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SHORT RESEARCH NOTES

Capsicum chlorosis virus infecting *Capsicum annuum* in the East Kimberley region of Western Australia

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Abstract. Capsicum chlorosis virus (CaCV) was detected in field grown *Capsicum annuum* from Kununurra in north-east Western Australia. Identification of the Kununurra isolate (WA-99) was confirmed using sap transmission to indicator hosts, positive reactions with tospovirus serogroup IV-specific antibodies and CaCV-specific primers, and amino acid sequence comparisons that showed >97% identity with published CaCV nucleocapsid gene sequences. The reactions of indicator hosts to infection with WA-99 often differed from those of the type isolate from Queensland. The virus multiplied best when test plants were grown at warm temperatures. CaCV was not detected in samples collected in a survey of *C. annuum* crops planted in the Perth Metropolitan area.

Additional keywords: tospovirus, identification, host range, symptomatology, sequence.

Capsicum chlorosis virus (CaCV; Family *Bunyaviridae*, Genus *Tospovirus*) causes a serious disease of capsicum (bell pepper) and sweet chilli (*Capsicum annuum*) in Queensland, where it also infects tomato (*Lycopersicon esculentum*) (McMichael *et al*. 2002). In July 2004, plants with symptoms of chlorotic mottle and deformation in young leaves and plant stunting were observed in a sweet chilli crop in the Ord river irrigation area at Kununurra in the East Kimberley region in north-east Western Australia. Two symptomatic plants were transported to Perth and transplanted into pots. Leaf sample extracts from each plant were tested by ELISA using antibodies specific to tospovirus serogroups I–III, *Tomato spotted wilt virus* (TSWV; Family *Bunyaviridae*, Genus *Tospovirus*) and *Cucumber mosaic virus* (CMV, Family *Bromoviridae*, Genus *Cucumovirus*). Although the symptoms present in both plants resembled those caused by TSWV, sample extracts from one of them failed to react with any of the antibodies while sample extracts from the other reacted only with antibodies to CMV. When leaf sap from the former was inoculated to plants of *Nicotiana benthamiana* and capsicum cv. Rialto, symptoms resembling those caused by CaCV developed. In *N. benthamiana*, symptoms consisted of chlorotic blotches in inoculated leaves and systemic chlorotic mottle, which was soon followed by wilting and plant death. In capsicum, they were chlorotic ringspots in inoculated leaves followed by systemic chlorotic mottle, interveinal chlorosis, leaf deformation and plant stunting. Leaf extracts from the field-infected sweet chilli plant were positive with tospovirus serogroup IV antibodies in ELISA, suggesting presence of a member of this serogroup, to which CaCV belongs (McMichael *et al*. 2002).

An 828 bp PCR product from the nucleocapsid (N) gene of WA-99 was amplified using primers CaCV.NPR (ATG TCT AAC GTT AGG CAA CTT A) and CaCV.NPF (TTA CAC TTC TAT AGA AGT ACT A), cloned using the pGEM-T Easy Vector System (Promega) and sequenced with an Applied Biosystems Inc. (ABI) automated sequencing system. After removal of the primers, this sequence (GenBank accession number AY839642) was compared with those from two already characterised isolates of CaCV from Queensland (GenBank accessions AY036057 and AY036058) using CLUSTALW (Thompson *et al*. 1994) and found to be 96% identical at the nucleotide level, and >97% identical at the amino acid level, over a 784 nt overlap.

Using infective *N. benthamiana* sap extracted in 0.05 M phosphate buffer, pH 7.2, containing 0.01 M sodium sulphite as inoculum and the abrasive 'celite', isolate WA-99 was

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Table 1. Comparative symptomatology of isolate WA-99 with that of CaCV type isolate 958

Host	Cultivar used in this research	Type isolate 958[A]		Isolate WA-99	
		Inoculated leaf	Systemic	Inoculated leaf	Systemic
Amaranthaceae					
Gomphrena globosa	–	NR[B]	NI	NS[C]	NI
Asteraceae					
Lactuca sativa (lettuce)	Green Mignonette	AS	Mo, NS	NS, NR[C]	NI
Chenopodiaceae					
Chenopodium amaranticolor	–	CS, NS	NI	NS[C]	NI
Chenopodium quinoa	–	CS, NS	Mo, W	NS[C]	NI
Convolvulaceae					
Ipomoea setosa	–	NT	NT	NS[D]	NI
Cruciferae					
*Brassica juncea (*mustard)	Tendergreen	NT	NT	NI	NI
Brassica napus (canola)	Pinnacle	NT	NT	NI	NI
Cucurbitaceae					
Citrullus lanatus (watermelon)	Sugar Baby	NS	NI	AS[C]	NI
Cucumis sativus (cucumber)	Pronto	CS	NI	NS[C]	NI
Cucurbita pepo (zucchini)	Blackjack	NT	NT	NS[C]	NI
Cucurbita pepo (squash)	Green Buttons	NI	NI	NS[D]	NI
Cucurbita maxima (Jarahdale pumpkin)	Queensland Blue	NT	NT	NI	NI
Fabaceae					
Arachis hypogaea (peanut)	–	AS	Mo, NS, St	NS[C]	NI
Cajanus cajan (pigeon pea)	–	NT	NT	NI	NI
Phaseolus vulgaris (French bean)	Purple King	NR, CS	NI	NS, CS[C]	NI
Pisum sativum (pea)	Dundale	NT	NT	NS[C]	NI
Vicia faba (faba bean)	Fiord	NT	NT	NS[C]	NI
Vigna unguiculata (cowpea)	–	NR	NI	CSR[C]	N1
Solanaceae					
Capsicum annuum (capsicum, −gene *Tsw*)	Rialto	AS	Mo, LD, IVC	FCR[C]	Mo, LD, IVC, St[C]
Capsicum annuum (capsicum, +gene *Tsw*)	Yatasto	AS	Mo, LD, IVC	FCR[C]	Mo, IVC, St[C]
Capsicum chinense PI 152225		AS	Mo, LD, St	CS[C]	CS, CR, LD, IVC, St[C]
Capsicum chinense PI 159236		AS	Mo, LD, St	NS[C]	NI
Capsicum chinense AVRDC 00943		AS	Mo, LD, St	NS[C]	NI
Lycopersicon esculentum (tomato, −*Sw-5*)	Grosse Lisse	NS	Mo, NS	FCB[C]	NI
Lycopersicon esculentum (tomato, +*Sw-5*)	Zodiac	AS	Mo, NS	FCB[C]	NI
Nicotiana benthamiana		CR	Mo, W, D	CS[C]	Mo, St, W, D[C]
Nicotiana debneyi		NT	NT	FNR[C]	NI
Nicotiana glutinosa		NR	NI	NS[C]	NI
Petunia hybrida (petunia)	Celebrity White	CS, NR	Mo	NS[C]	NI

[A]Type isolate data from McMichael *et al*. (2002).
[B]Coded symptom reactions: AS, asymptomatic infection; CR, chlorotic ringspots; CS, chlorotic spots; CSR, chlorotic blotches with red centres; FCB, faint chlorotic blotches; FCR, faint chlorotic ringsots; FNR, faint necrotic ringspots; NR, necrotic ringsots; NS, spreading necrotic spots; Mo, mosaic or mottle; IVC, interveinal chlorosis; LD, leaf deformation; St, stunting; W, wilting; D, death; NT, not tested; NI, not infected.
[C]Virus detected with serogroup IV antibodies in ELISA.
[D]Necrotic spot lesions developed in inoculated leaves but no virus was detected with serogroup IV antibodies in ELISA.

difficult to transmit from this host to most host species by sap inoculation, so plants were often inoculated repeatedly to establish infection. Also, in initial tests, symptoms developed slowly in plants grown in an air-conditioned glasshouse kept at ∼18°C after inoculation. Subsequently, the inoculated plants were always placed in a heated glasshouse kept at ∼25°C as symptoms developed rapidly under these conditions. This behaviour indicates that the virus is adapted to multiply best when infected plants are growing under warm conditions.

Using infective *N. benthamiana* sap, isolate WA-99 was inoculated to a range of indicator and crop species belonging to eight different plant families, most of which were tested previously by McMichael *et al*. (2002) in inoculations with CaCV type isolate 958 (Table 1). Symptom development was monitored and extracts from inoculated and tip leaves of each host were tested separately by ELISA using antibodies to tospovirus serogroup IV. Like isolate 958, WA-99 infected inoculated leaves of (i) a capsicum cultivar and three *C. chinense* accessions with TSWV resistance gene *Tsw*, and (ii) a tomato cultivar with TSWV resistance gene *Sw-5*. However, unlike isolate 958, WA-99 infected only two (*C. chinense* PI 152225 and capsicum cv. Yatasto) of these four TSWV-resistant genotypes systemically and did not spread out of inoculated leaves of the TSWV-susceptible tomato cultivar used. In addition, unlike isolate 958, isolate WA-99 caused only necrotic spots, which tended to enlarge considerably over time, in inoculated leaves of *Arachis hypogaea*, *C. chinense* PI 159236 and AVRDC 00943, *C. amaranticolor*, *Chenopodium quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *N. glutinosa* and *Petunia hybrida*. The results with *C. chinense* PI 159236 and AVRDC 00943 suggest that they may posses strain specific hypersensitive resistance to isolate 99 but not to isolate 958, possibly caused by an as yet unidentified strain specific, CaCV hypersensitivity gene. Isolate WA-99 also failed to infect *A. hypogaea*, *C. quinoa*, *Lactuca sativa* or *P. hybrida* systemically. Moreover, unlike isolate 958, it caused only asymptomatic infection, chlorotic spots or chlorotic blotches in inoculated leaves of *Citrullus lanatus*, tomato and *Vigna unguiculata*. In two instances when necrotic spots developed in inoculated leaves, serological confirmation of virus presence by ELISA was lacking. This was presumably because the virus broke down rapidly within the necrotic tissue. Necrotic local lesion development in *Ipomoea setosa* suggests that the *Convolvulaceae* may constitute an additional plant family that CaCV infects.

In November 1999, commercial capsicum plantings north and south of the Swan River in the Perth Metropolitan area were surveyed for tospovirus infection. For this, 100 leaf samples were collected at random from each of 11 capsicum crops and extracts from them tested by ELISA using antibodies specific to TSWV and tospovirus serogroup IV. TSWV was detected in six of the crops with individual infection incidences up to 40%. Extracts from freeze-dried material infected with CaCV (supplied by Dr Denis Persley, DPI&F, Brisbane), used as a control in the serogroup IV tests, reacted positively, but none of those from the capsicum survey samples did so.

This study confirms the presence of CaCV in the East Kimberley region of Western Australia based on symptomatology in capsicum, reactions in indicator plants, serology, RT–PCR amplification and cDNA sequencing. To establish its importance to the Western Australian vegetable industry, surveys are needed to determine its occurrence within the major vegetable growing areas outside the Perth Metropolitan area, especially those in the Ord river irrigation area, Broome and Carnarvon districts. CaCV is the third member of the genus *Tospovirus* to be found in Western Australia, the others being TSWV (Latham and Jones 1997) and *Iris yellow spot virus* (IYSV; Family *Bunyaviridae*, Genus *Tospovirus*) (Coutts *et al*. 2003).

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Note added in proof: CaCV is now known to occur in Thailand as well as Australia. *Reference*: Premachandra W. T. S. D. *et al*. (2005) *Ceratothripoides claratris*, a new vector of a Capsicum chlorosis virus isolate infecting tomato in Thailand. *Phytopathology* **95**, 659–663.

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