DIVERSITY OF TOSPOVIRUSES IN BRAZIL

R. de O. Resende, L. Posser, T. Nagata, I.C. Bezerra, M.I. Lima, E.W. Kitajima, and A.C. de Avila*

Departamento Biologia Celular, Universidade de Brasilia, 70910 Brasilia DF, Brazil

The genus *Tospovirus* within the Bunyaviridae family is comprised of five described virus species which use as the main descriptor the serological divergency of the nucleocapsid protein (N) and the primary nucleotide sequence of the nucleocapsid gene encoded by the S RNA. Surveys conducted in six states of Brazil and 150 samples of eight botanical species showing typical tospoviruses symptoms were randomly collected. The tospovirus species were identified by DAS-ELISA using a panel of polyclonal antisera against the N protein of tomato spotted wilt virus, tomato chlorotic spot virus, groundnut ring spot virus, and impatiens necrotic virus. The results showed that 69 isolates (46%) were identified as TSWV, 54 (36%) as TCSV, and 12 (8%) as GRSV. Fifteen isolates (10%) did not show any reaction in ELISA with the antisera used. Typical tospovirus particles were found in cells of plants infected with these viruses that may represent new tospovirus species. INSV isolates were not detected in this survey, maybe due to the very low number of ornamental plants tested. Although the three species were present in most of the states surveyed, TSWV was detected mostly in the Federal District and Paraná State while TCSV was found mainly in São Paulo State. In another recent survey in the northeastern part of Brazil only GRSV was present in more than 196 samples tested. This virus species distribution may indicate specific interactions among tospovirus species and the prevalent thrips species in different regions of Brazil.

Four possibly new tospovirus species were isolated from chrysanthemum (Chry 1), zucchini (1038), onion, and tomato (Tom), and are currently being characterized. The N protein of all four new isolates were purified and specific antisera were raised. The ELISA results and Western blot analyses demonstrated that they are serologically distinct from the already characterized tospovirus species. Using polymerase chain reaction employing two conserved primers in the genus, one covering the first 15 nucleotides of the nontranslated region of the N gene and the second one 18 nucleotides long located approximately 300 bases downstream from the AUG start codon, we were able to amplify a fragment of about 430 bases of all isolates except the onion isolate. The PCR amplified fragments from the isolates Chry 1, 1038, and Tom were cloned and sequenced. Comparisons of the serological results and the nucleotide sequence analysis among the characterized species and the new Brazilian tospoviruses isolates will be discussed.