DIVERSITY OF TOSPOVIRUSES IN BRAZIL

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Tomato spotted wilt virus (TSWV) has been reported in various crops in Brazil since the nineteen forties, causing the most frequently disease "vira-cabeça do tomateiro" on tomato [1]. Within the last decade the tospoviruses have caused significant crop losses in Brazil on tomato, sweet-pepper, lettuce and recently on ornamental crops. Previously, all tospovirus isolates studied in Brazil were referred to as TSWV. However, recent studies on several Brazilian tospovirus isolates have drastically changed this picture, revealing the occurrence of more species in Brazil than in any other part of the world. The main reasons for this might be due to the sub(tropical) and tropical climates which support high populations of thrips vectors the year around, as well as the large number of tospovirus plant hosts. So far, very limited information is available on tospovirus epidemiology, the population dynamics of the thrips species, and the relations between thrips and tospoviruses in Brazil. The thrips species, *Frankliniella schultzei*, has been reported as an important vector although other efficient thrips vector species are present in the country.

Divergence of the nucleocapsid protein (N) encoded by the S RNA, detected through serological and molecular approaches are the main parameters for tospovirus species classification, and has resulted, next to TSWV, in the identification of two other species, tomato chlorotic spot virus (TCSV) and groundnut ringspot virus (GRSV), in Brazil [2]. These two species comprise the serogroup II (Table 1) and display a broad host range, as described for TSWV. The homology of the N protein sequences was distinctly lower than that of TSWV (Table 1). Additionally, three new candidate tospovirus species have recently been characterised in Brazil: one (Chry 1) was isolated from *Chrysanthemum morifolium*, one (BR-09) from zucchini (*Cucurbita pepo*), and one (BR-10) from onion (*Allium cepa*). The Chry1 isolate was found in the State of São Paulo infecting chrysanthemum [3] and in tomato in the State of Minas Gerais. The chrysanthemum plants were found with necrotic lesions surrounded by yellow areas on the leaves, followed by necrosis on the stems, peduncles and floral receptacles. Its experimental host range was similar to that of TSWV. By contrast, the zucchini (BR-09) and onion (BR-10) isolates displayed a restricted experimental host range. In zucchini, BR-09 induces systemic chlorotic symptoms, necrosis of basal leaves and malformation with narrow and curled leaf blades and reduction in plant growth . This isolate induced local lesions on *Nicotiana occidentalis* and systemic infection on several other cucurbitaceae species, *N. benthamiana*, and *Gomphrena globosa*. tospovirus on onion was for the first time isolated 1981 [4] . Recently, a disease, named "Sapeca" and caused by a tospovirus, was detected was detected in the onion crop cultivar 'Granex 33, in the region of the SubMedio São Francisco, in the State of Pernambuco. The early symptoms displayed were systemic elliptical white lesions on leaves and later numerous necrotic eye-like spots on the flower stems which often collapsed. The flowers in the umbel wilted one by one and aborted. Total crop losses in several onion fields were often observed. Inoculation of *Chenopodium amaranticolor*, *C. quinoa*, *Datura stramonium*,

Gomphrena globosa and *Portulaca oleracea* resulted in only local lesions throughout and apart from onion only the species *N. benthamiana* and *N. rustica* became systemically infected. Polyclonal antisera raised against the (N) protein of each isolate strongly support the hypotheses that these three isolates form new serogroups within *Tospovirus* genus. In DAS-ELISA, they strongly reacted only with their homologous antigens (purified N protein and infected plant extract) (Table 1) with almost no reactions with heterologous antisera of TSWV, TCSV, GRSV, INSV, WSMV, Chry1, BR-09 and BR-10. The onion isolate shows a strong reaction in homologous and heterologous ELISA assays with a tospovirus, iris yellow spot virus (IYSV), isolated from *Iris* spp in the Netherlands [5] indicating they may be strains of the same virus. The results presented here shows that three new serogroups has to be added to the four established serogroups in the *Tospovirus* genus (Table 1). The host specificity (narrow and broad range), serology and amino acid sequence analysis of the (N) gene of these three isolates confirmed that they represent new tospovirus species (Table 1) (90% of amino acid homology as the threshold line between species and strains) as established at the last International Symposium on Tospoviruses held in Taiwan in 1995. Serological studies and comparison of the N protein sequence (90.5% amino homology) showed that the onion isolate and IYSV are strains of the same virus and form serogroup V. The zucchini isolate (serogroup VI) is proposed to be designated zucchini lethal chlorotic virus (ZLCV) and the Chry1 isolate (serogroup VII) *Chrysanthemum* stem necrosis virus (CSNV)(Table 1).

Table 1 - Serogroups and N gene amino acid identity/similarity (%) of nine tospovirus species.

identity (%)

Sero group	I	II		III	IV		V		VI	VII
	TSWV	TCSV	GRSV	INSV	GBNV	WSMV	$IYSV_{NL}$	$IYSV_{BR}$	ZLCV	CSNV
TSWV	100	77	78	55	32	33	35	35	72	77
TCSV	85	100	81	53	30	31	33	32	73	75
GRSV	86	87	100	54	33	33	33	33	75	75
INSV	63	64	62	100	31	32	31	30	52	58
GBNV	39	35	40	37	100	86	43	44	32	33
WSMV	40	38	40	38	92	100	42	43	31	32
$IYSV_{NL}$	41	40	42	39	51	52	100	90	32	35
$IYSV_{BR}$	42	41	42	39	51	52	93	100	32	34
ZLCV	79	80	80	61	38	39	40	39	100	82
CSNV	83	83	81	67	40	40	43	43	87	100

similarity (%)

(TSWV) tomato spotted wilt virus; (TCSV) tomato chlorotic spot virus; (GRSV) groundnut ringspot virus; (INSV) *Impatiens* necrotic spot virus; (CSNV) *Chrysanthemum* stem necrosis virus (partially sequenced) = Chry1 isolate from Brazil; (WSMV) watermelon silver mottle virus; (GBNV) groundnut bud necrosis virus, also reported as groundnut bud necrosis virus; ($IYSV_{NL}$) *Iris* yellow spot virus from the Netherlands, ($IYSV_{BR}$) = BR-10 onion isolate from Brazil; (ZLCV) zucchini lethal chlorotic spot virus = BR-09 zucchini isolate from Brazil.

References

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