**Natural Infection of Tomatoes (*Solanum lycopersicum*) by Euphorbia yellow mosaic virus Isolates across Four Brazilian States.**

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Severe yield losses induced by a complex of whitefly–transmitted *Begomovirus* species (family *Geminiviridae*) have been reported in tomatoes in Brazil (Reis et al. 2020). Nine isolates were obtained from tomato plants exhibiting begomovirus–like symptoms (*viz*. apical and interveinal chlorosis, yellow spots, and stunting) during independent field surveys: one isolate in Sumaré, São Paulo–SP State (isolate SP–066) in 2001, two in Serra Negra, Minas Gerais–MG (MG–012 and MG–016) in 2002, five in Caxias do Sul, Rio Grande do Sul–RS (RS–039, RS–045, RS–046, RS–047 and RS–058) in 2011 and one in Domingos Martins, Espírito Santo–ES (ES–148) in 2016. Disease incidence across all sampled fields ranged from 30% (in Domingos Martins–ES) to 90% in Sumaré–SP. Total DNA extraction was done by a modified CTAB method (Boiteux et al., 1999). Begomovirus infection was confirmed in all isolates by selective amplification of viral DNA–A segments using the primer pairs 'PAL1v1978 / PAR1c496' (Rojas et al., 1993) and 'BegomoAFor1' / 'BegomoARev1' (Ha et al., 2006), which produce two large and non–overlapping segments (≈1120 bp and ≈1205 bp, respectively). These PCR amplicons were initially characterized via direct Sanger dideoxy sequencing at CNPH. BLASTn analysis of the partial DNA–A genomes of these nine isolates indicated identity levels of 95–97% to three euphorbia yellow mosaic virus (EuYMV) reference isolates (= KY559532, JF756674, and KY559583) found infecting the weed *Euphorbia heterophylla* L. The entire DNA–A (2,609 nts = MN746971) and DNA–B (2,579 nts = MN746970) components of the MG–016 isolate were obtained via high–performance sequencing using Illumina HiSeq 2500 system (Macrogen Inc., South Korea). Sequences were assembled with the CLC Genomics Workbench program 10. Contigs were validated by BLASTx and BLASTn and compared to the ssDNA virus database at NCBI (www.ncbi.nlm.nih.gov). The fully–

characterized MG–016 isolate displayed identity levels ranging from 97 to 99% to the EuYMV reference isolates as well as similar genomic features such as the conserved TATA box, nonanucleotide, and iterons (that were in agreement with a cognate nature of the DNA–A and DNA–B components). A partial sequence of the DNA–B genome was also obtained for the MG–012 isolate (MT7831942). The isolates MG–012 and MG–016 were found in mixed infections with tomato severe rugose virus (ToSRV) and tomato golden vein virus (TGVV), respectively. In addition, the complete DNA–A genomes of ES–148 (MN746972) and SP–066 (MN782438) were also obtained via a combination of primer walking and Sanger dideoxy sequencing, displaying 96–98% identity to EuYMV isolates. To our knowledge, this is the first report of multiple and independent events of natural infection of tomatoes by EuYMV isolates. Our results confirm the natural host status of tomatoes to EuYMV isolates as indicated in previous infectivity assays using biolistic inoculation (Barreto et al., 2013). The weed *E. heterophylla* is widely disseminated and very often present within tomato fields due to its higher levels of tolerance to the major herbicide (metribuzin) employed in this crop. Therefore, this weed may act as a persistent reservoir of tomato–infecting EuYMV isolates, which may allow the selection of viral populations potentially more adapted to this vegetable crop.

***References:***

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