Identification of Eight Solanum (subgenus Leptostemonum) Species as Novel Natural Hosts of Tomato chlorosis virus in Brazil

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DISEASE NOTES

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Leaves of *Solanum* (subgenus *Leptostemonum*) species with crinivirus-like symptoms (interveinal to overall chlorosis and reduced vigor) were collected during field surveys in Brasília–DF, Brazil (2012 to 2017). Samples were obtained from *Solanum stramoniifolium* Jacq. (CR-073 and CR-076 isolates), *S. sessiliflorum* Dunal (CR-075), *S. subinerme* Jacq. (CR-079), *S. jamaicense* Mill. (CR-082), *S. mammosum* L. (CR-081), *S. scuticum* M. Nee (CR-198), and *S. velleum* Roem & Schult. (CR-199). Prevalence of symptomatic plants was high (80 to 100%), with mild symptoms in most of the sampled species except for early-infected *S. stramoniifolium* and *S. mammosum*,

in which symptoms were severe. A symptomatic *S. paniculatum* L. plant (found as a weed in a tomato field) was collected (CR-114) in Venturosa (Pernambuco State, Brazil). Leaves of tomato plants with crinivirus-like symptoms were also obtained (CR-115) in the same field in Venturosa. All samples were tested for the presence of two criniviruses known to infect solanaceous plants, *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV). Total nucleic acids were extracted using TRIzol from tissues of symptomatic and healthy controls (i.e., seedlings of all *Solanum* species maintained under controlled conditions). The universal primer pair HS-11/HS-12 (which amplifies a 587-bp RNA-2 segment encompassing the HSP70h protein gene) was used in the RT-PCR step (Dovas et al. 2002). A nested-PCR assay was performed with ToCV- (ToC-5/ToC-6) and TICV-specific (TIC-3/TIC-4) primers (Dovas et al. 2002). Only ToCV-specific amplicons (~463-bp) were detected in all symptomatic plants. Neither TICV- or ToCV-specific amplicons were detected in the healthy controls. Purified PCR products were directly sequenced at the Genomic Analysis Laboratory (CNPH, Brazil). Alignment of the sequences (419 nt) obtained for the eight *Solanum* isolates (GenBank accession nos. KY400116 to KY400119, KT727959, KX398666, KY400124, KY400132, and KY400133) and the tomato isolate CR-115 (KY400125) showed 99.2 to 100% identity among them and with a tomato-infecting ToCV isolate from Brazil (EU868927). ToCV infection was confirmed via dot-blot hybridization with a coat protein-derived RNA probe (436 nt fragment of RNA-2; primers MA-380/MA-381) (Fortes et al. 2012) labeled with digoxigenin (DIG)-11-UTP and with the chemiluminescent substrate CDP Star kit (Roche Diagnostics). Nucleic acid extracts of all isolates and their respective healthy controls were included in the dot-blot assays. Positive hybridization results were observed only in symptomatic samples. ToCV isolates from *S. stramoniifolium* and *S. mammosum* were transmitted to tomato 'TX468 RG' in controlled assays using adults of *Bemisia tabaci* MEAM1 as vectors. Even though Koch's postulates were not fulfilled for ToCV as causal agent of the disease syndrome observed in all the *Solanum* species sampled, this virus has already been reported infecting members of the subgenus *Leptostemonum* (Fonseca et al. 2016), suggesting a widespread susceptibility of the plants from this taxon. These host species are widespread in South America either as cultivated and weed species or as members of the native flora with occurrence near many tropical and semitropical tomato-producing areas across the

entire continent (Arruabarrena et al. 2014; 2015). Hence, these *Solanum* species (with long vegetative cycle) might have a significant epidemiological role as they might function as year-round reservoirs of ToCV.

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