Table 1 Suppl. Primers used in this study.

| | | | | | |
|---|---|---|---|---|---|
| Primers for the amplification of specific gene regions of the TYLCV-Mild genome | | | | | |
| TYLCV-Mild&IL-V2-F-T | F | GGGGATCCATGTGGGATCCACTTCTAAA | V2 155-505 | 367 | this study |
| TYLCV-Mild-V2-R-T | R | GGGGATCCTCAGTGCTTCGATACATTCT | | | |
| TYLCV-Mild-C4-F | F | GGGGATCCTAATGATTCTAAGAGCCTCTG | C4 2178-2471 | 310 | this study |
| TYLCV-Mild&IL-C4-R | R | GGGGATCCATGGGGAACCACATCTCCAT | | | |
| TYC1F | F | GGGCCTAGAGACCTGGCCCAC | part of C1 and part of the IR | 882 | Lapidot 2002 |
| TYC1R | R | CCGGTAATATTATACGGATGGC | | | |
| Primers for the detection of small interfering RNAs (siRNAs) deriving from TYLCV-Mild genome by stem-loop RT PCR | | | | | |
| siTYLCV_RNA-V2_325as-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACGACCAG | V2 | | this study |
| siTYLCV_RNA-V2_325as-F | F | GGCCGAAATGATTATATCGC | 345-325 | 60 | |
| siTYLCV_RNA-V2_352s-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACTTCGGC | V2 | | this study |
| siTYLCV_RNA-V2_352s-F | F | TTCCTCCGCCTCGAAGGTTC | 352-372 | 60 | |
| siTYLCV_RNA-C1_1596as-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACAGCCAC | C1 | | this study |
| siTYLCV_RNA-C1_1596as-F | F | GTCCGTGATACTTGCGAACA | 1596-1616 | 60 | |
| siTYLCV_RNA-C1_1616s-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACTGATAC | C1 | | this study |
| siTYLCV_RNA-C1_1616s-F | F | GTCCTAGCCACTGTTCGCAA | 1616-1596 | 60 | |
| siTYLCV_RNA-C1-C4_2471s-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACTTCTCG | C1-C4 | | this study |
| siTYLCV_RNA-C1-C4_2471s-F | F | GTCCTTTGCAGAGAACTCCA | 2491-2471 | 60 | |
| siTYLCV_RNA-C1-C4_2471as-RT | RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACTTGCAG | C1-C4 | | this study |
| siTYLCV_RNA-C1-C4_2471as-F | F | GTCCTTTCTCGTGGAGTTCT | 2471-2491 | 60 | |
| Universal reverse primer | R | GTGCAGGGTCCGAGGT | | | Varkonyi-Gasic *et al* 2007 |

Table 2 Suppl. Disease incidence, expressed as a percentage of infected plants upon topical application of dsRNAs (dsV2 and dsC4) against TYLCV-Mild in tomato. TYLCV-Mild was transmitted by agroinfiltration (see Materials and methods)

| Replicates of the bioassay against TYLCV-Mild | Disease incidence [%] | | |
|---|---|---|---|
| | TYLCV | TYLCV+dsV2 | TYLCV+dsC4 |
| I | 90 | 60 | not tested |
| II | 70 | 50 | 22.2 |
| III | 40 | 20 | 20 |
| IV | 50 | 30 | 10 |
| V | 70 | 70 | 40 |

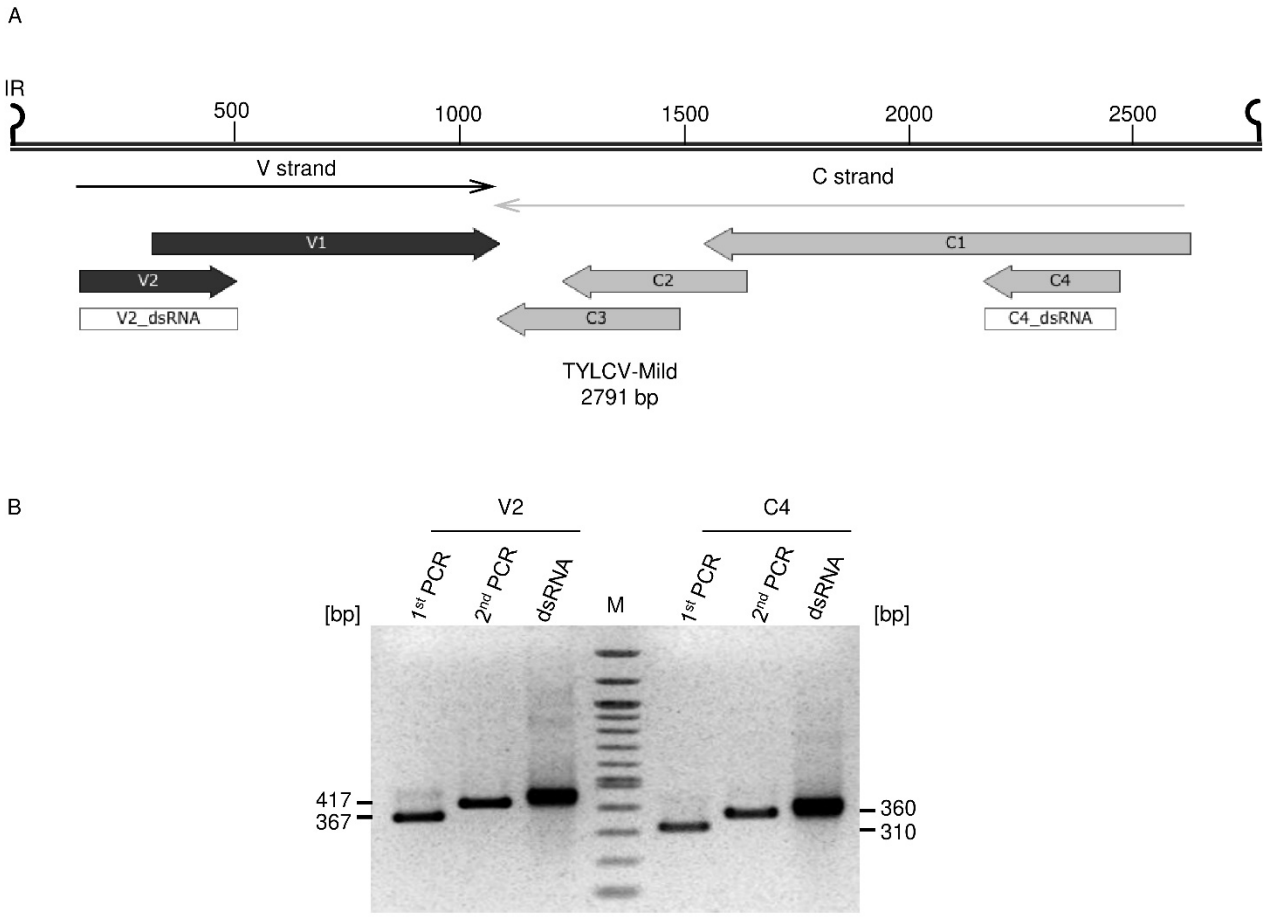


Fig. 1 Suppl. *In vitro* production of dsRNA-vaccines against TYLCV-Mild. *A* - TYLCV-Mild (AF071228.1) genome organization bidirectionally in two transcriptional units, divided by the intergenic region. The precise location of each gene and the target genes for dsRNA production are marked. *B* - Electrophoresis of the products of 1st PCR, 2nd PCR, and *in vitro* transcription for the genes V2 (whole gene, 351 bp) and C4 (almost the whole gene, 294 bp out of 303 bp) of TYLCV-Mild.

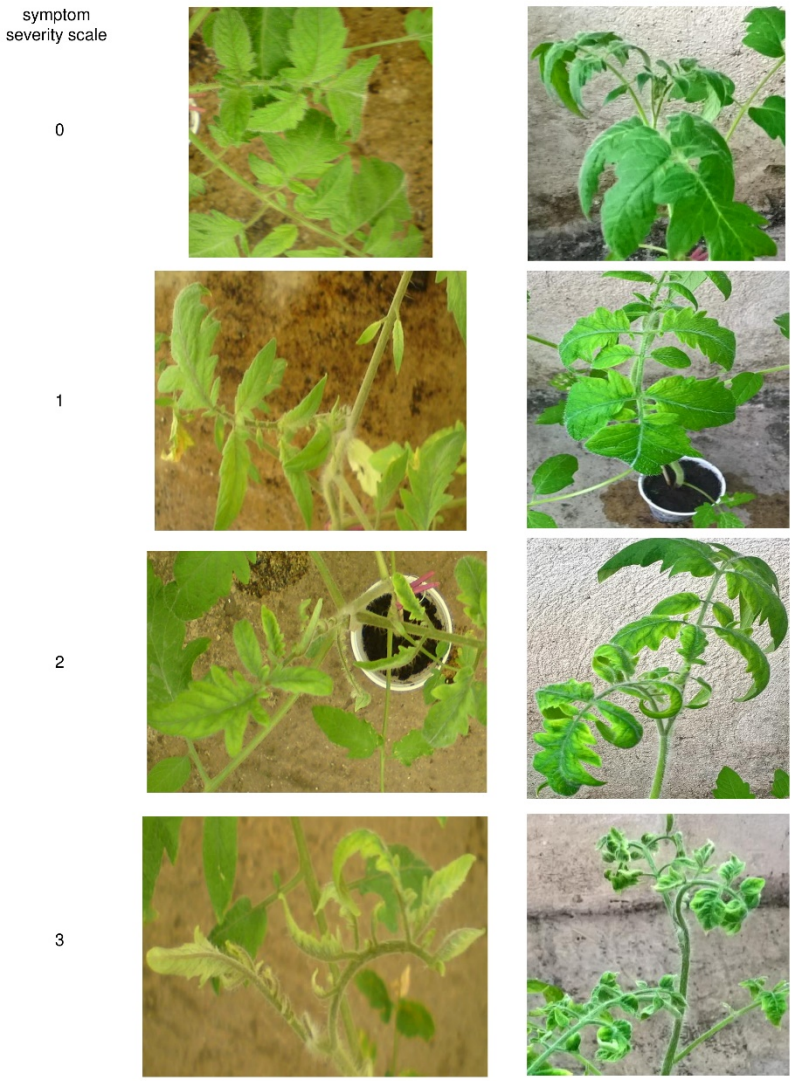


Fig. 2 Suppl. Evaluation of tomato yellow leaf curl disease development on tomato plants of the trials of dsRNA vaccination employing a symptom severity scale. Stage 0 - no symptoms; Stage 1 - appearance of mosaic/leaf curl at the top of the plant/threading of new leaves or mottling at one leaflet; Stage 2 - mosaic and leaf curl of the top of the plants/threading of newly developed leaves and mosaic/stunting and leaf curl of the top; Stage 3 - intense mosaic, leaf curl and stunting of the top of the plants.

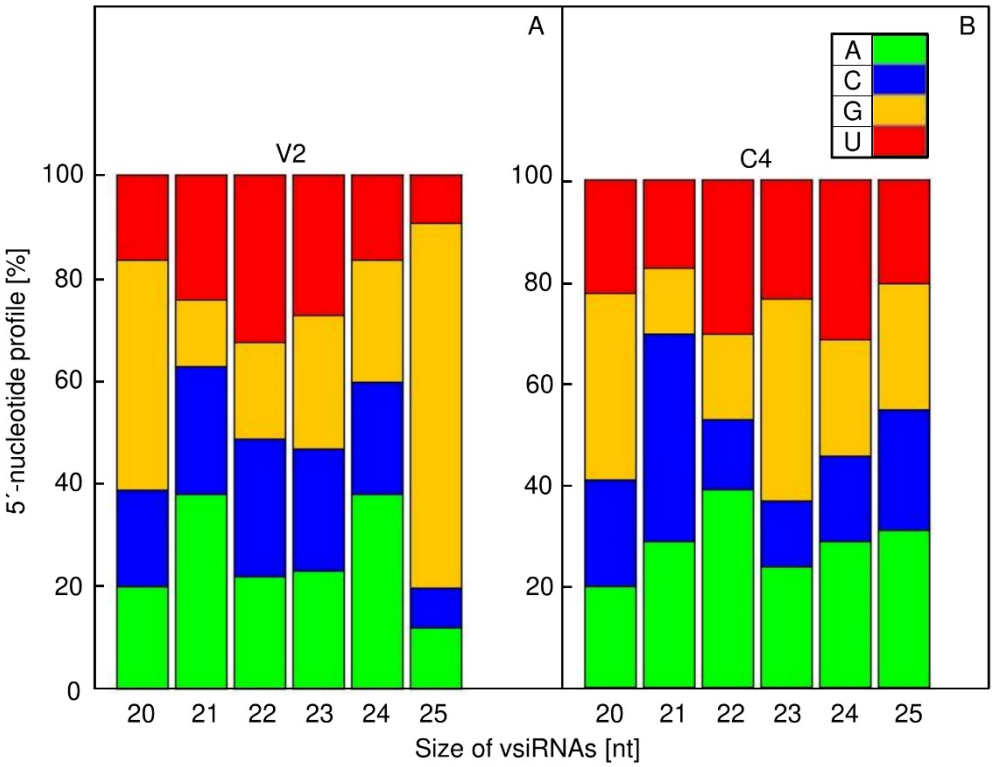


Fig. 3 Suppl. 5´-nucleotide profile of vsiRNAs (size 20-25 nt) as visualized by *MISIS* tool. *A* - V2 gene and *B* - C4 gene of TYLCV-Mild in sample from TYLCV-treated tomato exhibiting symptoms (replication 2).

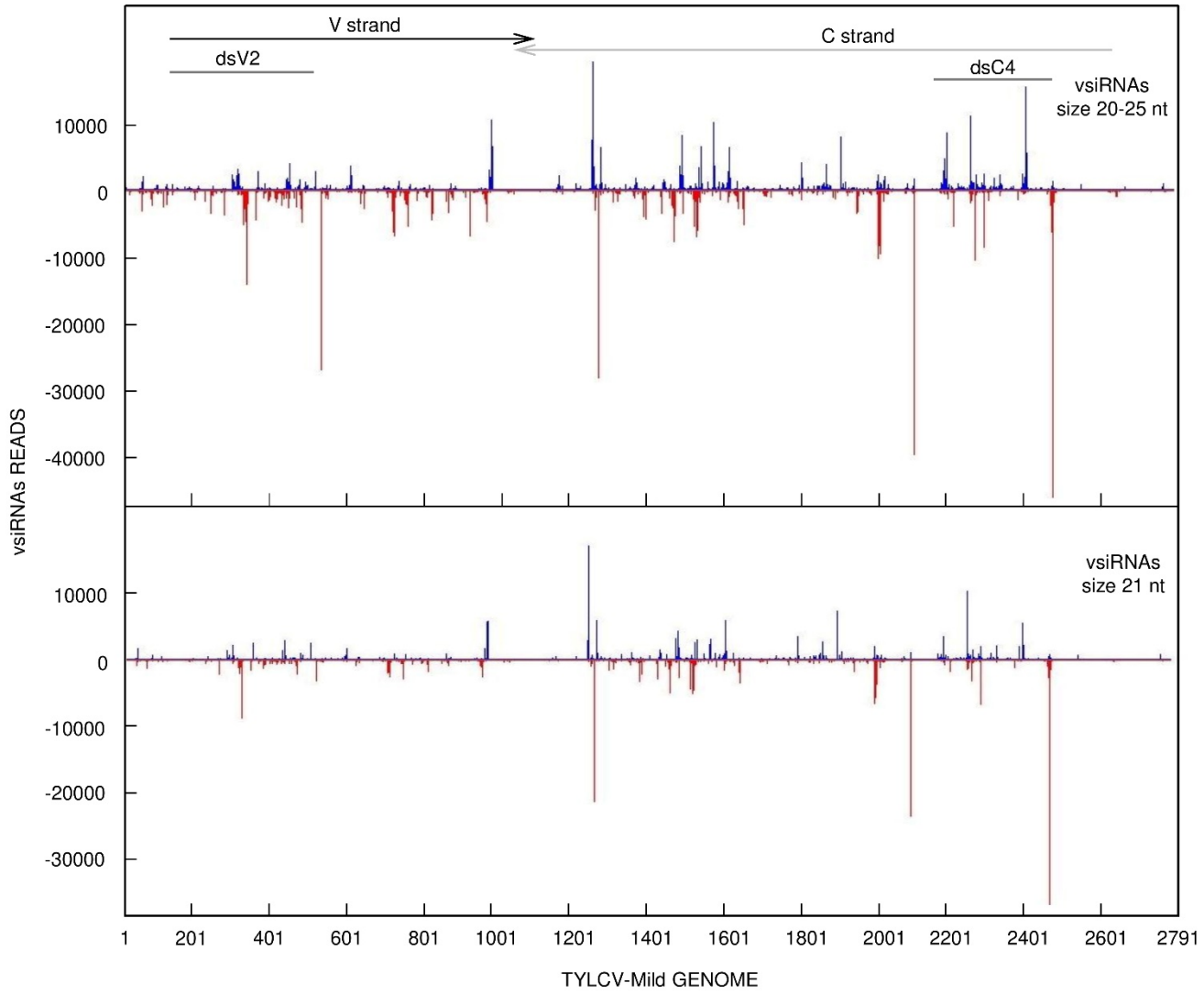


Fig. 4 Suppl. Mapping of 21 and 20-25 nucleotide siRNAs (total read counts) of TYLCV-Mild from tomato plants treated with TYLCV exhibiting symptoms (Replication 2), on the reference genome (TYLCV-Mild, AF071228.1). The peaks of the reads above and below the X-axis correspond to siRNAs of sense and anti-sense orientation respectively following the numbering order of the virus genome, according to MISIS software. In fact, due to the TYLCV genome organization, the peaks of the reads above and below the X-axis correspond to siRNAs of positive or negative polarity depending on the transcriptional unit they derive from. In addition, peaks above and below the X-axis represent reads starting and ending, respectively, at each nucleotide position.

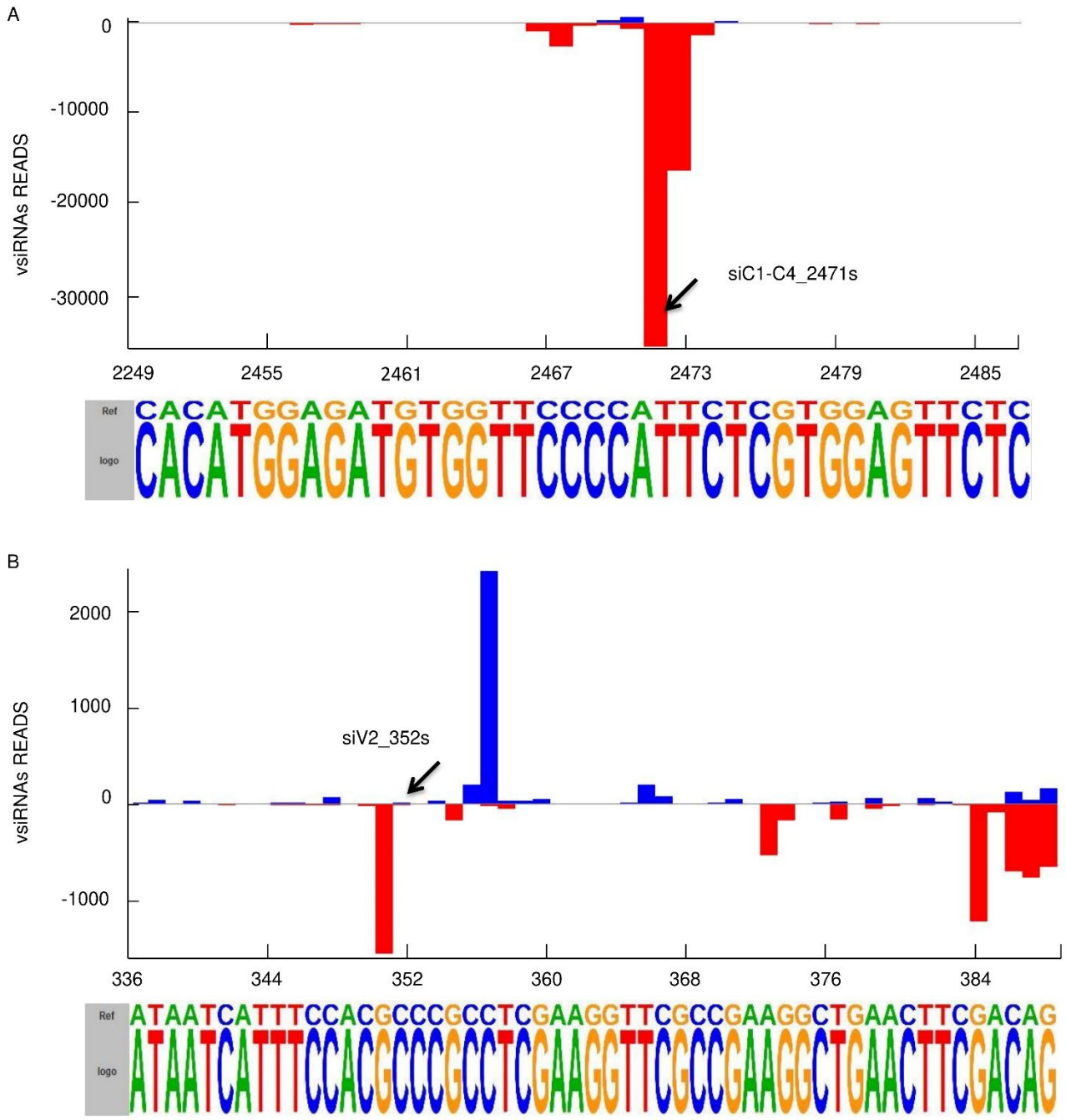


Fig. 5 Suppl. Visualization of two TYLCV-Mild-derived siRNAs. A - Histogram (*MISIS* software) representing TYLCV-Mild-derived siRNAs (21-nt, library from TYLCV-treated tomato, replication 2) focusing in a limited region where the siC1-C4_2471s, corresponding to the greatest hot spot for TYLCV-Mild for 21-nt point 2471 (L-Unit, 2491-2471, 36276 reads) is presented (indicated with an *arrow*). The y axis indicates the counts of vsiRNA reads (positive values represent vsiRNAs mapped to the sense of the viral genome, negative values represent vsiRNAs mapped in antisense direction to the viral genome). *Blue bars* show the 5'-end of each 21-nt vsiRNA and *red bars* show the 3'-end of each 21-nt vsiRNA. Due to the genome organization of TYLCV, the peaks of the reads above and below the x-axis correspond to siRNAs of positive or negative polarity depending on the transcriptional unit they derive from. The Ref line presents the reference genome (TYLCV-Mild, AF071228.1) and the logo line is the sequence as reported by next generation sequencing, which in our case are the same. *B* - Histogram (*MISIS* software) representing TYLCV-Mild-derived siRNAs (21 nt, library from TYLCV-treated tomato, replication 2) focusing in a limited region where the siV2_352s (352-372 nt) (indicated with an

*arrow*), corresponding to 511-531 nt hot spot for TYLCV-IL (X15656.1), is presented as a cold spot (2 reads). The y-axis indicates the counts of vsiRNA reads (positive values represent vsiRNAs mapped to the sense of the viral genome, negative values represent vsiRNAs mapped in antisense direction to the viral genome). *Blue bars* show the 5'-end of each 21-nt vsiRNA and *red bars* show the 3'-end of each 21-nt vsiRNA. Due to the genome organization of TYLCV, the peaks of the reads above and below the x-axis correspond to siRNAs of positive or negative polarity depending on the transcriptional unit they derive from. The Ref line presents the reference genome (TYLCV-Mild, AF071228.1) and the logo line is the sequence as reported by next generation sequencing, which in our case are the same.