

## SHORT RESEARCH NOTES

**First report of *Tomato spotted wilt virus* in chickpea (*Cicer arietinum*) in Australia**

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**Abstract.** *Tomato spotted wilt virus* (genus *Tospovirus*) is recorded on chickpea (*Cicer arietinum*) in Australia for the first time. It caused shoot tip symptoms of wilting, necrosis, bunching and chlorosis, followed by premature death of plants.

Chickpea (*Cicer arietinum*) is a major, cool-season pulse crop in Australia, and is grown in tropical, sub-tropical and temperate regions, mostly in rotation with cereals. Australia is the seventh largest producer in the world and the majority of the crop is exported (E. J. Knights, personal communication).

Virus diseases have been a significant production problem in chickpeas in northern New South Wales (NSW) in some years since 1992 (M W Schwinghamer, unpublished data). In Australia, ten viruses have been found to infect chickpea naturally: *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV) and *Cucumber mosaic virus* (CMV, Jones and Coutts 1996), *Pea seed-borne mosaic virus* (Latham and Jones 2001), *Beet western yellows virus* (BWYV), *Subterranean clover stunt virus*, at least one mastrevirus related to Chickpea chlorotic dwarf virus (M W Schwinghamer, M A Schilg, J N Parry, E K Dann and J E Thomas, unpublished data), *Bean leafroll virus*, *Subterranean clover red leaf virus* (SCRLV, Schwinghamer *et al.* 1999), and *Lettuce necrotic yellows virus* (LNYV, Behncken 1983). Symptoms of wilting and/or necrosis of shoot tips were associated with three of these viruses: AMV (Latham and Jones 2001), BYMV (McKirby *et al.* 2000) and LNYV (Behncken 1983).

In surveys of chickpeas in NSW, from 1992 onwards, tip-wilting symptoms first became noticeable in 1999 at some localities in the north of the State. The tip-wilting syndrome in chickpea included wilting and/or necrosis of shoot tips

(Fig. 1), or chlorosis and bunching of shoot tips (Fig. 2), accompanied by reddening, chlorosis, and subsequent death of entire plants. During surveys in central and northern NSW in 2001 and 2002 and southern Queensland in 2003, tip-wilting symptoms were noted again, though the incidence was usually less than 1%. Plants with tip wilting from the 2002 and 2003 surveys were indexed by ELISA, tissue blot immunoassay (TBIA) or RT-PCR for the viruses listed above and also *Broad bean wilt virus 1*, *Broad bean wilt virus 2* and *Clover yellow vein virus*, except that Queensland samples were not tested for SCRLV. As in 1999 and 2001, there was no clear correlation between the tip-wilting syndrome and presence of a particular virus.

A selection was made of 22 plants with tip wilting from three properties near Moree, one near Gunnedah, and five near Dubbo, NSW in 2002 and a further ten plants from Kingaroy Research Station, Queensland in 2003. These plants were tested for *Tomato spotted wilt virus* (TSWV) by ELISA (Bio-Rad PlantTest ELISA kit). TSWV was detected in 11/22 plants from NSW (ten from Moree, one from Gunnedah and none from Dubbo) and 6/10 plants from Queensland. Plants with other viruses were infrequent among the total of 32. Among the TSWV-negative plants, there was one with AMV, one with a mastrevirus and one with BWYV and SCRLV from NSW and three with CMV from Queensland. One TSWV-positive plant from NSW was also infected with BWYV and SCRLV. A further 74 plants from the Moree crops were tested by TBIA for TSWV. These plants were either healthy or had



**Fig. 1.** Wilting and necrosis of shoot tip in desi chickpea cv. Amethyst infected naturally with TSWV (field specimen DAR 76605).



**Fig. 2.** Chlorosis and bunching of shoot tips in desi chickpea cv. Jimbour infected with TSWV (field specimen DAR 76603).



**Fig. 3.** Wilting and necrosis of shoot tips and general plant collapse in desi chickpea cv. Amethyst graft-inoculated with TSWV-positive field specimen (DAR 76602) of chickpea.



**Fig. 4.** Small chlorotic leaves on shoot tip of desi chickpea cv. Amethyst mechanically inoculated with a TSWV isolate from chickpea (originally from field specimen DAR 76606).

virus symptoms other than tip wilting, and all were negative for TSWV.

Six of the NSW plants (DAR 76601 to 76606), which were positive for TSWV and negative for other viruses, were used as inoculum for mechanical and/or graft transmission to chickpea cv. Amethyst in the glasshouse. All produced severe shoot tip symptoms of wilting and necrosis (Fig. 3), or small chlorotic leaves (Fig. 4). Time to earliest appearance of symptoms was 19 days, but commonly 50 days or longer and in one instance, 80 days. Death of plants occurred within 5–9 days after first appearance of symptoms for the more severe isolates (DAR 76602, 76603 and 76605) and up to 50 days after for milder isolates (DAR 76601, 76604 and 76606). The presence of TSWV was confirmed by ELISA in source and inoculated plants for all six isolates, and in isolate DAR 76606 by TSWV-specific reverse transcription (RT) PCR. For RT-PCR, RNA was extracted from leaf tissue using the Concert RNA reagent (Invitrogen) and cDNA prepared using Superscript II (Invitrogen) as per the manufacturer's instructions. Primers TSWV.NPR (5' ATGTCTAAGGTTAAGCTCACTA 3') and TSWV.NPF (5' TTAAGCAAGTTCTGTGAG TT 3') were used at an annealing temperature of 56°C, which allowed amplification of the complete nucleocapsid gene of 777 nucleotides. The nucleotide sequence obtained (GenBank Accession AY611529) was > 98% identical to other TSWV sequences on GenBank.

Although TSWV was confirmed as causing tip wilting and top necrosis in chickpea, it was probably not the only cause of such symptoms. It was not detected in 15/32 field samples with tip wilting.

This is the first record of TSWV in chickpea from Australia. Apparently, the only other record of TSWV in chickpea is from Brazil (Boiteux *et al.* 1996), where symptoms of stunting, general chlorosis and necrosis of new growth were reported. Voucher specimens from NSW have been lodged with the Scientific Collections Unit, NSW Agriculture, Orange, as Accessions DAR 76601 to 76606.

Queensland isolates have been lodged in the Department of Primary Industries and Fisheries Culture Collection (Viruses) at Indooroopilly under Accession Numbers 1672 and 1674–1678.

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