Length Polymorphism) technique [6] and allows accessing the polymorphism at the transcriptional level, using the cDNA rather than genomic DNA in the digestion with restriction enzymes. The major advantage is that the cDNA-AFLP technique does not require prior knowledge of a sequence which makes it useful for discovery of new genes [7] [8]. The technique has been used in the detection of genetic polymorphisms at the transcriptional level (differentially expressed TDFs) between contrasting phenotypes under controlled conditions [9] [10]. Such polymorphisms can be used to identify candidate genes which can be applied in the development of genetic markers for sugarcane genome mapping. According to [11] differentially expressed cDNA-derived fragments identified during biotic stress have great potential as genetic markers for pests and disease resistance in sugarcane. The cDNA-AFLP technique was successfully applied to screen resistance to sugarcane leafhopper [12] and also important sugarcane diseases such as brown rust [13] and smut [9] [14]. In the present study, changes in the transcription profile of two SCMV resistances contrasting sugarcane varieties as well as the identification of differentially expressed TDFs obtained by cDNA-AFLP analysis were investigated when challenged with an aggressive SCMV strain, SCMV-Rib1 [4]. The identification of these differentially expressed TDFs could be further applied as potential candidate genes in genetic mapping for mosaic resistance.

## 2. Material and Methods

### 2.1. Material

Two sugarcane varieties from the IAC Sugarcane Breeding Program, Brazil, IACSP95-5000 and IAC91-1099, respectively resistant and susceptible to SCMV, were obtained by meristem tip culture at the Sugarcane Bio factory of the IAC Sugarcane Research Centre (Ribeirão Preto, Brazil). The virus indexing of the micro-propagated plantlets was done by molecular diagnosis via RT-PCR with specific primers to the SCMV coat protein [4] [15]. Virus inoculum was prepared from leaves of *Sorghum bicolor* (L.) "Rio", previously inoculated with the severe strain SCMV-Rib1 [4], originated from sugarcane collected in Ribeirão Preto, Brazil.

### 2.2. Experimental Design and Inoculations

Experiments were conducted in an aphid proof green-house under natural sunlight, with environment temperatures varying from 22˚C - 34˚C, in a randomized block experimental design at the IAC Sugarcane Research Centre between January and March 2012. The sugarcane plantlets from meristem tip culture were acclimated in the green-house during 20 days and transplanted to 1 liter plastic vessels. Fifteen days after transplanting six plants of each variety at each of the three sampling time-points (24, 48 and 72 hours post inoculation—hpi) were mechanically inoculated using as source of inoculum Sorghum "Rio" leaves infected with SCMV Rib-1 [4]. Sorghum leaves were ground in liquid nitrogen and homogenized in 0.01 M sodium phosphate buffer at pH 7.2 in a 1:10 ratio (leaf tissue/buffer) mixed with 600 meshes silicon carbide powder. Six plants were mock inoculated with phosphate buffer plus silicon carbide to serve as controls. In order to confirm the virus infection or its absence in the controls, RT-PCR with specific primers to the SCMV capsid protein, SCMVF4 and SCMVR3 [4] [15] was performed.

### 2.3. Tissue Sampling and RNA Extraction

The first leaf from the top to the bottom of the stalk with clearly visible dewlap (+1 leaf) of each replicate was individually collected from each treatment and respective controls and immediately stored in liquid nitrogen. Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, USA) following the manufacturer's instructions. The purity, integrity and quantification of the extracted RNA were evaluated through $A_{260}/A_{280}$ in a NanoDrop® (Promega, USA) along with electrophoresis in 1.5% agarose's gel.

### 2.4. cDNA-AFLP

The cDNA-AFLP analysis was conducted in bulks of equal quantities of total RNA extracted from 3 plants chosen randomly out of the 6 replicates. The first and the second strands cDNA synthesis was performed with the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Lithuania) following the manufacturer's instructions. Two hundred nanograms of double strand cDNA were double digested with the restriction enzyme combinations E*co*RI plus M*se*I and E*co*RI plus M*sp*I. The ligation of the adapters to the digested fragments, the pre-amplification and the selective amplification were performed as previously described [6]. The amplified fragments were resolved in 5% denaturing polyacrylamide gel electrophoresis and silver stained according to protocols [16].

### 2.5. Data Analysis

The number of TDFs observed between the varieties across the sampling time points (24, 48 and 72 hpi) was compared by the Chi-Square test contingency table analysis (hpi and variety). To test the equality of the frequency of TDFs within each variety the Chi-Square test was applied assuming equal frequencies (1/3 of the total TFDs) across the sampling time points.

### 2.6. Isolation, Cloning and Sequencing of TDFs

TDFs were excised from the gel, eluted in 50 µL of TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0) and incubated overnight at 4˚C. TDFs were re-amplified with their respective selective primers as used in the cDNA-AFLP analysis and purified with the Wizard® SV Gel and PCR Clean Up System Kit, cloned into pGEM-T Easy (Promega) and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit. The nucleotide sequences were submitted to Basic Local Alignment Search Tool (BLAST) from NCBI [17] to search for regions of local similarity between sequences already published in the GenBank. The E-value (the best Expect value of all alignments from the database sequence), query cover (percentage of query covered by alignment to the database sequence) and identity (the highest percent identity of all query-subject alignments) were obtained as the results of BLAST search. The 1e−5 was adopted as inferior threshold to the similarities analysis. The TDFs sequences showing similarity to hypothetical proteins were blasted to the protein data bank, UniProtKB (http://www.uniprot.org/) to verify the biological function annotations on Gene Ontology (GO).

## 3. Results

### 3.1. Symptomatology and Virus Detection by RT-PCR

One month after inoculation with SCMV Rib-1, plants from the susceptible variety (IAC91-1099) showed typical symptoms of infection, such as mosaic and reduced growth whereas plants from the resistant variety (IACSP95-5000) did not show any disease symptoms. The RT-PCR amplified a specific fragment of approximately 890 bp corresponding to the SCMV capsid protein in all inoculated plants, while no fragment was observed in the mock-inoculated ones (**Figure 1**).

### 3.2. Identification of TDFs along the Sampling Time-Points (hpi)

The number of TDFs observed for each variety, only in the inoculated treatments (I), which probably corresponds to induced genes, as well the number of TDFs observed only in the non-inoculated controls (R), which may correspond to repressed genes in the inoculated treatments is showed for the respective 16 selective primer combinations (**Table 1**).

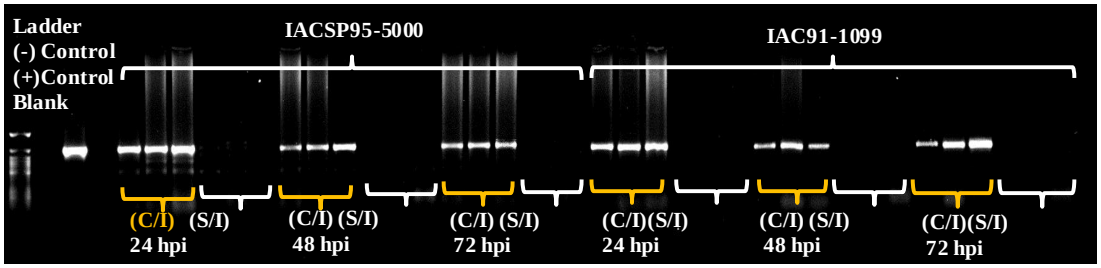


**Figure 1.** RT-PCR amplification from total RNA of sugarcane plants of varieties IACSP95-5000 and IAC91-1099 at time courses 24, 48 and 72 hpi. The 890 bp specific fragment is observed only in the inoculated treatments. C/I: Inoculated; S/I: Mock-inoculated controls.

**Table 1.** Number of TDF at 24, 48 and 72 hours after inoculation (hpi) in two sugarcane varieties contrasting to SCMV resistance using 16 selective primer combinations. I: TDFs exclusive to the inoculated plants (induction); R: TDFs exclusive to the mock-inoculated plants, *i.e.*, controls (repressed in the inoculated plants).

| Selective Primer Combination | Variety | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IACSP95-5000 | | | | | | IAC91-1099 | | | | | | |
| | 24 hpi | | 48 hpi | | 72 hpi | | 24 hpi | | 48 hpi | | 72 hpi | | |
| | I | R | I | R | I | R | I | R | I | R | I | R | |
| E-ACA/$M_1$CTT | 5 | 8 | 9 | 7 | 4 | 4 | 8 | 5 | 6 | 6 | 2 | 4 | 68 |
| E-AAA/$M_1$CTT | 7 | 8 | 7 | 8 | 6 | 3 | 0 | 4 | 6 | 7 | 6 | 4 | 66 |
| E-ACT/$M_1$CTA | 4 | 5 | 4 | 6 | 3 | 6 | 6 | 4 | 4 | 7 | 6 | 4 | 59 |
| E-ACC/$M_1$ CTT | 7 | 7 | 7 | 7 | 4 | 6 | 5 | 5 | 11 | 6 | 8 | 4 | 77 |
| E-AAG/$M_1$CTT | 3 | 5 | 2 | 4 | 3 | 3 | 1 | 1 | 4 | 6 | 2 | 1 | 35 |
| E-ACT/$M_1$CTT | 5 | 6 | 5 | 3 | 5 | 1 | 4 | 4 | 5 | 8 | 6 | 4 | 56 |
| E-ACT/$M_1$CAA | 6 | 5 | 1 | 3 | 1 | 0 | 4 | 3 | 3 | 6 | 1 | 3 | 36 |
| E-AAC/$M_1$CGT | 7 | 3 | 5 | 4 | 1 | 2 | 3 | 3 | 4 | 4 | 1 | 1 | 38 |
| E-AAG/$M_1$CGT | 1 | 2 | 2 | 4 | 3 | 3 | 4 | 1 | 3 | 6 | 3 | 3 | 35 |
| E-AAC/$M_2$ACT | 5 | 2 | 1 | 5 | 2 | 2 | 2 | 4 | 1 | 3 | 3 | 3 | 33 |
| E-AAC/$M_2$TCG | 1 | 1 | 3 | 1 | 2 | 2 | 0 | 0 | 2 | 1 | 2 | 2 | 17 |
| E-ACG/$M_2$ACT | 8 | 3 | 3 | 5 | 2 | 6 | 5 | 4 | 1 | 2 | 5 | 2 | 46 |
| E-AAC/$M_2$GAA | 4 | 2 | 2 | 3 | 2 | 1 | 3 | 1 | 4 | 4 | 3 | 2 | 31 |
| E-AAC/$M_2$ACA | 6 | 5 | 11 | 5 | 8 | 4 | 5 | 5 | 4 | 8 | 8 | 3 | 72 |
| E-ACG/$M_2$TTG | 9 | 6 | 3 | 1 | 3 | 5 | 4 | 3 | 6 | 5 | 9 | 7 | 61 |
| E-AGC/$M_2$ACA | 4 | 2 | 3 | 2 | 3 | 4 | 4 | 3 | 3 | 3 | 7 | 4 | 42 |
| Total (I/R) | 82 | 70 | 68 | 68 | 52 | 52 | 58 | 50 | 67 | 82 | 72 | 51 | 772 |
| Total (I) | 202 | | | | | | 197 | | | | | | 399 |
| Total (R) | 190 | | | | | | 183 | | | | | | 373 |
| Total/hpi | 152 | | 136 | | 104 | | 108 | | 149 | | 123 | | |
| Total/Variety | 392 | | | | | | 380 | | | | | | 772 |

E: three-base-selective primer E*co*RI (CTGCGTACCAATTC); $M_1$: three-base-selective primer M*se*I (GATGAGTCCTGAGTAA); $M_2$: three-base-selective primer M*sp*I (GATGAGTCCTGATCGG).

The selective primer combination E-AAC/$M_2$TCG produced the lowest number of TDFs (17), while the highest (77) was observed with the combination E-ACC/$M_1$CTT. In overall, 772 TDFs were obtained, of which 392 observed in the resistant (IACSP95-5000) and 380 in the susceptible (IAC91-1099) variety. Three hundred and ninety nine (52%) out of the 772 TDFs, were specifically observed only during the plant-pathogen interaction, *i.e.*, in the inoculated treatments.

The two varieties contrasting in their resistance to SCMV differed significantly in their expression profiles regarding the number of induced TDFs across the time points ($\chi^2$ = 7.29; P = 0.0262). This can be viewed by comparing the number of TDFs that may correspond to induced genes, along the different sampling time-points (**Figure 2**). The resistant variety expressed the highest number of TDFs at 24 hpi, which may correspond to the induced genes, relatively to the susceptible variety ($\chi^2$ = 6.29; P = 0.035). It's worthwhile to note that the general number of induced TDFs decreases along the time-points (hpi) in the resistant variety. On the other hand the two varieties did not differed significantly in their expression profiles regarding the number of repressed TDFs across the time points ($\chi^2$ = 4.52; P = 0.1044).

### 3.3. Identification of TDFs Exclusive to the Resistant Variety

The identify transcripts that could be directly related to mosaic resistance, two situations were considered: 1) for each sampling time-point (24, 48 and 72 hpi) TDFs present exclusively in the resistant variety (IACSP95-5000) inoculated treatment, but absent in the controls as well as in the susceptible variety treatments (IAC91-1099) and respective controls, were selected; 2) for each sampling time-point, TDFs present either in the resistant variety controls or in the susceptible variety (treatments and controls), but absent in the resistant variety inoculated plants were selected (**Figure 3**). The first situation probably reflects the genes induced exclusively by the resistant variety in response to the SCMV infection, while the second one probably represents the repressed genes in response to virus infection. Therefore, it was possible to identify 66 TDFs, of which 57 (86%) may correspond to genes exclusively induced in the resistant variety and 9 (14%) to repressed genes when challenged by SCMV Rib-1. It was also observed that most of the TDFs which may correspond to induced genes were expressed at 24 hpi (**Table 2**).

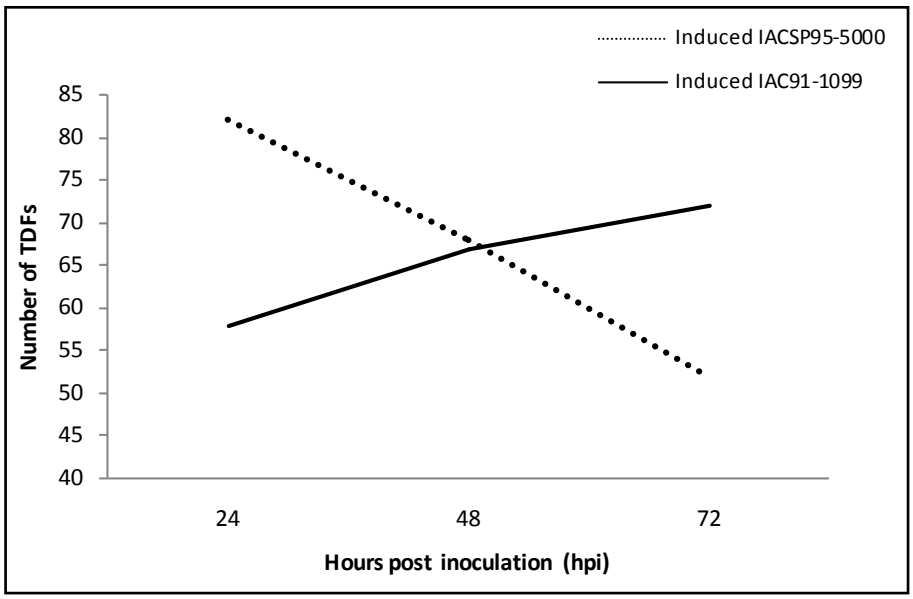


**Figure 2.** Distribution of the number of induced TDFs in the two sugarcane varieties over the time after inoculation (24, 48 and 72 hpi).

**Table 2.** Number of induced and repressed TDFs exclusive to IACSP95-5000 at different hours after inoculation (hpi) with SCMV.

| (hpi) | Induced | Repressed |
|---|---|---|
| 24 | 32 | 4 |
| 48 | 12 | 4 |
| 72 | 13 | 1 |
| Total | 57 | 9 |

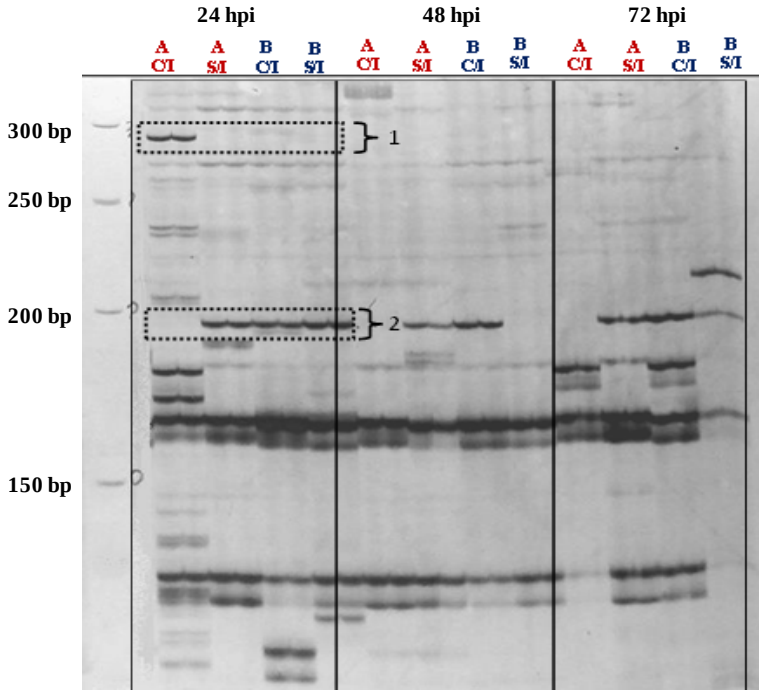


**Figure 3.** cDNA-AFLP profile after amplification and digestion with E*co*ACG/M*se*ACT combination revealed in 6% denaturing polyacrylamide gel showing the transcribed derived fragments (TDFs) after different sampling time points (hour post inoculation—hpi) in two situations: 1. Exclusive to inoculated plants of the resistant variety IACSP95-5000 (induced genes); and 2. Absent only in the inoculated plants of the resistant variety (repressed genes). A: Resistant variety (IACSP95-5000); B: Susceptible variety (IAC91-1099); C/I: Inoculated; S/I: Mock-inoculated.

### 3.4. Sequencing of the TDFs

Out of the 57 differentially expressed fragments, exclusively found in the resistant variety (**Table 2**), 23 were randomly selected for cloning and sequencing. Thirteen out of these 23 fragments were submitted to the algorithm Blast and showed E-value cut off 1e−5 (**Table 3**). The TDF identified as number 45, selected 24 hpi, showed 93% identity with a sugarcane retrotransposon sequence (JN800037.1) and the TDF number 52, also selected 24 hpi, showed 100% identity with a complete ribosomal RNA sequence of *Miscanthus* (JX457396.1). At the sampling time point 48 hpi, the TDFs number 41 and 52 showed 88% identity with SORBIDRAFT_06g022385 (XP_002448166.1) and SORBIDRAFT_01g024221 (XP_002467304.1), respectively. These sequences correspond to two sorghum hypothetical proteins which did not show any molecular functions when blasted against UniProt database. At 72 hpi, the TDF number 14 showed 80% identity with a VQ motif family protein (NP_001150511.1), while TDF 21 showed 65% identity with the sorghum hypothetical protein SORBIDRAFT_03g023980 (BT068454.2) which also has no information regarding its possible molecular function at UniProt.

## 4. Discussion

In this study two varieties which differ in response to resistance to SCMV were investigated at the transcriptional level through the cDNA-AFLP technique when challenged by a virus severe strain. The use of plantlets cleaned by meristem-tip tissue culture and indexed by RT-PCR ensured that the experiment was carried out with virus-free plants, discarding the possibility of cross-protection, *i.e.*, the occurrence of plant resistance to a virus strain induced by the previous systemic infection by a mild strain of the same virus species [18] which could interfere with the phenotypic manifestation of symptoms expected for these two varieties. In fact, after 30 days of inoculation, no symptoms were observed in the resistant variety IACSP95-5000 plants while typical mosaic

**Table 3.** Percentage of identity with GenBank sequences of the transcribed derived fragments-TDFs detected at different sampling time points after inoculation (hpi) with SCMV Rib-1.

| TDF[1] | Selective primer combination[2] | Fragment size (bp) | Accession No | Organism or gene | E-value[3] | Query cover[4] | Identity[5] |
|---|---|---|---|---|---|---|---|
| | | | | 24 hpi | | | |
| 45 | E-AAC/$M_1$CGT | 100 | JN800037.1 | Retrotransposon complete sequence | 7.00E−12˙ | 53% | 93% |
| 52 | E-AAC/$M_2$ACT | 300 | JX457396.1 | 23S ribosomal RNA (rrn23S) | 2.00E−60˙ | 79% | 100% |
| 63 | E-AAC/$M_2$GAA | 330 | XM_002453856.1 | Hypothetical protein, mRNA | 7.00E−77˙ | 83% | 87% |
| 82 | E-ACG/$M_2$ACT | 160 | XM_002444994.1 | Hypothetical protein, mRNA | 1.00E−51˙ | 83% | 93% |
| | | | | 48 hpi | | | |
| 29 | E-ACT/$M_1$CTA | 220 | – | None | – | – | – |
| 41 | E-AAC/$M_1$CGT | 450 | XP_002448166.1 | SORBIDRAFT_06g022385 | 1.00E−69† | 91% | 88% |
| 58 | E-AAC/$M_2$TCG | 200 | XP_002467304.1 | SORBIDRAFT_01g024221 | 6.00E−08† | 37% | 84% |
| 78 | E-ACG/$M_2$ACT | 350 | – | None | – | – | – |
| | | | | 72 hpi | | | |
| 14 | E-ACC/$M_1$CTT | 280 | NP_001150511.1 | VQ motif family protein [*Zea mays*] | 1.00E−14† | 41% | 80% |
| 21 | E-ACT/$M_1$CTT | 140 | XP_002455751.1 | SORBIDRAFT_03g023980 | 4.00E−14† | 47% | 65% |
| 28 | E-ACT/$M_1$CTA | 300 | BT068454.2 | *Zea mays* full-length cDNA clone | 3.00E−31˙ | 31% | 92% |
| 34 | E-ACA/$M_1$CTT | 290 | – | None | – | – | – |
| 81 | E-ACG/$M_2$ACT | 180 | AE009947.2 | *Saccharum* hybrid cultivar SP-80-3280 chloroplast, complete genome | 4.00E−72˙ | 83% | 100% |

[1]TDF derived from the AFLP selective primer combination; [2]E: E*co*RI; $M_1$: M*se*I; $M_2$: M*sp*I; [3]E-value attributed to blast search, the best (lowest) Expect value of all alignments from the database sequence; [4]Percentage of query covered by alignment to the database sequence; [5]The highest percent identity of all query-subject alignments; ˙Alignment performed with the BlastN tool; †Alignment performed with BlastX tool.

symptoms were observed in all inoculated plants of the susceptible variety IAC91-1099. Moreover, once the absence or presence of the virus in the micro-propagated plantlets and in the treatments in all-time courses was confirmed by RT-PCR, it is expected that the TDFs herein disclosed by cDNA-AFLP reflect changes exclusively derived from the plant-virus interaction in the transcription profiles within and between the contrasting sugarcane varieties. According to [19] the cDNA-AFLP technique is able to reveal alterations in the expression profile of any gene, since the transcripts of this gene have the restriction sites chosen for the analysis.

In our work, polymorphism obtained by digestion with the combination of the restriction enzymes E*co*RI/M*se*I and E*co*RI/M*sp*I was explored using primers with three selective bases. As pointed out by [20] [21] the choice of the enzyme combination is crucial to the success of the cDNA-AFLP technique since they should recognize and cut each cDNA molecule derived from the genome under study in order to investigate the expression pattern of the genome as a whole. If the transcribed genes are not recognized by the chosen restriction enzymes the chances to discover new genes will be lost. In our study, the use of selective primers with three bases contributed to minimizing the likelihood of non-specific amplifications, as in the AFLP analysis's selective PCR products can be avoided by increasing the length of the selective primers. On average, the number of TDFs generated with E*co*RI/M*se*I combination was higher than that with E*co*RI/M*sp*I. Such difference may be related to the frequency of rare cutting restriction sites, TTAA and GGCC, recognized respectively by the M*sp*I and M*se*I in the sampled transcripts. In overall, the cDNA-AFLP technique was effective in exhibiting an extensive profile of TDFs during the interaction between the two sugarcane varieties and SCMV.

The resistant variety presented a very different expression profile in comparison to the susceptible one, particularly in relation to induction TDFs over the time-course samplings after inoculation. Similar results were also reported by [19] when studying genes induced and repressed in resistant sugarcane somaclones after inoculation with *Ustilago scitaminea* and *Bipolaris sacchari*. These authors found a greater number of induced genes compared to repressed ones in response to independent inoculations with these two fungi species. Cheng *et al.* (2010) [22] used the same technique to identify differentially expressed genes in *Nicotiana benthamiana* infected by *Bamboo Mosaic Virus* (BaMV) and also found an increased number of activated TDFs in relation to

the repressed ones. These reports may be related to the ability of genotypes carrying resistance genes to early recognize the pathogen in the initials steps of the infectious process preparing an effective defense response.

According to [23] plant-pathogen interactions are complex and the defense response is initiated immediately after the recognition of the virus by the plant. Additionally, in our work the distribution of the TDFs along the hours after inoculation in the resistant variety, suggests that sampling in the first 24 hours after virus inoculation may be more appropriate for gene expression studies, once it led to a greater number of differentially expressed transcripts. The preliminary sequence analysis of the TDFs here identified showed sequence identity with genes involved in various molecular events, somewhat related to plant responses to stress. In fact, at 24 hpi sampling in the resistant variety, it identified one TDF with 93% identity to a sugarcane retrotransposon sequence. Similarly, [14] also identified a TDF with similarity to a LTR (Long Terminal Repeat) retrotransposon *gypsy* type in a smut resistant sugarcane variety in response to infection with *Ustilago scitaminea*, the smut causal agent. Transposable elements were initially associated with biotic stress responses by [24] [25] and can affect nearby genes by generating a diverse set of small RNAs that trigger the mechanisms of gene silencing [26]. In a similar way the primary mechanism of defense against plant viruses is also believed to be based mainly on RNA silencing [27]. Among the three TDFs (identified 48 hpi), special attention must be given to the TDF associated to the protein named SORBIDRAFT_06g022385, whose molecular functions displayed by the Gene Ontology (GO) comprise hydrolase activities along with carbohydrate metabolic processing. According to [28] the hydrolases, which promote cellular disorganization in pathogens, fall into the category of PR (Pathogenesis Related) proteins. The PR proteins are produced by plants in response to the infection by several pathogens. In tobacco, group 1 PR proteins were intimately associated with the location of the virus in the cell and were found in greater amounts in tissues where the spread of the virus was interrupted [29]. According to [30] the family of proteins containing the VQ motif can act as a link in the connection of signaling defense mechanism. Hence, the TDF detected 72 hpi in the resistant variety showing 80% identity to the corn protein with a VQ motif, which may be involved in the signaling defense cascade against infection by SCMV. Our study allowed the identification, sequencing and analysis of limited number of TDFs, and some of them clearly involved in resistance to SCMV. Nevertheless, the TDFs that did not show significant sequence similarities in the investigated databases may correspond to unknown genes related to important functions in the plant defense mechanism to SCMV or even other biotic factors.

The suitability of the experimental design and of the cDNA AFLP technique for these analysis offers the possibility of its application for the generation and sequencing of a greater number of TDFs, which will allow not only a better understanding of the resistance mechanisms involved in SCMV infection, but also the identification of candidate genes to be applied in the development of molecular markers aiming the genetic mapping for virus resistance in sugarcane.

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